Quantifying changes in tree physiology after amelioration to reduce sunburn on apples

by

Stephan Hermann Daiber

Thesis submitted in partial fulfilment of the requirements for the Master of Science in the Faculty of Agriculture at Stellenbosch University

Supervisor: Dr Elmi Lötze

Co-supervisor: Prof Stephanie J. E. Midgley

March 2017

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 sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature: _______________ Date: _________________

Summary

Research was done in response to initial findings that combined calcium (Ca) and boron (B) reduced sunburn damage in apples. With the mode of action for this amelioration being unclear, current knowledge of this new sunburn-reducing strategy needed to be increased. With other strategies that are already able to eradicate or reduce sunburn incidence, like kaolin-film sprays or shade nets respectively, another alternative now exists in combined Ca and B. The motivation behind this sunburn-reducing strategy lies in its ease of use, economic viability due to low infrastructural expenses as well as its sustainability.

Continued research endeavours into this field are especially motivated by the changing climate, which confronts the deciduous fruit industry with increased temperatures. This could further exacerbate sunburn incidence which has already been up to 50% in some orchards. Future water restrictions, with more severe droughts and sporadic rainfall can give rise to water stress in the orchard, which increases sunburn.

In this study 'Cripps' Pink' and 'Granny Smith' apples were studied in addition to 'Golden Delicious' apples and alternative combined Ca and B formulations were evaluated to compare the success of the initial study on 'Golden Delicious' with other formulations and cultivars. In the first season, 'Cripps' Pink' apples on Welgevallen Experimental Farm, Stellenbosch, South Africa, were sprayed with weekly foliar applications of Ca and B for six weeks from 40 days after full bloom. Mineral analysis was done on the peels two weeks after the final application, as well as at harvest. Treatments containing Calcinit™, a calcium nitrate product, gave the highest B concentrations in the peel and no significant differences were observed for Ca concentrations. The Spraybor® Calcinit™ treatment had a significantly higher amount of B in the peel than the Spraybor® Manni-Plex® Ca treatment as well as the control, at harvest. This, however, did not reflect in a significant reduction in sunburn incidence at harvest. In an attempt to exacerbate sunburn damage, the 'Cripps' Pink' apples were harvested relatively late, which gave rise to pink colour development of the bicolour cultivar. Masking of sunburn, especially of the milder sunburn classes like class 1 and class 2 sunburn occurred.

In the second season, a separate trial was done on 'Golden Delicious' and 'Granny Smith' apples in Grabouw, Elgin Valley, South Africa. The 'Cripps' Pink' trial was repeated with alternative Ca and B formulations. In light of the first season's results, the 'Cripps' Pink apples were harvested earlier to prevent excessive masking of sunburn. Significant

differences were observed between the control and all Ca B foliar treatments, which all brought about a significant reduction in class 1 sunburn incidence. Significant differences were also recorded between treatments in 'Golden Delicious' apples. The 0.5 $g.L^{-1}$ Spraybor® Calsol® treatment was the only treatment that had significantly lower (5.7 %) Class 1 fruit than the control (16.4 %) and Manni-Plex Cal-Zn Manni-Plex B treatment (17.4 %). The same treatment also had a significantly lower Class 2 sunburn incidence than the control. The poor performance of the Manni-Plex® Cal-Zn Manni-Plex® B treatment was unexpected and was perhaps due to the alteration of the original (2011/13) Manni-Plex® Ca formulation during 2015.

Another objective of this study was to elucidate the mode of action of Ca and/or B ameliorating sunburn incidence, by looking at cell wall thicknesses below the peel. It was hypothesised that thicker cell walls due to Ca B applications resulted in sunburn reduction. Initial observations showed a trend of increased cell wall thicknesses when Ca and B formulations were applied, specifically especially with the Spraybor® Calcinit™ treatment. Cell walls were also thicker in the peel of sunburnt fruit than fruit with no sunburn.

Additionally, the final objective of this study was to determine how these foliar applications affected fruit physiology like chlorophyll fluorescence and fruit surface temperature. Even though there were significant differences between treatments with respect to all physiological parameters quantified in this study at some point, none of the effects could be related directly to a reduction in sunburn incidence; neither could the physiological changes as a result of the treatments explain the mode of action of the significant reduction of sunburn incidence observed.

Even though the efficacy of Ca and B applications could be established on 'Cripps' Pink' apples, a clear mode of action could not be established for this sunburn ameliorating strategy on a physiological level. More research therefore needs to be done into a possible biochemical and/or metabolic reaction caused by the combination of Ca and B in reducing sunburn on apples.

Opsomming

Navorsing is uitgevoer ter opvolging van aanvanklike bevindings dat ‘n kombinasie van kalsium (Ca) en boor (B) die voorkoms van sonbrand in appels verminder. Die metode van werking vir hierdie vermindering van sonbrand voorkoms is onduidelik, dus moet daar meer navorsing gedoen word om duidelikheid te kry. Met ander strategieë wat reeds sonbrand effektief onderskeidelik uitwis of ten minste verminder, soos kaolien-film of skadunette, bestaan daar nou ‘n alternatief in die blaarbespuiting van ’n Ca B bespuiting. Die motivering agter hierdie sonbrand verminderingsstrategie is die gemak van toediening, ekonomiese lewensvatbaarheid as gevolg van lae infrastruktuur uitgawes asook volhoubaarheid.

