Post-harvest rind pitting studies on 'Valencia' orange

by

Jacques Louis Ehlers

Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Agriculture (Horticultural Science) at the University of Stellenbosch

Supervisor: Dr P.J.R. Cronjé

Co-Supervisor: Prof K.I. Theron

Faculty of Agriscience

Dept. of Horticultural Science

Stellenbosch University

South Africa

Co-Supervisor: Dr F. Alférez

Citrus Research and Education Center

Institute of Food and Agricultural Sciences

University of Florida

United States of America

March 2016

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: **March 2016**

SUMMARY: Post-harvest rind pitting on 'Valencia' Oranges

Post-harvest rind pitting is a non-chilling related physiological rind disorder that affects various citrus cultivars and reduces fruit value. This disorder is characterised by the collapse of the flavedo sub-epidermal cells, however the main cause of this disorder is unknown, but it is aggravated by changes in relative humidity (RH) and rind water status. Studies were conducted on 'Turkey' and more susceptible 'Benny' valencia oranges in Limpopo and Mpumalanga South Africa. The effect of fruit position, maturity and size on fruit susceptibility to this disorder was investigated and it was found that fruit from the outside of the canopy are more susceptible to this disorder probably due to greater exposure to variation in environmental conditions than fruit from the inside of the canopy. More mature fruit were also found to be slightly more susceptible, however size did not influence incidence of this disorder. Various plant growth regulators were also evaluated to prevent pitting. The application of the synthetic auxins 2,4-dichlorophenoxy acetic acid (2,4-D) and 3,5,6 trichloro-2-pyridiloxyacetic acid (3,5,6 TPA) at 50 % petal drop (2,4-D) or after physiological fruit drop (2,4-D or 3,5,6-TPA) reduced the incidence of post-harvest pitting. Application of s-abscisic acid 1 week before harvest was also found to reduce incidence of this disorder, however gibberellic acid applied in January did not reduce the incidence of post-harvest rind pitting. A systemic fungicide thiabendazole (TBZ) which reduces the incidence of chilling injury also reduced post-harvest pitting incidence if applied before fruit were subjected to stress inducing environmental conditions. Pre-harvest foliar application of TBZ 1 week before harvest and post-harvest dip treatments directly after harvest reduced post-harvest weight loss and incidence of this disorder. A citrus industry survey was conducted to estimate the financial impact of this disorder at foreign and local markets on producers. Markets generating higher prices had a lower tolerance for incidence of post-harvest rind pitting than lower priced markets and are therefore seen as high-risk. Due to this large reduction in market price for fruit with the disorder, treatments found during this study might be cost effective.

OPSOMMING: Na-oes gepokteskil van 'Valencia' lemoene

Gepokteskil is 'n na-oes fisiologiese skildefek wat nie met koueopberging by lae temperature gedurende geassosieer word nie en kan verskeie sitruskultivars affekteer en die waarde van vrugte verminder. Die defek word gekenmerk deur die ineenstorting van die sub-epidermale flavedo selle en alhoewel die hoof oorsaak van die defek nie bekend is nie word dit vererger deur variasie in relatiewe humiditeit (RH) en skil water status in die na-oes omgewing. Hierdie studie was op 'Turkey' en die meer vatbaar 'Bennie' valencia lemoene in Limpopo en Mpumalanga Suid-Afrika gedoen. Die effek van voor-oes faktore soos vrug posisie, -volwassenheid en -grootte op die vatbaarheid van vrugte vir hierdie defek is ondersoek. Dit is bevind dat die vrugte aan die buitekant van die blaardak meer vatbaar is vir hierdie defek, waarskynlik as gevolg van groter blootstelling aan variasie in omgewings toestande as vrugte vanaf die binnekant van die blaardak. Daar is ook gevind dat meer volwasse vrugte ʼn hoër vatbaarheid vir die defek het, maar dat vruggrootte dit nie beïnvloed nie. Die effektiwiteit van verskeie plant groei reguleerders om die voorkoms van gepokteskil te verminder is geëvalueer. Die toediening van sintetiese ouksiene 2,4-dichlorofenoksie asynsuur (2,4-D) en 3,5,6 trichloro-2-piridiloksi asynsuur (3,5,6 TPA) by 50 % blomblaarval (2,4-D) of na fisiologiese vrug val (2,4-D en 3,5,6-TPA) verminder die voorkoms van na-oes gepokteskil. So ook het die toediening van s-absisiensuur een week voor-oes die voorkoms van hierdie defek verlaag, maar daarteenoor het gibberelliensuur in Januarie geen effek op die voorkoms van na-oes gepokteskil gehad nie. Dit is al voorheen bewys dat 'n sistemiese swamdoder thiabendasool (TBZ) koueskade kan verminder, en TBZ het ook die voorkoms van na-oes gepokteskil verminder mits dit voor stres-induserende omgewings toestande aangewend word. TBZ toediening een week voor-oes as ʼn blaar bespuiting of as ʼn doop behandeling direk na oes verminder gewig verlies en die voorkoms van die afwyking. ʼn Sitrusbedryf opname was gedoen om die geskatte finansiële impak van gepokteskil in buitelandse en plaaslike markte op produsente te bepaal. Markte wat hoër pryse aanbied het ʼn laer toleransie vir die voorkoms van na-oes gepokteskil as markte wat laer pryse aanbied en word gesien as hoë risiko. As gevolg van ʼn drastiese afname in markprys vir vrugte met gepokteskil kan van die behandelings in die studie moontlik koste effektief wees.

ACKNOWLEDGEMENTS

I thank the following people and institutions for their various contributions throughout the completion of this study:

My Heavenly Farther, for blessing me with this opportunity and His guidance leading me through my studies and endeavours.

My supervisor, Dr Paul Cronje, for his invaluable expertise, guidance and encouragement throughout this study.

My co-supervisors, Prof Karen Theron and Dr Fernando Alferez for their support and expert contribution to this study.

My Family, especially Hennie and Dulcie Ehlers for their love, support, encouragement and input throughout my studies.

Citrus Research International, Post-harvest innovation fund for financial support

Henk van Rooyen (Groep 91), Eddie Voster and Hugo Enderman (Mahela) and Graham Pinner (Croc valley) for making trial sites available and assistance with trials.

Agriculture Research Council Nelspruit, Dr Nhlanhla Mathaba for his facilities and assistance.

Bureau for Food and Agriculture Policy (BFAP), Dr Marnus Gouse and Johann Boonzaaier for their expertise and invaluable contribution.

Freshgro, Danie and Hanli Stander for their invaluable assistance with the Citrus industry survey.

Respondents of the Citrus industry survey, for your honesty and invaluable information you supplied

Staff and fellow students at the Lombardi building for the coffee breaks, good laughs and memories made.

CRI staff in Nelspruit, for use of your facilities and equipment and making my stay so enjoyable.

The ''Vlakvarke'' for your invaluable contributions around the braai.

TABLE OF CONTENTS

1. GENERAL INTRODUCTION

The South African citrus industry is predominantly focused on export markets, exporting over 70 % of the production. Citrus fruit quality an important factor determining price in these markets. Situated far from most export markets, fruit from South Africa takes up to 4 weeks to reach their destination. During this time, due to numerous reasons such as an unfavourable post-harvest environment, post-harvest physiological rind disorders could develop that reduce the quality of fruit and diminishes producer's financial returns.

One such physiological rind disorder is non-chilling post-harvest rind pitting that has been reported in Navel oranges (*Citrus sinensis* L. Osb.) (Agusti et al., 2001) and 'Marsh' grapefruit (*C. paradisi* Macf.) (Alférez and Burns, 2004), however no research has been done on the factors impacting on susceptibility of 'Valencia' oranges to this disorder. This disorder is characterized by a collapse of the sub- epidermal rind cells with no discoloration taking place during early stages of development. As the disorder progresses, oil glands are affected and release intercellular content that changes colourless lesions to a bronze/brown colour due to enzymatic oxidation (Agusti et al., 2001). The primary cause of this disorder is unknown, however variation in relative humidity (RH) and especially changes from low to high RH intensified incidence of this disorder (Alférez et al., 2003).

Plant growth regulators (PGRs) or synthetic phytohormones are used in citriculture pre- and post-harvest to improve fruit quality and reduce certain physiological rind disorders. One such group of PGR's, namely auxins increase fruit size by thinning fruitlets (Mesejo et al., 2012) and increasing fruit sink strength (Agustí et al., 1994). Auxin is also used in citriculture as pre-harvest sprays to increase rind strength, reduce navel-end opening and fruit split or post-harvest to preserve a green and intact calyx which is an important quality factor for fresh markets(Cronjé et al., 2005; Greenberg et al., 2006; Stander et al., 2014; Mupambi et al., 2015). Gibberellic acid is used by citrus producers to improve fruit set and reduce incidence of physiological rind disorders such as creasing and puffiness (Fidelibus et al., 2002). Abscisic acid (ABA) has never been used in commercial citruculture however, it seems to affect certain physiological rind disorders as 'Pinalate' orange, an ABA-deficient mutant, is more susceptible to post-harvest pitting and less susceptible to chilling injury (CI) (Alférez et al., 2005).

A systemic fungicide, thiabendazole (TBZ) is used during post-harvest handling of fruit in citrus pack-houses to reduce decay controlling green [*Penicillium digitatum* (Pers.:Fr.) Sacc] and blue (*P. italicum* Wehmer.) mould. Pre- and post-harvest application of TBZ reduced the incidence of CI during cold storage of 'Tarocco' orange (Schirra and Mulas, 1995; Schirra et al., 2002), however the mode of action is unknown and has never been evaluated for efficacy on pitting. Due to *Penicillium* spp. resistance, TBZ is used in combination with other chemicals during post-harvest applications and little research has been done on pre-harvest application of this fungicide.

In this study we first examined the effect of pre-harvest factors, i.e. fruit position, maturity and size as well as variation in post-harvest environmental conditions on post-harvest pitting incidence. Secondly we examined the effect of pre-harvest application of different PGR's and TBZ on the susceptibility of 'Valencia' fruit to this disorder and thirdly we investigated the effect of timing of TBZ application on pitting. The aim of this study was to provide producers with pre- and post-harvest management strategies to reduce fruit susceptibility and reduce environmental stress to minimise incidence of this disorder. Lastly an investigation was done to estimate the financial impact of this disorder on grower returns to determine cost effectiveness of management strategies.

References

Agustí, M., Almela, V., Aznar, M., El-Otmani, M., Pons, J., 1994. Satsuma mandarin fruit size increased by 2, 4-DP. HortScience 29, 279-281.

Agusti, M., Almela, V., Juan, M., Alférez, F., Tadeo, F., Zacarias, L., 2001. Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. Annals of Botany 88, 415-422.

Alférez, F., Agusti, M., Zacarías, L., 2003. Postharvest rind staining in Navel oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143-152.

Alférez, F., Sala, J.M., Sanchez-Ballesta, M.T., Mulas, M., Lafuente, M.T., Zacarias, L., 2005. A comparative study of the postharvest performance of an ABA-deficient mutant of oranges: I. Physiological and quality aspects. Postharvest Biol. Technol. 37, 222-231.

Alférez, F., Burns, J.K., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79-87.

Cronjé, P., Crouch, E., Huysamer, M., 2005. Postharvest calyx retention of citrus fruit. Proc. Int. Postharvest Symposium 5, 369-376.

Fidelibus, M.W., Davies, F.S., Campbell, C.A., 2002. Gibberellic acid application timing affects fruit quality of processing oranges. HortScience 37, 353-357.

Greenberg, J., Kaplan, I., Fainzack, M., Egozi, Y., Giladi, B., 2006. Effects of auxins sprays on yield, fruit size, fruit splitting and the incidence of creasing of Nova mandarin. Acta Hort. 727, 249-254.

Mesejo, C., Rosito, S., Reig, C., Martínez-Fuentes, A., Agustí, M., 2012. Synthetic auxin 3, 5, 6-TPA provokes citrus clementina (Hort. ex Tan) fruitlet abscission by reducing photosynthate availability. J. Plant Growth Regul. 31, 186-194.

Mupambi, G., Verreynne, J.S., Stander, O.P.J., Cronje, P.J.R., 2015. Optimal timing of application of 2,4-D on 'Navel' sweet orange [*Citrus sinensis* (Osbeck)] reduces the size of the navel-end. J. Hortic. Sci. Biotec. 90, 619-625.

Schirra, M., Cabras, P., Angioni, A., D'hallewin, G., Pala, M., 2002. Residue uptake and storage responses of Tarocco blood oranges after pre-harvest thiabendazole spray and postharvest heat treatment. J. Agric. Food Chem. 50, 2293-2296.

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

Stander, O., Theron, K., Cronje, P., 2014. Foliar 2, 4-Dichlorophenoxy Acetic Acid (2, 4-D) Application after Physiological Fruit Drop Reduces Fruit Splitting and Increases Fruit Size in Mandarin. Acta Hort. 1042, 43-50.

2. Literature Review: Effect of plant growth regulators on citrus fruit quality.

2.1. Introduction

Citrus fruit is produced in large parts of the world including South Africa (FAO, 2013). Producing fruit of high quality is important in order to stay competitive. Fruit quality is a broad term and the quality parameters that are important differ between consumers. However quality, to a large extend, determines the price of fruit in the market and therefore profitability. Fruit quality as perceived by the customer can be influenced by a wide range of biotic, and abiotic-factors at any time during the pre-harvest fruit growth and developmental stages and post-harvest handling.

Fruit quality can be improved by utilizing pre-harvest technologies such as genotype selection, pruning and nutrition and irrigation management. Post-harvest technologies include application of fungicides to avoid decay, ethylene gas for degreening and wax to enhance shelf life (Chien et al., 2007). Plant growth regulators (PGRs) (synthetic phytohormones) are used pre- and post-harvest to improve fruit quality. The synthetically produced PGR's, auxin, abscisic acid (ABA), cytokinin, ethylene and gibberellin (GA) can be used to improve fruit quality and increase yield. The main focus of this literature review was to examine the use of PGRs to improve fruit quality in citrus production.

2.2. Citrus fruit quality

Fruit quality is not easy to define as the parameters used to determine fruit quality differ between markets. The choice of what to measure and how to measure depends on the proposed use of the product, i.e. for instance, for fruit intended for the fresh market, external and internal quality are both important, whereas fruit destined for processing needs only high internal quality (Abbott, 1999). Different quality aspects are focused on along the value chain of export fruit. A pack house manager categorises good quality as blemish free fruit of uniform size, while an exporter wants large fruit with good appearance and long shelf life. Most importantly, fresh citrus consumers prefer large, seedless fruit with a high sugar to acid ratio and good external appearance (Blasco et al., 2007). The price obtained for fruit on the market is directly correlated with quality and availability of that fruit at a given time. Therefore income depends on the yield and the quality of the products in order to consistently produce profitably (Guardiola and García-Luis, 2000; Blasco et al., 2007). Inferior quality fruit in some instances do not cover the cost of harvesting and handling (Guardiola and García-Luis, 2000).

A broad definition of fruit quality includes all the fruit characteristics used by the consumer to evaluate fruit, which determines superiority and degree of consumer acceptance (Zeithaml, 1988; Castle, 1995; Ladanyia, 2010). The methods used to determine fruit quality can be either subjective or objective. Subjective methods make use of human senses, for instance taste or smell. Objective methods make use of instruments to determine e.g. acidity or firmness. Both methods have advantages and disadvantages as subjective methods are easier with less expensive equipment needed, but results are variable, while objective methods are more accurate, but it can take longer and may involve expensive equipment (Mitcham et al., 1996). Furthermore methods used to evaluate quality can be destructive or non-destructive. If the fruit is destroyed during analysis it is considered a destructive method as for instance juice presentation or total soluble solids concentration (TSS) to total acid (TA) ratio. If the fruit is still intact after evaluation it is considered a non-destructive method such as colour or size measurements (Ladanyia, 2010). Lastly methods can be divided into chemical, physical or physiological depending on the analytical process and principals involved (Ladanyia, 2010).

2.2.1. External fruit quality

External fruit quality is very important for fresh markets as costumers primarily buy fruit based on appearance and past experience (El-Otmani et al., 2000; Agustí et al., 2002a). Even though the external quality might seem unimportant for processing needs as the rind is discarded during processing and does not directly affect the processed product's quality, fruit that are damaged or has a soft rind are also discarded before fruit are processed (Fidelibus et al., 2002). External quality equates to fruit appearance and can be observed without peeling the fruit; therefore the geometric and chromatic attributes like size, colour, rind blemishes and shape of the fruit.

Fruit size

Fruit size is determined through fruit growth which is the result of the accumulation of dry matter and water. The rate of fruit growth is inversely proportional to fruitlet abscission as fruit size is dependent on the sink capacity of the fruitlet and the assimilate supply of the source (Guardiola and García-Luis, 2000; Agustí et al., 2002a; Garcia-Luis et al., 2002). The size of fruit is determined early during development, as a shortage in supply of metabolites during the early stages of development may irreversibly impair sink strength of fruitlets. However the fruitlet growth limiting factor changes during the development stages of fruit. At maturity individual fruit weight is inversely related to fruit count per tree. This indicates a source limiting effect while total yield is directly related to fruit count per tree indicating a sink limiting effect during the late period of fruit development. Besides sink and source relations the transport capacity of the sieve tubes and vessels can also be a limiting factor as there is a direct relationship between pedicel size and final fruit size (Guardiola and García-Luis, 2000). Horticultural practices which manipulate sink: source balance such as pruning, thinning and the use of plant growth regulators can increase fruit size (El-Otmani et al., 2000; Agustí et al., 2002b; Garcia-Luis et al., 2002) and will be discussed in greater detail.

Rind colour

Rind colour is crucial in determining quality of citrus fruit and market value. Rind colour develops late in the season and is a result of chlorophyll degradation and changes in the carotenoid profile (El-Otmani et al., 2000; Ladanyia, 2010). Carotenoids are synthesised via the carotenoid biosynthesis pathway from two geranylgeranyl pyrophosphate (C_{20}) (GGPP) molecules. During the first three steps in the carotenoid biosynthesis pathway GGPP is converted to lycopene, a carotenoid that gives 'Star ruby' grapefruit (*Citrus paradisi* Macfad) its characteristic red pulp colour. In immature fruit lycopene is converted by lycopene ε-cyclase (ε-LCY) and lycopene β-cyclase (β-LCY) to α-carotene, which is later converted to lutein, the main carotenoid in the rind of immature fruit. While in mature fruit the activity of ε-LCY declines and the activity of β-LCY increases to convert lycopene to β-carotene the precursor for β-xanthophylls, as 9-Z-violaxanthan which is the main carotenoid present in the rind of mature orange fruit (Alquezar et al., 2009). Approximately 115 different carotenoids have been reported, each one contributing to the range of different colours found in citrus fruit (Xu et al., 2006). Certain colour changes are influenced by climatic conditions, PGR usage and nutrient levels (Iglesias et al., 2007; Ladanyia, 2010). Hence, the final desired colour and intensity thereof, of each citrus variety, depends on selecting the right cultivar for an area as well as applying correct manipulation techniques.

Rind lesions, decay and disorders

A fruit with high quality rind should be intact and free of physical damage such as bruises, cuts, oleocellosis, pests, chemical burns and foreign matter. In addition, it should be able to withstand handling and transport and arrive at its final destination in a satisfactory condition (OECD, 2010). The rind is affected by physiological and pathological disorders that blemishes and deforms the rind leading to diminished external quality (El-Otmani et al., 2000; Alférez et al., 2003; Ladanyia, 2010). Pathological decay, which also negatively affects quality, such as green- [*Penicillium digitatum* (Pers.:Fr.) Sacc] and blue- (*P. italicum* Wehmer) mould can however be controlled with post-harvest fungicides application, while physical rind disorders are more difficult to predict and control (Porat et al., 2002; Porat et al., 2004; Wardowski, 2006).

Physical rind disorders can be divided into chilling related and non-chilling related rind disorders. Chilling injury (CI) develop in storage at low, sub-optimal storage temperatures and are amplified when fruit are transferred to room temperature. Symptoms of CI vary between cultivars; sinking of the rind below the surface and browning/blackening of rind tissue may be observed with watery breakdown of the flesh and rind (Reuther, 1989). Non chilling related rind disorder may develop at low, sub-optimal temperatures, but low temperature is not required for development of non-chilling related disorders. Post-harvest rind pitting is a non-chilling related rind disorder that is characterised by the collapse of the sub-epidermal cells that progresses to affect the surrounding oil glands leading to browning/bronzing of rind tissue due to oxidation (Alférez et al., 2005b). The cause of this disorder is thought to be due to variation in post-harvest relative humidity (Alférez et al., 2005b). Stem-end rind breakdown (SERB) is another non-chilling related rind disorder that can lead to diminished external fruit quality. This disorder involves the collapse of tissue around the stem-end side of the fruit with the tissue directly around the stem being unaffected (Dou et al., 2001; Ritenour et al., 2004). As with post-harvest pitting of navel orange this disorder seems to also be affected by water stress (Romero et al., 2013).

2.2.2. Internal fruit quality

Internal quality is important for both fresh consumption and processing. For processing, internal quality is more important than external quality (El-Otmani et al., 2000). Any factor influencing taste is regarded as part of internal quality, i.e. juice content, TSS and TA.

The TSS consists 80 % of carbohydrates and the rest of a mixture of organic acids, lipids, proteins and minerals (Iglesias et al., 2007; Ladanyia, 2010). TSS contributes to the overall flavour and palatability of citrus fruit (Al-Jaleel et al., 2005). Therefor factors influencing the photosynthesis capacity of a tree will influence the TSS of the fruit and overall flavour (Jifon and Syvertsen, 2001). TSS is measured as °Brix using a refractometer and readings should be taken at 20°C to be accurate or if not possible, the International Correction Table should be used (OECD, 2009).

Citrus internal quality is greatly influenced by TA, one of the most distinctive characteristics of citrus fruit. TA plays a major role in flavour and consumer acceptability of fruit (Fang et al., 1997). During the early growth and development of citrus fruit, TA increases up to a certain level and then stays constant (Bain, 1958). As the fruit grows and develops it seems to decrease in total TA, but this is due to a dilution effect as the fruit increase in size and juice content (Ladanyia, 2010)

In citrus, internal quality is often expressed as the TSS:TA ratio. For navel oranges a minimum TSS:TA ratio of 6.5:1 is required (OECD, 2010). This measurement alone does not give a very clear indication of quality as fruit with low acidity have an insipid taste (El-Otmani et al., 2000; Ladanyia, 2010). As sugar and acid have opposite effects on flavour and the tongue is more sensitive to acid, (Jordan et al., 2001) suggested subtracting TA from TSS after multiplying TA with a constant *k* that differs with fruit type and named in BrimA. BrimA is closely related to flavour likeability (Obenland et al., 2009). In April 2012, California adopted the California Standard, which is derived from BrimA, as determinant for internal quality and maturity for 'Navel' orange (C. *sinensis* Osb.). The California Standard uses the relationship between TSS and acidity, but in a different formula to BrimA (California Citrus Mutual, 2015):

$$California\ Standard = [(TSS - (4 \times TA)) \times 16]$$

The California Standard score should be above 90 before oranges are harvested.

High juice content is associated with high internal quality (Al-Jaleel et al., 2005). Juice content is determined as percentage of volume or weight (Ladanyia, 2010). For export, navels have a minimum juice requirement of 33 % (OECD, 2010).

Seed content also affects the overall quality of fruit. Consumers prefer seedless fruit which are easier to eat (Campbell et al., 2004). This has resulted in breeding or selecting seedless cultivars and/or avoid cross pollination.

2.3. Citrus fruit growth and development

The period in which citrus fruit grow and develop from flowering until maturity differs between cultivars. 'Satsuma' mandarin (C. *unshiu* Marcow.) could developed from a flower till a mature fruit in 5-7 months while 'Valencia' oranges can take to between 12 and 14 months to develop and mature (Bain, 1958).

Anthesis first occurs in the warm quadrant of the tree with budbreak of buds on leafless inflorescence occurring four to seven days before buds on leafy inflorescence (Krajewski and Rabe, 1995).Flowering is followed by fruit set. Leafy inflorescences have a greater ability to set in most cultivars than leafless inflorescences (Goldschmidt, 1999). This could be related to the leaf carbohydrate supply. During anthesis and fruit set, carbohydrates can be a limiting factor as girdling can improve fruit set, but this is not true in all cases and for each cultivar there can be additional factors also influencing fruit set (Goldschmidt, 1999). Iglesias et al. (2007) identified three levels of regulation of fruit growth and abscission namely; genetic, metabolic and environmental.

The full development and maturation process of citrus fruit is divided into three stages.

Stage I is characterised by slow growth rate as cell division predominates, the rind develops and differentiates into flavedo and albedo and the juice sacs develop inside the pulp. The marginal growth that takes place during stage I can mostly be attributed to rind growth. After stage I is completed cell division stops in the fruit with the exception of the outer rind that still continues dividing (Bain, 1958). Fruit abscission continues throughout stage I (Iglesias et al., 2007). Stage I starts at anthesis and normally ends with December fruit drop Southern Hemisphere (SH) in 'Valencia' oranges (Bain, 1958).

Stage II is regarded as the cell enlargement stage as fruit growth increases rapidly with water uptake (Iglesias et al., 2007). Morphological, anatomical and physiological changes inside the fruit occur quickly as the cells expand with no cell division taking place except in the outer rind (Bain, 1958). Fruit become storage sinks and factors influencing supply of water and carbohydrates are very important (Bain, 1958; Iglesias et al., 2007). Fruit diameter increases 3 fold during stage II which can primarily be attributed to increased pulp growth, while the rind volume also increases and the ratio of rind to pulp decreases (Bain, 1958). Anatomical changes also occur in the rind during stage II, as the central albedo cells enlarge and sponginess increases (Bain, 1958). During this and the subsequent stage, fruit abscission is low

(Iglesias et al., 2007). As mentioned earlier, fruit size is an important external quality characteristic (Goldschmidt, 1999). During fruit enlargement carbohydrates seem to be the limiting factor as fruit thinning and girdling can improve fruit size (Goldschmidt, 1999; Guardiola and García-Luis, 2000; Agustí et al., 2002b). In South Africa applying gibberellic acid (GA_3) in January during stage II is recommended to prevent creasing since it prevents the reduction of polysaccharides, specifically pectin and hemi-cellulose, during stage III of fruit development thus reducing incidence of creasing (Jona et al.,1989)

Stage III is characterised by slow growth and fruit maturation taking place (Bain, 1958). The rates of morphological, anatomical and physiological changes decrease as fruit start maturing until harvest (Bain, 1958). Growth rate decreases but still continues while fruit are on the tree, the rind colour changes, respiration rate decreases, sugar content increase and titratable acidity decreases (Bain, 1958). Citrus fruit are non-climacteric and mature on the tree (Iglesias et al., 2007). Although the rind and pulp of citrus fruit mostly mature together, they are two separate organs and could mature at different times (Iglesias et al., 2007). Rind maturation is characterised by a loss of chlorophyll and changes in carotenoid profile to give citrus fruit its characteristic orange or yellow colour. These changes are influenced by environmental conditions, nutrient availability and PGRs (Iglesias et al., 2007). The degreening is enhanced by short days with cold nights. Nutrients have the ability to enhance or delay degreening and regreening. Sucrose promotes degreening and inhibits regreening while nitrogen inhibits degreening (Huff, 1983; Huff, 1984; Iglesias et al., 2007). PGRs are used in citriculture to increase colour break or delay it and will be discussed in more detail later.

2.4. Plant growth regulators used to improve citrus fruit quality

Fruit development and growth is influenced by various external factors i.e. temperature, mineral nutrient and water availability, as well as internal factors i.e. carbohydrate status and nutrient availability and allocation thereof (El-Otmani et al., 2000; Ladanyia, 2010). One of the factors which can alter fruit development is PGRs. PGRs have been widely used in citrus production to improve yield and fruit quality (El-Otmani et al., 2000). The use of PGRs may differ between growing regions due to differing environmental conditions. PGRs are used to manipulate fruit development at different stages of growth and the end result depends on the PGR used as well as at what stage of development (Iglesias et al., 2007).

2.4.1. Auxins

Auxin was the first PGR to be studied and is involved in almost every aspect of plant growth and development (Taiz and Zeiger, 2010). Synthetic auxin can have contrasting effects on fruit growth and development depending on the type of auxin, time and rate of the application. It can either delay or enhance fruit abscission, and promote cell enlargement during stage II of fruit growth (Agustí et al., 2002b; Iglesias et al., 2007).

The number of fruit on a tree affects fruit size and synthetic auxins can be used to control abscission i.e. thinning. Depending on the type of auxin, time and rate of the application it has contrasting effects on abscission. The synthetic auxin, 3, 5, 6-trichloro-2-pirydiloxyacetic acid (3, 5, 6-TPA) is used as a thinning agent during the cell division stage (Stage I) of fruit development. Mesejo (2012) reported that 15 mg l^{-1} of 3, 5, 6-TPA applied during the cell division stage of 'Clementine' mandarin (C. *clemintina* Hort. *ex* Tan.) increased fruit abscission with 30 %. Fruit growth rate was also temporarily decreased on treated trees from 3 days after treatment (DAT) until 8 DAT, however at 16 DAT the treated trees overcame the negative effect of 3,5,6-TPA and exhibited an increased growth rate, producing larger fruit than the untreated control. When 3, 5, 6-TPA was applied at the onset of the cell enlargement stage the thinning effect of this auxin was reduced compared to the earlier treatment during the cell division stage and higher rates of 3,5,6-TPA was needed for the same abscission response (Agustí et al., 2007). In addition, 2,4-dichlorophenoxypropionic acid 2-ethylhexyl ester (2,4-DP) applied to 'Navel' oranges before colour break and during pre-harvest drop decreased abscission of mature fruit with the response increasing with concentration until 15 mg l^{-1} after which higher concentrations had no effect (Agustí et al., 2006).

Auxins do not only increase fruit size through abscission i.e. reducing sink numbers, but also increase sink strength. As indicated earlier the time and concentration of treatments play a significant role in the plants response. Auxin has the ability to increase fruit size without thinning of fruit and 2, 4-DP applied after physiological fruit drop increases fruit size without any effect on fruit number per tree. When 2,4-DP was applied 15 days after physiological fruit drop, a higher concentration was needed to have the same effect on fruit size. Treated fruit also had higher juice percentage and rind weight (Agustí et al., 1994).

Some mandarin cultivars are prone to fruit splitting which reduces yield and increases labour cost to sanitise orchards. The application of 2,4-dichlorophenoxy acetic acid (2,4-D) after physiological fruit drop decreased this disorder on 'Marisol' clementine and 'Nova' mandarin (Greenberg et al., 2006; Stander et al., 2014). The time of 2,4-D application plays an important role as application just after physiological fruit drop at the onset of stage 2 of fruit development was optimal. Later applications were less effective at reducing splitting on the early maturing ‘Marisol’ clementine (Stander et al., 2014). It was also found that 2,4-D applied at full bloom reduced the size of navel-end opening and increased the percentage of fruit with closed navel-ends of ‘Washington’ navel oranges without affecting fruit quality characteristics such as TSS, TA or rind colour (Mupambi et al., 2015).