Addisionele navorsing in hierdie gebied in die sagtevrugtebedryf word veral gemotiveer deur klimaatsverandering wat manifesteer as verhoogde. Dit kan verder sonbrand voorkoms vererger wat reeds so hoog as 50 % in sommige appelboorde voorkom. Toekomstige waterbeperkings, met meer intensiewe droogtes en sporadiese reënval, kan aanleiding gee tot waterstres in boorde wat sonbrand verder kan verhoog.

'Cripps' Pink‘ en 'Granny Smith' appels is in hierdie studie bestudeer en alternatiewe Ca en B formulasies geëvalueer om die sukses van die eerste studie oor 'Golden Delicious’ met ander formulerings en kultivars te vergelyk. In die eerste seisoen, is 'Cripps' Pink' appels op die Welgevallen-proefplaas, Stellenbosch, Suid-Afrika gespuit met weeklikse blaartoedienings van Ca en B vir ses weke, vanaf 40 dae na volblom. Mineraal-analises is gedoen op die skil twee weke na die finale toediening, asook tydens oes. Behandelings met Calcinit™, 'n kalsiumnitraat produk, het die hoogste B konsentrasie in die skil gehad. Geen beduidende verskille is waargeneem vir Ca nie. Die Spraybor Calcinit™ behandeling het betekenisvol hoër B konsentrasie in die skil as die Spraybor Manni-Plex Ca behandeling sowel as die kontrole getoon tydens oes. Dit is egter nie weerspieël in 'n aansienlike vermindering in sonbrand voorkoms tydens oes nie. In 'n poging om voorkoms van sonbrandskade te verhoog, is die 'Cripps' Pink' appels relatief laat geoes, wat na pienk kleurontwikkeling van die tweekleurige kultivar gelei het. Maskering van sonbrand, veral van ligter sonbrand klasse soos Klas 1 en Klas 2 sonbrand, het plaasgevind.

In die tweede seisoen, het ons ‘n addisionele proef op 'Golden Delicious’ en 'Granny Smith' appels in Grabouw, Elgin Valley, Suid-Afrika uitgevoer. Die 'Cripps' Pink’ proef te Welgevallen proefplaas is herhaal met alternatiewe Ca en B formulasies. Om oormatige maskering van sonbrand te voorkom, het ons die 'Cripps' Pink’ appels vroeër geoes.

Beduidende verskille is waargeneem tussen die kontrole en al die Ca B behandelings met 'n aansienlike vermindering in klas 1 sonbrand voorkoms. Beduidende verskille is ook waargeneem tussen behandelings in 'Golden Delicious' appels. Die 0.5 g.L-1 Spraybor Calsol was die enigste behandeling wat beduidenisvolle laer (5.7 %) Klas 1 vrugte as die kontrole (16.4 %) en Manni-Plex Cal-Zn Manni-Plex B behandelings (17.4 %) gehad het. Dieselfde behandeling het ook 'n beduidenisvol laer Klas 2 sonbrand voorkoms as die kontrole gehad. Die onverwagte swak prestasie van die Manni-Plex Cal-Zn Manni-Plex B behandeling, na vroeër sukses op ‘Golden Delicious’, was moontlik gedeeltelik te wydte aan die aanpassing van die aanvanklike (2011/13) Manni-Plex Ca formulasie in 2015.

Die effek van Ca en B op selwand verdikking van selle onder die skil as metode van werking vir die vermindering van sonbrand op appels is evalueer. Aanvanklike waarnemings wys 'n tendens van toenemende selwand diktes wanneer Ca en B toegedien word, spesifiek die Spraybor® Calcinit™ behandeling. Selwande onder die skille van vrugte met sonbrand is dikker as skille van vrugte sonder sonbrand.

Daarbenewens was die laaste doelwit van die studie om vas te stel hoe hierdie blaartoedienings vrugfisiologie soos chlorofilfluoressensie en oppervlaktemperatuur beïnvloed. Al was daar beduidende verskille tussen behandelings met betrekking tot al die fisiologiese parameters wat gekwantifiseer is, kon nie een van die gevolge direk na 'n vermindering in sonbrand voorkoms lei nie. Die fisiologiese veranderinge wat waargeneem is na behandelings verduidelik ook nie die metode van werking wat aanleiding gegee het tot ’n aansienlike vermindering van sonbrand nie.

Selfs al kon die effektiwiteit van Ca en B bevestig word op ‘Cripps’ Pink’, kon 'n duidelike metode van werking nie vasgestel word vir hierdie sonbrand-vermindering strategie op 'n fisiologiese vlak nie. Meer navorsing moet dus gedoen word op 'n moontlike biochemiese en/of metaboliese reaksie wat ‘n vermindering van sonbrand op appels veroorsaak na ‘n blaartoediening van Ca en B.

Acknowledgments

I am greatly indebted to the following people and institutions:

My supervisor, Dr. Elmi Lötze for her guidance, patience and advice during my studies, as well as her unmatched sense of humour. My co-supervisor, Prof. Stephanie J. E. Midgley for her assistance and advice during my studies.

Mr. G. Lötze for his assistance in the lab, as well as the technical staff, with special mention to Mr. A. Swartz for the early hours of travelling and spraying on the farms.

The Department of Horticultural Science for maintaining such high standards of teaching and research.

Nulandis (Pty) Limited, South Africa for funding my research.

The owners at Applethwaite farm for their assistance and accommodation of the trial on their farm.

Mr. Willem van Kerwel and the staff on the Welgevallen Experimental Farm for their assistance during spraying and harvesting.

My fellow postgraduate students, for their assistance, encouragement and friendship; with special mention to Anthony Mwije, Helen Marais and Imke Kritzinger who has helped me on countless times while writing up this thesis.

My family for their encouragement, prayers and support throughout my studies.