An important attribute of citrus fruit destined for fresh use is a green intact calyx, not only is it an indication of freshness to consumers, but fruit with an abscised calyx are more prone to fungal decay during storage and transport. In South Africa 2, 4-D is used post-harvest to prevent abscission of the calyx and stem-end rot by keeping the button fresh (van Zyl, 2014). The use of the auxin 2, 4-D in post-harvest dips, drenches or wax treatments reduces calyx browning and abscission (Cronjé et al., 2005). Calyx abscission is a major problem when fruit needs to be degreened as in one study 98 % of degreened ‘Oronules’ mandarin had abscised or damaged calyxes (Salvador et al., 2010). The use of auxin before degreening reduced calyx abscission, but also reduced the effect of ethylene on the rind colour (Sdiri et al., 2013). In most cases the reduction in colour change had no effect as all fruit treated with auxin before degreening reached a commercially accepted colour (Cronjé et al., 2005; Salvador et al., 2010).

2.4.2. Abscisic acid

Abscisic acid is a phytohormone first thought to control abscission hence the name, but the role of ABA in abscission remains controversial and since its discovery it has been found to play a more direct role in stomatal regulation, growth and seed maturity and dormancy (Taiz and Zeiger, 2010). Abscisic acid influences vegetative growth and citrus trees with low ABA and high auxin levels normally grow vigorously while trees with high ABA and low auxin levels are dwarfed (Bertling and Lovatt, 1996). The use of ABA on citrus tree, stimulates ethylene production in mature citrus leaves, not by accelerating senescence, but by stimulating 1-aminocylopropane-1-carbocylic acid (ACC) synthesis (Riov et al., 1990)

ABA is used in viticulture to enhance red colour development especially in seedless grape cultivars. Often GA_3 is applied to increase berry size and to meet market standards in particular in growing regions with a warm summer climate GA_3 increases berry size, but inhibits red colour development (Peppi et al., 2006; Cantín et al., 2007). The application of exogenous ABA at the onset of ripening (veraison) increases anthocyanin content in the grape skin of 'Flame Seedless' (*Vitis vinifera* L.) and 'Crimson Seedless' grapes (Peppi et al., 2006; Cantín et al., 2007). It was also reported that the application of ABA to bell pepper seedlings (*Capsicum annuum* L.) increased yield and reduced transplant shock by increasing leaf resistance and plant water potential (Berkowitz and Rabin, 1988). Buran et al. (2012) found that ABA delayed ripening in southern highbush blueberries (*Vaccinium darrowii* camp). This effect was attributed to the ability of ABA to induce stomatal closure and inhibiting the activity of ribulose 1,5-bisfosfate carboxylase resulting in reduced photosynthetic CO_2 assimilation (Seemann and Sharkey, 1987).

In recent years studies on an ABA-deficient mutant of 'Navelate' orange, 'Pinalate' has shown that ABA has an effect on citrus rind disorders. The ABA-deficient mutant was more susceptible to rind pitting, but more resistant to CI compared to a control 'Navelate' orange (Alférez et al., 2005a). However the resistance of 'Pinalate' to CI is thought to not only be attributed to reduced ABA, but the total difference in carotenoid profile of 'Pinalate' fruit (Alférez et al., 2005a). The quality was also affected as 'Pinalate' fruit had higher transpiration rate, due to reduced stomatal control, leading to less firm fruit and higher weight loss during storage (Alférez et al., 2005a).

2.4.3. Cytokinins

Cytokinin (CK) affects a wide array of physiological and developmental processes such as stimulating cell division (cytokinesis), retarding leaf senescence and overcoming apical dominance (Taiz and Zeiger, 2010). CK stimulate cell division and are produced in the seeds of developing fruitlets (Hernandez Minana and Primo-Millo, 1990). CK increase sink strength, but are not commercially used to enhance fruit set in citrus (Iglesias et al., 2007). Exogenous applied CK enhances fruit development of parthenocarpic cultivars (Iglesias et al., 2007). The application of 6-benzyladenine (6-BA) to 'Satsuma' mandarins increased phloem formation in the pedicle (Guardiola and García-Luis, 2000).

Root produced CK regulates vegetative shoot growth and influences flowering (Oslund and Davenport, 1987). CK also play a role in alternate bearing as a heavy crop in an "on year" results in low levels of CK due to low carbohydrate and other nutrient reserves, leading to poor flowering (Davenport, 1990)

2.4.4. Ethylene

Ethylene is used worldwide on a variety of horticultural crops, as a ripening hormone as in citriculture for de-greening when internal maturity has been reached without adequate rind colour. Ethylene is also used for other agricultural practices such as stimulating seed germination, retarding plant growth and thinning of flowers and fruit (Abeles et al., 2012).

Ethylene enhances rind colour development of citrus by increasing the degradation of chlorophyll through increasing chlorophyllase activity (Shimokawa et al., 1978) and changing the carotenoid profile of the rind (Stewart and Wheaton, 1972). During exposure to ethylene the activity of chlorophyllase is unchanged, however 6 hours after exposure to ethylene the activity of chlorophyllase starts to increase, and can last up to 92 hours (Shimokawa et al., 1978). Stewart and Wheaton (1972) reported that 2 weeks after ethylene treatment, the rind of 'Robinson' tangerine (C. *reticulate* Blanco) fruit changed from green to yellow, then later to orange and lastly to red, while untreated fruit remained yellow. Treated fruit had increased levels of cryptoxanthin and *β*-citraurin, the main two carotenoids contributing to the orange rind colour of citrus fruit.

In contrast to the positive colour change, ethylene negatively affects fruit quality. De-greened 'Valencia' fruit has a higher sensitivity to CI and increased susceptibility to pathogens. If stored at 0°C, fruit became more susceptible to CI when exposed to ethylene, even at very low ethylene concentrations (Yuen et al., 1995). Pre-treating fruit with ethylene was also found to reduce non-chilling rind staining of 'Navelina' oranges. When fruit where pre-treated before storage with 0.1 or 10 $\mu l \cdot l^{-1}$ ethylene for 4 days, the incidence of this disorder was reduced during storage, with increasing efficacy at higher concentrations or time of exposure. This decrease seems not to be attributed to the increase in ABA levels as ABA levels did not increase with higher ethylene concentrations and after 14 days of storage there was no significant difference between ethylene and air treated fruit (Lafuente and Sala, 2002). Cajuste and Lafuente (2007) attributed the decrease in sensitivity for non-chilling rind pitting of 'Navelate' orange fruit to an increase in the phenolic metabolism after ethylene pre-treatments before storage, but suggested that ethylene induced other additional defence mechanisms which reduced non-chilling rind pitting.

2.4.5. Gibberellic acid

Gibberellic acid (GA) is used on a number of horticultural crops for different reasons as GA influences seed germination, stem and root growth, plant phase changes, floral initiation, pollen development, fruit set and parthenocarpy (Taiz and Zeiger, 2010) Dwarfing rootstocks such as 'C35' citrange (C. *sinensis* × *Poncirus trifoliata*.) are widely used in citrus production as smaller trees are easier to spray and harvest. Reduced levels of GA_1 in actively growing shoot modify the architecture of trees resulting in dwarfed trees (Fagoaga et al., 2007). GA_3 reduced flower intensity if applied during flower initiation. 'Satsuma' mandarins (*C. unshui* Marc.) treated with GA_3 during winter had significant fewer inflorescences with earlier treatments being more effective (Iwahori and Oohata, 1981). GA_3 applied to shoots during winter at different stages of inflorescences and shoot development reduced flower intensity, with earlier application during the dormant state of buds more effective that application at microscopic and macroscopic budbreak (Lord and Eckard, 1987).

GA_3 improved fruit set by stimulating translocation of macro-nutrients thus stimulating early fruit growth on 'Clementine' mandarin after treatment (García-Martínez and Garcia-Papi, 1979). Application of GA_3 reduced leaf nitrogen (N), phosphate (P) and potassium (K) concentration, but subsequently increased the concentration of these minerals inside fruit tissue. As this application of GA_3 also increased the activity of two auxin like substances, this translocation of minerals could not be attributed to GA_3 alone. (García-Martínez and Garcia-Papi, 1979). The average size of treated fruit that abscised during June-drop, [Northern hemisphere (NH)], were smaller than that of untreated fruit indicating that the stimulation of growth reduced abscission (García-Martínez and Garcia-Papi, 1979). GA improved set in 'Bearss' lime (C. *latifolia* Tan), 'Eureka' lemon (C. *limon* Nurm. f.) and 'Washington navel' orange (*C. sinensis* Osb.) (Hield et al., 1958). In South Africa different formulations and concentrations of GA_3 is used on navel, Valencia orange and mandarin to increase fruit set (van Zyl, 2014).

The external quality can be improved by GA_3 as it reduces the incidence of some rind disorders such as creasing (Fidelibus et al., 2002). GA_3 applied to 'Satsuma' mandarins at the onset of chlorophyll degradation has been shown to prevent puffiness, a disorder related to the disintegration of the albedo. Unfortunately, GA_3 also delays colour development (Garcia-Luis et al., 1985) and should not be applied close to colour break i.e. February in South Africa.

Gibberellin delays chloroplast breakdown and carotenoid accumulation (Fidelibus et al., 2002). This delay can be beneficial as it extends the storage life of fruit and delays rind senescence. The timing of GA_3 application affects the magnitude of the response, the best response to delay chlorophyll degradation and carotenoid accumulation is after the onset of chlorophyll degradation and before the onset of carotenoid accumulation (García-Luís et al., 1992). The effect of GA_3 on rind strength is longer lasting than the GA_3 effect on colour and could therefore be used to produce fruit with good colour quality and rind strength (Fidelibus et al., 2002).

2.5. Additional chemical compounds used to improve citrus fruit quality

In citriculture other non-PGR chemical compounds are also used to improve fruit quality. These compounds are either sprayed on the tree before harvest of applied post-harvest through drenches, dip tanks or in the wax during pack-house treatments.

2.5.1. Arsenate

High TA in mature fruit negatively affects fruit quality and tools to manage high acidity are limited. Lead arsenate (As_2O_3) improves internal fruit quality by decreasing total acidity, resulting in earlier commercially acceptable maturity (Deszyck and Sites, 1954). 'Ruby Red' grapefruit treated with lead arsenate had reduced TA and increased TSS. This response was enhanced with increasing concentration of lead arsenate application (Deszyck and Sites, 1954). Arsenic compounds reduce TA by reducing citric acid accumulation and temporarily decreases the citrate synthase activity (Sadka et al., 2000).

2.5.2. Thiabendazole (TBZ)

Citrus fruit are very susceptible to pathogens when fruit are high in nutrients and have a low pH and high moisture content such as at the time of maturity (harvest) (Chien et al., 2007). The use of the systemic fungicide TBZ is there for a routine practice in citrus pack-houses around the world to prevent post-harvest decay that is caused by green mould and blue mould (Smoot and Melvin, 1970; Gutter, 1985). TBZ increases post-harvest quality and shelf life of citrus fruit (Chien et al., 2007).

The duration and temperature of TBZ dip treatments were investigated by Cabras et al. (1999) who found TBZ was more effective when applied in hot water as greater deposits of the fungicide were found, however the duration of treatment had no significant effect. The uptake of TBZ is also affected by pre-and post-harvest factors, dosage, method of application, storage conditions and cultivar (Cabras et al., 1999).

TBZ reduces chilling injury of grapefruit, but during application water temperature of the treatment should be carefully regulated, as temperatures above 58°C for 2 min damages the rind, and below 40°C the treatment is ineffective (Wild, 1993; Schirra et al., 2002). Schirra et al. (2002) showed that a single pre-harvest application 14 days before harvest can reduce decay and incidence of chilling injury, but a much higher concentration of fungicide is needed to be equally effective as post-harvest treatments.

The extensive use of TBZ in pack-houses has led to development of some resistant strains of *P. digitatum* that cause green mould (D'Aquino et al., 2013). *P. digitatum* has a short disease cycle (3 to 5 days at 25°C) and one infected fruit can produce 1 to 2 billion conidia that infect other fruit during handling, storage and transport (Holmes and Eckert, 1999). To combat *Penicillium* spp. resistance, TBZ is used in combination with other chemicals such as Imazalil (IMZ) and Gauzatine (Dodd et al., 2010)

2.6. Pre-harvest horticultural practises that impact on citrus fruit quality

Producers can manipulate fruit quality by using different horticultural practices such as changing irrigation scheduling or nutrition. Aspects such as fruit size is influenced by both water and mineral availability and changing irrigation schedule can be used to improve fruit quality i.e. high TSS

2.6.1. Irrigation

Irrigation is an important part of the production process of any horticultural crop. Water is required in plants for photosynthesis and many other essential processes (Taiz and Zeiger, 2010). In citrus production, manipulation of irrigation scheduling can be used to increase citrus fruit quality and decrease water usage of citrus orchards (Zekri et al., 2009; García-Tejero et al., 2010)

Various models exist to quantify the impact of irrigation. Water use efficiency (WUE) is an indication of the amount of water used to produce 1 kg of fruit (Hutton et al., 2007). WUE can be improved by either decreasing water usage or increasing yield while the other stays constant (Fereres et al., 2003; Hutton et al., 2007). Even though the WUE is a good indication of water use, it does not give an indication of the quality of fruit being produced, which is critical for sustainable citrus sales.

Deficit irrigation has contrasting effects (negative and positive) on fruit quality depending on the physiological stage it is implemented, the duration of treatment, water quality and plant genotype (García-Tejero et al., 2010). A strategy to obtain high quality fruit with less water, is to implement deficit irrigation in such a way that the positive outweigh the negative effects (Hutton et al., 2007). During flowering (Stage I) in October and November (SH) moisture stress can lead to a decrease in final yield due to more fruit abscission. In addition, the spring flush is also sensitive to moisture stress and a reduction in vegetative growth can be realised (Syvertsen, 1984). The reduction in vegetative growth is likely to decrease the yield potential of following season (Syvertsen, 1984) due to lack of 1-year-old bearing shoots.

Hutton et al. (2007) evaluated the effects of irrigation scheduling during stage II (Cell elongation) and III (TSS accumulation and maturation) of fruit development on tree response and fruit quality. By irrigating at 3, 10 or 17 day intervals, the canopy size was not affected by the irrigation scheduling. The longer interval irrigation scheduling reduced growth in spring, but this effect was counteracted by increased shoot growth in late summer with trees that received deficit irrigation. This summer flush increased fruiting positions for the subsequent season. More inflorescences led to higher set in water stressed plants. The yield (t/ha) was not affected as yields differed more between years than between treatments. The fruit size was reduced with longer interval irrigation, however it was a relatively small reduction compared to the increase in WUE. The TSS improved with longer irrigation intervals, however the higher TA meant a lower TSS:TA ratio which is undesirable for fresh markets. The higher TSS per ton of fruit is however an advantage for the processed markets (Hutton et al., 2007).

Deficit irrigation and partial root drying had no effect on the rind thickness at maturity of 'Navel' orange, however rind thickness did differ significantly between seasons and rootstocks, but no effect was seen between irrigation strategies (Treeby et al., 2007). The timing of deficit irrigation had an impact on rind thickness of 'Salustiana' orange. Deficit irrigation applied during flowering and fruit set (stage I) resulted in thinner or similar rind thickness while deficit irrigation during fruit maturation (stage III) increased rind thickness (Castel and Buj, 1990).

2.6.2. Mineral Nutrition

Citrus trees use a variety of mineral elements to produce high quality fruit and plants growing under optimal nutrient conditions tend to better tolerate pest and diseases among others (Zekri et al., 2009). Essential elements are elements that are involved in the structure or metabolism of the plant, which causes abnormalities when absent (Taiz and Zeiger, 2010). These essential elements can be divided into macronutrients and micronutrients according to their relative concentration in the plant tissue (Taiz and Zeiger, 2010). Nutrients are obtained from the soil via the plants root system or by foliar application.

The main elements used in citrus production are N, P and K while other macronutrients such as calcium (Ca), magnesium (Mg) and sulphur (S) are also used, but in smaller quantities. It is important to note that application of mineral nutrients will only have an effect if plant tissue concentration is less than optimal. The risk exists that toxic levels are reached when plants are over supplied with any nutrient, which will lead to negative effects on plant growth and fruit quality.

Nitrogen forms part of amino acids, amides and proteins, among others. Therefore N is important for vegetative growth, fruit quality and yield. An N application at the correct time increases yield, TSS, TA and juice percentage and enhance juice colour (Zekri et al., 2009; Lovatt, 1999). A foliar application of N as urea applied pre-bloom can increase inflorescence per tree and flowers per inflorescence, as it increases tissue concentrations of ammonia, arginine and polyamines (Lovatt, 1999). Polyamines promote growth and urea increases polyamine synthesis (Lovatt, 1999). However a late application of N during February in South Africa can delay colour break extending harvest date (Koo, 1979; Ladanyia, 2010). It is thought that excess N also reduces the storability of citrus fruit (Ladanyia, 2010).

Phosphorus has no effect on TSS, but rather reduces TA and increases the TSS/TA ratio. When applied to P-deficient soils, it also increases yield. High levels of P can increase wind scar expression and green fruit percentage but reduces rind thickness (Zekri et al., 2009). Trees growing under P deficiencies produce fruit with a thick, rough textured rind, which mature later than fruit from optimal P supply (Ladanyia, 2010).

Potassium increases yield, fruit size and weight but decreases TSS, juice content and juice colour and increases TA (Koo, 1979; Zekri et al., 2009). Potassium can also decrease the incidence of creasing in citrus fruit and stem-end rot during storage. Under K deficiency trees produce fewer and smaller fruit per tree, however K applied often can reduce splitting in trees with initial low K (Ladanyia, 2010). It is interesting to note that a double foliar application of potassium phosphate at the end of cell division (stage I) increased TSS (Lovatt, 1999).

Calcium is involved in many cell functions including cell wall structure and hydrolysis of ATP and phospholipids. In addition, Ca plays an important role as a secondary messenger in intercellular spaces (Taiz and Zeiger, 2010). Deficiencies normally occur in acidic soils as natural occurring Ca has been leached out and in high alkaline soils because of high levels of sodium (Na) (Zekri and Obreza, 2003a). Trees growing under Ca deficient conditions may deliver a reduced yield with misshapen fruit and shrivelled juice vesicles. The juice percentage decreases, but TSS and TA increase under Ca deficient conditions (Zekri and Obreza, 2003a).

Magnesium is mobile in the plant xylem and deficiency symptoms do not normally show during the spring flush, but during summer symptoms are more apparent on older leaves as Mg is translocated from older leaves towards fruit and younger leaves (Zekri and Obreza, 2003a). A sufficient supply of Mg to previously Mg deficient trees increased TSS, TA and yield without affecting fruit size or juice percentage (Quaggio et al., 1992). Mg deficiency leads to twig die-back and defoliation. This results in fewer bearing positions and fewer leaves to supply fruit with carbohydrates (Zekri and Obreza, 2003a).

Like N, Sulphur (S) also plays an important role in plant growth as it is a component of the amino acids cysteine, cysteine and methionine. The big difference between N and S is their mobility inside plant tissue. N is mobile in plants and can be translocated while S is not (Taiz and Zeiger, 2010). As N and S are both needed for plant growth a balance between the supply N and S is important as S deficiencies are more pronounced with increasing levels of N (Zekri and Obreza, 2003a).

Micronutrients are needed in small amounts by the plant and is mostly at adequate concentration levels in soils. When producers focus on macronutrient fertilization only it can lead to micronutrient deficiencies (Zekri and Obreza, 2003b). Micronutrients deficiencies such as Manganese (Mn), Zinc (Zn) Boron (B) and Iron (Fe) can decrease fruit quality (Zekri and Obreza, 2003b). Zn and Mn deficiencies may lead to a lower yield with smaller fruit with low juice content and thin, smooth rind that is lightly coloured (Zekri and Obreza, 2003b). Fe deficiency does not only affect yield but also quality. Fe deficiency decreases shoot growth leading to reduced yield while fruit have lower TSS and TA (Zekri and Obreza, 2003b).

2.6.3. Rootstock selection

Rootstocks have been used in citrus production for centuries, but their ability to overcome production obstacles and improve fruit quality has only been studied in the last century. Producers make a rootstock selection depending on environmental conditions e.g. soil pathogen status in their orchard, keeping in mind that a rootstock selection is critical and cannot be changed after orchard establishment. The way rootstocks affect fruit quality is not always clear, but plant hormone balance, nutrition and water relations seem to play key roles (Castle 1995).

Popular rootstocks worldwide are the two sister hybrids 'Carrizo' and 'Troyer' citrange (C. *sinensis* x P. *trifoliate*), which are tolerant to *Tristeza* and *Phytophthora* and grow well on a wide range of soil types and produce moderately vigorous trees. They are frost tolerant, however other rootstocks such as 'Cleopatra' mandarin (C. *reshni*), Trifoliate (P. *trifoliata*) and Sour orange (C. *aurantium*) perform better in cold conditions. They are also vulnerable to blight and burrowing nematodes. Troyer was once the more popular of the two, but as Carrizo has performed better it is now the more popular selection of the two. Both of these two rootstocks produce fruit of good quality comparable to fruit produces on Swingle (C. *paradisi* × P. *trifoliate*) and Sour orange rootstocks. Unfortunately Carrizo and Troyer are incompatible with 'Eureka' lemon and 'Murcott' mandarin (Saunt, 1990)

'C35' citrange was developed by the University of California and grows well in sandy, loam and clay soils, but is sensitive to calcareous soil. It produces trees of medium size and should be planted at close spacing. It is also tolerant to *Phytophthora* and *Tristeza* and resistant to nematodes. In South Africa 'C35' citrange is effective with grapefruit and orange scions, producing fruit comparable in size and quality to 'Carrizo' citrange (Saunt, 1990).

The vigorous rootstock 'Rough Lemon' (C. *jambhiri* Lush) is popular in South Africa and mainly used for 'Valencia' oranges. It results in large trees that are drought tolerant because of the well-developed root system. It is tolerant to *tristeza*, but susceptible to *Phytophthora*. Trees on this rootstock produce large fruit with low internal quality, low juice content and low TSS:TA ratio, and thick, coarse rind. This rootstock combines well with kumquats (*Fortunella crassifolia* Swingle) as the thick rind is sweeter tasting (Saunt, 1990).

In 1907 Swingle was bred, a hybrid of Duncan grapefruit and trifoliate orange. An estimated 40 % of all rootstocks used in South Africa is Swingle. It produces good yields with high quality fruit, comparable to Carrizo, Troyer and Sour orange. The trees are cold-hardiness and tolerance to tristeza and phytophthora. Trees on Swingle rootstock in sandy soils tend to be smaller, so closer spacing is recommended for this combination. Swingle does well on waterlogged soils, but do not grow well in calcareous soils, heavy clay soils or high pH soils making it unpopular in the Mediterranean countries (Saunt, 1990).

In conclusion, there is no 'one size fits all' option when it comes to rootstocks and citrus fruit production and quality. Each has advantages and disadvantages. The decision remains with the producer and should be thoroughly researched and discussed before planting. There are however very limited information available on the impact of rootstocks on rind disorders

2.7. Conclusion

Fruit quality is not always an easy concept to define or measure as different aspects are important to different consumers. The highest prices are normally obtained on fresh markets where consumers buy fruit based on appearance, making external quality such as rind condition, size and colour very important. During the growth and development of fruit various factors influence these quality aspects. During fruit set, flowering, fruitlet abscission, cell division, fruit growth and maturation producers use PGR to improve fruit quality.

Depending on when it is applied, auxin is used to increase fruit size by reducing fruitlet competition and increasing fruit sink strength, or to increase rind strength, reducing fruit split in mandarins or for the prevention of post-harvest calyx abscission. In citriculture CKs are not regularly used but do enhance fruit development in parthenocarpic cultivars and play a role in alternate bearing. Ethylene is mostly used post-harvest for degreening when internal maturity has been reached and rind colour is still lacking. One of the most widely used PGRs in citriculture is GA_3 as it influences a wide range of factors during fruit growth and development depending on time and concentration of treatment. GA_3 can be used to reduce flower intensity and increase fruit set and also reduces rind disorders such as creasing and puffiness. GA_3 affects rind colour by preventing chlorophyll degradation and carotenoid accumulation. ABA influences vegetative growth, but in recent years has been found to influence rind quality and sensitivity toward CI and rind pitting a subject that needs more research.

The use of PGRs is not the only tools producers have to increase fruit quality and arsenic compounds can be used to lower TA in fruit. In addition TBZ is a chemical compound mostly used post-harvest to prevent fungal decay and increase shelf-life but indication exist of a positive impact on rind quality. Other horticultural practices such as rootstock selection, fertilization and irrigation scheduling also affect fruit and possibly rind quality.

Numerous research has been done on the effect of PGRs on citrus fruit quality, yet disorders such as creasing, CI and post-harvest rind pitting still lead to losses for the citrus industry. Thus more research is needed on the effect of PGRs on rind quality of citrus fruit.

2.8. References

Abbott, J.A., 1999. Quality measurement of fruits and vegetables. Postharvest Biol. Technol. 15, 207-225.

Abeles, F.B., Morgan, P.W., Saltveit Jr, M.E., 2012. Ethylene in Plant Biology, 2nd ed. Academic press, Oval Road, London, 24-28.

Agustí, M., Almela, V., Aznar, M., El-Otmani, M., Pons, J., 1994. Satsuma mandarin fruit size increased by 2, 4-DP. HortScience 29, 279-281.

Agustí, M., Martinez-Fuentes, A., Mesejo, C., 2002a. Citrus fruit quality. Physiological basis and techniques of improvement. Agrociencia 6, 1-16.

Agustí, M., Juan, M., Martínez-Fuentes, A., Mesejo, C., Reig, C., Almela, V., 2006. Application of 2, 4-dichlorophenoxypropionic acid 2-ethylhexyl ester reduces mature fruit abscission in Citrus navel cultivars. J. Hort. Sci. Biotecnol. 81, 532-536.

Agustí, M., Juan, M., Almela, V., 2007. Response of 'Clausellina' Satsuma mandarin to 3, 5, 6-trichloro-2-pirydiloxyacetic acid and fruitlet abscission. Plant Growth Reg. 53, 129-135.

Agustí, M., Zaragoza, S., Iglesias, D.J., Almela, V., Primo-Millo, E., Talón, M., 2002b. The synthetic auxin 3, 5, 6-TPA stimulates carbohydrate accumulation and growth in citrus fruit. Plant growth Reg. 36, 141-147.

Alférez, F., Agusti, M., Zacarías, L., 2003. Postharvest rind staining in Navel oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143-152.

Alférez, F., Sala, J.M., Sanchez-Ballesta, M.T., Mulas, M., Lafuente, M.T., Zacarias, L., 2005. A comparative study of the postharvest performance of an ABA-deficient mutant of oranges: I. Physiological and quality aspects. Postharvest Biol. Technol. 37, 222-231.

Alférez, F., Zacarias, L., Burns, J.K., 2005. Low relative humidity at harvest and before storage at high humidity influence the severity of postharvest peel pitting in citrus. J. Am. Soc. Hort. Sci. 130, 225-231.

Al-Jaleel, A., Zekri, M., Hammam, Y., 2005. Yield, fruit quality, and tree health of 'Allen Eureka' lemon on seven rootstocks in Saudi Arabia. Scientia Hort. 105, 457-465.

Alquezar, B., Zacarias, L., Rodrigo, M.J., 2009. Molecular and functional characterization of a novel chromoplast-specific lycopene beta-cyclase from Citrus and its relation to lycopene accumulation. J. Exp. Bot. 60, 1783-1797.

Bain, J.M., 1958. Morphological, anatomical, and physiological changes in the developing fruit of the Valencia orange, Citrus sinensis (L) Osbeck. Aust. J. Bot. 6, 1-23.

Berkowitz, G.A., Rabin, J., 1988. Antitranspirant associated abscisic Acid effects on the water relations and yield of transplanted bell peppers. Plant Physiol. 86, 329-331.

Bertling, I., Lovatt, C., 1996. A possible hormonal basis for vigour/dwarfing in commercially important citrus rootstocks. Proc. Int. Soc. Citriculture. 8, 12-17.

Blasco, J., Aleixos, N., Gómez, J., Moltó, E., 2007. Citrus sorting by identification of the most common defects using multispectral computer vision. J. Food Eng. 83, 384-393.

Buran, T.J., Sandhu, A.K., Azeredo, A.M., Bent, A.H., Williamson, J.G., Gu, L., 2012. Effects of exogenous abscisic acid on fruit quality, antioxidant capacities, and phytochemical contents of southern high bush blueberries. Food Chem. 132, 1375-1381.

Cabras, P., Schirra, M., Pirisi, F.M., Garau, V.L., Angioni, A., 1999. Factors affecting imazalil and thiabendazole uptake and persistence in citrus fruits following dip treatments. J. Agric. Food Chem. 47, 3352-3354.

Cajuste, J.F., Lafuente, M.T., 2007. Ethylene-induced tolerance to non-chilling peel pitting as related to phenolic metabolism and lignin content in 'Navelate'fruit. Postharvest Biol. Technol. 45, 193-203.

California Citrus Mutual, 2015. The new california navel. http://www.cacitrusmutual.com/market-info/the-california-standard. html 2015.

Campbell, B.L., Nelson, R.G., Ebel, R.C., Dozier, W.A., Adrian, J.L., Hockema, B.R., 2004. Fruit quality characteristics that affect consumer preferences for Satsuma mandarins. HortScience 39, 1664-1669.

Cantín, C.M., Fidelibus, M.W., Crisosto, C.H., 2007. Application of abscisic acid (ABA) at veraison advanced red color development and maintained postharvest quality of 'Crimson Seedless' grapes. Postharvest Biol. Technol. 46, 237-241.

Castel, J., Buj, A., 1990. Response of Salustiana oranges to high frequency deficit irrigation. Irrig. Sci. 11, 121-127.

Castle, W.S., 1995. Rootstock as a fruit quality factor in citrus and deciduous tree crops. N. Z. J. Crop Hortic. Sci. 23, 383-394.

Chien, P., Sheu, F., Lin, H., 2007. Coating citrus (Murcott tangor) fruit with low molecular weight chitosan increases postharvest quality and shelf life. Food Chem. 100, 1160-1164.

Cronjé, P., Crouch, E., Huysamer, M., 2005. Postharvest calyx retention of citrus fruit. Proc. Int. Postharvest Symposium 5, 369-376.

D'Aquino, S., Fadda, A., Barberis, A., Palma, A., Angioni, A., Schirra, M., 2013. Combined effects of potassium sorbate, hot water and thiabendazole against green mould of citrus fruit and residue levels. Food Chem. 141, 858-864.

Deszyck, E., Sites, J., 1954. The effect of lead arsenate sprays on quality and maturity of Ruby Red grapefruit. Proc. Fla. State Hort. Soc. 67, 38-42.

Davenport, T., 1990. Citrus flowering. Hort. Rev. 12, 349-408.

Dodd, M., Cronje, P., Taylor, M., Huysamer, M., Kruger, F., Lotz, E., Van der Merwe, K., 2010. A review of the post harvest handling of fruits in South Africa over the past twenty five years. South African J. of Plant and Soil: 25th anniversary edition 27, 97-116.

Dou, H., Zhang, J., Ismail, M.A., Ritenour, M., 2001. Postharvest factors influencing stem-end rind breakdown (SERB) of 'Valencia'oranges. Proc. Fla. state. Hort. Soc 114, 164-169.