My Lord and Saviour Jesus Christ.

Dedication

Dedicated to my father and fellow Maties Alma Mater.

Danke Papa.

Table of Contents

Note

This dissertation has been compiled in such a way that each chapter is an individual entity and as thus some repetitions across chapters has been unavoidable.

General Introduction

Sunburn is a physiological disorder related to solar radiation and/or radiant heating of the apple's fruit surface, resulting in damage to the peel (Racsko and Schrader, 2012). Ambiguity exists around the term "sunburn" since it has often been used interchangeably with the term "sun scald", which is described as an injury to the bark caused by freezing (Schrader et al., 2001). Other terms referring to the fruit disorder, that will be referred to in this thesis include "heat injury", "solar injury" or "sun blush".

Sunburn incidence under South African conditions has accounted for up to 50% reductions in exportable fruit numbers (Makeredza et al., 2013). This has significant effects on the profitability of South African apple production, even more so with predicted increasing sunburn-conducive conditions in the future for local apple production areas (IPCC, 2007). Global climate change will not only result in temperature increases, but also change South African rainfall patterns (Benhin, 2008). This will affect sunburn incidence in various ways. Firstly, a rise in ambient temperatures will result in an increase in fruit surface temperatures (FST) which put fruit more at risk of reaching threshold FST levels at which sunburn occurs. Secondly, extreme weather conditions will become more sporadic and sudden exposure to higher temperatures will result in sunburn to the unacclimated apple peel. Thirdly, less rainfall or changing rainfall patterns will result in more frequent drought conditions, which can enhance the problem even further, as water stress increases sunburn incidence (Makeredza et al., 2013).

Various sunburn-reducing strategies have been used and researched in South Africa. For example, over-tree evaporative cooling (Gindaba and Wand, 2005; Van den Dool, 2006), kaolin clay application (Gindaba and Wand, 2005; Le Grange et al., 2004; Wand et al., 2006), and shade nets (Fouché, 2009; Gindaba and Wand, 2005; Smit, 2007). Of these, shade nets performed the best in a comparative study, with a complete eradication of sunburn incidence under shade nets (Gindaba and Wand, 2005). However, to date no significant and consistent reduction in sunburn damage has been recorded on a commercial scale (Lötze and Hoffman, 2014).

An alternative approach to existing management practices was reported by Lötze and Hoffman (2014), who were able to reduce sunburn using a foliar application of mineral nutrients. A significant reduction of sunburn incidence was achieved with eight foliar applications of a Manni-Plex® Ca Manni-Plex® B formulation early in the season on 'Golden Delicious' apples. This was the first report of a sunburn reduction by means of a mechanism that is based on enhancing the mineral nutrition. This alternative versus those mentioned above seems promising due to its low infrastructural expenses and its simplicity of use.

Research for this thesis was performed in response to the proposal for future research made by Lötze and Hoffman (2014), which was to "investigate the mode of action of B and Ca foliar applications in reducing the incidence of sunburn in 'Golden Delicious' apples". It was also proposed that similar foliar applications of B and Ca be made to other cultivars to possibly obtain comparable results.

The main objective of this thesis was to further assess the efficacy of the foliar application of B and Ca in decreasing sunburn. This was done by including 'Granny Smith' and 'Cripps' Pink' apples to increase the cultivar range, but also by evaluating alternative formulations of Ca and B. As part of the main objective of this study, minerals were analysed within the peel of the fruit after foliar applications to quantify their uptake; but also at harvest to determine if these minerals were still relevant at that stage. Sunburn classification was based on the classification guide developed by Felicetti and Schrader (2008) for 'Fuji' apples and re-created for 'Cripps' Pink', 'Golden Delicious' and 'Granny Smith'. This was done in an attempt to standardise the classification of sunburn, but also to give an indication of the degree to which the sunburn has developed. In previous studies, sunburn has been classified, as being present or not (Fouche, 2009) or into three classes (Makeredza, 2011; Marais, 2005; Smit, 2007).

Another objective was to correlate the concentrations of these minerals in the fruit peel with the cell wall thickness of individual cells in the peel. This was done using scanning electron microscopy, in order to elucidate a possible mode of action the application of B and Ca might have in ameliorating sunburn. Ca application to fruit reinforces cell walls through calcium bridges between polymers and a partial reduction of cell wall-modifying enzyme activity (Ortiz et al., 2011). The mineral B is also involved in maintaining cell wall and membrane structure, by binding to glycoproteins and glycolipids (Bolaños et al., 2004). An increase in cell wall thickness due to supplementation of Ca and B through foliar application could therefore be a mode of action to reduce sunburn. This has not been documented. Since this sunburn ameliorating strategy is a recent area into which more research needs to be done. However, cell wall thickening has been observed in response to severe sunburn damage (Hao and Huang, 2004; Racskó et al., 2005; Racsko and Schrader, 2012), but also prior to visible sunburn symptom appearance (Andrews and Johnson, 1996).