El-Otmani, M., Coggins Jr, C.W., Agustí, M., Lovatt, C.J., 2000. Plant growth regulators in citriculture: world current uses. Crit. Rev. Plant Sci. 19, 395-447.

Fagoaga, C., Tadeo, F.R., Iglesias, D.J., Huerta, L., Lliso, I., Vidal, A.M., Talon, M., Navarro, L., García-Martínez, J.L., Peña, L., 2007. Engineering of gibberellin levels in citrus by sense and antisense overexpression of a GA 20-oxidase gene modifies plant architecture. J. Exp. Bot. 58, 1407-1420.

FAO. Pesticide residues in food 2012. FAO, Rome. 2013. ISBN 9789251075586

Fang, D., Federici, C., Roose, M., 1997. Development of molecular markers linked to a gene controlling fruit acidity in citrus. Genome 40, 841-849.

Fereres, E., Goldhamer, D.A., Parsons, L.R., 2003. Irrigation water management of horticultural crops. HortScience 38, 1036-1042.

Fidelibus, M.W., Davies, F.S., Campbell, C.A., 2002. Gibberellic acid application timing affects fruit quality of processing oranges. HortScience 37, 353-357.

Garcia-Luis, A., Agusti, M., Almela, V., Romero, E., Guardiola, J., 1985. Effect of gibberellic acid on ripening and peel puffing in 'Satsuma'mandarin. Scientia Hort. 27, 75-86.

García-Luís, A., Herrero-Villén, A., Guardiola, J.L., 1992. Effects of applications of gibberellic acid on late growth, maturation and pigmentation of the Clementine mandarin. Scientia Hort. 49, 71-82.

Garcia-Luis, A., Oliveira, M.E., Bordon, Y., Siqueira, D.L., Tominaga, S., Guardiola, J.L., 2002. Dry matter accumulation in citrus fruit is not limited by transport capacity of the pedicel. Ann. Bot. 90, 755-764.

García-Martínez, J., Garcia-Papi, M., 1979. Influence of gibberellic acid on early fruit development, diffusible growth substances and content of macronutrients in seedless Clementine mandarin. Scientia Hort. 11, 337-347.

García-Tejero, I., Romero-Vicente, R., Jiménez-Bocanegra, J., Martínez-García, G., Durán-Zuazo, V., Muriel-Fernández, J., 2010. Response of citrus trees to deficit irrigation during different phenological periods in relation to yield, fruit quality, and water productivity. Agric. Water Manage. 97, 689-699.

Goldschmidt, E.E., 1999. Carbohydrate supply as a critical factor for citrus fruit development and productivity. HortScience 34, 1020.

Greenberg, J., Kaplan, I., Fainzack, M., Egozi, Y., Giladi, B., 2006. Effects of auxins sprays on yield, fruit size, fruit splitting and the incidence of creasing of Nova mandarin. Acta Hort 727, 249-254.

Guardiola, J., García-Luis, A., 2000. Increasing fruit size in Citrus. Thinning and stimulation of fruit growth. Plant Growth Reg. 31, 121-132.

Gutter, Y., 1985. Combined treatment with thiabendazole and 2-aminobutane for control of citrus fruit decay. Crop Protection 4, 346-350.

Hernandez Minana, F., Primo-Millo, E., 1990. Studies on endogenous cytokinins in Citrus. J. Hort. Sci. 65, 595-601.

Hield, H., Coggins, C., Garber, M., 1958. Gibberellin tested on citrus: Fruit set on Bearss lime, Eureka lemon, and Washington navel orange increased by treatments in preliminary investigations. Calif. Agric. 12, 9-11.

Holmes, G.J., Eckert, J.W., 1999. Sensitivity of Penicillium digitatum and P. italicum to postharvest citrus fungicides in California. Phytopathology 89, 716-721.

Huff, A., 1984. Sugar regulation of plastid interconversions in epicarp of citrus fruit. Plant Physiol. 76, 307-312.

Huff, A., 1983. Nutritional control of regreening and degreening in citrus peel segments. Plant Physiol. 73, 243-249.

Hutton, R., Landsberg, J., Sutton, B., 2007. Timing irrigation to suit citrus phenology: a means of reducing water use without compromising fruit yield and quality. Animal Production Science 47, 71-80.

Iglesias, D.J., Cercós, M., Colmenero-Flores, J.M., Naranjo, M.A., Ríos, G., Carrera, E., Ruiz-Rivero, O., Lliso, I., Morillon, R., Tadeo, F.R., 2007. Physiology of citrus fruiting. Brazilian Journal of Plant Physiology 19, 333-362.

Iwahori, S., Oohata, J., 1981. Control of flowering of Satsuma mandarins (Citrus *unshiu* Marc.) with gibberellin. Proc. Int. Soc. Citriculture. 1, 247-249.

Jifon, J., Syvertsen, J., 2001. Effects of moderate shade on Citrus leaf gas exchange, fruit yield and quality. Proc. Fla. State Hort. Soc. 114, 177-181.

Jona, R., Goren, R., Marmora, M., 1989. Effect of gibberellin on cell-wall components of creasing peel in mature 'Valencia'orange. Scientia Hort. 39, 105-115.

Jordan, R.B., Seelye, R.J., McGlone, V.A., 2001. A sensory-based alternative to Brix/acid ratio. Food Technol. 55, 36-44.

Koo, R., 1979. The influence of N, K and irrigation on tree size and fruit production of 'Valencia' orange. Proc. Fla. state. Hort. Soc 92, 10-13.

Krajewski, A., Rabe, E., 1995. Citrus flowering: a critical evaluation. J. Hortic. Sci. 70, 357-374.

Ladanyia, M., 2010. Citrus Fruit: Biology, Technology and Evaluation, 1ste ed. Academic press, ICAR Research Complex for Goa.

Lafuente, M.T., Sala, J.M., 2002. Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rindstaning of 'Navelina' orange (*Citrus sinensis* L. Osbeck) fruit. Postharvest Biol. Technol. 25, 49-57.

Lord, E., Eckard, K., 1987. Shoot development in Citrus sinensis L.(Washington navel orange). II. Alteration of developmental fate of flowering shoots after GA3 treatment. Bot. Gazette , 17-22.

Lovatt, C.J., 1999. Timing citrus and avocado foliar nutrient applications to increase fruit set and size. HortTechnology 9, 607-612.

Mitcham, B., Cantwell, M., Kader, A., 1996. Methods for determining quality of fresh commodities. Perishables handling newsletter 85, 1-5.

Mesejo, C., Rosito, S., Reig, C., Martínez-Fuentes, A., Agustí, M., 2012. Synthetic auxin 3, 5, 6-TPA provokes citrus clementina (Hort. ex Tan) fruitlet abscission by reducing photosynthate availability. J. Plant Growth Regul. 31, 186-194.

Mupambi, G., Verreynne, J.S., Stander, O.P.J., Cronje, P.J.R., 2015. Optimal timing of application of 2,4-D on 'Navel' sweet orange [*Citrus sinensis* (Osbeck)] reduces the size of the navel-end. J. Hort. Sci. Biotec. 90, 619-625.

Obenland, D., Collin, S., Mackey, B., Sievert, J., Fjeld, K., Arpaia, M.L., 2009. Determinants of flavor acceptability during the maturation of navel oranges. Postharvest Biol. Technol. 52, 156-163.

OECD, 2010. Citrus Fruits. International Standards for Fruit and Vegetables.

OECD, 2009. OECD Fruit and Vegetables Scheme. Citeseer.

Oslund, C., Davenport, T., 1987. Seasonal enhancement of flower development in Tahiti limes by marcottage. HortScience 22, 498-501.

Peppi, M.C., Fidelibus, M.W., Dokoozlian, N., 2006. Abscisic Acid Application Timing and Concentration Affect Firmness, Pigmentation and Color of 'Flame Seedless' Grapes. HortScience 41, 1440-1445.

Porat, R., Daus, A., Weiss, B., Cohen, L., Droby, S., 2002. Effects of combining hot water, sodium bicarbonate and biocontrol on postharvest decay of citrus fruit. J. of Hort. science & biotec. 77, 441-445.

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

Quaggio, J., Teófilo Sobrinho, J., Dechen, A., 1992. Magnesium influences on fruit yield and quality of ‘Valencia’ sweet orange on 'Rangpur' lime. Proc. Int. Soc. Citric. 2, 633-637.

Reuther, W., 1989. The Citrus Industry, Rev. ed. UCANR Publications, Oakland,California.: University of California, Division of Agriculture and Natural Resources.

Riov, J., Dagan, E., Goren, R., Yang, S.F., 1990. Characterization of abscisic Acid-induced ethylene production in citrus leaf and tomato fruit tissues. Plant Physiol. 92, 48-53.

Ritenour, M.A., Stover, E., Boman, B.J., Dou, H., Bowman, K.D., Castle, W.S., 2004. Effect of rootstock on stem-end rind breakdown and decay of fresh citrus. HortTechnology 14, 315-319.

Romero, P., Rodrigo, M.J., Lafuente, M.T., 2013. Differential expression of the Citrus sinensis ABA perception system genes during postharvest fruit dehydration. Postharvest Biol. Technol. 76, 65-73.

Sadka, A., Artzi, B., Cohen, L., Dahan, E., Hasdai, D., Tagari, E., Erner, Y., 2000. Arsenite reduces acid content in citrus fruit, inhibits activity of citrate synthase but induces its gene expression. J. Am. Soc. Hort. Sci. 125, 288-293.

Salvador, A., Sdiri, S., Navarro, P., Monterde, A., MARTINEZ-JAVEGA, J., 2010. The use of auxins to maintain postharvest quality of citrus fruit. Acta horticulturae .

Saunt, J., 1990. Citrus Varieties of the World. an Illustrated Guide., 2nd ed. Sinclair International Ltd., Norwich,United Kingdom.

Schirra, M., Cabras, P., Angioni, A., D'hallewin, G., Pala, M., 2002. Residue uptake and storage responses of Tarocco blood oranges after pre-harvest thiabendazole spray and postharvest heat treatment. J. Agric. Food Chem. 50, 2293-2296.

Sdiri, S., Navarro, P., Salvador, A., 2013. Postharvest application of a new growth regulator reduces calyx alterations of citrus fruit induced by degreening treatment. Postharvest Biol. Technol. 75, 68-74.

Seemann, J.R., Sharkey, T.D., 1987. The Effect of Abscisic Acid and Other Inhibitors on Photosynthetic Capacity and the Biochemistry of CO(2) Assimilation. Plant Physiol. 84, 696-700.

Shimokawa, K., Shimada, S., Yaeo, K., 1978. Ethylene-enhanced chlorophyllase activity during degreening of Citrus *unshiu* Marc. Scientia Hort. 8, 129-135.

Smoot, J.J., Melvin, C., 1970. Decay control of Florida citrus fruits with packinghouse applications of thiabendazole. Food Chem. 18, 681-684.

Stewart, I., Wheaton, T.A., 1972. Carotenoids in citrus. Their accumulation induced by ethylene. J. Agric. Food Chem. 20, 448-449.

Stander, O., Theron, K., Cronje, P., 2014. Foliar 2, 4-Dichlorophenoxy Acetic Acid (2, 4-D) Application after Physiological Fruit Drop Reduces Fruit Splitting and Increases Fruit Size in Mandarin. Acta Hort. 1042, 43-50.

Syvertsen, J., 1984. CO2 assimilation and water use efficiency of young expanding citrus leaves. Acta. Hort. 171, 229-236.

Taiz, L., Zeiger, E., 2010. Plant Physiology, 5th ed. Sinauer Associates, Sunderland, USA.

Treeby, M., Henriod, R., Bevington, K., Milne, D., Storey, R., 2007. Irrigation management and rootstock effects on navel orange (C. sinensis (L.) Osbeck) fruit quality. Agric. Water Manage. 91, 24-32.

Van Zyl, K., 2014. A Guide to the use of Plant Growth Regulants, Defoliants and Desiccants., 1ste ed. Avcasa, South Africa.

Wardowski, W.F., 2006. Fresh Citrus Fruits, 2nd ed. Florida Science Source, Inc., Longboat Key, Fla.

Wild, B., 1993. Reduction of chilling injury in grapefruit and oranges stored at 1°C by pre-storage hot dip treatments, curing, and wax application. Animal Production Sci. 33, 495-498.

Xu, C., Fraser, P.D., Wang, W., Bramley, P.M., 2006. Differences in the carotenoid content of ordinary citrus and lycopene-accumulating mutants. J. Agric. Food Chem. 54, 5474-5481.

Yuen, C., Tridjaja, N.O., Wills, R.B., Wild, B.L., 1995. Chilling injury development of 'Tahitian' lime,'Emperor' mandarin,'Marsh' grapefruit and 'Valencia' orange. J. Sci. Food Agric. 67, 335-339.

Zeithaml, V.A., 1988. Consumer perceptions of price, quality, and value: a means-end model and synthesis of evidence. The Journal of marketing , 2-22.

Zekri, M., Obreza, T.A., 2003a. Macronutrient deficiencies in citrus: calcium, magnesium, and sulfur. Citrus Veg. Mag. 68, 8-11.

Zekri, M., Obreza, T.A., 2003b. Micronutrient deficiencies in citrus: iron, zinc, and manganese. Fla. Coop. Ext. Serv. Doc SL 204, 1-4.

Zekri, M., Obreza, T.A., Koo, R., 2009. Irrigation, nutrition, and citrus fruit quality. Fla. Coop. Ext. Serv. Doc SL 207, 1-3.

3. Paper 1: Pre-harvest factors and plant growth regulators affecting the incidence of post-harvest rind pitting in 'Valencia' orange (*Citrus sinensis* L. Osb.)

Abstract

Post-harvest pitting is a non-chilling related physiological rind disorder that reduces external quality which lead to reduced fruit market value. The disorder affects various citrus cultivars including 'Navel' orange and 'Benny' valencia. The primary cause of the disorder is not known, but it is aggravated by changes in post-harvest relative humidity (RH) and rind water status. Incidence of this disorder is erratic due to a wide array of factors influencing fruit susceptibility. The aim of the experiment was to ascertain the impact of various pre-harvest factors on rind pitting of 'Benny' and 'Turkey' valencia orange. Fruit position in the canopy and fruit maturity were found to affect 'Benny' valencia fruit susceptibility to this disorder with outside fruit exposed to greater variation in environmental conditions and more mature fruit being more susceptible. Pre-harvest application of plant growth regulators was also evaluated to prevent pitting. Application of 10 mg·L^{-1} synthetic auxins, 2,4-dichlorophenoxy acetic acid at 50 % petal drop or after physiological fruit drop and 3,5,6 trichloro-2-pyridiloxyacetic acid after physiological fruit drop, reduced this disorder. One week before harvest a foliar application of 400 mg·L^{-1} s-abscisic acid as well as 4000 mg·L^{-1} thiabendazole reduced the incidence of this disorder by an average of 30 % and 50 %, respectively. These results indicate a definite impact of pre-harvest conditions in deterring susceptibility and offer some possible management actions to decrease the impact of this disorder.

Additional keywords: Physiological disorder, flavedo, fruit position, maturity, auxins, abscisic acid, gibberellic acid, thiabendazole.

Introduction

South Africa is the second largest exporter of fresh citrus fruit in the world and exported 1.7 million tons 'Valencia' oranges during the 2014 season (CGA, 2014). To remain competitive and obtain premium prices on markets fruit need to be of a high physical and physiological quality and lesion free. Non-chilling post-harvest pitting is a physiological rind disorder that diminishes external fruit quality resulting in economic losses. The disorder originates in the transitional zone of the flavedo-albedo with epidermal cells of the flavedo, oil glands and deeper layers of the albedo initially unaffected (Agusti et al., 2001). As the disorder progresses, affected areas turn bronze in colour, most likely due to the oil gland content being released into intercellular spaces resulting in enzymatic oxidation (Agusti et al., 2001).

The primary cause or mechanism of the disorder is unknown, however variation in relative humidity (RH) during post-harvest handling and resultant changes in the rind water status seem to be involved in the susceptibility to develop this disorder in 'Navel' oranges (Agusti et al., 2001; Alférez et al., 2005b) and 'Marsh' grapefruit (C. *paradisi* Macf.) (Alférez and Burns, 2004). Alférez and Burns, (2004) reported a significant positive correlation between cumulative weight loss and change in water balance in the rind with an increase in post-harvest pitting index (PPI). The microclimate in a tree canopy or orchard, RH, temperature and light affect citrus fruit quality (Sites and Reitz, 1949; Fallahi and Moon Jr, 1989). In addition, Fallahi and Moon Jr (1989) reported that inside fruit, not exposed to direct radiation, had a significant higher fruit weight, juice %, rind thickness and rind weight (dry and fresh) relative to fruit from the outside of the canopy. Canopy position can also affect citrus fruit rind biochemical properties as well as susceptibility to physiological disorders such as rind breakdown of 'Nules Clementine' mandarin (*C. reticulate* Blanco), where fruit from the inside of the canopy have less carotenoids and were more susceptible to this disorder (Cronje et al., 2011). In contrast, 'Valencia' oranges from the outside of the canopy were reported to be more susceptible to pre-harvest rind staining compared to fruit from the inside of the canopy (Arpaia et al., 1991).

In citriculture plant growth regulators (PGRs) or synthetic phytohormones are used pre- and post-harvest to improve fruit set, reduce creasing incidence. (gibberellic acid, GA_3) (Fidelibus et al., 2002) improve fruit size (auxin) (Agustí et al., 1994) and to improve rind colour development (ethylene) (Stewart and Wheaton, 1972). The synthetic auxin, 3,5,6 trichloro-2-pyridiloxyacetic acid (3,5,6 TPA), is used as a commercial chemical thinning agent, but also reduces the incidence of creasing and fruit splitting in 'Nova' mandarin (*C. reticulate* × (*C. paradisi* × *C. reticulate*) (Greenberg et al., 2006). Another synthetic auxin, 2,4-dichlorophenoxy acetic acid (2,4-D), when applied after physiological fruit drop, is known to reduce fruit splitting in mandarin (Greenberg et al., 2006; Stander et al., 2014). In addition, when applied during full bloom to 'Navel' orange, 2,4-D reduces the size of navel-end opening and increases the percentage of 'Washington' navel fruit with closed navel-ends (Mupambi et al., 2015)

GA_3 is used in citriculture to improve fruit set (van Zyl, 2014) and also reduces the incidence of multiple rind disorders, such as creasing in 'Valencia' and 'Navel' orange fruit (Fidelibus et al., 2002) and puffiness in 'Satsuma' mandarin (C. *unshiu* Marc.) (Garcia-Luis et al., 1985). If applied early during fruit development, GA_3 increases rind strength, whilst application at colour break delays colour development by delaying chloroplast breakdown and carotenoid accumulation (Fidelibus et al., 2002). A GA biosynthesis inhibitor, prohexadione-calcium used on pome fruit trees to reduce vegetative growth (Miller, 2002; Stover et al., 2004) has been reported to enhance rind colour development of citrus fruit (Barry and Le Roux, 2010).

Exogenous application of s-abscisic acid (s-ABA) is not used in citriculture, but in viticulture $0.2g{\cdot}L^{-1}$ is applied at veraison to enhance berry colour development (Peppi et al., 2006; Cantín et al., 2007; van Zyl, 2014). However, it has been reported that the ABA level in the fruit rind influences the incidence of certain physiological rind disorders such as post-harvest pitting and chilling injury (CI) (Alférez et al., 2005a). 'Pinalate' orange, an ABA-deficient mutant, was more susceptible to post-harvest pitting and less susceptible to chilling injury (CI). It is possible that the lack of ABA impacted on the higher transpiration rate of the cultivar, due to poor stomatal control, resulting in increased weight loss and less firm fruit (Alférez et al., 2005a).

The first objective of this study was to identify pre-harvest, fruit position, maturity and size, factors that contribute to the susceptibility of a fruit to post-harvest pitting. Secondly, various PGRs were evaluated for their efficacy in reducing pitting of 'Valencia' oranges.

Materials and methods

Sites and plant material

Experiments were conducted over two seasons 2013/14 (1^{st} season) and 2014/15 (2^{nd} season) in the Letsitele valley in Limpopo, the main 'Valencia' orange production area of South Africa. Two cultivars prone to post-harvest pitting, 'Benny' and 'Turkey' valencia orange, were investigated and orchard details are summarised in Table 1.

'Turkey' valencia orchards were selected at The Plains (23°50'46.91S 30°27'03.81E) and La Gratitude (23°48'37.69S 30°25'59.48E), one on either side of the Letaba river (Table 1;

Fig. 1. A and B). While another orchard was selected further south at Novengilla (23°54’07.17S 30°24’09.95E) (Table 1; Fig. 1. C).

‘Benny’ valencia orchards were selected at Riverside (23°48’02.51S 30°27'27.20E), The Plains (23°50’23.94S 30°25'55.82E) and Letaba Orange 23°48’27.50S 30°26'06.13E) in the Letsitele valley (Fig. 1. D, E and F) and two additional orchards close to Mbombela, Mpumalanga at Croc Valley (25°27’43.25S 31°03'29.97E) was also used for sampling of fruit (Table 1.) These different sites were selected to account for soil and microclimatic differences throughout the Letsitele valley.

Treatments, experimental design and sampling procedure

Postharvest stress treatments

To determine if the postharvest stress of dehydration followed by rehydration could be used to induce pitting (Alférez et al 2005b) to use this as a test of efficacy of treatments, the following experiment was conducted prior to large scale testing. Two samples of twenty fruit were harvested per tree from 10 healthy and uniform ‘Benny’ valencia trees in each orchard at Riverside and The Plains. One sample received a stress treatment before being placed into storage (4.5°C) and the other was placed into cold storage directly after harvest. The stress treatment involved dehydrating fruit at 25°C and 50 % RH (VPD of 1.55 kPa) for three days followed by 1 day at 20°C with 99 % RH before being placed in cold storage. Further sampling was done 1 week later from Riverside, again two samples of 20 fruit were harvested from each tree. A similar stress treatment was carried out; however in this experiment one sample from each tree either received wax after stress treatment while the other was left unwaxed prior to being placed in cold storage. In Figure 3 the negative impact of dehydration followed by rehydration is illustrated confirming that this stress can be used to test other treatment efficacies on pitting incidence.

Fruit canopy position and harvest maturity

During the 1st season 10 uniform and healthy trees were selected in the ‘Turkey’ valencia orchards at La Gratitude, The Plains and Novengilla and in ‘Benny’ valencia orchards at Riverside and The Plains. While during the 2nd season orchards were selected on the same farms except for Novengilla.

For three consecutive weeks during the commercial harvest window, 20 fruit from the outside (within the first 30 cm from the outside of the canopy) and 20 fruit from the inside (deeper than 30 cm from the outside of canopy) of the canopy were harvested from each tree at the selected orchards. The 'Turkey' valencia orchards were harvested on June 4, 11 and 18 during the 1st season and on June 1, 8 and 15 during the 2nd season. The 'Benny' valencia, later maturing cultivar, were harvested later during each season on June 24, July 1 and 9 during the 1st season and on June 22, 29 and July 6 during the 2nd season. Harvest dates were determined from historical records of a producer's commercial harvest window, however maturity was not measured due to the lack of correlation between internal and external maturity in citrus fruit.

Fruit size

During the 1st season 20 fruit with a diameter smaller than 69 mm and 20 fruit with a diameter larger than 73 mm were harvested from 10 uniform and healthy 'Benny' valencia trees in Bend 1 and Umdoni 2 at Croc valley. After harvest fruit received the stress treatment and were waxed before being placed in cold storage at 4.5°C for 21 days prior to evaluations.

Foliar sprays

During the 1st season healthy and uniform 'Benny' valencia trees were selected at Riverside (block 3.5) and The Plains (block 7) with at least 2 buffer trees between each selected tree and a buffer row between rows. Each foliar treatment was applied to ten replicate trees (n=10) in a randomised complete block design. The following products where applied: 2,4-D [2,4-D, Dow AgroSciences (Pty) Ltd.], Gibberellic acid (GA_3) [Progibb®, Philargo SA (Pty) Ltd.], 2,4-dichloroprop P (2,4-DP)[Coracil-P®, Nufarm Agriculture (Pty) Ltd.] and prohexadione-calcium [Regalis®, BASF SA (Pty) Ltd]. A non-ionic wetting agent [Break-Thru®, Villa Crop Protection (Pty) Ltd.] was also added to spray solutions of 2,4-D, GA_3 and 2,4-DP , while the non-ionic wetting agent [Dash®, BASF SA (Pty) Ltd.] was added to spray solutions of prohexadione-calcium. Water with Break-Thru® was used as the control and applied to 10 trees (Table 2)

During the 2nd season, healthy uniform 'Benny' valencia trees were selected at Riverside (block 3.13) and in the same orchard at The Plains as during the previous season, however not the same trees, with buffer trees between selected trees and buffer rows between rows. Each treatment was applied as foliar spray to eight replicate trees (n=8) in a randomised complete block design. The treatments as summarised in Table 3 were applied. In this season 3,5,6 trichloro-2-pyridiloxyacetic acid (3,5,6 TPA) [Maxim®, ARYSTA Lifescience SA (Pty) Ltd.] and the plant growth regulator s-abscisic acid (s-ABA) (Protone®, Valent

BioSciences) and thiabendazole (TBZ) [ICA-TBZ, ICA international chemicals (Pty) Ltd.] were also included in the trial while prohexadione-calcium was excluded during this season.

During the 1st season 40 fruit were harvested from each replicate tree on June 14 at Riverside and July 1 at The Plains. During the 2nd season 30-40 fruit per tree were harvested on the 22 and 29 June at Riverside and The Plains, respectively.

Little information is available on the use of ABA in citriculture and therefore 32 healthy and uniform 'Benny' valencia trees were selected at Letaba Orange with buffer trees between selected trees and buffer rows between rows and ABA was applied at three different concentrations (200 $mg \cdot l^{-1}$), (400 $mg \cdot l^{-1}$) (dosage used in grape industry to improve berry coloration) and (800 $mg \cdot l^{-1}$) mixed with 5 ml per 100 L Break-Thru® to eight trees (n=8) each in a randomised complete block design. Control trees were sprayed with only water and 5 ml per 100 L Break-Thru®. Treatments were applied on 21 June. Ten fruit were harvested per tree 1 day after application (DAA) as well as, 8 and 15 DAA.

All foliar treatments were applied using a Stihl mist blower (SR 450, ANDREAS STIHL AG & Co., KG, Germany) at approximately 3 L of the mixture per tree.

Waxing

Fruit were waxed in the 1st season with 18 QDP wax [John Bean Technologies (Pty) Ltd.]. The bottom of an open top box lined with 36 shoe brushes (Academy Brushware (Pty) Ltd) with brush-side facing upwards was used. Before fruit were placed inside the box, brushes were sprayed with wax using a hand-held (Racomaster Enterprsises Corp.) spray bottle until wet enough to cover entire hand when brushed. Fruit were placed inside the box on top of brushes and sprayed a further 5 times, the box was shook in a horizontal manner to roll fruit back and forth over the brushes until fruit were fully covered by wax. Fruit were then left to dry for two hours at room temperature (18°C).

Data collection and evaluation

Fruit weight was measured directly after harvest, after dehydration and after storage with a balance (Kern KB 3600-2N, KERN & SOHN GmbH | Ziegelei 1 | 72336 Balingen - Germany).

Fruit size of 150 'Benny' valencia fruit per tree from the control, 2,4-D and dichloroprop-P treated trees at The Plains and Riverside were measured just prior to harvest during the 1st season using an electronic calliper (CD-6"C, Mitutoyo Corp, Tokyo, Japan).

Pitting incidence evaluation of the fruit was done after 21 days storage at 4.5°C. Fruit were inspected and rated from 0 (no post-harvest pitting) to 4 (severe post-harvest pitting) (Fig. 2), and a post-harvest pitting index (PPI) was calculated as follows:

$$\text{PPI (0-4)} = \sum \frac{Rating\ (0-4) \times number\ of\ number\ fruit\ within\ each\ class}{Total\ number\ of\ fruit}$$

Residue analysis was performed by an accredited analytic laboratory (Hearshaw and Kinnes Analytical Laboratory, Westlake, Cape Town, South Africa) on fruit from TBZ treated trees (n=4) at Riverside and The Plains. Six fruit per replicate were cut into pieces, weighed and pulped using a blender and stored at -5°C until samples were sent for residue analysis. The samples were extracted by using acetonitrile and a matrix solid phase dispersion extraction. The analysis of the extracts was conducted using liquid chromatography mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, CA, USA).

Data analysis

PPI (ranked) data and weight loss (%) were analysed using the one-way Anova, two-way Anova (test interaction) and non-parametric one-way Anova (Kruskal-wallis test, when data was not normally distributed) with SAS Enterprise guide v.5.1 (SAS institute, Cary, NC, USA). Each treatment was compared with the other using Fischer least significance differences (LSD), and *P*-values smaller than 0.05 were deemed significant unless otherwise indicated.

Results

Post-harvest pitting and weight loss

Postharvest water stress of 'Benny' valencia fruit resulted in significantly higher PPI and total weight loss after 21 days storage at 4.5°C and higher weight loss three days after harvest at both farms (Fig. 3), while waxing fruit increased PPI (*p*: 0.5549), although not significantly (Fig. 4).

Canopy position did not significantly affect PPI (Fig. 5) or weight loss (data not shown) during dehydration between outside and inside 'Turkey' valencia fruit at The Plains and La

Gratitude during both seasons except at La Gratitude in the 1st season where inside fruit had higher weight loss during dehydration than outside fruit (data not shown). In contrast the 'Benny' valencia fruit PPI was significantly affected by canopy position. During the 1st season the inside fruit had significantly higher PPI than the outside fruit at Riverside even though PPI was low, with non-significant difference at The Plains (*p*: 0.766) (Fig. 6). In the 2nd season fruit from the outside of the canopy had significantly higher PPI than fruit from the inside of the canopy at both farms (Fig. 6). The weight loss during dehydration was not significantly affected by fruit position in either season (data not shown).

Fruit maturity significantly influenced PPI and weight loss of 'Turkey' valencia at The Plains during both seasons regardless of low incidence of post-harvest pitting (Fig. 7.). During the 1st season fruit harvested the second week (week 24) had significantly lower PPI than fruit harvested the first week (week 23), however the last week of harvest (week 25) did not differ from any other week (Fig. 7.). During the 2nd season very low post-harvest pitting was found during the first week of harvest (week 23) and no post-harvest pitting was found the following two weeks (week 24 and 25)(Fig. 7). During the 1st season the weight loss during dehydration was reduced with each week of harvest, with the first week of harvest being significantly higher than the second and last weeks of harvest which did not differ from each other (Fig. 7). In contrast during the 2nd season the fruit harvested the first week (week 23) had significantly lower weight loss during dehydration than the following two weeks (week 24 and 25) which also did not differ from each other (Fig. 7). At La Gratitude maturity had no effect on PPI of 'Turkey' valencia during the two seasons but % weight loss during dehydration increased significantly each week from week 23 until week 25 the 2nd season (date not shown).