The final objective of this study was to determine how these foliar applications affected some fruit physiology aspects. Parameters that were measured included chlorophyll fluorescence measurements (Fv/Fm ratios), or maximal photochemical efficiency. The Fv/Fm ratio is used to indicate stress to chlorophyll a in response to sunburn damage caused by high irradiance and/or FST (Chen et al., 2008; Hengari et al., 2014). The motivation behind measuring this parameter that has been found to successfully indicate sunburn damage (Makeredza et al., 2015), was to identify a relation between Fv/Fm and Ca and B application and how it relates to sunburn incidence. The other physiological parameter was FST, which alongside irradiance is the other direct factor causing sunburn formation on apples (Racsko and Schrader, 2012). FST is simply a reflection of the heat exchange between the fruit surface and the atmosphere and is affected by other factors such as wind velocity which affects the boundary layer, relative humidity and solar radiation (Racsko and Schrader, 2012; Zhang et al., 2007). These indirect factors should therefore also be taken into consideration when the FST measurements are related to sunburn incidence. A high FST does not necessarily result in sunburn incidence, under Washington State (USA) weather conditions, an increase of wind velocity from 0.5 to 3.5m.s^{-1} reduces FST by about 5˚C (Schrader et al., 2003).

In general, this thesis will investigate possible modes of actions in this new area of research - that of an amelioration of sunburn incidence of apples due to the application of combined foliar Ca and B formulations. This was done both at a physiological level by studying the Fv/Fm ratios and also to some extent on a physical level, by examining cell wall thickness in relation to mineral concentrations of the elements of interest in the peel. Essentially, this thesis will also attempt to evaluate the efficacy of Ca and B on a broader scope of cultivars than ‘Golden Delicious’ apples (Lötze and Hoffman, 2014) and thus included the addition of ‘Cripps’ Pink’ and ‘Granny Smith’ apples.

References

Andrews, P.K., Johnson, J.R., 1996. Physiology of sunburn development in apples. Good Fruit Grow. 47, 33–36.

Benhin, J.K.A., 2008. South African crop farming and climate change: An economic assessment of impacts. Glob. Environ. Chang. 18, 666–678. doi:10.1016/j.gloenvcha.2008.06.003

Bolaños, L., Lukaszewski, K., Bonilla, I., Blevins, D., 2004. Why boron? Plant Physiol. Biochem. 42, 907–912. doi:10.1016/j.plaphy.2004.11.002

Chen, L.S., Li, P., Cheng, L., 2008. Effects of high temperature coupled with high light on the balance between photooxidation and photoprotection in the sun-exposed peel of apple. Planta 228, 745–756. doi:10.1007/s00425-008-0776-3

Felicetti, D., Schrader, L., 2008. Changes in pigment concentrations associated with the degree of sunburn browning of "Fuji" apple. J. Am. Soc. Hort. Sci. 133, 27–34. doi:10.1016/j.plantsci.2008.09.013

Fouché, J.R., 2009. Increasing class one fruit in 'Granny Smith' and 'Cripps' Pink' apple. MSc Agric thesis. Faculty of AgriScience. Stellenbosch University.Stellenbosch.

Gindaba, J., Wand, S.J.E., 2005. Comparative effects of evaporative cooling, kaolin particle film, and shade net on sunburn and fruit quality in apples. HortSci. 40, 592–596.

Hao, Y.Y., Huang, W.D., 2004. Changes in antioxidative system and cell ultrastructure in the fruit peels of apple during sunburn development. J. Plant Physiol. Mol. Biol. 30, 19–26.

Hengari, S., Theron, K.I., Midgley, S.J.E., Steyn, W.J., 2014. Response of apple

(*Malus domestica Borkh.*) fruit peel photosystems to heat stress coupled with moderate photosynthetic active radiation at different fruit developmental stages. Sci. Hortic. 178, 154–162. doi:10.1016/j.scienta.2014.08.019

IPCC, 2007. Climate Change 2007: impacts, adaptation and vulnerability: contribution of Working Group II to the fourth assessment report of the Intergovernmental Panel, Genebra, Suíça. Cambridge. doi:10.1256/004316502320517344

Le Grange, M., Wand, S.J.E., Theron, K.I., 2004. Effect of kaolin applications on apple fruit quality and gas exchange of apple leaves. Acta Hort. 636, 545–550.

Lötze, E., Hoffman, E.W., 2014. Foliar application of calcium plus boron reduces the incidence of sunburn in 'Golden Delicious' apple. J. Hortic. Sci. Biotechnol. 89, 607–612.

Makeredza, B., Marais, H., Schmeisser, M., Lötze, E., Steyn, W.J., 2015. Ripening associated red color development masks sunburn browning in apple peel. HortSci. 50, 814–818.

Makeredza, B., Schmeisser, M., Lötze, E., Steyn, W.J., 2013. Water stress increases sunburn in 'Cripps' Pink’ apple. HortSci. 48, 444–447.

Makeredza, B., 2011. Studies towards understanding sunburn in apple. MSc Agric thesis. Faculty of AgriScience. Stellenbosch University.Stellenbosch.

Marais, S., 2005. Sunburn control in apple fruit. MSc Agric thesis. Faculty of AgriScience. Stellenbosch University. Stellenbosch.

Ortiz, A., Graell, J., Lara, I., 2011. Preharvest calcium applications inhibit some cell wall-modifying enzyme activities and delay cell wall disassembly at commercial harvest of 'Fuji Kiku-8' apples. Postharvest Biol. Technol. 62, 161–167. doi:10.1016/j.postharvbio.2011.04.014

Racsko, J., Schrader, L.E., 2012. Sunburn of Apple Fruit: Historical Background, Recent Advances and Future Perspectives. CRC. Crit. Rev. Plant Sci. 31, 455–504. doi:10.1080/07352689.2012.696453

Racskó, J., Szabó, Z., Nyéki, J., 2005. Importance of the supraoptimal radiance supply and sunburn effects on apple fruit quality. Acta Biol. Szeged. 49, 111–114. doi:10.1007/s00425-008-0776-3

Schrader, L., Zhang, J., Sun, J., 2003. Environmental stresses that cause sunburn of apple. Acta Hort. 618, 397–405.