Fruit maturity and harvest date affected PPI and weight loss of 'Benny' valencia. During the 1st season the last harvested fruit (week 28) had significantly higher PPI and weight loss during dehydration as well as total weight loss compared to the previous two weeks at The Plains (week 26 and 27) (Fig. 8). During the 2nd season the first and last harvested fruit (week 26 and 28) had higher PPI than the second week (week 27) while the last week (week 28) also had significantly higher weight loss, total and during dehydration than the previous two (week 26 and 27) (Fig. 8). At Riverside maturity of 'Benny' valencia significantly affected PPI and weight loss during dehydration the 1st season, but only total weight loss was affected the 2nd season (Fig. 9). Incidence of post-harvest pitting remained constant between the first two weeks of harvest (week 26 and 27) while incidence of this disorder increased in the following week (week 28) (Fig. 9). The second week (week 27) had the lowest weight loss during dehydration and total weight loss followed by the first week (week 26) then the last week (Fig. 9).

Fruit size had no effect on PPI in either of the two orchards sampled at Croc Valley (Fig. 10).

Foliar applied treatments affected PPI in orchards if high PPI were evident in the season, viz., The Plains (*p*: 0.0007) in the 1st season and Riverside (*p*: 0.059) the 2nd season (Fig. 11 and 12). During the 1st season at The Plains 2,4-D was the only treatment which significantly reduced PPI. Due to low PPI at Riverside in that season no treatment effect was seen in PPI and weight loss (Fig. 11). In the 2nd season at Riverside (Fig. 12) both treatments with 2,4-D as well as 3,5,6-TPA and TBZ, but not GA3 reduced PPI compared to the control (p=0.059). GA_3 and TBZ significantly increased weight loss during dehydration at The Plains while at Riverside TBZ reduced weight loss during dehydration and GA_3 did not have an effect (Fig. 12). Total weight loss at The Plains was increased by all treatments except 2,4-D at 50 % PD and s-ABA, which did not differ from the control (Fig. 12).

A general reduction in post-harvest pitting was found with each increase in concentration of s-ABA over all three harvesting dates, however no interaction was found between concentration and DAA the fruit were harvested. The two higher concentrations of 400 $mg \cdot L^{-1}$ and 800 $mg \cdot L^{-1}$ s-ABA reduced pitting incidence, however only significantly so if applied at 800 $mg \cdot L^{-1}$ and harvest 15 DAA (Fig. 13). Also 400 $mg \cdot L^{-1}$ reduced the disorder at this harvesting date, however a significant difference was only found at a 90 % significance level (data not shown). The time of harvest after application followed a similar trend with the two later harvest dates reducing incidence of this disorder. Fruit harvest 8 DAA and 15 DAA had significantly lower incidence of this disorder than fruit harvested 1 DAA at a concentration of 400 $mg \cdot L^{-1}$. At the higher concentration of 800 $mg \cdot L^{-1}$ this disorder was reduced in fruit harvested 15 DAA however at 8 DAA this disorder was less than at 1 DAA, although not significant.

Weight loss during dehydration was only affected 15 DAA with a slight decrease in weight loss with each increase in concentration (Fig. 14). Only 0.8 $g \cdot L^{-1}$ significantly reduced the weight loss during dehydration as well as total weight loss from the control while all other treatments did not differ. Total weight loss was only significantly affected 1 DAA with similar trend as mentioned above.

Fruit size and rind thickness

Dichloroprop-P and 2,4-D had no significant effect on fruit diameter during the 1^{st} season at The Plains or Riverside (Fig. 15).

Residue analysis

Thiabendazole residues of the foliar sprays were 56% higher at Riverside than at The Plains during the 2^{nd} season (Fig. 16).

Weather

Throughout both seasons maximum daily temperature and RH remained relatively constant while the minimum temperature declined in winter and increased in summer and large variation was seen in the minimum daily RH (Fig. 17). A closer look at the weather before each harvest also showed a large variation in specifically RH (Fig. 18).

Discussion

Cultivar difference in susceptibility to postharvest pitting was recorded with 'Benny' valencia being consistently more susceptible than 'Turkey' valencia. In addition it was documented for the first time for any 'Valencia' orange cultivar that change in RH during post-harvest storage increased post-harvest pitting of 'Benny' valencia oranges, as fruit that were dehydrated for three days at low RH then transferred to high RH for one day before storage had 90 % higher pitting incidence than fruit that were kept at constant high RH. These results concur with results on 'Navel' orange (Alférez et al., 2003) and grapefruit (Alférez and Burns, 2004).

Alferez et al. (2003) postulated that when fruit were stored at low RH, a high vapor pressure deficit (VPD) between epidermal cells and the atmosphere results in water loss and reduction in water potential (Ψ_w) of albedo and flavedo cells, when such dehydrated fruit are transferred to high RH the VPD is reduced and the water movement is changed, water moves from the air to cells in the flavedo and outer albedo. The Ψ_w of the flavedo and outer albedo recovers faster than that of the corresponding inner albedo and this difference in Ψ_w creates a suction force between cells in the rind that could be the reason for the increase in post-harvest pitting of 'Benny' valencia oranges when fruit are transferred from low to high RH. It has been reported that the transfer of dehydrated 'Navel' orange fruit to high RH results in a transient increase in respiration rate and ethylene production, this increase appears to be related to changes in cell wall turgor or cellular damage (Alférez et al., 2003; Alférez et al., 2005b). Thus this transfer of dehydrated 'Benny' valencia fruit from low to high RH could result in similar cellular damage that initiated post-harvest pitting. In contrast to (Alférez et al., 2005b) the wax application did not significantly increase the pitting incidence. This slight increase could be attributed to a decrease in internal O_2 and increase in internal CO_2 within the rind (Petracek et al., 1998). Thus wax is not the primary cause of post-harvest pitting, however it can enhance the disorder.

Large variation of PPI was found between seasons, orchards, trees within an orchard and fruit on the same tree from different positions, similar to pre-harvest rind stain incidence of 'Valencia' oranges (Arpaia et al., 1991) and post-harvest rind staining of 'Navelina' oranges (Lafuente and Sala, 2002). This variation attests to the impact of environmental conditions which could be a major factor affecting fruit susceptibility to post-harvest pitting between seasons. The prevailing microclimate - temperature and RH- and the variation thereof inside the orchard during fruit growth and development is thought to influence fruit susceptibility to this disorder. Citrus fruit physiology is known to be influenced by position on the tree due to slight change in microclimate (Sites and Reitz, 1949; Sites and Reitz, 1950; Boswell et al., 1982). Arpaia et al. (1991) reported higher pre-harvest rind stain incidence on the exposed Valencia fruit on the outer side of the tree canopy than fruit from the inside concurring with the general results in this study. It is expected that fruit grown on the outer parts of the tree canopy are more exposed to fluctuations in environmental conditions, thus being exposed to more stress as the VPD increases during the day and decreases during the night leading to water-stress similar to water stress induced by the stress treatment applied in this experiment (Agusti et al., 2001). This influence of microclimatic variation on the exposed 'Benny' valencia fruit could possibly explain the higher susceptibility to post-harvest pitting compared to fruit inside the canopy (Fig. 6).

The influence of environmental conditions in the 7 to 10 days prior to harvest could possibly explain the difference in pitting incidence between harvest dates. It is possible that condition leading to high vapour pressure deficit (VPD) prior to and at harvest could induce more stress than the previous or following week thus resulting in more sensitive fruit. However despite this possible microclimatic variation, maturity seem to be a factor, although minor, influencing fruit susceptibility as a general trend of more mature fruit having higher susceptible to post-harvest pitting were recorded in five out of six orchards monitored (Fig. 7-

9). A positive relationship was seen between PPI and weight loss during dehydration at both farms during the 1st season, similar to results reported by Alférez et al., (2005b) for 'Fallglo' tangerine (*C. reticulate* × *C. reticulate* × *C. paradisi*) and cumulative weight loss %. Alférez et al. (2003) also reported a similar trend between maturity of 'Navel' orange fruit and % weight loss. Fruit maturity could also be another factor contributing to the effect of fruit position on pitting susceptibility as Boswell et al. 1982 reported that 'Navel' orange fruit exposed to higher temperature and light, mature earlier than unexposed fruit.

It has been shown that epicuticular wax structure and morphology affects physiological rind disorders such as non-chilling pitting of 'Navelina' oranges (Sala et al., 1992). Prevailing environmental conditions alter citrus fruit epicuticular wax morphology (El-Otmani et al., 1989). El-Otmani et al. 1989 found a relation between epicuticular wax morphology of citrus fruit and the incidence of pre-harvest rind stain of 'Valencia' oranges. This could explain difference in susceptibility between fruit on the same tree, but not in maturation as wax formation is affected over a long time span, thus the micro climatic conditions could affect post-harvest pitting through changes in wax morphology, however this is possibly not the only manner in which the micro climate influences fruit susceptibility to this disorder.

The efficiency of foliar sprays to reduce the incidence of post-harvest pitting of 'Benny' valencia depends on the active ingredient and concentration of chemical used. The efficacy is further depended on orchard susceptibility to this disorder, as a treatment effect only became evident in susceptible orchards (Fig. 11-13). Foliar applied synthetic auxins 2,4-D and 3,5,6-TPA reduced post-harvest pitting of 'Benny' valencia while another synthetic auxin, dichloroprop-p had no effect (Fig. 11 and 12). No comparable studies have been done on the effect of synthetic auxins on post-harvest pitting of Valencia oranges, but these synthetic auxins have been shown to influence rind development and reduce splitting in mandarin (Greenberg et al., 2006; Stander et al., 2014), creasing in 'Nova' mandarin (Greenberg et al., 2006) and to reduce the percentage of Navel oranges with open navel-ends (Mupambi et al., 2015). At a significance level of 10 %, application of 10 $mg \cdot L^{-1}$ 2,4-D at either 50 % PD or after physiological fruit drop reduce post-harvest pitting of 'Benny' valencia whereas 3,5,6-TPA also reduced this disorder when applied after physiological fruit drop at the same concentration. The 2,4-D application had no effect on fruit size nor was any increase in rind coarseness noticed (Fig. 15). The reduction of pitting by these auxins could be attributed to their ability to change plant and fruit growth and development. Auxins increase fruit sink strength and dry mater accumulation (Agusti et al., 1995; Agustí et al., 2002) as well as increase the fruit peduncle diameter (Mesejo et al., 2003) which enhances transport capacity of the vascular system, to transport water and nutrients (Bustan et al., 1995). 2,4-D also promotes growth of rind cells during stage II of fruit development and can lead to increased rind thickness (Duarte et al., 2006; Stander et al., 2014). Thus any one or a combination of these effects of auxin on rind development and fruit growth could contribute to a stronger, less susceptible rind due to a possible improved nutrient accumulation of minerals and carbohydrates in the flavedo.

Gibberellic acid have been shown to reduce multiple citrus rind disorders such as creasing in oranges (Fidelibus et al., 2002) and puffiness in 'Satsuma' mandarin (Garcia-Luis et al., 1985), but did not reduce post-harvest pitting of 'Benny' valencia oranges when applied at 20 mg·l^{-1} after physiological fruit drop (Fig. 11 and 12). In most instances there was a slight reduction in this disorder on GA_3 treated fruit however this reduction was never significant.

Application of the postharvest fungicide TBZ as a pre-harvest spray to reduce incidence of this disorder have not been documented in literature. In this study foliar application of TBZ one week before harvest at 4000 mg·L^{-1} significantly reduced the incidence of post-harvest pitting of 'Benny' valencia (Fig. 12) without exceeding the maximum residue level (MRL) for TBZ of 5 ppm. Even though the effect of pre-harvest applied TBZ on post-harvest pitting has not been previously studied the effectiveness of TBZ to reduce chilling injury during cold storage has been reported (Schiffmann-Nadel et al., 1972). Subsequently it was shown that application of TBZ as 1 % active ingredient 14 days before harvest reduced chilling injury of 'Tarocco' blood oranges (Schirra et al., 2002). The mode of action through which TBZ reduces post-harvest pitting in unknown, however higher TBZ residues were found at Riverside, where a reduction in post-harvest pitting incidence and weight loss during dehydration was found. Thus TBZ could possibly reduce the incidence of pitting by reducing water stress during dehydration. More research will be needed to understanding mode of action of TBZ in affecting the incidence of rind pitting.

In this first documented study pre-harvest foliar application of s-ABA reduced pitting incidence. The concentration of 200 mg·L^{-1} s-ABA, -recommended for use in viticulture to improve colour development, - was too low to be effective, however the higher concentrations of 400 mg·L^{-1} and 800 mg·L^{-1} reduced PPI (Fig. 13). Even though 800 mg·L^{-1} had a greater reduction in PPI than 400 g·L^{-1}, there was no significant difference. Indicating that 800 mg·L^{-1} could be an over application. Time of application was also found to be important as the efficacy in reducing pitting improve from one day after application to 8 and 15 DAA (Fig.

13). This time delay affect was also evident in the ability of s-ABA to reduce fruit weight loss post-harvest. As a slight decrease in weight loss (total and during dehydration) was found with every increase in concentration, but this weight loss reduction was only significant during dehydration of fruit harvested 15 DAA and in total weight loss of fruit harvested 1 DAA (Fig 14). These results indicate that the effect of s-ABA on weight loss was only temporary as total weight loss of fruit harvested 1 DAA was reduced while there was no difference in total weight loss of fruit harvested 15 DAA. The slight reduction in weight loss could be a result of increased stomatal control or as Alférez et al. (2003) noted, a reduction in respiration. Alférez et al. (2003) found similar results on 'Pinalate' orange, where the incidence of post-harvest pitting was reduced with post-harvest ABA application. The mode of action of s-ABA seems to be increased water potential due to enhanced stomatal control that reduced water stress, as incidence of the disorder was reduced when weight loss was reduced during dehydration. There however seems to be an additional effect of s-ABA on pitting incidence reduction as the effect on weight loss was only marginal. Application of this PGR has not been document for this purpose in citriculture and could be a novel technology to control postharvest pitting of 'Valencia' orange.

In conclusion, post-harvest pitting is a physiological rind disorder aggravated by changes in post-harvest RH and temperature. Waxing of fruit is not the cause, but can enhance the incidence of the disorder. Large variation exists between cultivars, seasons, and farms, within orchards and within trees. The 'Benny' valencia was more susceptible than 'Turkey' valencia while more mature fruit on the outside of the canopy seemed to be mostly affected, possibly due to being more exposed to changes in environmental conditions resulting in increased water stress pre-harvest. This aspect should receive more focused research attention. The synthetic auxins 2,4-D applied at 50 % PD of after physiological fruit drop and 3,5,6-TPA applied after physiological fruit drop significantly reduced the disorder when applied at 10 $mg \cdot L^{-1}$ possibly due to increased sink strength, increased transport of water and nutrients and increased cell growth of the rind. In a novel approach the application of s-ABA and the fungicide TBZ reduced the disorder. An application of 10 $mg \cdot L^{-1}$ 2,4-D or 3,5,6-TPA after physiological fruit drop is recommended to reduce susceptibility of fruit. In addition fruit should be packed and placed within the cold chain as soon possible to minimise post-harvest environmental variation and water stress resulting in dehydration followed by rehydration of the rind which results in pitting development in susceptible fruit.

References

Agustí, M., Almela, V., Aznar, M., El-Otmani, M., Pons, J., 1994. Satsuma mandarin fruit size increased by 2, 4-DP. HortScience 29, 279-281.

Agusti, M., Almela, V., Juan, M., Alferez, F., Tadeo, F., Zacarias, L., 2001. Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. Ann. of Bot. 88, 415-422.

Agusti, M., El-Otmani, M., Aznar, M., Juan, M., Almela, V., 1995. Effect of 3, 5, 6-trichloro-2-pyridyloxyacetic acid on clementine early fruitlet development and on fruit size at maturity. HortScience 70, 955-962.

Agustí, M., Zaragoza, S., Iglesias, D.J., Almela, V., Primo-Millo, E., Talón, M., 2002. The synthetic auxin 3, 5, 6-TPA stimulates carbohydrate accumulation and growth in citrus fruit. Plant growth Reg. 36, 141-147.

Alférez, F., Agusti, M., Zacarías, L., 2003. Postharvest rind staining in Navel oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143-152.

Alférez, F., Sala, J.M., Sanchez-Ballesta, M.T., Mulas, M., Lafuente, M.T., Zacarias, L., 2005a. A comparative study of the postharvest performance of an ABA-deficient mutant of oranges: I. Physiological and quality aspects. Postharvest Biol. Technol. 37, 222-231.

Alférez, F., Zacarias, L., Burns, J.K., 2005b. Low relative humidity at harvest and before storage at high humidity influence the severity of postharvest peel pitting in citrus. J. Am. Soc. Hort. Sci. 130, 225-231.

Alférez, F., Burns, J.K., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79-87.

Arpaia, M., Kahn, T., El-Otmani, M., Coggins, C., DeMason, D., O'Connell, N., Pehrson, J., 1991. Pre-harvest rindstain of 'Valencia' orange: histochemical and developmental characterization. Scientia Hort. 46, 261-274.

Barry, G.H., Le Roux, S., 2010. Preharvest Foliar Sprays of Prohexadione–calcium, a Gibberellin-biosynthesis Inhibitor, Induce Chlorophyll Degradation and Carotenoid Synthesis in Citrus Rinds. HortScience 45, 242-247.

Boswell, S., Nauer, E., Atkin, D., 1982. Effect of tree density on fruit quality, temperature, light penetration, growth, and production of old-line 'Atwood' navel orange trees [California]. J. Am. Soc. Hort. Sci. 107, 60-65.

Bustan, A., Erner, Y., Goldschmidt, E., 1995. Interactions between developing citrus fruits and their supportive vascular system. Ann. of Bot. 76, 657-666.

Cantín, C.M., Fidelibus, M.W., Crisosto, C.H., 2007. Application of abscisic acid (ABA) at veraison advanced red colour development and maintained postharvest quality of 'Crimson Seedless' grapes. Postharvest Biol. Technol. 46, 237-241.

Citrus Growers Association of Southern Africa (CGA) 2014. Annual report 2014.

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

Duarte, A.M., García-Luis, A., Molina, R.V., Monerri, C., Navarro, V., Nebauer, S.G., Sánchez-Perales, M., Guardiola, J.L., 2006. Long-term Effect of Winter Gibberellic Acid Sprays and Auxin Applications on Crop Value of 'Clausellina' Satsuma. J. Am. Soc. Hort. Sci. 131, 586-592.

El-Otmani, M., Arpaia, M.L., Coggins Jr., C.W., Pehrson Jr., J.E., O'Connell, N.V., 1989. Developmental changes in 'Valencia' orange fruit epicuticular wax in relation to fruit position on the tree. Scientia Hort. 41, 69-81.

Fallahi, E., Moon Jr, J.W., 1989. Fruit quality and mineral nutrient from exposed verses internal canopy positions of four scitrus varieties. J. Plant Nutr. 12, 523-534.

Fidelibus, M.W., Davies, F.S., Campbell, C.A., 2002. Gibberellic acid application timing affects fruit quality of processing oranges. HortScience 37, 353-357.

Garcia-Luis, A., Agusti, M., Almela, V., Romero, E., Guardiola, J., 1985. Effect of gibberellic acid on ripening and peel puffing in 'Satsuma' mandarin. Scientia Hort. 27, 75-86.

Greenberg, J., Kaplan, I., Fainzack, M., Egozi, Y., Giladi, B., 2006. Effects of auxins sprays on yield, fruit size, fruit splitting and the incidence of creasing of Nova mandarin. Acta Hort. 727, 249-254.

Lafuente, M.T., Sala, J.M., 2002. Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rindstaning of 'Navelina' orange (Citrus sinensis L. Osbeck) fruit. Postharvest Biol. Technol. 25, 49-57.

Mesejo, C., Martínez-Fuentes, A., Juan, M., Almela, V., Agustí, M., 2003. Vascular tissues development of citrus fruit peduncle is promoted by synthetic auxins. Plant growth regulation 39, 131-135.

Miller, S.S., 2002. Prohexadione-calcium controls vegetative shoot growth in apple. J. of tree fruit production 3, 11-28.

Mupambi, G., Verreynne, J.S., Stander, O.P.J., Cronje, P.J.R., 2015. Optimal timing of application of 2,4-D on 'Navel' sweet orange [*Citrus sinensis* (Osbeck)] reduces the size of the navel-end. J. Hort. Sci. Biotec. 90, 619-625.

Peppi, M.C., Fidelibus, M.W., Dokoozlian, N., 2006. Abscisic Acid Application Timing and Concentration Affect Firmness, Pigmentation and Colour of 'Flame Seedless' Grapes. HortScience 41, 1440-1445.

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

Sala, J., Lafuente, T., Cuñat, P., 1992. Content and chemical composition of epicuticular wax of 'Navelina' oranges and 'Satsuma' mandarins as related to rindstaining of fruit. J. Sci. Food Agric. 59, 489-495.

Schiffmann-Nadel, M., Chalutz, E., Waks, J., Lattar, F., 1972. Reduction of pitting of grapefruit by thiabendazole during long-term cold storage. HortScience 4, 394-395.

Schirra, M., Cabras, P., Angioni, A., D'hallewin, G., Pala, M., 2002. Residue uptake and storage responses of Tarocco blood oranges after pre-harvest thiabendazole spray and postharvest heat treatment. J. Agric. Food Chem. 50, 2293-2296.

Sites, J.W., Reitz, H.J., 1950. The variation in individual Valencia oranges from different locations of the tree as a guide to sampling methods and spot-picking for quality. Part II. Titratable acid and the soluble solids/titratable acid ratio of the juice. Proc. Am. Soc. Hort. Sci. 55, 73-71.

Sites, J., Reitz, H., 1949. The variation in individual Valencia oranges from different locations of the tree as a guide to sampling methods and spot-picking for quality. I. Soluble solids in juice. Proc. Am. Soc. Hort. Sci. 54, 1-10.

Stander, O., Theron, K., Cronje, P., 2014. Foliar 2, 4-Dichlorophenoxy Acetic Acid (2, 4-D) Application after Physiological Fruit Drop Reduces Fruit Splitting and Increases Fruit Size in Mandarin. Acta Hort. 1042, 43-50.

Stewart, I., Wheaton, T.A., 1972. Carotenoids in citrus. Their accumulation induced by ethylene. J. Agric. Food Chem. 20, 448-449.

Stover, E., Ciliento, S., Myers, M., 2004. Response of six citrus genotypes to Prohexadione-Ca. Plant Growth Reg. Soc. Amer. 32, 86.

Van Zyl, K., 2014. A Guide to the use of Plant Growth Regulants, Defoliants and Desiccants., 1ste ed. Avcasa, South Africa.

Table 1: Summary of orchard sites used during the two seasons (2013/14 and 2014/15).

Farm	Location	Block	Cultivar	Row Orientation	Rootstock	Plant density (m)	Planting year
Croc valley	Mbombela	Bend 1 Umdoni 2	Benny	East - West	Rough Lemon	6 × 8	1996
The Plains	Letsitele	Block 15	Turkey	North - South	Swingle	2.5 × 6	2002
		Block 7	Benny	North - South		2.7 × 6.7	1997
La Gratitude	Letsitele	Block 7.22	Turkey	North - South	Swingle	3 × 7	2002
Letaba Orange	Letsitele	Block 6.2	Benny	East - West	Swingle	3 × 7	1994
Novingilla	Letsitele	Block 6	Turkey	North east – South west	Swingle	3 × 7.3	2002
Riverside	Letsitele	Block 3.5	Benny	East - West	Swingle	3 × 7.5	1994
		Block 3.13		North - South	Rough Lemon		

Table 2: Foliar applied synthetic plant growth regulators, type, concentrations and date applied during 1st season.

Type	Trade name	Active ingredient	Concentration (Active ingredient)	Wetting agent (Concentration)	Date
Auxin	2,4-D amine	2,4-D	10 $mg \cdot L^{-1}$	Break-Thru® (0.05 $ml \cdot L^{-1}$)	4 Dec. 2013
Auxin	Coracil P®	Dichloroprop P	800 $mg \cdot L^{-1}$	Break-Thru® (0.05 $ml \cdot L^{-1}$)	4 Nov. 2013
Gibberellic acid	Progibb®	Gibberellin A_3	20 $mg \cdot L^{-1}$	Break-Thru® (0.05 $ml \cdot L^{-1}$)	11 Jan. 2014
Anti-gibberellic acid	Regalis™	Prohexodione-calcium	3.5 $g \cdot L^{-1}$	Dash® (0.6 $ml \cdot L^{-1}$)	4 Dec. 2013 11 Jan. 2014

Table 3. Foliar applied synthetic plant growth regulators and fungicide, type, concentrations and date applied during 2nd season.

Type	Trade name	Active ingredient	Concentration (Active ingredient)	Wetting agent (Concentration)	Date
Auxin	2,4-D amine	2,4-D	10 mg·L^{-1}	Break-Thru® (0.05 ml·L^{-1})	23 Sept. 2014/ 25 Nov. 2014
Auxin	Maxim®	2,5,6-TPA	10 mg·L^{-1}	Break-Thru® (0.05 ml·L^{-1})	25 Nov. 2014
Gibberellic acid	Progibb®	Gibberellin A_3	20 mg·L^{-1}	Break-Thru® (0.05 ml·L^{-1})	14 Jan. 2015
Abscisic acid	Protone®	s-Abscisic acid	400 mg·L^{-1}	Break-Thru® (0.05 ml·L^{-1})	22 June 2015[a]
Fungicide	ICA-TBZ®	Thiabendazole	4000 mg·L^{-1}	Break-Thru® (0.05 ml·L^{-1})	16 June 2015[b] 22 June 2015[c]

[a] ABA only applied at The Plains block 7.
[b] TBZ applied at Riverside block 3.13.
[c] TBZ applied at The Plains block 7.

Fig. 1. Map of the different sites on either side of the Lataba River where ‘Turkey’ and ‘Benny’ valencia fruit were harvested in the Letsitele valley Limpopo. A: The Plains block 15(‘Turkey’ valencia). B: La Gratitude (‘Turkey’ valencia). C: Novingilla (‘Turkey’ valencia). D: Riverside Block 3.5 and 3.13 (‘Benny’ valencia). E: The Plains block 7 (‘Benny’ valencia). F: Lataba Orange (‘Benny’ valencia).

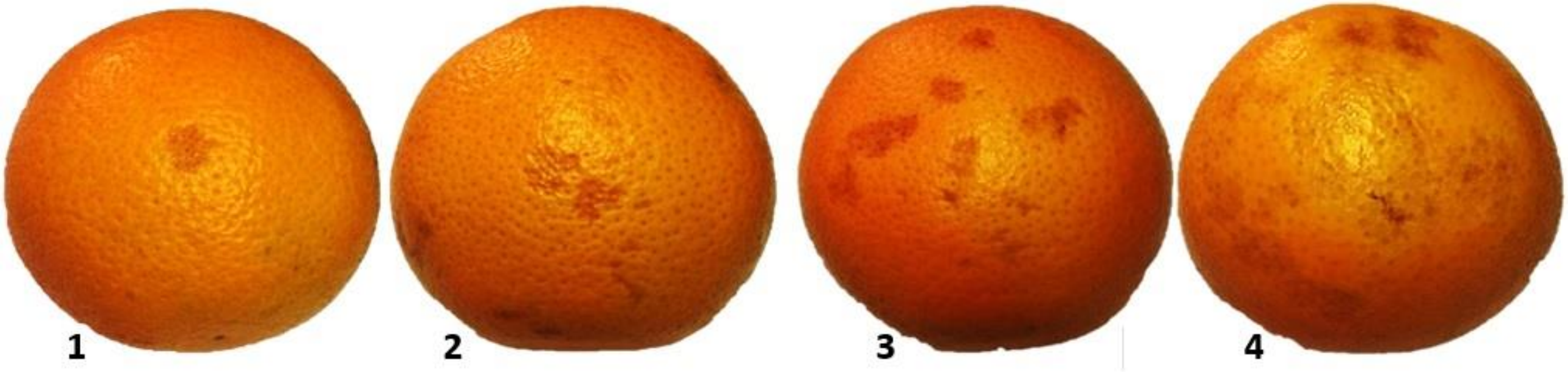

Fig. 2. Post-harvest pitting rating sheet. 0 = No post-harvest pitting (Not shown). 1 = Low post-harvest pitting. 2 = low to moderate post-harvest pitting. 3 = moderate to high pitting. 4 = high post-harvest pitting.

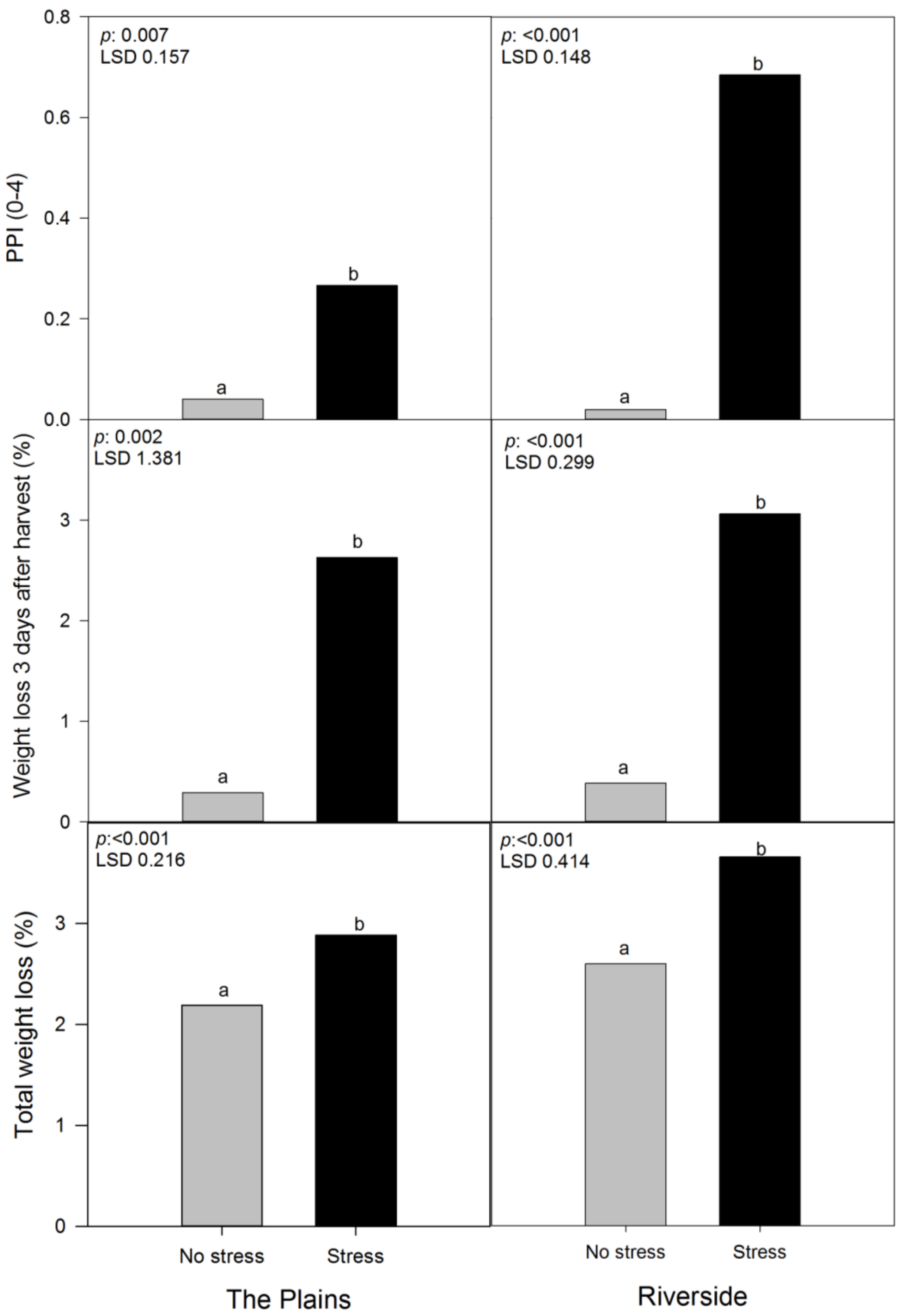

Fig. 3. Post-harvest pitting index (PPI) (0-4) of 'Benny' valencia fruit evaluated after 21 days storage at 4.5°C. Weight loss 3 days after harvest (%) and total weight loss (%) after 21 days storage at 4.5°C of fruit that received the stress treatment (stress) and fruit which did not receive the stress treatment (no stress). Different letters indicate significant difference at 95 % level ($p \leq 0.05$).