Schrader, L.E., Zhang, J., Duplaga, W.K., 2001. Two Types of Sunburn in Apple Caused by High Fruit Surface (Peel) Temperature. Plant Heal. Prog. doi:10.1094/PHP-2001-1004-01-RS

Smit, A., 2007. Apple tree and fruit responses to shade netting. MSc Agric thesis. Faculty of AgriScience. Stellenbosch University. Stellenbosch.

Van den Dool, K., 2006. Evaporative cooling of apple and pear orchards. MSc Agric thesis. Faculty of AgriScience. Stellenbosch University.Stellenbosch.

Wand, S.J.E., Theron, K.I., Ackerman, J., Marais, S.J.S., 2006. Harvest and post-harvest apple fruit quality following applications of kaolin particle film in South African orchards. Sci.Hort. 107, 271–276. doi:10.1016/j.scienta.2005.11.002

Zhang, J., Di, B., Li, Y., Zhang, J., Sun, J., Liu, Y., 2007. Effect of microenvironments and exogenous substance application on 5'-nucleotidase activities in apple peel. Front. Agric. China 1, 67–71. doi:10.1007/s11703-007-0012-0

Literature Review

The role of plant-available water in fruit quality

1. Introduction

Water availability is declining worldwide and due to rising competition for water allocation from other industries as well as global warming concerns, the deciduous fruit industry needs to adopt water productive practices in orchards. This review focuses on agricultural water usage prospects in South Africa, the effect this will have on the agricultural sector, and strategies to counter water deficits in the near future. This is looked at in general, but also more specific within the deciduous fruit industry and pome fruit in particular.

The trend to grow high yielding apple cultivars in often high density orchards has resulted in yields of up to 130 tons per hectare (Wünsche and Lakso, 2000). The number of orchards recording such yields is also increasing and in response to these heavier crop loads, trees require significantly more water, due to higher transpiration rates. If these trends persist, more irrigation is therefore required for the deciduous fruit for these yields to be maintained.

Cultivated low rainfall areas as well as semi-arid farming practices, which depend on irrigation; necessitate emphasis on water use efficiency practices (Huang et al., 2008; Ramos et al., 2011; Ruiz Sánchez et al., 2010). Water efficiency is improved by irrigation scheduling (Fereres and Soriano, 2007) as well as reducing water losses through evaporation by using cultural practices such as mulching, which conserve soil moisture (Huang et al., 2008).

To use water effectively, biochemical and physiological stress indicators need to be evaluated carefully (Šircelj et al., 2007), so as to achieve optimum water use efficiency in an ever increasingly water scarce world.

2. Prospects of water restrictions in South Africa

According to Taylor and Gush (2014), available water resources in South Africa are continuing to be under pressure, resulting in more stringent monitoring of water distribution and application of irrigated water. In 2007, 10 468 million cubic meters of water was used per year on 1.5 million hectares of land. Sprinkler systems are the main form of irrigation (31%), followed closely by centre pivot (24%) as well as micro-irrigation (22%) and flood systems (14%).

South Africa is a water scarce country (Kiker, 1999; Taylor and Gush, 2014). A threshold of water availability of 1500 cubic meters per capita per year was determined by Yang et al. (2003). If water availability falls below this, a country tends to have cereal imports that are inversely correlated with its renewable water resources. By 2030 South Africa will be one of various countries in Africa and Asia, that will fall below this threshold (Rijsberman, 2006; Yang et al., 2003).

Specifically for the Western Cape region, a drying trend is predicted with weaker winter rainfall and increasing temperatures. Water resources, which experience an increased demand from various sectors, are already fully committed. Some water catchments are already experiencing a water deficit, which threaten the integrity of the ecosystems that form part of these catchments (Midgley et al., 2005).

2.1 Climate change

According to the South African Department of Water and Sanitation, climate change has increased in importance with regard to water resource management strategies (Department of Water and Sanitation, 2009). They found that higher temperatures and more extreme weather would result in an increase in rainfall intensity in some areas of the country while causing extreme droughts in other parts. Farmers across the spectrum of the agricultural sector will see an impact of climate change on their crop yield (Benhin, 2008; Müller et al., 2011; Wiid and Ziervogel, 2012).

Researchers looked at the vulnerability to climate change on a local level and defined it as “a function of the severity of the impacts experienced, as well as the sensitivity to change of a farm and its practices and the degree of adaptive capacity” (Adger, 2006;

Eakin and Luers, 2006; Wiid and Ziervogel, 2012). Some authors have argued that extreme climate conditions or moisture extremes, like droughts and floods, have the greatest impact on agricultural systems (Fischer et al., 2005; IPCC, 2007; Midgley et al., 2005; Wiid and Ziervogel, 2012).

Climate change will cause an increase in temperatures and a change in rainfall patterns in South Africa, which will have a significant impact on all sectors of the economy, given the already scarce water resources available to the country (Benhin, 2008). The agricultural sector is the most vulnerable to changes in temperature and precipitation. The National Department of Agriculture highlighted three factors that would contribute to this, namely: water scarcity, frequency of droughts and semi-arid nature of the country, and increased marginal land farming (NDA, 2013). In assessing the impact of these changes, the fact that a farm was irrigated or not was far more important than the scale at which farming occurs (Benhin, 2008). More research therefore needs to be done in terms of generating heat tolerant and water stress resistant crop varieties, as well as improving farming technologies.