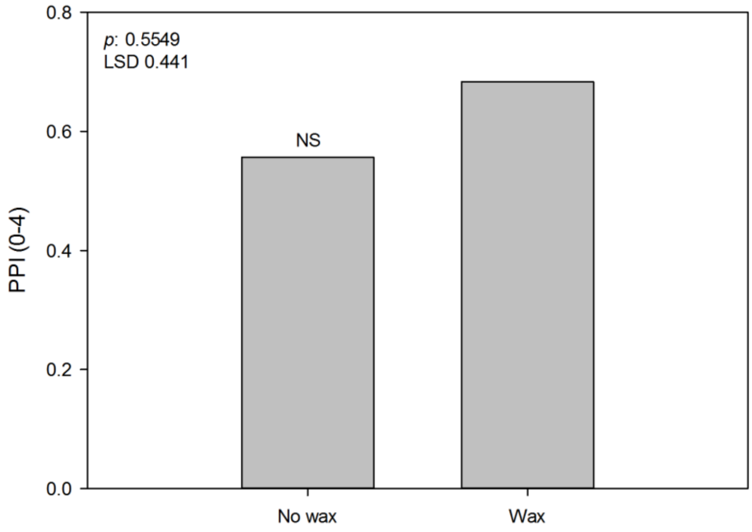

Fig. 4. Post-harvest pitting index (PPI) (0-4) after 21 days storage at 4.5°C of un-waxed and waxed 'Benny' valencia fruit. NS=No significant difference at 95 % level ($p \leq 0.05$).

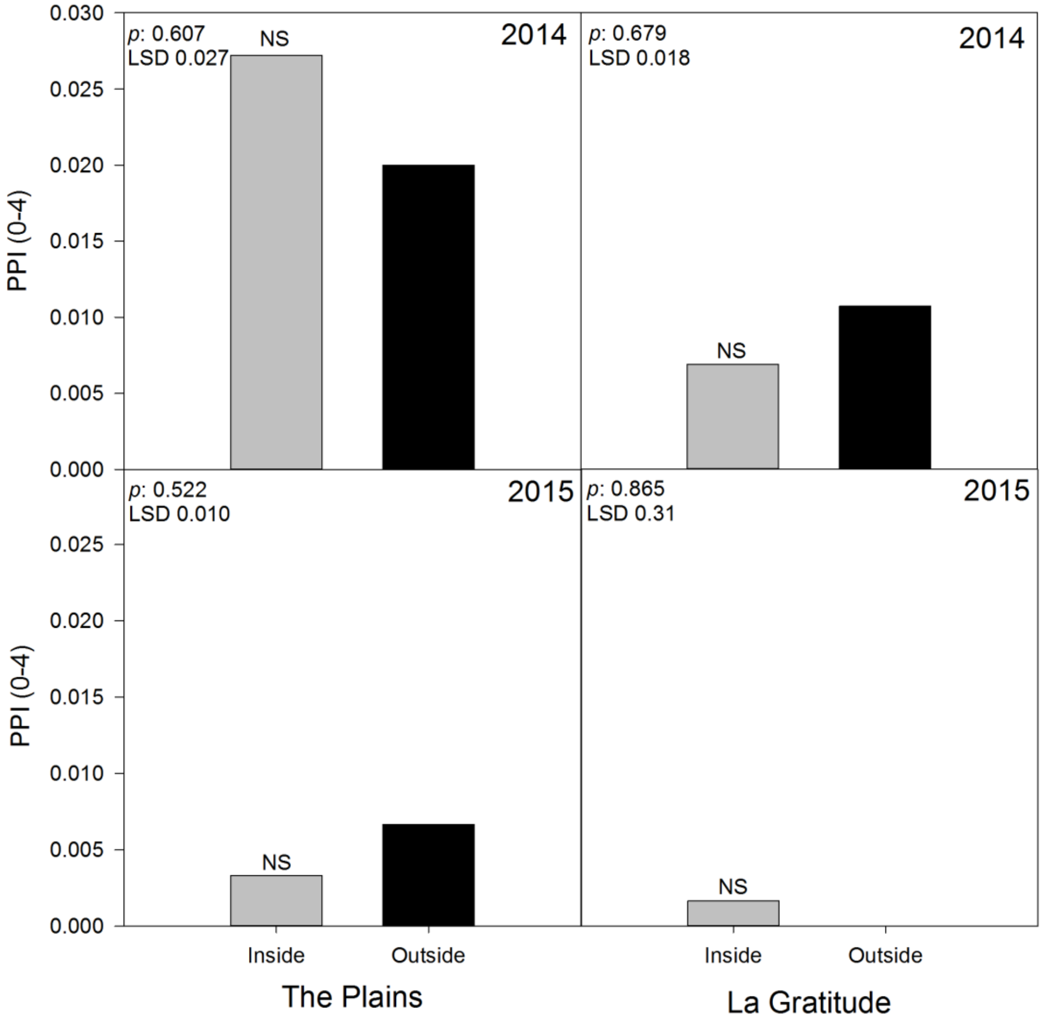

Fig. 5. Post-harvest pitting index (PPI) (0-4) evaluated after 21 days storage at 4.5°C of 'Turkey' valencia fruit from the inside and outside canopy positions during both seasons at The Plains and La Gratitude. NS=No significant difference at 95 % level (p≤0.05).

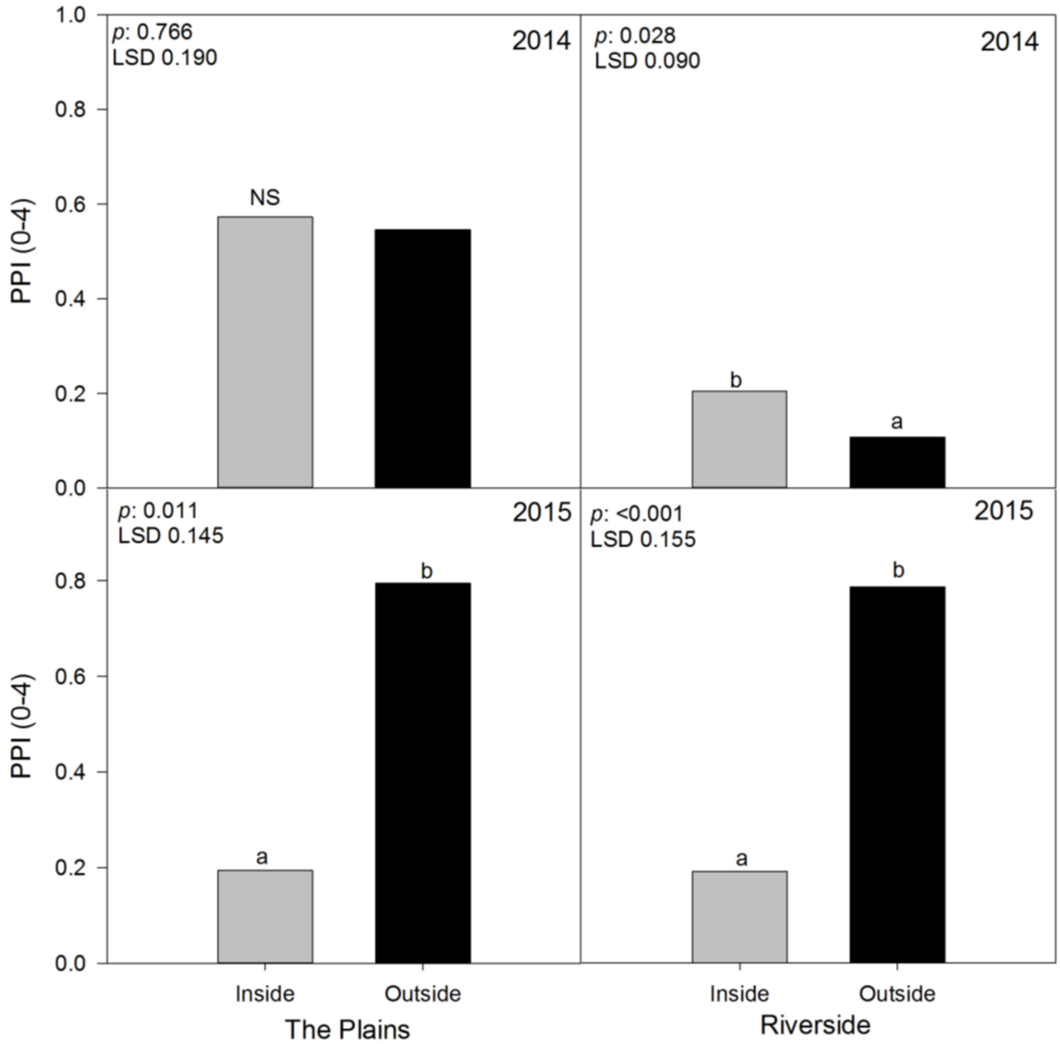

Fig. 6. Post-harvest pitting index (PPI) (0-4) evaluated after 21 days storage at 4.5°C of 'Benny' valencia fruit from the inside and outside canopy positions the 1st and 2nd seasons at The Plains and Riverside. Different letters indicate significant difference at 95 % level (p≤0.05). NS=No significant difference at 95 % level (p≤0.05).

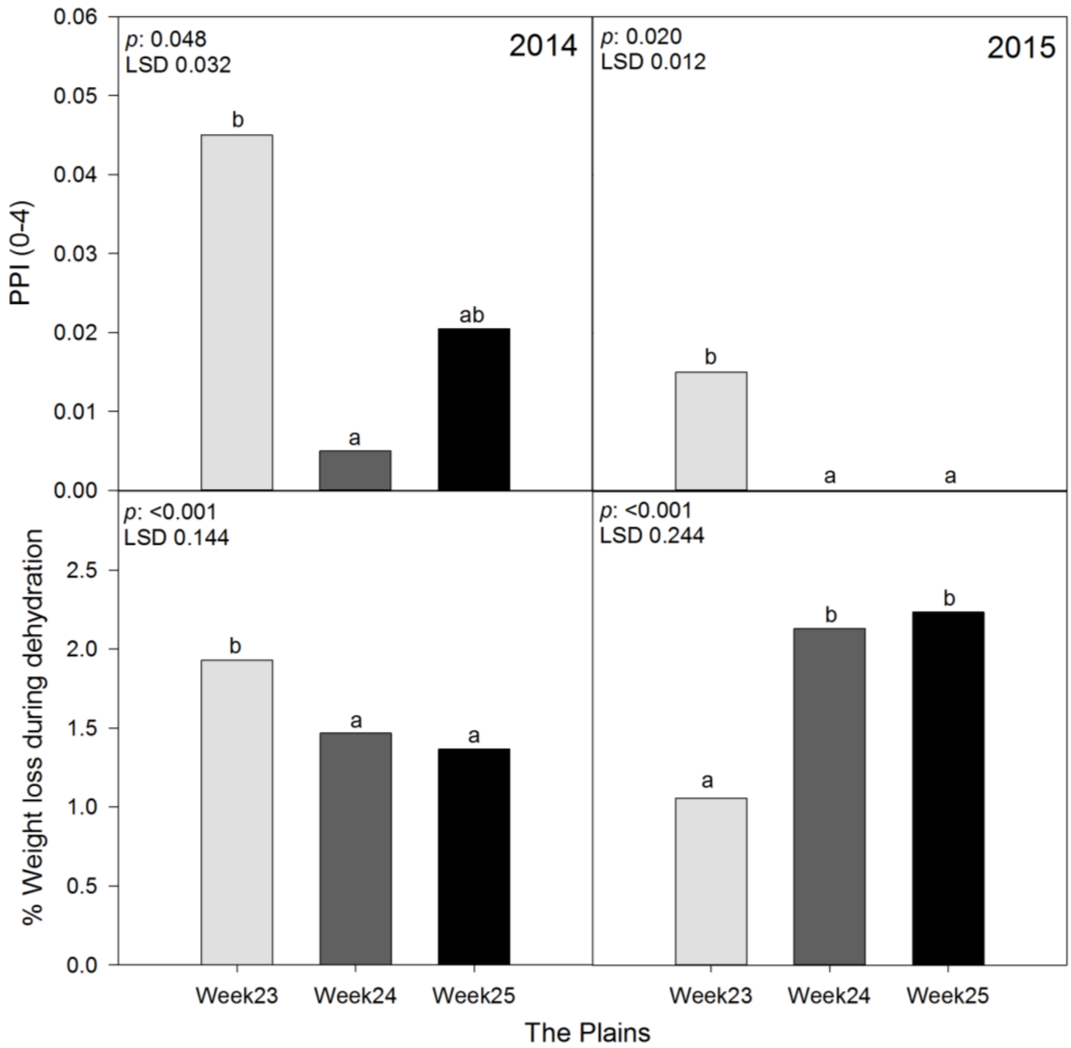

Fig. 7. Post-harvest pitting index (PPI) (0-4) evaluated after 21 days storage at 4.5°C and weight loss during dehydration (%) of 'Turkey' valencia fruit harvested for three consecutive weeks during both seasons at The Plains. Different letters indicate significant difference at 95 % level (p≤0.05).

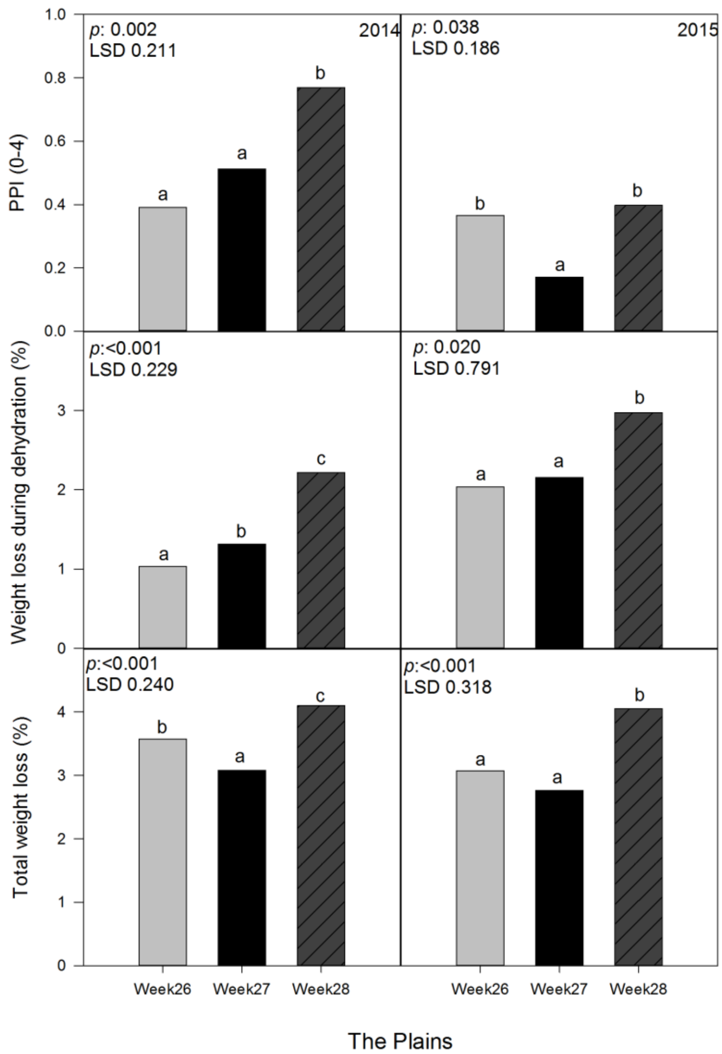

Fig. 8. Post-harvest pitting index (PPI) (0-4) of 'Benny' valencia fruit. Weight loss (%) during dehydration and total weight loss 'Benny' valencia fruit harvested for three consecutive weeks the 1st and 2nd season from The Plains. Different letters indicate significant difference at 95 % level (p≤0.05).

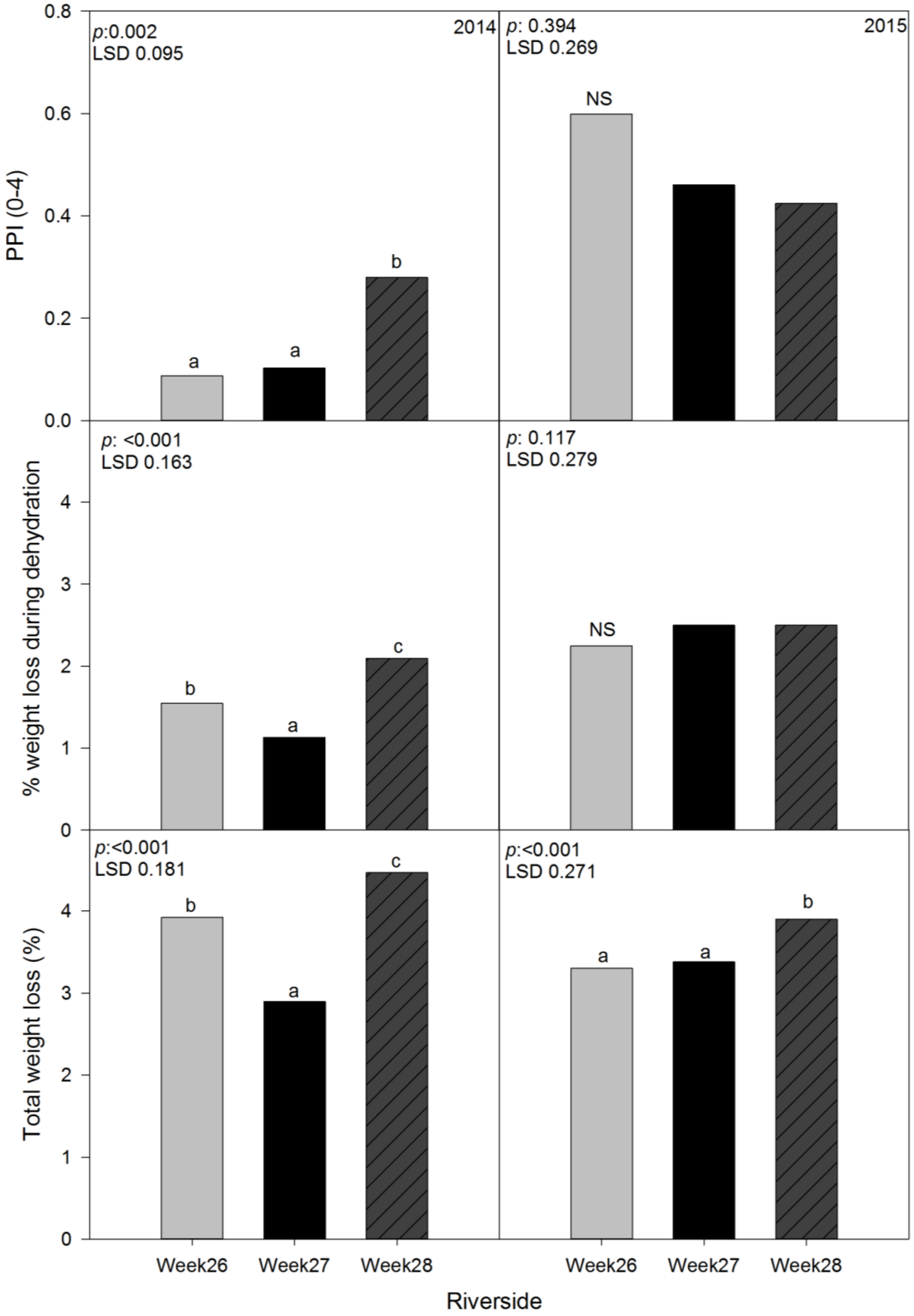

Fig. 9. Post-harvest pitting index (PPI) (0-4), weight loss (%) during dehydration and total weight loss of 'Benny' valencia fruit harvested for three consecutive weeks the 1st and 2nd season from Riverside. Different letters indicate significant difference at 95 % level (p≤0.05). NS=No significant difference at 95 % level (p≤0.05).

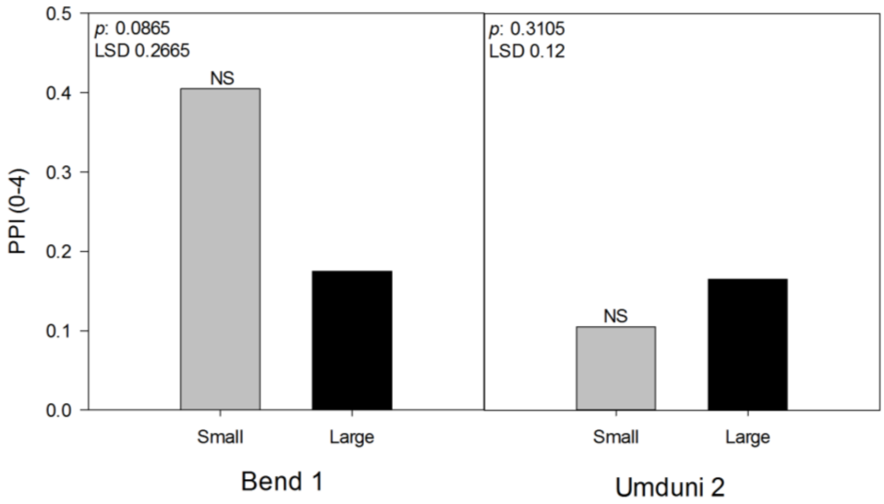

Fig. 10. Post-harvest pitting index (PPI) (0-4) of small (< 69mm) and large (>73mm) 'Benny' valencia fruit harvested from Croc Valley the 1st season. Different letters indicate significant difference at 95 % level (p≤0.05). NS=No significant difference at 95 % level (p≤0.05).

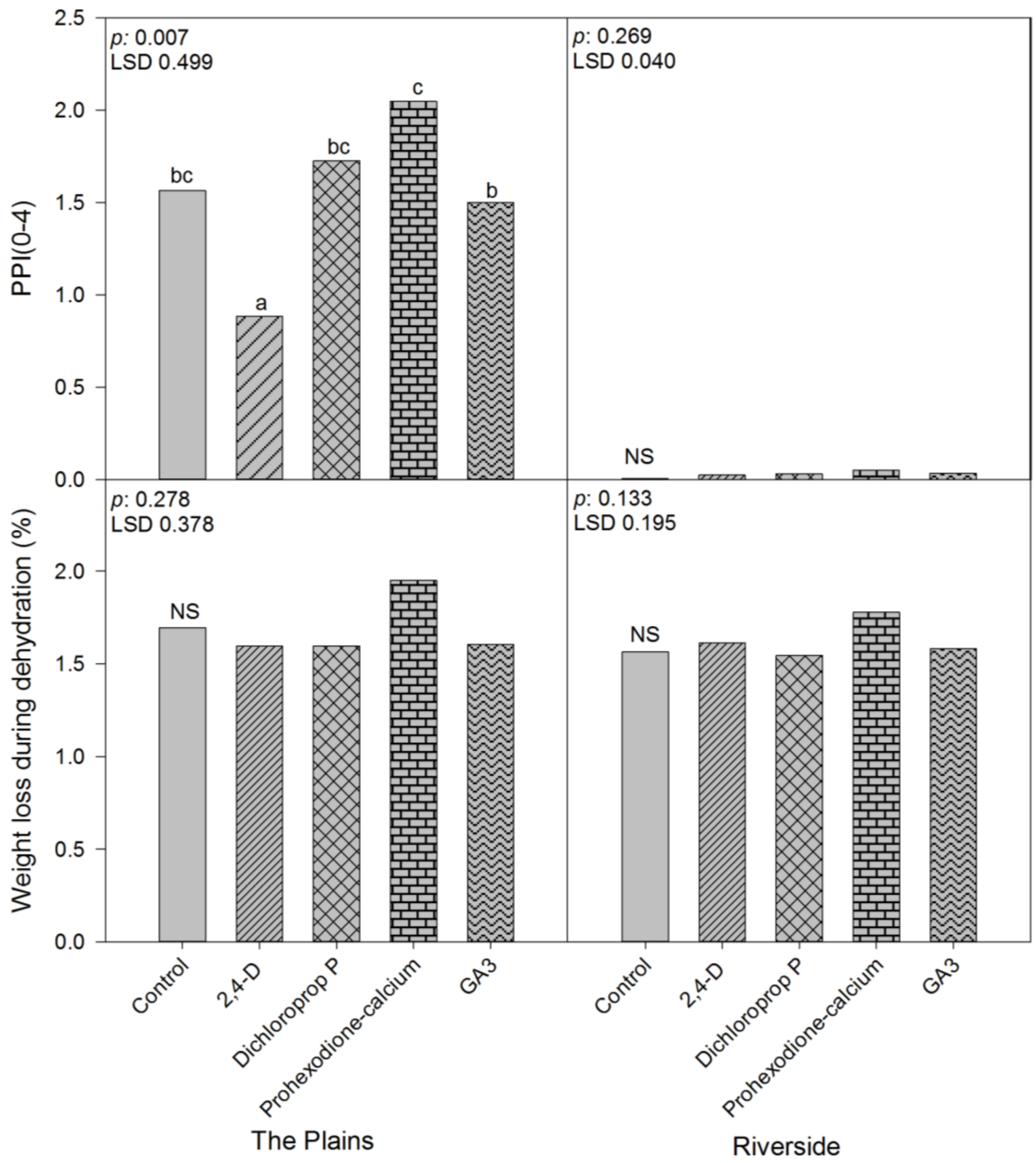

Fig. 11. Post-harvest pitting index (PPI) (0-4) and weight loss during dehydration (%) of 'Benny' valencia fruit treated with foliar applied PGR's the 1st season at The Plains and Riverside. Different letters indicate significant difference at 95 % level ($p \leq 0.05$). NS=No significant difference at 95 % level ($p \leq 0.05$).

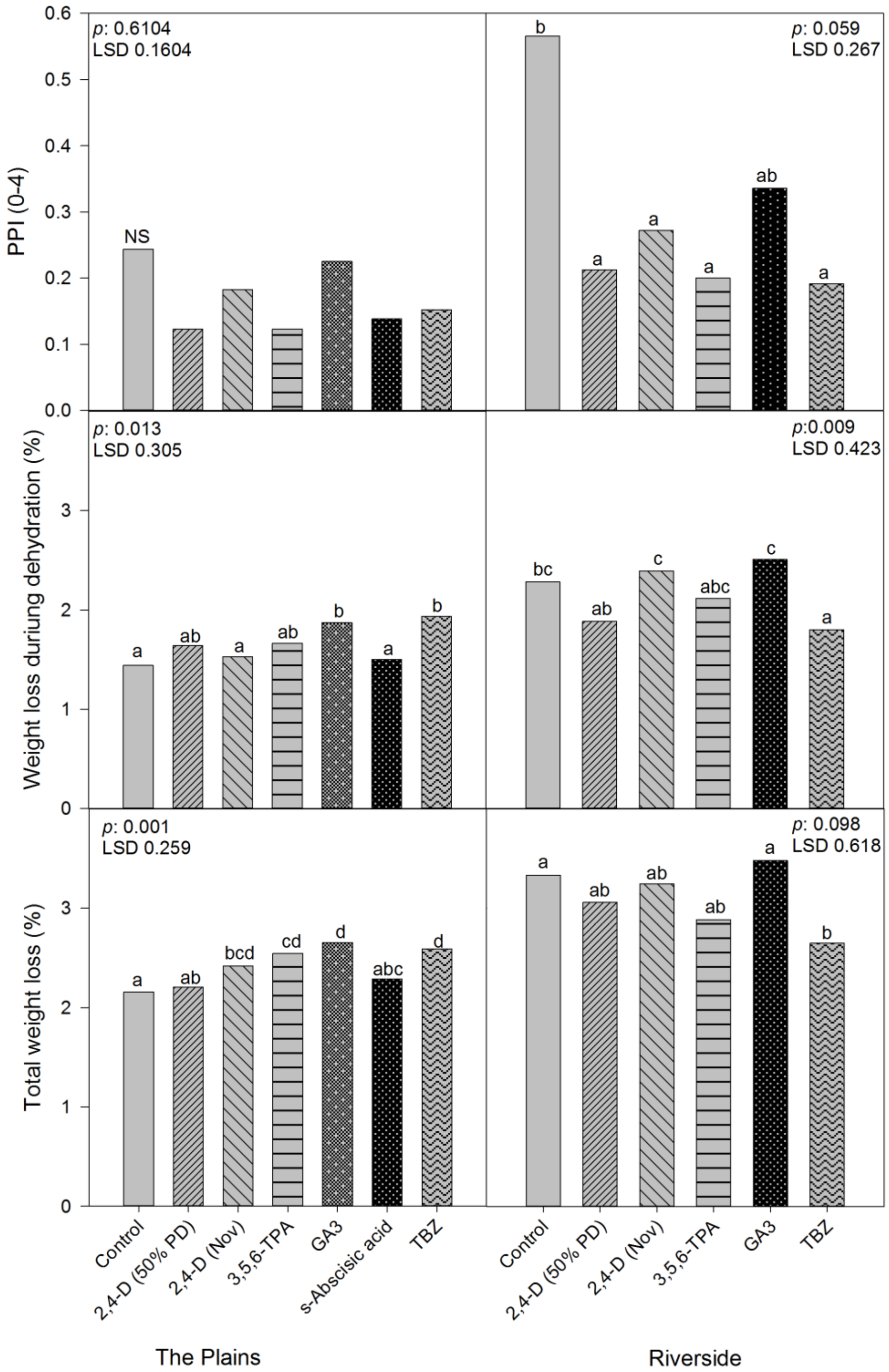

Fig. 2. Post-harvest pitting index (PPI) (0-4), weight loss (%) during dehydration and total weight loss of 'Benny' valencia fruit treated with foliar applied PGR's the 2nd season at The Plains and Riverside. Different letters indicate significant difference at 90 % level (p≤0.1). NS=No significant difference at 90 % level (p≤0.1).