2.2 Irrigation management

Irrigation water is applied in response to a lack of water supply from rainfall, especially in semi-arid or winter-rainfall regions, to prevent water deficits that impact negatively on crop yield. Varying reports show that about half of food production worldwide is done on irrigated land, which amounts to more than 280 million hectares (Afzal et al., 2016). According to Fereres and Soriano (2007), 40% of global food production is from irrigated agriculture. However, irrigated land only uses 17% of the land area allocated to food production globally.

Irrigation management implies that water is administered correctly in terms of availability and plant requirements to acquire acceptable yields. It is of that much greater importance, when insufficient water supply for irrigation will become the norm rather than the exception. Irrigation management will shift towards maximizing production per unit of water (i.e. water productivity), as opposed to production per unit area (Fereres and Soriano, 2007). Irrigated agriculture, in some parts of the world, is done with complete disregard for conserving water resources. To maximize productivity and save water, irrigation management needs to be applied with great efficiency. It is clear that future prospects in terms of water scarcity and technological availability require an optimisation of irrigation management to ensure water productivity and thereby food security.

3. Water use of apple trees

This section looks at some water use requirements of apple trees. To irrigate apple orchards sustainably with a high water use efficiency, it would seem important to understand the water use of the crop.

3.1 Water requirements of apple orchards

At the turn of this millennium, researchers focused on determining the environmental factors that influence tree water-use as well as the irrigation needs of the plant. This was done in an effort to "develop equitable and sustainable irrigation strategies for orchard trees" (Green et al., 2003).According to Green et al. (2003) apple trees use up to 70 litres of water per day during mid-summer, from midnight to midnight. The average over the year is about 45 litres a day. The main factors that determine apple tree water consumption are the atmospheric demand of evaporation, canopy size and crop load. A higher transpiration rate, and therefore water use, is found in larger trees. Trees with large crop loads also have a higher transpiration rate, due to greater sink strength for assimilates as well as high stomatal conductance so that a high photosynthesis rate can be maintained (Dzikiti et al., 2014). High yielding orchards will therefore most likely require higher irrigation rates.

Studies like that of Green et al. (2003) determined water requirements of apple orchards elsewhere in the world. However, no accurate information on water use of apple orchards in Mediterranean climates like those in South Africa is available (Gush et al., 2014). In a study on 'Cripps' Pink' trees planted in well drained gravel soil with a high sand and stone content, water use values were modelled over two seasons. Total evaporation over both seasons was 952 mm per year for the first season and 966 mm per year for the second season. Of this, more than two thirds was used for transpiration. It is important to note that both yield and water use of apple orchards varies between cultivars, rootstocks, plant densities, irrigation systems and management practices (Dzikiti et al., 2014).

3.2 Measuring water use

To increase water use efficiency, especially in cultivated arid and semi-arid regions, water losses by crops should be evaluated correctly (Rana and Katerji, 2000). Whole-tree estimates of water use have become more popular in forestry studies (Wullschleger et al., 1998). This is in response to unreliable methods of inferring rates of canopy water-use using data from removed leaves and branches and as well as extrapolating water use estimates from potted seedlings to large trees. Such studies failed to reproduce the climate conditions in which these respective trees were grown.

Some measurements suited for whole-tree water-use, include weighing lysimeters, large-tree photometers, ventilated chambers, radio-isotopes, stable isotopes as well as various heat balance and dissipation methods (Rana and Katerji, 2000; Wullschleger et al., 1998). With a vast number of measurement methodologies available for estimating evapotranspiration, the three most popular methods used in horticulture are; soil water balance method, the micrometeorological method and a combination of the soil evaporation and plant transpiration method (Taylor and Gush, 2014).

Soil water balance methods calculate the total evaporation from a water balance equation, which takes the contribution and deduction of water to the soil into consideration. A simplified version of this water balance equation was developed due to the difficulty of measuring some of the components (Rana and Katerji, 2000).

$$Precipitation + Irrigation - Total\ Evaporation = \pm[\Delta Plant\ available\ water]$$

This equation however seems to be unsuitable for precise evaporation measurements (Taylor and Gush, 2014). However, continuously monitoring soil water content within and below the rooting zone can enable optimal irrigation scheduling to minimize effects of water stress on the plants and leaching of soil minerals below the root zone, which can result in negative environmental effects (Fares and Alva, 2000).

Micro-meteorological methods include the Bowen ratio, Eddy correlation and scintillometry methods; which directly measure transpiration and thus give insight to forest hydrological processes (Wullschleger et al., 1998). These methods consider evapotranspiration, the latent heat measured as energy flux needed for transporting water from inner leaves to the atmosphere, as water (Rana and Katerji, 2000). Due to canopy heterogeneity, some of these methods have encountered difficulty, with the exception of scintillometry due to spatial averaging (Gong et al., 2007).

Soil evaporation and transpiration combination methods using the productivity estimate from crop transpiration, with unproductivity estimates from soil evaporation, gives an indication of the different components of total evaporation (Taylor and Gush, 2014). For this, a heat-pulse velocity technique is used to measure sap flow rates (transpiration) in trees, which is then combined with micro-lysimeters in soil. This method seems to be more accurate than the previous two methods mentioned above (Gong et al., 2007).

4. Plant response to water stress

Water stress occurs in response to either too much water or too little water. For the purpose of this review, the focus will be on water stress associated with water deficit. Some of the physiological and biochemical perturbations include water potential reduction, cellular dehydration, reduced cell expansion, and stomatal closure (Taiz and Zeiger, 2010). In apple orchards, a yield decline is a common cause of water stress. This is due to a suppression of processes like respiration and photosynthesis which rely on a sufficient water supply. Other effects of water stress on apple trees include smaller stem diameters and average leaf area (Yuncong et al., 2010).

4.1 Biochemical response to water stress

A water deficit is known to trigger both physiological and biochemical responses in plants (Šircelj et al., 2005). An increase of free radicals and reactive oxygen species (ROS) are only some of the biochemical effects that result from drought, which in turn affect plant metabolism and causes damage on a cellular level (Chaves et al., 2003; Reddy et al., 2004; Šircelj et al., 2007, 2005; Smirnoff, 1993). A non-enzymatic response to the ROS is the formation of anti-oxidant defence components like ascorbic acid, glutathione, tocopherol and carotenoids. These react differently depending on species, drought stress duration and antioxidants investigated (Boo and Jung, 1999;

Munné-Bosch and Peñuelas, 2004; Zagdańska and Wiśniewski, 1996; Zhang and Kirkham, 1996). This variation in observed reaction to drought stress can be because of the "temporal sequence of antioxidant responses" (Šircelj et al., 2005).

Another biochemical response to water stress is the metabolism of soluble carbohydrates, which act as antioxidants and compatible solutes (Šircelj et al., 2005; Smirnoff, 1993). Free amino acids, like proline, also increase in response to water stress and appear to significantly affect osmotic adjustments (Šircelj et al., 2007, 2005). The free amino acids appear to increase proteolysis or inhibit protein synthesis (Šircelj et al., 2007). Proline also seems to play a role as an antioxidant (Reddy et al., 2004; Šircelj et al., 2007). Solutes are actively accumulated during water stress, which decreases leaf osmotic potential (potential of the leaf to take up water) for leaf turgor pressure (pressure water exerts on plasma membrane) to be conducive for cellular expansion (Wang and Stutte, 1992).

4.2 Physiological response of plants to water stress

The hydraulic conductivity decreases as soil dries and this occurs sharply towards the permanent wilting point (the minimal point of soil moisture at which plants will not be able recover from wilting) this occurs sharply, after which the water delivery to the roots is too slow for the plant to rehydrate itself overnight (Taiz and Zeiger, 2010). As plants cells lose water in response to a lack of water uptake, cells start to shrink which in turn is caused by cytorrhysis (cell wall collapse). Severe water loss in cells results in cellular dehydration and the water potential of the apoplast becoming more negative than that of the symplast. The effects of cellular dehydration can also have secondary effects of cytotoxicity. Since cell growth is driven by turgor pressure, a plant under water stress will exhibit stunted growth, which in turn will adversely affect yield.

Furthermore, for efficient tree functioning, stomata conductance needs to ensure optimal gas exchange in response to the environment and the leaf's photosynthetic rate, including the hydraulic characteristics of both soil and tree (Pretorius and Wand, 2003). Stomatal responses of apple leaves are regulated primarily by the photosynthetic rate (i.e. light) and not so much by leaf water potential when the plant is not under stress. Prolonged soil moisture deficit, or a rapid one, will result in stomatal closure. The water stress does however need to be at an advanced stage in mature leaves, because the leaves have developed osmotic adjustment capabilities (the ability to lower osmotic potential by accumulating solutes) in order to maintain leaf turgor (Lakso, 1979; Pretorius and Wand, 2003). In apple trees, these water deficits are often experienced by stomata even under optimal soil water conditions due to poor hydraulic conductivity of the root systems (Jones et al., 1985). However, this problem is solved, specifically in apple trees, by the highly effective ability of mature leaves to make osmotic adjustments (Faust, 1989). Apple trees can therefore show resilience during mild to moderate droughts by maintain canopy photosynthesis (Pretorius and Wand, 2003). This ability of the apple tree could be harnessed to reduce irrigation water costs, reduce late vegetative growth as well as conserve water (Jerie et al., 1989); however not at the detriment of reproductive buds (Pretorius and Wand, 2003).

In terms of osmotic adjustment, some plant species have the ability to counter water stress by actively accumulating solutes during water stress (Hsiao et al., 1976). This decreases leaf osmotic potential so that leaf turgor potential can be maintained at levels sufficient for turgor-mediated processes and for cellular expansion (Hsiao, 1973; Hsiao et al., 1976; Turner, 1979; Wang and Stutte, 1992). Leaf osmotic potential decreases gradually and leaf water potential decreases from about -0.6 to -2.6 MPa (Wang and Stutte, 1992). The turgor potential also decreases up to 1 MPa, and is then maintained at this level through osmotic adjustment mechanisms. If the leaf water potential is above -1.6 MPa, the leaf turgor pressure can be maintained by the osmotic adjustments, after which it becomes insufficient and turgor mediated processes cannot be maintained (Wang and Stutte, 1992).

4.3 Physical response to water stress

One of the most obvious and severe physical responses of trees to water stress is tree mortality. Even though the exact mechanisms for tree mortality have been a subject for debate (Gonzalez-Rodriguez et al., 2016), two dominant mechanisms were identified: carbon starvation and hydraulic failure (McDowell et al., 2008). Carbon starvation occurs in response to reduced carbon assimilation due to an inhibition of photosynthesis. Photosynthesis is reduced in response to inefficient stomatal conductance due to a lack of water as discussed in the section above. As drought progresses for a longer period of time, the carbon reserves will eventually be depleted and metabolism will thus be impaired (Gonzalez-Rodriguez et al., 2016; McDowell et al., 2008). Pathogens have also been identified as a contributing factor to tree mortality as the trees become more susceptible to pathogenic attack as water stress with accompanying symptoms progresses (McDowell et al., 2008). Hydraulic failure, as the other mechanism for tree mortality, involves cavitation (vaporization of xylem sap forming small liquid-free zones) of the xylem vessels and reduces sap flow and hydraulic conductivity (Jones, 2004; McDowell et al., 2008). This occurs in response to very severe drought conditions during which the hydraulic pressures in the xylem become very negative. The increasing negative hydraulic pressure allows cavitation to occur, thereby progressively decreasing stomatal conductance (Gonzalez-Rodriguez et al., 2016; McDowell et al., 2008).