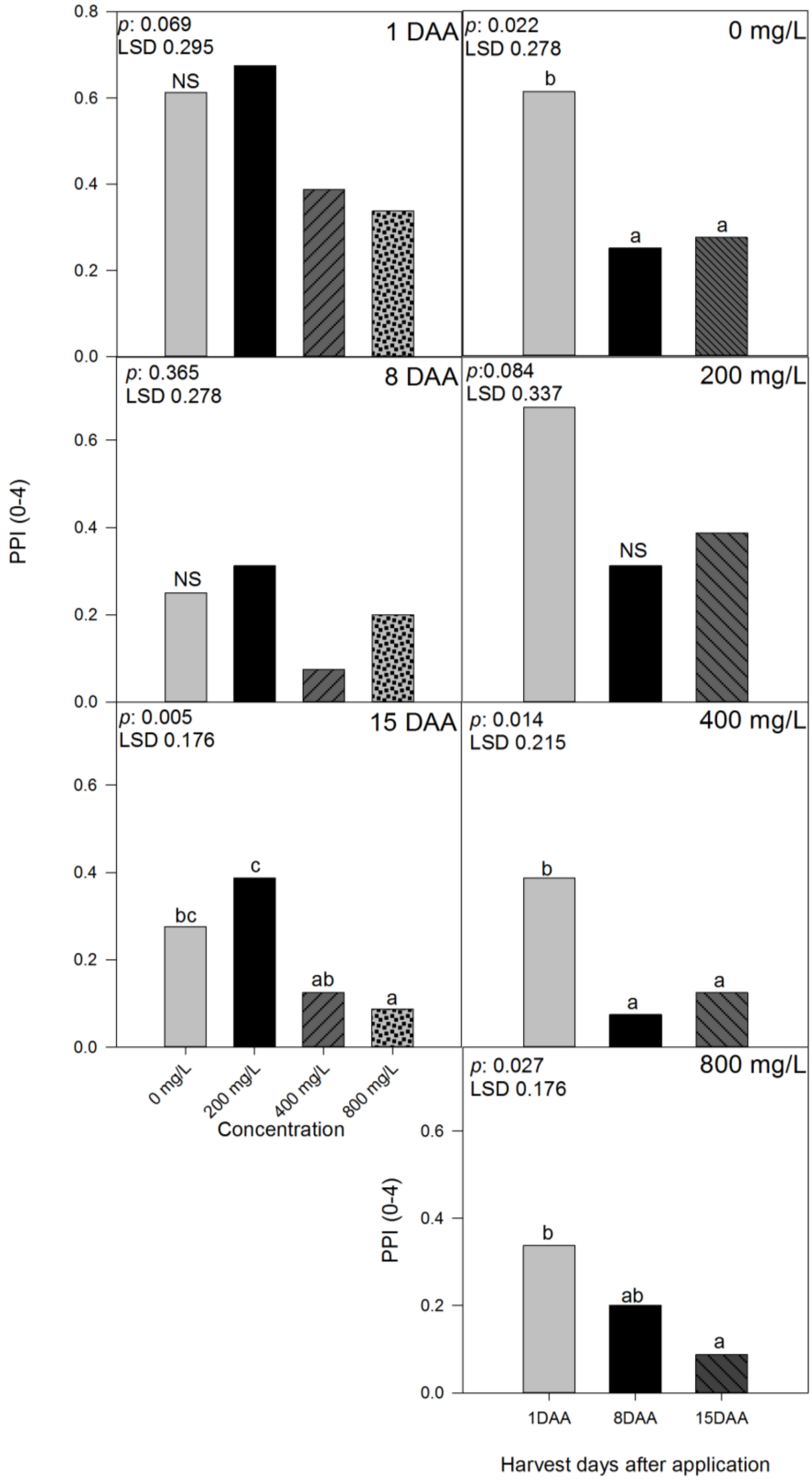

Fig. 3. Post-harvest pitting index (PPI) (0-4) of 'Benny' valencia fruit harvested 1 Day after application (DAA), 8 DAA and 15 DAA, treated with four different concentrations of s-ABA $0.0g{\cdot}L^{-1}$, $0.2g{\cdot}L^{-1}$, $0.4g{\cdot}L^{-1}$ and $0.8g{\cdot}L^{-1}$. Different letters indicate significant difference at 95 % level ($p \leq 0.05$). NS=No significant difference at 95 % level ($p \leq 0.05$).

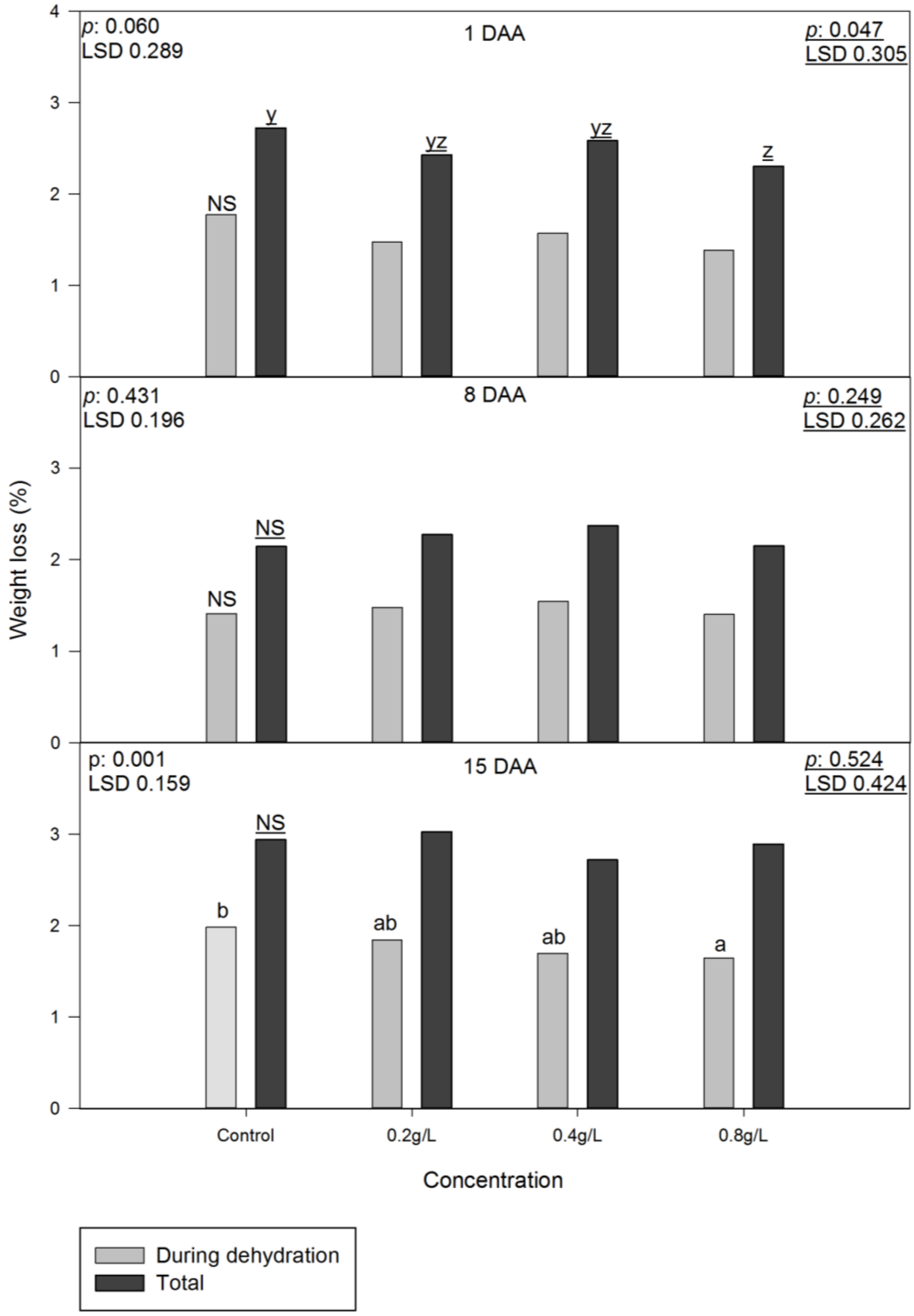

Fig. 4. Weight loss (%) during dehydration and total weight loss after 21 days storage of 'Benny' valencia fruit harvested 1 DAA, 8 DAA and 15 DAA, treated with three different concentrations of s-ABA $0.0g \cdot L^{-1}$, $0.2g \cdot L^{-1}$, $0.4g \cdot L^{-1}$ and $0.8g \cdot L^{-1}$. Different letters indicate significant difference at 95 % level ($p \leq 0.05$). NS=No significant difference at 95 % level ($p \leq 0.05$).

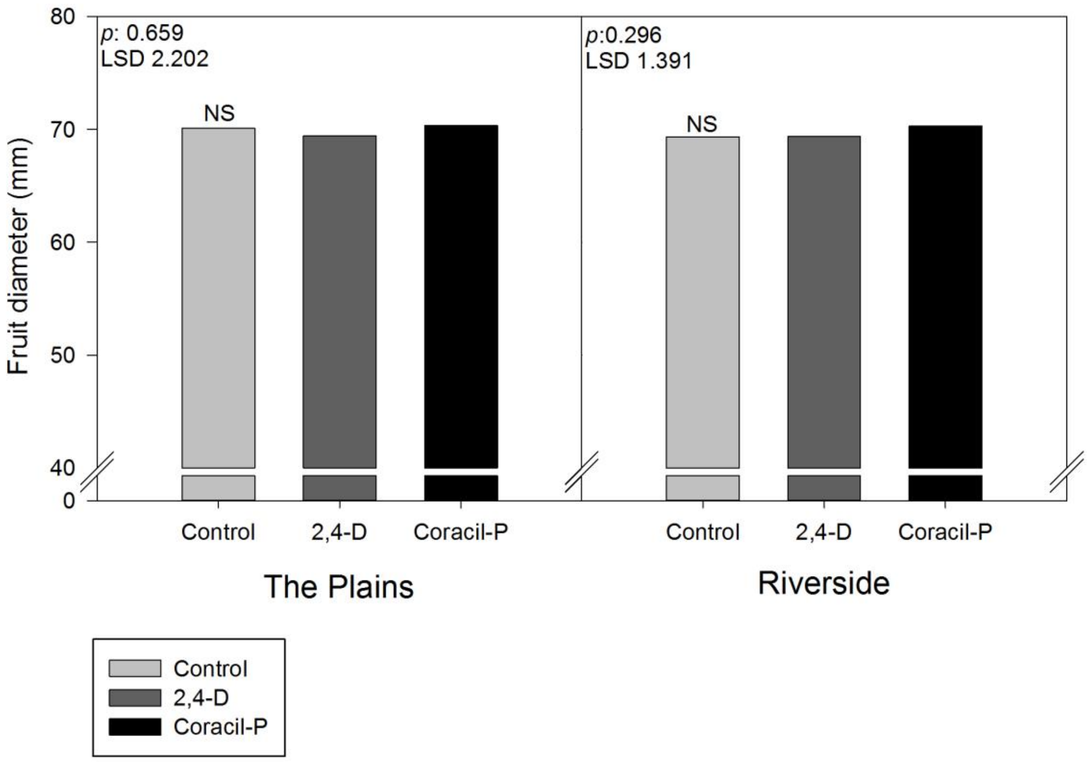

Fig. 5. Effect of 2,4-D and Coracil-P on fruit diameter at The Plains and Riverside during 1st season. NS=No significant difference at 95 % level (p≤0.05).

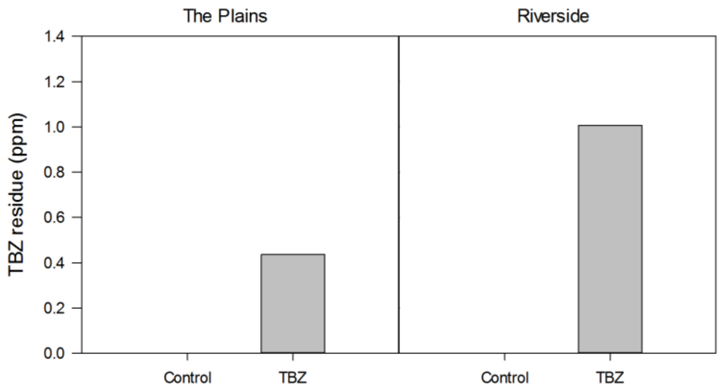

Fig. 6. Residue analysis (average of 2 replicates) of fruit treated with TBZ one week before harvest at The Plains and Riverside during the 2nd season. The Maximum residue level (MRL) for TBZ on Citrus for European Union member countries is 5 ppm.

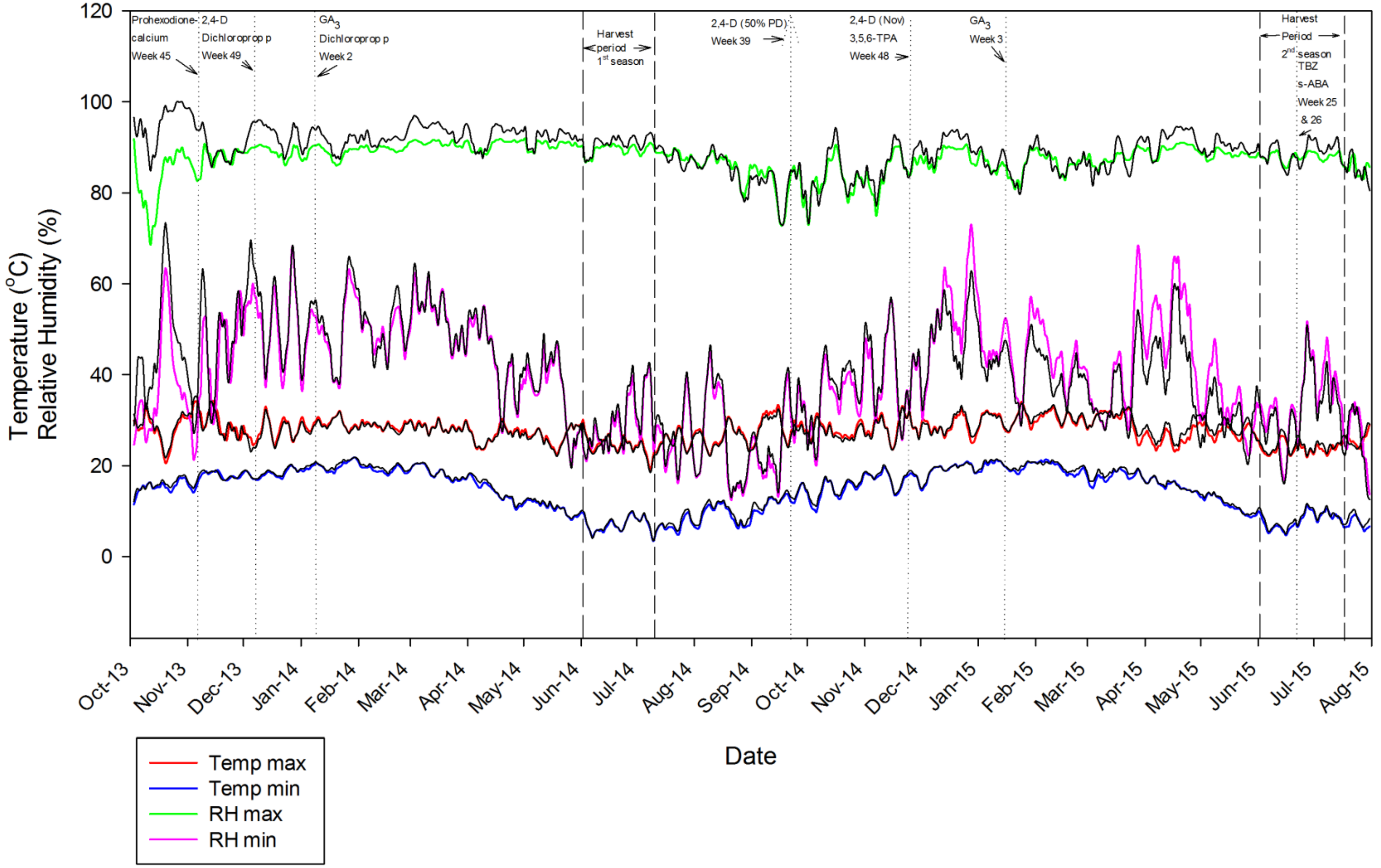

Fig. 7. Daily minimum and maximum temperature and RH at Riverside (coloured) and The Plains (Black) over two seasons.

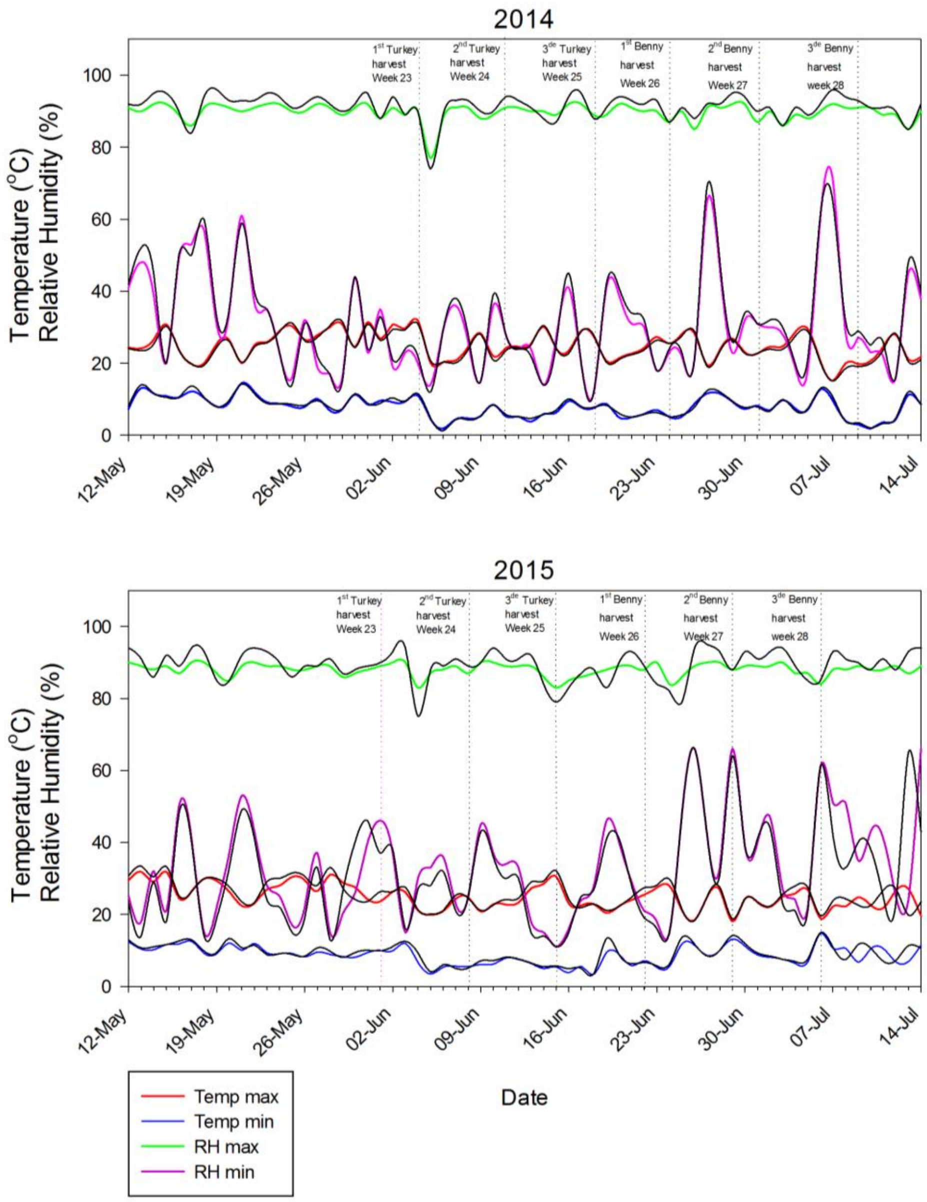

Fig. 8. Daily maximum and minimum temperature and RH at Riverside (Coloured) and The Plains (Black) each season over the harvest period.

4. Paper 2: Pre- and post-harvest thiabendazole application affect post-harvest pitting incidence of 'Benny' valencia oranges (*Citrus sinensis* L. Osb.).

Abstract

External quality of citrus fruit is reduced by physiological rind disorders such as chilling injury (CI) and post-harvest rind pitting. Rind pitting manifests post-harvest and is aggravated by variation in post-harvest environmental conditions which results in water related stress in rind cells. Post-harvest application of thiabendazole (TBZ) reduces incidence of CI in various citrus cultivars. The objective of this study was to examine the effect of TBZ on incidence of post-harvest rind pitting. Pre-harvest spray application of 4000 µg·mL^{-1} TBZ one week before harvest and post-harvest TBZ dip for one minute in 2000 µg·mL^{-1} TBZ solution before post-harvest stress reduced the incidence of this disorder. The reduction of this disorder could be attributed to reduced weight loss during dehydration and morphological and structural changes in the epicuticle wax by TBZ and adjuvants added to the TBZ mixture. However TBZ applied in a wax treatment after stress treatment did not reduce incidence of this disorder.

Keywords: Physiological disorder; TBZ residue; epicuticle wax; fruit weight loss, pitting

Introduction

The fresh market value of citrus fruit is dependent on internal and external fruit quality. An intact, blemish-free rind is an important factor determining external quality which can be reduced by physiological rind disorders such as post-harvest rind pitting which reduces market value. This disorder is characterised by sunken areas of the rind which result from the collapse of subepidermal cells. As pitting progresses, oil glands become affected and release their content intercellularly which leads to oxidation of rind cells turning lesion into a bronze/brown colour (Agusti et al., 2001; Alférez et al., 2003; Alférez and Burns, 2004; Alferez et al., 2005). Post-harvest pitting manifests at non-chilling storage temperatures on a variety of citrus cultivars including 'Benny' Valencia oranges (Cronjé, 2012).

The primary cause of the disorder is not known, however a change in rind water status through post-harvest variation in environmental conditions such as relative humidity (RH) and temperature seem to aggravate the disorder in 'Navelate' (Alférez and Zacarias, 2001) and 'Navelina' oranges (Lafuente and Sala, 2002). Such stress could be induced between harvest and cold storage where fruit are exposed to high temperature and low RH for an extended period of time before being packed and placed in cold storage at low temperature and high RH. Large variation in the incidence of this disorder occurs between seasons, orchards and trees in 'Navelina' oranges (Lafuente and Sala, 2002). In Paper 1 of this study similar variation was found in the incidence of this disorder on 'Benny' valencia. This variation is attributed to variation in micro climatic conditions within orchards and between seasons. Currently there is no commercial pre- or post-harvest treatment to reduce post-harvest pitting of 'Benny' valencia, however it was reported in Paper 1 that pre-harvest foliar applications of the auxins, 3,5,6 trichloro-2-pyridiloxyacetic acid (3,5,6 TPA) and 2,4-dichlorophenoxy acetic acid (2,4-D) and s-abscisic acid (s-ABA) reduced incidence of this disorder.

Thiabendazole is a systemic benzimidazole fungicide that is used post-harvest in citrus to control green [*Penicillium digitatum* (Pers.:Fr.) Sacc] and blue (*P. italicum* Wehmer.) mould. Post-harvest application of TBZ through dip treatments reduced the physiological rind disorder, chilling injury (CI) in 'Tarocco' oranges (Schirra and Mulas, 1995; Schirra et al., 1998) and 'Star ruby' grape fruit (*C. paradisi* Macf.) (Schirra et al., 2000). Also supplying TBZ in a wax treatment reduced CI in grapefruit (Schiffmann-Nadel et al., 1972), and 'Valencia' oranges (Kellerman et al., 2014). According to *Codex alimentarius* the maximum residue level (MRL) for TBZ on citrus fruit is 7 $\mu g \cdot g^{-1}$ for non-European union member countries and 5$\mu g \cdot g^{-1}$ for European Union member countries (FAO 2013). Commercially TBZ is applied post-harvest in a drench treatment prior to packing or within a dip or wax treatment during the packing process (Kellerman et al., 2014). Pre-harvest foliar application of TBZ has received little attention due to *P. digitatum* rapidly developing a TBZ resistant population (Dodd et al., 2010) as well as the commercial feasibility (Schirra et al., 2002) as a much higher concentration of TBZ need to be applied pre-harvest to obtain similar residue levels as post-harvest applications. Nonetheless, Schirra et al. (2002) reported that pre-harvest foliar application of 1% active ingredient TBZ two weeks before harvest reduced CI with and without post-harvest heat-treatment but TBZ residue levels were still evident 7 weeks after harvest. TBZ's mode of action in reducing CI is unknown (Schirra et al., 2002).

Thiabendazole residue loading is affected by numerous factors such as application method and fruit type (Kellerman et al., 2014). Wax applications load higher TBZ residues than dip treatments (Brown, 1984; El-Tobshy et al., 1982; Kellerman et al., 2014) however El-

Tobshy et al. (1982) proposed that wax application of TBZ limits its bioavailability as TBZ becomes encapsulated in the wax. Schirra et al., (2008) reported that TBZ penetration inside fruit tissue is affected by application parameters, fruit dipped for 3 min in 50°C TBZ water solution had significantly higher TBZ residue compared with fruit dipped for 1 min in 50°C or 3 min in 20°C TBZ water solution.

In this study we examined the efficacy of pre- and post-harvest TBZ application on reducing post-harvest pitting, with the focus on timing of TBZ application in relation to post-harvest environmental stress.

Materials and methods

Pitting inducing stress

The fruit received the same dehydration/rehydration stress treatment to induce pitting as described in Paper 1. In short, after harvest fruit were dehydrated at 25°C and 50% RH for three days followed by rehydration for one day at 20°c and 99% RH. After stress treatment fruit were waxed and placed in cold storage at 4.5°C for three weeks.

Pre-harvest

Uniform and healthy 'Benny' valencia trees where selected on two commercial farms, at Riverside (23°48'02.51S 30°27'27.20E) and The Plains (23°50'23.94S 30°25'55.82E) pre-harvest TBZ application. Orchard information was summarised in Paper 1, Table 1.

Thiabendazole was applied at (4 $g{\cdot}L^{-1}$) [ICA-TBZ®, ICA international chemicals (Pty) Ltd.] with 5 ml per 100 L non-ionic wetting agent Break-Thru® [Villa Crop Protection (Pty) Ltd.] on eight trees (n=8) at each farm 1 week before harvest, June 16 and 22, 2015 (2nd season) at Riverside and The Plains, respectively. The control treatments only received a water and wetting agent application with no TBZ.

At commercial harvest, 22 June at Riverside and 29 June at The Plains, 40 fruit from each replicate tree were harvested.

Post-harvest

Fruit for post-harvest treatments were harvested from three commercial 'Benny' valencia orchards in the Letsitele valley, Limpopo, South Africa. The three orchards are located on Riverside (23°48’02.51S 30°27'27.20E), The Plains (23°50’23.94S 30°25'55.82E) and Letaba Orange (23°48’27.50S 30°26'06.13E). Orchard information is summarised in Paper 1, Table 1. During 2014 (1st season), fruit were harvested on 14 July 2014 at The Plains while during the 2nd season fruit were harvested from Riverside farm and Letaba Orange on 6 July 2015.

During the 1st season fruit were divided into 6 treatments with each treatment having ten replicates (n=10) consisting of 10 fruit per replicate. For the 2nd season only eight replicates (n=8) were used to reduce the large quantity of fruit needed for the experiment. Both seasons, each treatment contained twelve additional fruit for residue analysis.

Thiabendazole was applied as a post-harvest dip bath and/or wax treatment according to recommended rates on product label. A bath was filled with 50 L of water and 200 ml TBZ for a concentration of 2000 $\mu g \cdot mL^{-1}$. The bath was heated to 35°C and thoroughly mixed before and during each dip treatment to prevent any precipitation of TBZ. Fruit were dipped in the bath for 1 minute before placed at ambient (18°C) for 2 hours to dry.

Thiabendazole was applied at 4000 $\mu g \cdot mL^{-1}$ in the wax application for the required treatment. The wax treatments were applied as in Paper 1, however for the two treatments with TBZ in the wax, 8 ml TBZ (4000 $\mu g \cdot mL^{-1}$) was added to 1 L of 18 QDP wax [John Bean Technologies (Pty) Ltd.] during the 1st season and Poly Orange wax [Citrashine (Pty) Ltd.] the 2nd season due to availability at time of experiment. The wax application with and without TBZ were done with separate boxes and brushed as to avoid TBZ contamination.

In order to determine the most opportune timing, TBZ was applied at four different stages during the post-harvest stress treatment. A control which was not treated with TBZ and a best practice treatment was also evaluated. Furthermore an ideal practice was compared and reported separately to the control. The treatments were as follows:

- Directly *after harvest* fruit were dipped before undergoing the pitting inducing stress treatment and waxed afterwards without TBZ and placed into cold storage*.
- During the pitting inducing stress treatment *after dehydration* for 3 days at 25°C and 50% RH fruit were dipped before being rehydrated for 1 day at 20°c at 99% RH. After rehydration fruit were waxed without TBZ and placed into cold storage*.
- *After rehydration* fruit were dipped and waxed without TBZ and placed into cold storage*.
- After the pitting inducing stress treatment fruit were waxed with *TBZ in the wax* and placed into cold storage*.

- The *control fruit* underwent the pitting inducing stress treatment and was waxed afterwards without TBZ and placed into cold storage*.
- The *ideal practice* fruit were dipped, waxed and placed into cold storage*directly after harvest, this treatment did not receive the pitting inducing stress treatment.

*All fruit received similar cold storage conditions and were placed into cold storage at 4.5°C for 21 days before evaluation for post-harvest pitting incidence after cold storage.

Data Collection and Evaluation

Fruit fresh weight was determined directly after harvest, after dehydration and after storage with a balance (Kern KB 3600-2N, KERN & SOHN GmbH | Ziegelei 1 | 72336 Balingen - Germany).

Pitting incidence evaluation of the fruit was done after 21 days storage at 4.5°C. Fruit were inspected and rated from 0 (no post-harvest pitting) to 4 (severe post-harvest pitting) (Paper 1, Fig. 3) and a post-harvest pitting index (PPI) was calculated as follows:

$$\text{PPI (0-4)} = \sum \frac{Rating\ (0-4) \times number\ of\ number\ fruit\ within\ each\ class}{Total\ number\ of\ fruit}$$

Residue analysis. Six fruit from two replicates (n=2) at each orchard of the pre-harvest treatments and two replicates (n=2) of six fruit per replicate from each of the post-harvest treatments were chopped, weighed and pulped using a blender and stored at -5°C until samples were sent for residue analysis by (Hearshaw and Kinnes Analytical Laboratory, Westlake, Cape Town, South Africa) an accredited analytic laboratory. The samples were extracted by using acetonitrile and a matrix solid phase dispersion extraction. The analysis of the extracts was conducted in liquid chromatography mass spectrometry (LCMS/MS; Agilent 6410, Agilent Technologies Inc., Santa Clara, CA, USA).

Data analysis

PPI (ranked) data and weight loss (%) was analysed using the one-way Anova and non-parametric one-way Anova (Kruskal-wallis test, when data was not normally distributed) with SAS Enterprise guide v.5.1 (SAS institute, Cary, NC, USA). Each treatment was compared with the other using Fischer least significance differences (LSD). *P*-values smaller than 0.05 were deemed significant.

Results

Pre-harvest TBZ foliar application had a slight reduction in post-harvest pitting severity, at both farms, Riverside (p=0.07) and The Plains (p=0.276) (Fig. 1). The large tree to tree variation in pitting severity could have contributed to this lack of significance level, specifically at Riverside where TBZ reduced pitting by nearly 60%. Weight loss during dehydration and total weight loss was significantly increased by pre-harvest TBZ application at The Plains, but at Riverside the control fruit lost significantly less total weight while weight loss during dehydration was unaffected, but followed a similar trend (*p*: 0.06) (Fig. 1). Low residue levels were found at The Plains while Riverside had 56 % higher TBZ residue levels than at The Plains. No explanation for this could be identified except for canopy size as The Plains had considerably larger tree canopy size compared to trees at Riverside which could have influenced foliar application.

Post-harvest dip application of TBZ at The Plains the 1st season (2014) significantly reduced post-harvest pitting compared to the control. Fruit treated with TBZ directly after harvest, after dehydration and after rehydration did not differ from each other (Fig. 2). Fruit treated with TBZ in wax after the stress treatment did not differ from control fruit and had higher PPI than fruit treated with TBZ directly after harvest (Fig. 2). Time of TBZ application did not affect post-harvest pitting incidence at Riverside (*p*: 0.387) and Letaba orange (*p*: 0.573) the 2nd season (Fig. 3 and 4). The lower PPI of the 2nd season (0.4-0.5) compared to the PPI in 1st season (0.9-1.0) could have influenced efficacy. In all instances the ideal practice fruit had significantly lower post-harvest pitting than control fruit (Fig. 5).

Weight loss was affected at all three experimental sites. At The Plains, TBZ application after harvest reduced weight loss during dehydration more than all other treatments except fruit that received TBZ application after dehydration which did not differ from any other treatment (Fig. 3). Fruit treated after harvest also suffered significantly less from total weight loss at The Plains (Fig. 4). At Riverside weight loss during dehydration was significantly higher in the control fruit than any other treatments which did not differ from each other. Total weight loss followed a similar trend (*p*: 0.142), but results were not significant. At Letaba Orange fruit treated immediately after harvest had lower weight loss during dehydration than fruit treated after rehydration and in wax, however no differences were found in total weight loss (*p*: 0.67) (Fig. 4).

The *Ideal practice* significantly reduced incidence of post-harvest pitting at all 3 farms tested. During 2014 low incidence of the disorder was found on fruit treated with the ideal practice treatment while during 2015 the ideal practice treatment completely preventing the disorder (Fig.5).

Residue analysis. Residues for dip treatments were relatively constant at The Plains during the 1st season while waxed fruit had double the amount of TBZ residue. At Riverside the 2nd season a similar trend were found with dip treatments loading low TBZ residue levels around and below 1 $\mu g{\cdot}g^{-1}$ while wax treatment loaded considerably higher TBZ residue levels.

Discussion

The application of TBZ reduced post-harvest rind pitting when applied before the dehydration/rehydration stress treatment. This trend was evident in all five experiments, however, only when the severity of this disorder was sufficiently high enough did TBZ significantly reduce this disorder. This could be seen with post-harvest treatment at The Plains the 1st season while a similar pattern was evident with pre-harvest sprays at Riverside the 2nd season (p: 0.07). The severity of this disorder was low at the remaining farms during the 2nd season which resulted in a masking of efficacy in fruit with a better rind condition.

Pre harvest foliar applications of 4000 $\mu g{\cdot}mL^{-1}$ TBZ 1 week before harvest reduced incidence of pitting. The largest difference was seen at Riverside where severity of post-harvest pitting was higher and where considerably higher TBZ residues were found on the fruit. In the only similar research done and reported on in literature on the effect of pre-harvest TBZ sprays on physiological rind disorders, Schirra et al. (2002) found a significant reduction in CI of 'Tarocco' orange fruit treated with a TBZ pre-harvest foliar application two weeks before harvest.

All the post-harvest TBZ dip treatments reduced pitting in the 1st season, with the greatest difference found between fruit dipped directly after harvest and control fruit. A similar pattern, although not significant was evident the 2nd season at Riverside (p: 0.387) and Letaba Orange (p: 0.573). Fruit that were treated after harvest, before the stress treatment also had lower weight loss during dehydration in every experiment. The cumulative weight loss during this dehydration period was previously correlated with PPI in 'March' grapefruit (Alférez and Burns, 2004). This might be part of an explanation as to the mode of action of TBZ in reducing pitting, however no reports could be found on the effect of TBZ on weight loss of citrus fruit.

Palma et al. (2013) showed that 2000 μg·mL^{-1} TBZ dip treatments at 20°C affected epicuticle wax structure and morphology of 'Tarocco' oranges. Treated fruit had a more homogenous wax layer than control fruit, with little visible raised edges of the platelets and granules "relaxed" just after treatment. After 3 weeks at 1°C this effect was less noticeable as the wax of treated fruit became rough and lifting around the edges of wax plates increased, treated fruit however had less of the rind surface area uncovered by epicuticle wax than control fruit. This could explain the reduction in water loss after TBZ application. The structure and morphology of epicuticle wax has been reported to influence non-chilling peel pitting of 'Navel' oranges (Sala et al., 1992) and pre-harvest rind stain of 'Valencia' oranges (El-Otmani et al., 1989). Palma et al. (2013) attributed this effect of TBZ dip treatment to the adjuvants added to TBZ to enhance effectiveness against *Penicillium*. Therefore it is possible that the reduction in post-harvest pitting of 'Benny' valencia oranges could be attributed to the effect of the added adjuvants to the TBZ mixture on the epicuticle wax which reduced fruit weight loss and thus water stress of rind cells and not the fungicide itself.

There seem to be an additional effect of TBZ on fruit susceptibility besides the reduction in weight loss during dehydration as the pre-harvest sprays at the Plains slightly reduced the disorder, but weight loss was higher in TBZ treated fruit. It has also been shown that TBZ reduces rind senescence of grapefruit (Schiffman-Nadel et al., 1975) which could explain reduction in post-harvest pitting incidence and also contribute to the reduced weight loss during dehydration.

Wax application with TBZ after stress treatment does not reduce incidence of this disorder even though wax treatments loaded much higher TBZ residues than dip treatments. The TBZ residue levels did not exceed MRL set by *Codex alimentarius* of 7 μg·g^{-1} nor did it exceed the MRL set for European Union countries of 5 μg·g^{-1}. It seems that the TBZ application at this time is too late as the cells had already been damaged by the stress treatment that induce post-harvest pitting. TBZ applied in wax could also not be as readily available to cells as with dip treatments as suggested by El-Tobshy et al. (1982).

In all incidences the ideal practice -dip, wax with TBZ and placed into cold storage the same day as harvest- significantly reduced post-harvest pitting. This fact illustrates how this disorder could be reduced or completely prevented when fruit are not stressed -dehydrated/rehydrated- and treated with TBZ directly after harvest. However unfortunately in order to commercially reduce moisture loss to this level would not always be practical due to logistical issues and packhouse design, fruit will always undergo some sort of environmental stress during post-harvest handling.

To conclude, pre- or post-harvest application of the systemic fungicide TBZ before stress related environmental conditions reduced post-harvest pitting of 'Benny' valencia oranges. Pre-harvest foliar application at 4000 $\mu g \cdot mL^{-1}$ 1 week before harvest and post-harvest dip treatment at 2000 $\mu g \cdot mL^{-1}$ for 1 minute directly after harvest reduced incidence of this disorder. In contrast TBZ applied in wax after stress treatment did not reduce incidence of this disorder. The mode of action of TBZ to reduce this disorder is unknown; however a reduction in weight loss was found when fruit were treated before dehydration. This could be due to changes in epicuticle wax structure and morphology brought on by adjuvants added to TBZ solution. In future more attention should be given to the effect of TBZ on cuticle wax, as well as how to implement TBZ application in commercial practices currently used to have greatest possible reduction of post-harvest pitting.

References

Agusti, M., Almela, V., Juan, M., Alferez, F., Tadeo, F., Zacarias, L., 2001. Histological and physiological characterization of rind breakdown of 'Navelate' sweet orange. Ann. Bot. 88, 415-422.

Alférez, F., Zacarias, L., 2001. Postharvest pitting in navel oranges at non-chilling temperature: influence of relative humidity. Acta Hort. 533, 307-308.

Alférez, F., Agusti, M., Zacarías, L., 2003. Postharvest rind staining in Navel oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biol. Technol. 28, 143-152.

Alférez, F., Zacarias, L., Burns, J.K., 2005. Low relative humidity at harvest and before storage at high humidity influence the severity of postharvest peel pitting in citrus. J. Am. Soc. Hort. Sci. 130, 225-231.

Alférez, F., Burns, J.K., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biol. Technol. 32, 79-87.

Brown, G.E., 1984. Efficacy of citrus postharvest fungicides applied in water or resin solution water wax. Plant Dis. 68, 415-418.

Cronjé, P., 2012. Postharvest rind disorders of 'Nadorcott' mandarin are affected by rootstock in addition to postharvest treatments. Acta Hort. 1007, 111-117.

Dodd, M., Cronje, P., Taylor, M., Huysamer, M., Kruger, F., Lotz, E., Van der Merwe, K., 2010. A review of the post harvest handling of fruits in South Africa over the past twenty five years. South African J. of Plant and Soil: 25th anniversary edition 27, 97-116.

El-Otmani, M., Arpaia, M.L., Coggins Jr., C.W., Pehrson Jr., J.E., O'Connell, N.V., 1989. Developmental changes in 'Valencia' orange fruit epicuticular wax in relation to fruit position on the tree. Scientia Horticulturae 41, 69-81.

El-Tobshy, Z., Abdel-Latif, M., Mahmoud, M., 1982. Influence of chemical application time on green mould control of navel orange. Agricultural Research Review 60, 233-243.

FAO. Pesticide residues in food 2012. FAO, Rome. 2013. ISBN 9789251075586

Kellerman, M., Erasmus, A., Cronjé, P.J.R., Fourie, P.H., 2014. Thiabendazole residue loading in dip, drench and wax coating applications to control green mould and chilling injury on citrus fruit. Postharvest Biol. Technol. 96, 78-87.

Lafuente, M.T., Sala, J.M., 2002. Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rindstaning of 'Navelina' orange (*Citrus sinensis* L. Osbeck) fruit. Postharvest Biol. Technol. 25, 49-57.

Palma, A., D'Aquino, S., Vanadia, S., Angioni, A., Schirra, M., 2013. Cold quarantine responses of 'Tarocco'oranges to short hot water and thiabendazole postharvest dip treatments. Postharvest Biol. Technol. 78, 24-33.

Sala, J., Lafuente, T., Cuñat, P., 1992. Content and chemical composition of epicuticular wax of 'Navelina' oranges and 'Satsuma' mandarins as related to rindstaining of fruit. J. Sci. Food Agric. 59, 489-495.

Schiffman-Nadel, M., Chalutz, E., Waks, J., Dagan, M., 1975. Reduction of chilling injury in grapefruit by thiabendazole and benomyl during long-term storage. J. Am. Soc. Hort. Sci. 100, 270-272.

Schiffmann-Nadel, M., Chalutz, E., Waks, J., Lattar, F., 1972. Reduction of pitting of grapefruit by thiabendazole during long-term cold storage. HortScience 4, 394-395.

Schirra, M., Cabras, P., Angioni, A., D'hallewin, G., Pala, M., 2002. Residue uptake and storage responses of Tarocco blood oranges after pre-harvest thiabendazole spray and postharvest heat treatment. J. Agric. Food Chem. 50, 2293-2296.

Schirra, M., D'Aquino, S., Palma, A., Angioni, A., Cabras, P., 2008. Factors affecting the synergy of thiabendazole, sodium bicarbonate, and heat to control postharvest green mold of citrus fruit. J. Agric. Food Chem. 56, 10793-10798.

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

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

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

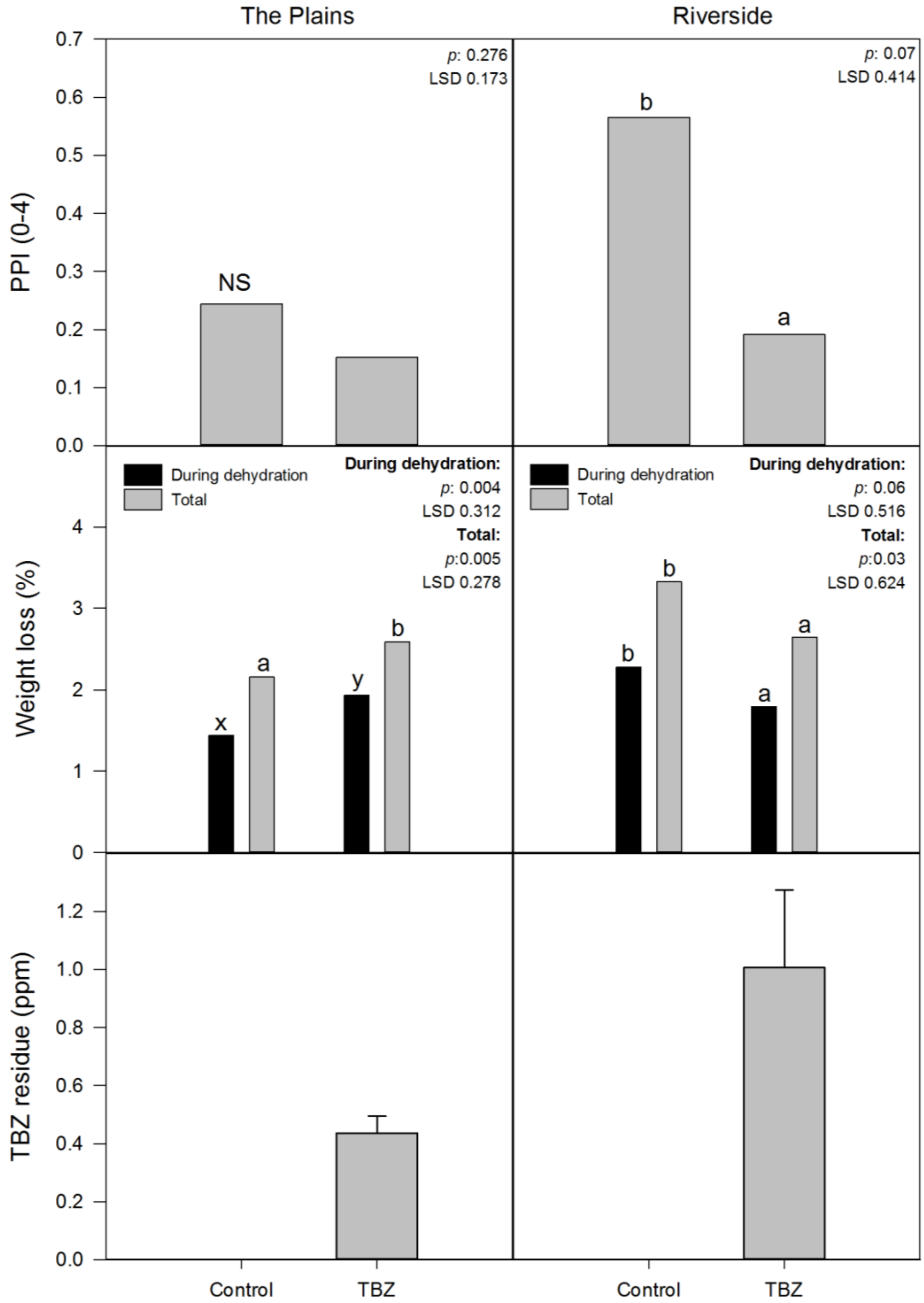

Fig. 1. Post-harvest pitting index (PPI) (0-4), weight loss (%) during 3 days dehydration, total weight loss (%) during post-harvest handling and thiabendazole (TBZ) residue analysis with standard error of fruit treated 1 week before harvest with a TBZ spray and control fruit. Harvested at The plains and Riverside during the 2nd season. Different letters indicate a significant difference at 90 % level ($p \leq 0.1$). NS=No significant difference at 90 % level ($p \leq 0.1$).

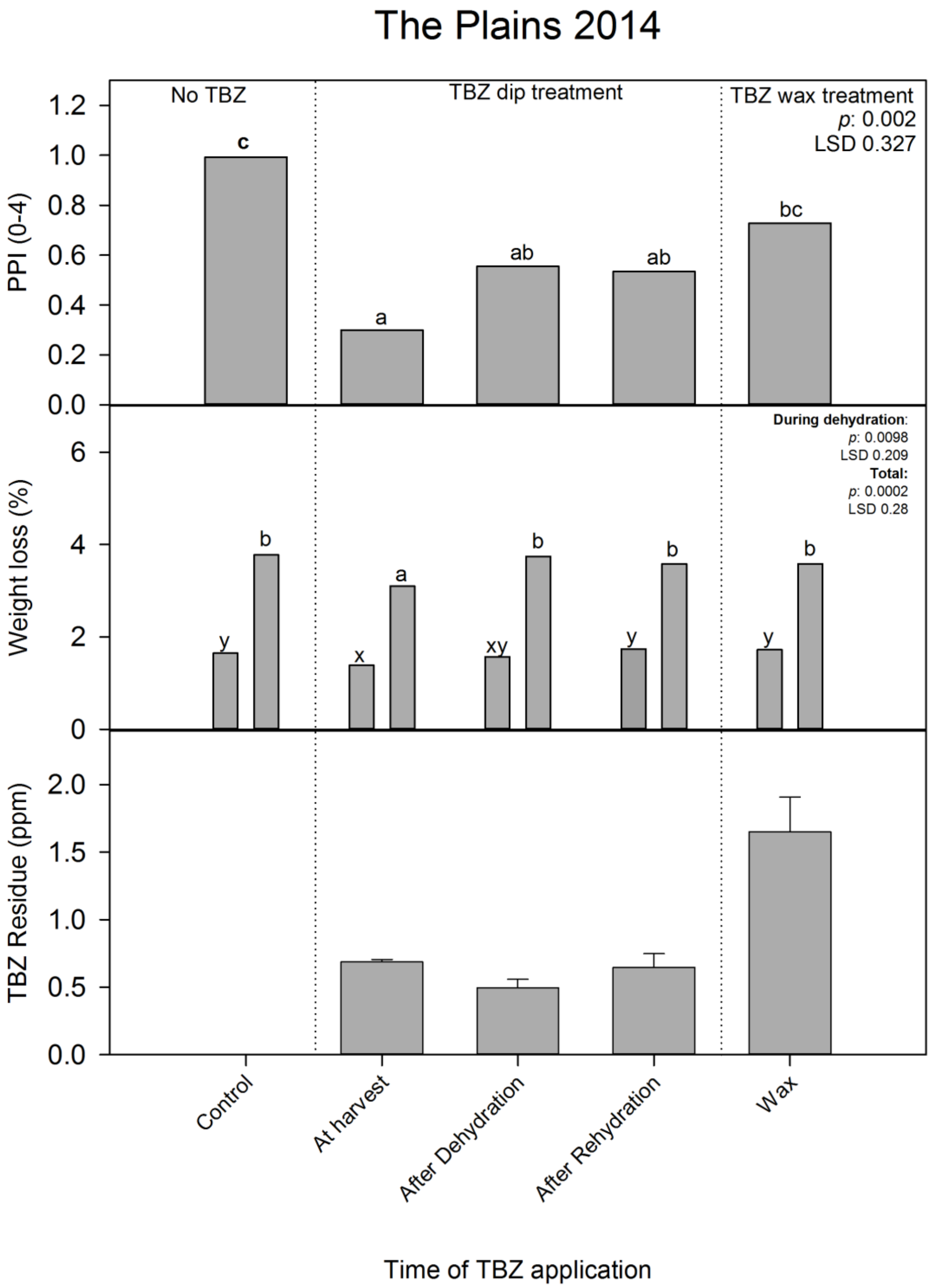

Fig. 2. Post-harvest pitting index (PPI) (0-4), weight loss (%) during three days dehydration, total weight loss (%) during post-harvest handling and thiabendazole (TBZ) residue analysis with standard error of fruit treated with TBZ post-harvest at different times throughout a stress treatment. Fruit harvested at The Plains 2014. Different letters indicate significant difference at 95 % level ($p \leq 0.05$).

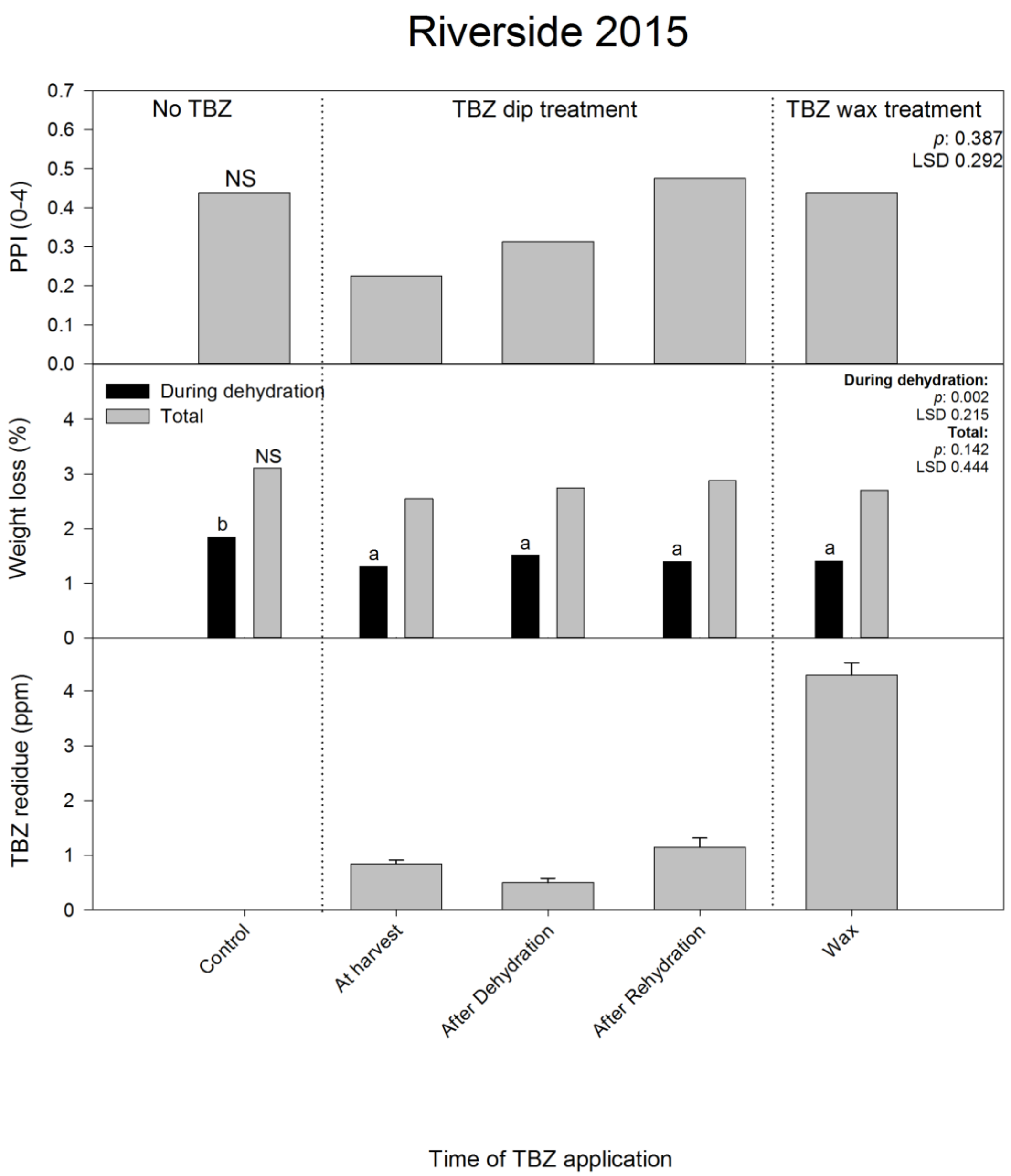

Fig. 3. Post-harvest pitting index (PPI) (0-4), weight loss (%) during three days dehydration, total weight loss (%) during post-harvest handling and thiabendazole (TBZ) residue analysis with standard error of fruit treated with TBZ post-harvest at different times throughout a stress treatment. Fruit Harvested at Riverside 2015. Different letters indicate a significant difference at 95 % level (*p*≤0.05). NS=No significant difference at 95 % level (*p*≤0.05).

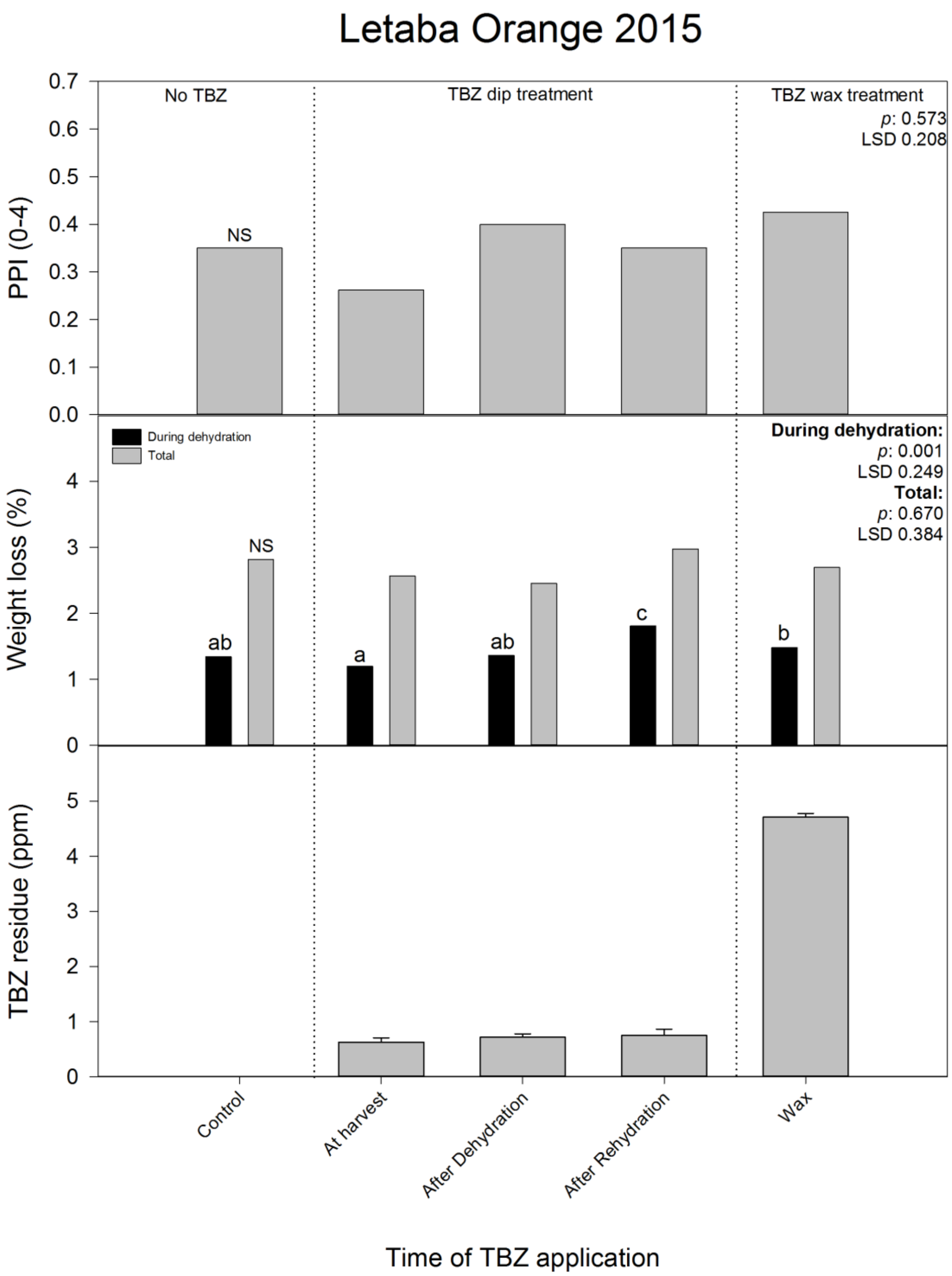

Fig. 4. Post-harvest pitting index (PPI) (0-4), weight loss (%) during three days dehydration, total weight loss (%) during post-harvest handling and thiabendazole (TBZ) residue analysis with standard error of fruit treated with thiabendazole post-harvest at different times throughout a stress treatment. Fruit harvested at Letaba Orange 2015. Different letters indicate a significant difference at 95 % level ($p \leq 0.05$). NS=No significant difference at 95 % level ($p \leq 0.05$).

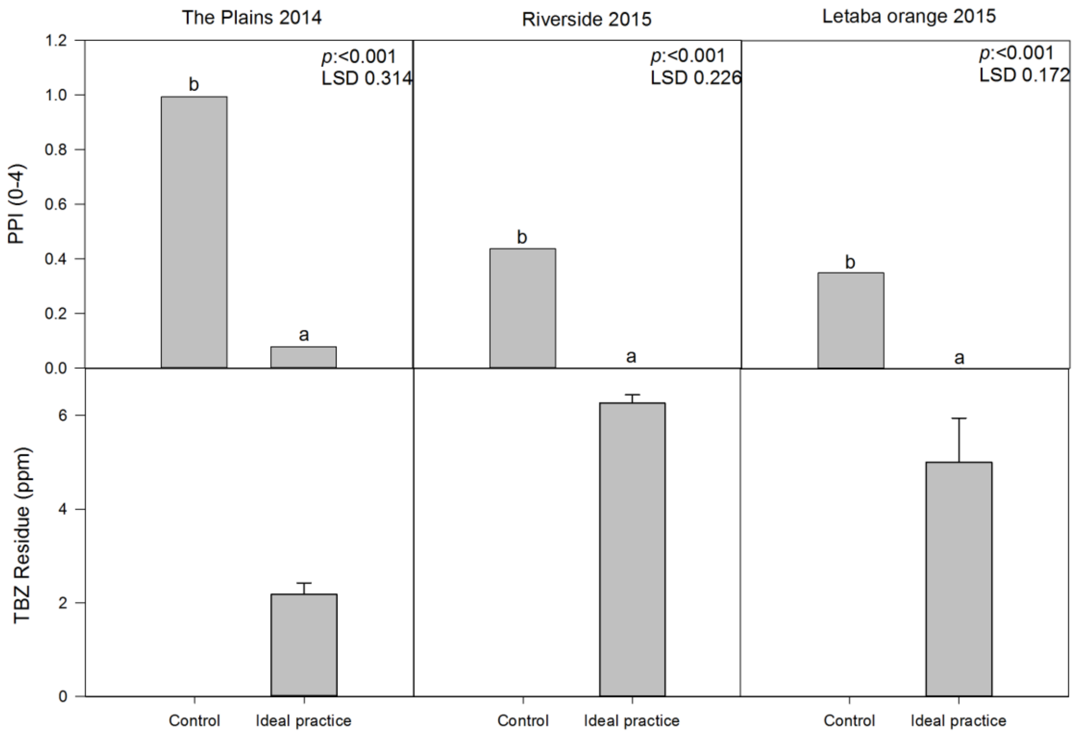

Fig. 5. Post-harvest pitting index (PPI) (0-4) and thiabendazole (TBZ) residue analysis with standard error of (fruit dipped and waxed with TBZ which did not receive stress treatment) and control (fruit which wasn't treated with TBZ and received the stress trestment). Fruit harvested at The plains 2014 and Riverside 2015 and Letaba Orange 2015. Different letters indicate a significant difference at 95 % level (p≤0.05).

5. Paper 3: A survey of the estimated financial impact of post-harvest pitting of 'Valencia' oranges in foreign and local markets on South African producers.

Abstract

Post-harvest pitting incidence diminishes the market price of oranges thus reducing producer income. However little is known about the loss of income incurred due to this disorder. A survey was set up to determine the estimated cost associated with this disorder in certain markets and a questionnaire was sent to producers, pack-house managers and exporters. The information received was pooled to determine the potential loss of income when low (15 %) and high (30 %) pitting incidence was evident in the 'Valencia' fruit rind. It was found that in markets with higher prices for good quality pitting-free fruit the price reduced rapidly when pitting incidence was found, however in markets which offer lower initial prices for pitting-free fruit the price reduction was smaller when pitting incidence was evident. In some instances lower losses were incurred if fruit were sold locally before the disorder manifested, thus it could make financial sense to rather sell high risk fruit on local markets. In Paper 1 and 2 of this study, treatments were identified that could reduce the occurrence of this disorder, however some of the treatments are costly. This study showed that these treatments can be cost effective in sensitive orchards but it is up to the producer to evaluate risk and manage fruit pre- and post-harvest in order to reduce the risk of potential losses to this physiological rind disorder.

Introduction

South Africa is the 8th largest citrus producer in the world, producing citrus on over 62 000 ha mostly in Limpopo, Eastern Cape, Mpumalanga and Western Cape. South Africa exports over 70 % of the production making the country the 2nd largest citrus exporter in the world after Spain (CGA, 2013). The small South African local market and weak local currency (Rand) contributes to the industry being export focussed. Most of other large citrus producing countries, with the exception of Argentina and Brazil, are situated in the northern Hemisphere thus South Africa also has the advantage of producing and supplying fresh citrus in the Northern Hemisphere's off-season and therefore not competing directly (FAO, 2003).

The main export markets for South African citrus are Europe (EU) 32 %, Middle East (ME) 20 %, Russia 12 %, Asia 7 % and the United Kingdom (UK) 10 %. Exports to these five countries represented over 80 % of South Africa's citrus exports during 2012 (CGA, 2013). These markets offer premium prices, but demand the highest quality fruit which is constituted by optimum size and colour and a blemish free rind. A constant threat that can reduce the value of citrus fruit is the development of physiological rind disorders during exports. Post-harvest pitting (Fig. 1) is such a physiological rind disorder which reduces external quality, impacting negatively on financial returns for producers. This disorder manifests in the post-harvest cold chain and on most occasions symptoms appear after the fruit have been shipped. This results in financial losses as high capital investments are required to export fruit and there is a considerable loss due to opportunity cost as low prices are realised due to inferior quality fruit in the markets.

There exists a lack of information about the severity of financial losses that could occur due to high incidence of this physiological disorder. This is the first study of this nature attempting to place an estimated value on the cost of post-harvest pitting at a farm level and identify sensitivity to an incidence of pitting in the market.

Methodology

A citrus industry survey (CIS) was done through a questionnaire (Appendix A) which was designed to identify the cost of production, packing and transport of fruit to the various markets. All the questions related to costs where posed on a Likert scale to shorten answering time, however prices at markets were open ended questions. Seven markets were chosen to include most of South Africa's major export markets, i.e. Canada, China, EU, Hong Kong, ME, Singapore and the UK. Citrus fruit are exported to these markets at 4°C and therefore at conditions not related to chilling injury. The United States of America (USA) was exclude from this study as not all South African citrus production regions are allowed to export to the USA all fruit are exported at -0.6°C for 32 days and there for exposed to conditions that could lead to chilling injury.

In order to facilitate calculations a few assumptions were made:

- an exchange rate of R 14.00 to the US Dollar
- a bin of fruit weighs 380 kg
- a pallet consists of 80 boxes and box weight of 15.5 kg

- an average pack out percentage of 71 %
- 4.6 bins of fruit are needed to produce 1 pallet of fruit.

The data from the CIS 2015 were obtained and pooled to give an overall industry view of cost and prices. Unfortunately only enough information was obtained for EU, ME and UK markets and these results are reported further in this study. The findings are however relevant as in 2013 over 50 % of South Africa's oranges were exported to these three markets (CGA, 2013) and there is little reason to believe that volume per country exports for 2015 was much different. The information obtained was used to calculate the production, packing and transport costs to deliver a pallet of fruit to the inland port. Prices were obtained at delivered in port (DIP) level and net income was estimated for each market per carton of fruit for the following pitting severities: zero, low (15 %) or high (30 %). The prices for these fruit were also estimated for local markets at the pack-house level.

With this information a hypothetical 'Valencia' farm of 100 ha was created that produced 251 935 cartons of exportable fruit annually (Table 1) and four hypothetical scenarios were created as follows:

- The 1st scenario 100 % of fruit had no pitting incidence
- The 2nd scenario 80 % of fruit had no pitting incidence, while 10 % of fruit had low and 10 % high pitting incidence
- The 3rd scenario 60 % of fruit had no pitting incidence while 20 % of fruit had low and 20 % high pitting incidence
- The 4th scenario 50 % had no pitting incidence while 30 % of fruit had low and 20 % of fruit had high pitting incidence.

Results

Based on assumptions and information obtained from the CIS 2015, the average cost to produce a carton of 'Valencia' oranges for export was R 63.31 during the 2014 season. This included costs associated with production, packing and transport to an inland port (Table 2). The average cost to produce a bin of fruit for the local market was R 522.50.

The estimated DIP price and income for a carton of Valencia orange fruit in September without pitting, with low or high pitting incidence is summarised in Table 3. The highest price for a carton of fruit with no pitting was obtained in the UK market followed by the EU and lastly the ME market. The price realised per carton dropped below the cost to produce and deliver a carton if fruit had low pitting incidence, with the highest reduction in price in the UK market. As pitting incidence increased the realised price was reduced even more and higher loses were incurred (Fig. 2). On the local market an estimated loss of R 97.50 and R 137.50 occurred when a bin of fruit was sold for fresh or processing use, respectively (Table 4). This is a loss of R 5.62 or R 10.78 per carton fruit, respectively.

As the percentage pitting increased so did the potential losses that occurred at markets. In the severe scenarios 3 and 4, more than half of the potential income of the hypothetical farm was lost due to pitting incidence. However even though the price reduction was greatest in the UK market the highest income for each scenario was when 100 % of fruit were sent to the UK (Table 5).

Discussion

Post-harvest pitting can lead to significant financial losses at the farm level depending on severity of this disorder as well as the market destination. From the results of the CIS it was found that markets with higher prices such as the UK were higher risk markets with a low tolerance to any pitting incidence. In this market high penalties are paid as seen in price decline when any incidence of pitting is evident. In contrast in less sensitive markets with lower initial prices such as the ME and EU the drop in price was not as substantial when pitting incidence was found on delivery of the fruit (Fig. 2). In this example the UK offered the highest price for fruit with no pitting, however if the fruit had any incidence of pitting prices were reduced below that of the ME and EU markets for similar fruit.

With lower pitting incidence, a smaller loss was obtained in the EU and ME markets comparable to the local market, however sending fruit to these foreign markets when pitting is expected could result in higher losses than in local markets when severity of this disorder is higher than anticipated. This could reduce South Africa's market share in foreign markets as buyers will search for other suppliers if they receive fruit with pitting on a regular basis. If fruit have a high risk of developing high pitting incidence, selling fruit locally before the disorder has manifested could minimise potential losses and loss in market share. Thus producers should use historical orchard data to evaluated and flag sensitive, high risk orchards. These fruit should be sent to lower risk markets such as the ME or the EU or fruit should be sold locally before the disorder manifests to reduce potential losses and market damage that could occur.

In the four scenarios tested even a low % of pitting incidence could result in a large reduction in expected income. In Paper 1 and 2 of this study some preventative treatments were found to reduce the incidence of this disorder, however these treatments can be costly as applying (40 $g{\cdot}L^{-1}$) s-abscisic acid (s-ABA)(Protone®, Valent BioSciences) before harvest could cost up to R 5 000 ha^{-1} for the product. Application of s-ABA acid one week before harvest resulted in a 30 % reduction of the disorder, thus this product could potentially be cost effective when applied to high risk orchards with a history of developing post-harvest rind pitting when sensitive markets are targeted.

To conclude, to reduce potential financial losses due to post-harvest pitting producers should first determine the likelihood or susceptibility of fruit to develop post-harvest pitting. This should be done for each orchard by factoring in knowledge on cultivar, orchard history and environmental conditions and then attempt to minimise this risk through management practices such as reducing time between harvest, application of Thiabendazole and optimising the cold chain or applying s-ABA before harvest. Secondly, after the risk of fruit to develop pitting has been determined, the producer and exporter should formulate a strategy in order to manage supply of fruit to markets with a certain risk attached to them. This is necessary as the highest rate of return was found in a high risk market i.e. the UK, with a known zero to little tolerance for this disorder. Fruit from orchards with a history of developing pitting should then rather be exported to the ME of EU markets as even though lower prices are realised in these markets the penalty is less if the fruit develop pitting prior to arrival. If a specific orchard has been identified as a very high risk it would make more financial sense to sell the fruit at the pack-house to the local market for substantially lower loss than what would be incurred if fruit were exported.

References

Citrus Growers Association of Southern Africa (CGA) 2013. Key industry statistics for citrus growers 2013.

FAO. 2003 Medium-term prospects for agricultural commodities. Rome www.fao.org

Table 1. Hypothetical 'Valencia' orange farm. a) Production area (ha), production (T/ha), total production and pack out (kg). b) Production exportable fruit as bins, pallets and cartons.

Farm	
Area (ha)	100
Yield (Ton Ha^{-1})	55
Total Yield (Tons)	5 500
Pack-out (71%) (kg)	3 905 000
Exportable fruit (units)	
Bins	10 276
Pallets	3 149
Cartons	251 935

Source: Own calculations from CIS, 2015.

Table 2. Estimated cost of production packing and transport of Valencia orange fruit for a) an export carton and b) a bin for local market.

a) Export carton	
Costs	R/Carton
Production	16.39
Packing	33.06
Transport till inland port	13.86
Total	**63.31**
b) Bin	
Costs	R/Bin
Production	285.00
Packing costs	237.50
Total	**522.50**

Source: Own calculations from CIS, 2015.

Table 3. a) Estimated delivered in port (DIP) price per carton of fruit at EU, ME and UK markets and b) Net income of loss occurred per carton of fruit at EU, ME and UK markets.

a) DIP price in export markets (R/Carton)			
Market	**No pitting**	**Low pitting**	**High pitting**
EU	78.98	60.88	53.82
ME	76.19	62.5	52.50
UK	93.02	48.49	45.53
b) Net Income/loss (R/Carton)			
Market	**No pitting**	**Low pitting**	**High pitting**
EU	15.67	-2.43	-9.49
ME	12.88	-0.81	-10.81
UK	29.71	-14.82	-17.78

Source: CIS, 2015

Table 4. Estimated local price per bin and net income per bin and per carton.

	Local Market Price(R/Bin)	**Net Income (R/Bin)**	**Net Income (R/Carton)**
Fresh	425	-97.50	-5.61
Processed	335	-187.50	-10.78

Source: CIS, 2015

Table 5. Estimated income and potential losses incurred in four hypothetical scenarios due to pitting at EU, ME and UK markets.

	Scenario			
Pitting severity	**1**	**2**	**3**	**4**
Zero	100	80	60	50
Low (15 %)	0	10	20	30
High (30 %)	0	10	20	20
	Income (Rand)			
EU	3 946 987	2 857 534	1 768 081	1 312 287
ME	3 245 031	2 303 360	1 361 688	1 016 819
UK	7 485 001	5 166 732	2 848 464	1 726 721
	Losses (Rand)			
EU	0	1 089 453	2 178 906	2 634 699
ME	0	941 672	1 883 344	2 228 212
UK	0	2 318 268	4 636 537	5 758 279

Source: Own calculations from CIS, 2015.

Fig.1. 'Benny' valencia oranges with symptoms of post-harvest rind pitting upon arrival in the export market.

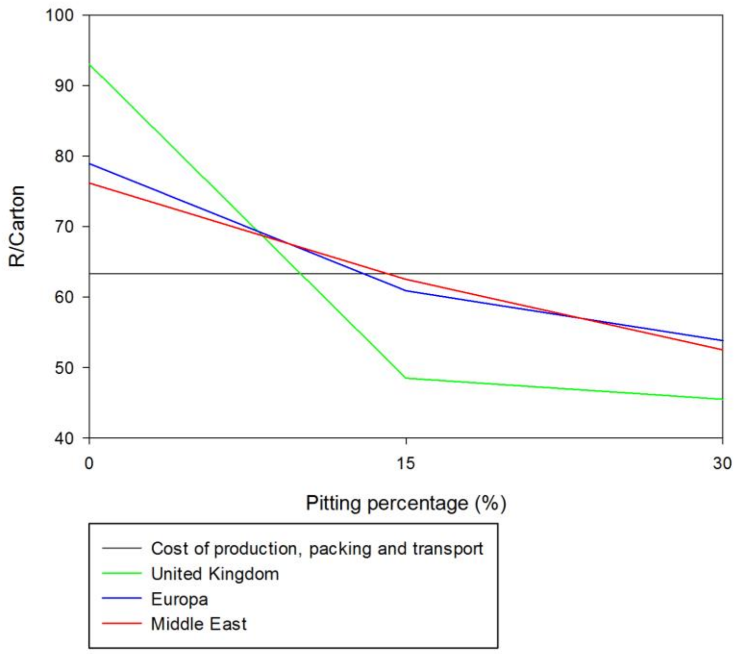

Fig. 2. Cost of production, packing and transport of one carton 'Valencia' orange until inland harbour. Delivered in port (DIP) price of one carton of 'Valencia' oranges at United Kingdom, Europa, and Middle East markets with different levels of pitting incidence.

6. General conclusion

Post-harvest rind pitting in 'Valencia' oranges was shown to be aggravated by a change from low to high relative humidity (RH) during post-harvest handling. Commercially all citrus fruit are to some extent subjected to similar conditions as we used during this study to induce the disorder, where fruit were dehydrated for three days at low RH at then rehydrated for one day at high RH. In addition, during the period before harvest fruit are subjected to daily variation in temperature and RH followed by the post-harvest fluctuation in RH, which may lead to a higher incidence of pitting.

The first major finding in this study was that 'Benny' valencia fruit are far more susceptible to this disorder than 'Turkey' valencia and in addition large variation existed between seasons, orchards, trees and between fruit from the same tree. This variation can be attributed to the major influence of pre-harvest environmental conditions on fruit susceptibility. Exposed fruit from the outside canopy are significantly more susceptible to this disorder due to being more exposed to a variating climate which could induce increased cell water stress and influence cuticle wax morphology. However the effect of the environmental conditions on epicuticle wax morphology and the water potential of rind cells was not examined during this study and should receive attention in future research. The use of shade netting to reduce climatic variation should also be investigated, however due to marginal income with this cultivar and high investment cost of shade netting this might possibly not be commercially feasible. Fruit maturity was found to only slightly impacted fruit susceptibility to pitting as more mature fruit were marginally more susceptible, however in future a longer harvesting period should be tested for a more conclusive answer.

Foliar applied plant growth regulators (PGR's) reduced severity of this disorder, however with varying efficacy, dependent on type, concentration, time of application and inherent susceptibility of the orchard to this disorder. Generally, higher treatment efficacy was recorded in orchards with higher susceptibility. The synthetic auxins 3,5,6 trichloro-2-pyridiloxyacetic acid (3,5,6 TPA) and 2,4-dichlorophenoxy acetic acid (2,4-D) consistently reduced the severity of rind pitting, especially when 10 $mg \cdot L^{-1}$ 2,4-D was applied at 50 % petal drop or after physiological fruit drop (2,4-D and 3,5,6-TPA). Gibberellic acid (GA3) which reduces a number of other physiological rind disorders and is known to decrease rind senescence did not affect the incidence of post-harvest rind pitting. S-abscisic acid (s-ABA) (Protone®), applied for the first time to our knowledge for this purpose, 1 to 2 weeks before harvest also reduced this disorder. As this was the first study on rind pitting in 'Valencia' oranges, attention was given to the effects of PGRs on post-harvest pitting incidence and weight loss, while in future more attention should be given to the mode of action of the PGRs that reduced this disorder as well as the dosage and timing used and the effect of these PGRs on other fruit quality aspects.

The application of thiabendazole (TBZ) pre- and post-harvest reduced the severity of post-harvest pitting if applied before fruit were subjected to environmental stress. In most instances a transient reduction in the rate of weight loss was observed after application of TBZ. This could be due to the effect of TBZ and/or the adjuvants added to the TBZ on epicuticle wax. This needs to be investigated in the future as a possible mode of action of TBZ to reduce physiological rind disorders. Even though TBZ was applied as a pre-harvest spray in this study, this method of application is not advised due to the high probability of *Penicillium* spp. developing resistance against TBZ which could be problematic for post-harvest decay control. The effect of TBZ applied within the wax before stress treatment, TBZ concentration and duration of the dip treatment were not examined and needs attention in future research projects. A specific protocol for TBZ treatments should be developed in order to incorporate it in current commercial treatments as during this study only the effect of TBZ was investigated without taking into consideration other commercial practices being used such as chlorine baths which could wash off the TBZ residue. It is possible that an increase in dwell time between TBZ dip and chlorine baths could lead to a higher efficacy due to better TBZ penetration into the rind.

During this study a large number of samples consisting of low fruit numbers were evaluated in order to screen as many treatments as possible as this was the first study of its kind on 'Valencia' oranges. This resulted in large variation in the incidence of this disorder leading to statistically non-significant difference even if such differences in pitting would be commercially significant.

Pre- and post-harvest treatments were identified during this study that reduced post-harvest rind pitting incidence, however all the treatments were evaluated alone and these results should serve as a base-line for future research to evaluate the effect of different treatment combinations on managing this disorder. In order to develop a producer management strategy for a whole season various combinations need to be evaluated e.g. applying 2,4-D after physiological fruit drop and s-ABA one week before harvest as well as treating the fruit with TBZ directly after harvest and minimising environmental stresses post-harvest

Appendix A

UNIVERSITEIT•STELLENBOSCH•UNIVERSITY
jou kennisvennoot • your knowledge partner

Dear Sir/Madam,

I am Jacques Ehlers, a student at the Stellenbosch University enrolled for a Master's degree in Horticulture. The purpose of the study is to analyse the cost producers occur due to post-harvest rind pitting on Valencia oranges at different markets. The title of study is: "Analysis of the cost of post-harvest rind pitting", under the supervision of Doctor Paul Cronje from Citrus Research International funded by the South African citrus industry in collaboration with Bureau for Food Agricultural Policy (BFAP). The questionnaire is broken down into four sections, section 1 aims to identify the cost of production, section 2 aims to identify packing cost of fruit destined for processing, local markets and export markets, section 3 aims to identify price obtained for fruit destined for processing and on local markets while section 4 aims to identify high, medium and low risk export markets in respect to post-harvest rind pitting. The questionnaire will take approximately 20 minutes to complete.

Your contribution and participation is very valuable and crucial to obtain the required result. Your expert opinion is essential to highlight the cost producers can occur when making risky decisions in regard to post-harvest pitting incidence.

All information provided will be treated confidentially; no individual information will be published under any circumstances. If there are any uncertainties please do not hesitate to contact me directly. We would sincerely appreciate it if you can e-mail the completed form directly to 15993574@sun.ac.za or paulcronje@sun.ac.za. The completed form can also be faxed to 021 808 2121.

Thank you in advance for taking time to complete the questionairre.

Kind regards

Jacques Ehlers
E-mail: 15993574@sun.ac.za Contact number: 072 240 2289

Dr. Paul Cronje 084 447 1047
E-mail: paulcronje@sun.ac.za

Fakulteit AgriWetenskappe
•
Faculty of AgriSciences

Kantoor van die Dekaan • Office of the Dean
Privaatsak/Private Bag X1 • Matieland 7602 • Suid-Afrika/South Africa
Tel: +27 21 808 4792 • Faks/Fax: +27 21 808 2001
jer@sun.ac.za • www.sun.ac.za/agric

1) Production cost.

Tick the appropriate box corresponding to your view of yield and production costs of Valencia oranges.

1. Indicate average yield/ha (Tons)

<30	30-40	40-50	50-60	60-70	70<

2. Production Cost/Ha (R/ha)

<20 000	20 000-25 000	25 000-30 000	30 000-35 000	35 000-40 000	40 000<

3. Total Area under production (ha)

4. Production area.(Province)

2) Packing Cost (Export, Local and Processed) of Valencia orange fruit

Tick the appropriate box corresponding to your view of packaging cost where needed or give percentage where asked.

1. Average tipping cost (Rand/Bin).

<200	200-225	225-250	250-275	275-300	300<

2. Average pack out %.

<50	50-60	60-70	70-80	80-90	90<

Fakulteit AgriWetenskappe
•
Faculty of AgriSciences

Kantoor van die Dekaan • Office of the Dean
Privaat Sak/Private Bag X1 • Matieland 7602 • Suid-Afrika/South Africa
Tel: +27 21 808 4737 • Faks/Fax: +27 21 808 2001
agric@sun.ac.za • www.sun.ac.za/agric

3. Average % fruit destined for local fresh use markets. (%)

4. Average % fruit destined for processed market (%)

5. Average packaging material cost for A15C carton (Rand/Carton).

<18	18-19	19-20	20-21	21-22	22<

6. Average drench cost (Rand/Bin)

<20	20-30	30-40	40-50	50-60	60<

7. Average degreening cost (Rand/Bin)

<20	20-30	30-40	40-50	50-60	60<

8. Average % Valencia bins degreened (%)

9. Give a percentage of packaging type used for exporting Valencia oranges.

Packaging	Volume (%)
E10D	
E15D	
A15C (Standard)	
SuperVent (A15C)	

10. Total packing cost (R/Pallet) Overall total packing cost if cost breakdown could not be given.

3) Income (Local and processed)

Tick the appropriate box corresponding to your view of price obtained for fruit sold for processing and on local markets.

1. Average income for processed Valencia orange fruit (Rand/Bin) (2.5 Bins = Ton)

<200	200-250	250-300	300-350	350-400	400<

2. Average income for Valencia orange fruit sold in local markets (Rand/Bin)

<200	200-250	250-300	300-350	350-400	400<

4) Cost of exports.

4.1) Market view

1. Give the percentage of Valencia oranges exported to the following markets.

Market	Volume (%)
Canada	
China	
Europe	
Hong Kong	
Middle east	
Singapore	
United Kingdom	

2. From previous season's experience, rank these following markets from highest to lowest risk regarding the pitting incidence of Valencia oranges and indicate if the markets are seen as high, medium or low risk in your opinion.

Market	Risk(1-7)	High, Medium or Low
Canada		
China		
Europe		
Hong Kong		
Middle east		
Singapore		
United Kingdom		

Kantoor van die Dekaan • Office of the Dean
Privaat Sak/Private Bag X1 • Matieland 7602 • Suid-Afrika/South Africa
Tel: +27 21 808 4737 • Faks/Fax: +27 21 808 2001
agric@sun.ac.za • www.sun.ac.za/agric

4.2) Cost till inland port.

Tick the appropriate box or give estimated amount where asked.

1. Average transport cost from pack house till port. (R/pallet)

<200	201-300	301-400	401-500	500<

2. Distance from pack house to port. (Km)

3. Port of export

CPT	PE	DBN	MPT

4. Cost of Cold Storage

<150	151-250	251-350	351-450	450<

5. Container haulage (Non –steri)

<1500	1500-2000	2000-2500	2500-3000	3000<

6. Container haulage (Steri)

<4500	4500-5000	5000-5500	5000-6000	6000<

7. PPECB Inspection cost (R/Pallet)

8. Landside port cost (R/pallet)

<6500	6500-7000	7000-7500	7500-8000	8500<

9. Export Documentation (Courier fee, PPECB certificate, EUR 1/FORM A)(R/Pallet)

<800	800-1200	1200-1600	1600-2000	2000<

10. Marine insurance cost for Valencia oranges (R/pallet)

<40	41-50	51-60	61-70	70<

11. Credit guarantee cost for Valencia oranges (R/pallet)

<40	41-50	51-60	61-70	70<

12. Exporter commission percentage.

4%	6%	8%	10%	12%	14%

13. Exporter commission at what level.

CIF	FOB	DIP

14. Add exporting cost before freight.

Description	R/pallet

15. Total cost till inland port.) Overall total FOB cost if cost breakdown could not be given.

Fakulteit AgriWetenskappe
•
Faculty of AgriSciences

Kantoor van die Dekaan • Office of the Dean
Privaat Sak/Private Bag X1 • Matieland 7602 • Suid-Afrika/South Africa
Tel: +27 21 808 4737 • Faks/Fax: +27 21 808 2001
agric@sun.ac.za • www.sun.ac.za/agric

4.3) Market specific price and cost (Canada)

Cost to market

Tick the appropriate box or give estimated amount where asked.

1. Freight cost ($/container) (**20 Pallets/container**)

<5500	5500-6000	6000-6500	6500-7000	7000<

2. Average duration of shipping. (days)

<10	10-15	15-20	20-25	25<

3. Importing port cost ($/container)

4. Importing tax (%)

<4	5-9	10-14	15-19	20-25

5. Importers commission (%)

<4	5-9	10-14	15-19	20<

6. Add cost (grey channel costs) (R/container)

Description	R/pallet

Income

a. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**No** pitting incidence 0%)

June	July	August	September	October	November

b. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**Low** pitting incidence 15%)

June	July	August	September	October	November

c. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**High** pitting incidence 30%)

June	July	August	September	October	November

Fakulteit AgriWetenskappe
•
Faculty of AgriSciences

Kantoor van die Dekaan • Office of the Dean
Privaat Sak/Private Bag X1 • Matieland 7602 • Suid-Afrika/South Africa
Tel: +27 21 808 4737 • Faks/Fax: +27 21 808 2001
agric@sun.ac.za • www.sun.ac.za/agric

4.4) Market specific price and cost (China)

Cost to market

Tick the appropriate box or give estimated amount where asked.

1. Freight cost ($/container) (**20 Pallets/container**)

<3500	3500-4000	4000-4500	4500-5000	5000<

2. Average duration of shipping. (days)

<10	10-15	15-20	20-25	25<

3. Importing port cost ($/container)

4. Importing tax (%)

<4	5-9	10-14	15-19	20-25

5. Importers commission (%)

<4	5-9	10-14	15-19	20<

6. Add cost (grey channel costs) (R/container)

Description	R/pallet

Income

a. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**No** pitting incidence 0%)

June	July	August	September	October	November

b. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**Low** pitting incidence 15%)

June	July	August	September	October	November

c. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**High** pitting incidence 30%)

June	July	August	September	October	November

Fakulteit AgriWetenskappe
•
Faculty of AgriSciences

Kantoor van die Dekaan • Office of the Dean
Privaat Sak/Private Bag X1 • Matieland 7602 • Suid-Afrika/South Africa
Tel: +27 21 808 4737 • Faks/Fax: +27 21 808 2001
agric@sun.ac.za • www.sun.ac.za/agric

4.5) Market specific price and cost (Europe)

Cost to market

Tick the appropriate box or give estimated amount where asked.

1. Freight cost ($/container) (**20 Pallets/container**)

<5000	5000-5500	5500-6000	6000-6500	6500<

2. Average duration of shipping. (days)

<10	10-15	15-20	20-25	25<

3. Importing port cost ($/container)

4. Importing tax (%)

<4	5-9	10-14	15-19	20-25

5. Importers commission (%)

<4	5-9	10-14	15-19	20<

6. Add cost (grey channel costs) (R/container)

Description	R/pallet

Income

a. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**No** pitting incidence 0%)

June	July	August	September	October	November

b. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**Low** pitting incidence 15%)

June	July	August	September	October	November

c. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**High** pitting incidence 30%)

June	July	August	September	October	November

Fakulteit AgriWetenskappe
•
Faculty of AgriSciences

Kantoor van die Dekaan • Office of the Dean
Privaat Sak/Private Bag X1 • Matieland 7602 • Suid-Afrika/South Africa
Tel: +27 21 808 4737 • Faks/Fax: +27 21 808 2001
agric@sun.ac.za • www.sun.ac.za/agric

4.6)Market specific price and cost (Hong Kong)

Cost to market

Tick the appropriate box or give estimated amount where asked.

1. Freight cost ($/container) (**20 Pallets/container**)

<2000	2000-2500	2500-3000	3000-3500	3500<

2. Average duration of shipping. (days)

<10	10-15	15-20	20-25	25<

3. Importing port cost ($/container)

4. Importing tax (%)

<4	5-9	10-14	15-19	20-25

5. Importers commission (%)

<4	5-9	10-14	15-19	20<

6. Add cost (grey channel costs) (R/container)

Description	R/pallet

Income

a. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**No** pitting incidence 0%)

June	July	August	September	October	November

b. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**Low** pitting incidence 15%)

June	July	August	September	October	November

c. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**High** pitting incidence 30%)

June	July	August	September	October	November

Kantoor van die Dekaan • Office of the Dean
Privaat Sak/Private Bag X1 • Matieland 7602 • Suid-Afrika/South Africa
Tel: +27 21 808 4737 • Faks/Fax: +27 21 808 2001
agric@sun.ac.za • www.sun.ac.za/agric

4.7)Market specific price and cost (Middle East)

Cost to market

Tick the appropriate box or give estimated amount where asked.

1. Freight cost ($/container) (**20 Pallets/container**)

<3000	3000-3500	3500-4000	4000-4500	4500<

2. Average duration of shipping. (days)

<10	10-15	15-20	20-25	25<

3. Importing port cost ($/container)

4. Importing tax (%)

<4	5-9	10-14	15-19	20-25

5. Importers commission (%)

<4	5-9	10-14	15-19	20<

6. Add cost (grey channel costs) (R/container)

Description	R/pallet

Income

a. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**No** pitting incidence 0%)

June	July	August	September	October	November

b. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**Low** pitting incidence 15%)

June	July	August	September	October	November

c. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**High** pitting incidence 30%)

June	July	August	September	October	November

4.8)Market specific price and cost (Singapore)

Cost to market

Tick the appropriate box or give estimated amount where asked.

1. Freight cost ($/container) (**20 Pallets/container**)

<2000	2000-3500	3500-4000	4000-4500	5500<

2. Average duration of shipping. (days)

<10	10-15	15-20	20-25	25<

3. Importing port cost ($/container)

4. Importing tax (%)

<4	5-9	10-14	15-19	20-25

5. Importers commission (%)

<4	5-9	10-14	15-19	20<

6. Add cost (grey channel costs) (R/container)

Description	R/pallet

Income

a. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**No** pitting incidence 0%)

June	July	August	September	October	November

b. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**Low** pitting incidence 15%)

June	July	August	September	October	November

c. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**High** pitting incidence 30%)

June	July	August	September	October	November

4.9)Market specific price and cost (United Kingdom)

Cost to market

Tick the appropriate box or give estimated amount where asked.

1. Freight cost ($/container) (**20 Pallets/container**)

<5000	5000-5500	5500-6000	6000-6500	6500<

2. Average duration of shipping. (days)

<10	10-15	15-20	20-25	25<

3. Importing port cost ($/container)

4. Importing tax (%)

<4	5-9	10-14	15-19	20-25

5. Importers commission (%)

<4	5-9	10-14	15-19	20<

6. Add cost (grey channel costs) (R/container)

Description	R/pallet

Income

a. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**No** pitting incidence 0%)

June	July	August	September	October	November

b. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**Low** pitting incidence 15%)

June	July	August	September	October	November

c. Average price ($/Pallet) obtained at the market for relevant months (R/pallet) (**High** pitting incidence 30%)

June	July	August	September	October	November