In terms of stress response, various authors have brought forward two different strategies observed in isohydric and anisohydric trees (Gonzalez-Rodriguez et al., 2016; McDowell et al., 2008; Sade et al., 2012). Isohydric trees regulate the stomatal opening by reducing the variation of leaf water potential, thereby reducing transpiration (i.e. water loss) and limiting the risk of xylem cavitation (Gonzalez-Rodriguez et al., 2016; Sade et al., 2012). However, this poses a risk of carbon starvation as carbon assimilation from photosynthesis will be limited due to stomatal closure (Gonzalez-Rodriguez et al., 2016). Anisohydric plants have more variable leaf water potential and keep stomata open for longer and thereby extend periods of high photosynthetic rates (Sade et al., 2012). These plants have been termed as “opportunistic risk-takers”, showing positive drought responses under minimal and moderate stress. This is however not the case when drought conditions are prolonged (Sade et al., 2012). Isohydric plants seem to regulate their relative water content in leaves more strictly than their water potential (Sade et al., 2012). It is suggested that a higher cell wall elasticity cause plants to be more sensitive, so that a minimum change in relative water content (RWC) results in a maximum response in leaf water potential adjustments (Kramer and Boyer, 1995; Sade et al., 2012).

Another physical response to water stress that is of economic concern is sunburn. Water stressed trees bear apples that are more prone to sunburn (Makeredza et al., 2013, Makeredza, 2011; Schrader et al., 2003b; Van den Ende, 1999). Sunburn on apples occurs via three different mechanisms, the most frequent mechanism being that of sunburn browning. This occurs in response to a combination of high irradiance and a high fruit surface temperature. To cool fruit surface temperature, water evaporation from the fruit peel dissipates energy. A water deficit experienced by the tree therefore results in increased fruit surface temperatures and therefore an increase in sunburn development (Makeredza, 2011; Woolf and Ferguson, 2000).

Another physical response is that of bitter pit, even though reportedly a calcium disorder (Perring, 1986; Saure, 2005), increased incidences in bitter pit were recorded by Schrader et al. (2003) when 'Jonagold' apples were exposed to high temperatures and water stress. This agrees with results of Failla et al. (1990), which showed a reduction of bitter pit in response to increasing degrees of water stress on 'Granny Smith' apples. Calcium distribution in the tree is regulated by transpiration rates, due its low mobility in phloem (Failla et al., 1990) and thus it was suggested that bitter pit would likely be intensified by water stress if a certain transpiration threshold is reached hence leading to insufficient amounts calcium reaching the apple fruit (Failla et al., 1990).

4.5 Root response to water stress

Extensive research has been done on osmotic adjustments, antioxidant protection and stomatal movement, however little research had been done on the root response to water stress (Ji et al., 2014) more so especially the ability of root systems to reprogram in an attempt to maximize water absorption for survival.

Water uptake is primarily a passive mechanism caused by a water potential gradient formed by transpiration pull through the apoplast (Bengough et al., 2011). Water shortage and transpirational pull of shoots also influences the variability of hydraulic conductance in roots. Water stress in combination with mechanical impedance, causing rapid penetration of roots in soil to be reduced, greatly limits root elongation. During a water deficit, an apoplastic barrier is developed and the anatomy of the root tissue changes, preventing water and ion flow (Steudle, 2000; Taleisnik et al., 1999). Water channels or aquaporins in root cell membranes also undergo closure, reducing hydraulic conductivity (Steudle, 2000).

In response to water stress, root growth is strongly inhibited (Sharp and Davies, 1989; Westgate and Boyer, 1985). A mild to moderate osmotic stress induced on wheat

(*Triticum aestivum*) by polyethylene glycol results in early differentiation of the root apical meristem (Ji et al. 2014). This “conserved adaptive mechanism” is utilised by various plants in response to osmotic stress. The premature root differentiation resulted in growth of lateral roots and a cessation of primary root growth.

Water stress is also able to increase specific root length, a good indicator for determining the tolerance of different rootstocks to water stress (Psarras and Merwin, 2000). Root to shoot ratio is also increased in response to water deficit and is more pronounced in vigorous rootstocks like M111 versus an M9 dwarfing rootstock.

5. Mechanisms to reduce water stress in plants

5.1 Mulching

Changes in soil nutrient availability, reduced soil temperature through shading and reduction in raindrop-impact are some of the effects to which effective mulching practices are attributed (Buerkert et al., 2000; Salau et al., 1992).

Mulching is used to conserve soil moisture and is particularly beneficial when new land is cultivated in increasingly low rainfall areas, like the subtropical Australian hardwood plantations where water stress is increasingly a limiting factor. In this study a significant increase in water use was recorded for spotted gum trees '*Eucalyptus maculaa'* which were grown in soils that were mulch (Huang et al., 2008).

Earlier studies by Baxter (1970) showed an increased yield of 20 tonnes of apples and 10 tonnes of peaches per hectare over two years, as a result of mulching. Mulching can greatly increase the yield of apple trees, due to a reduction of moisture stress between irrigations and a reduction of evaporation from soil. Even though the initial cost of mulching is quite high, the increased yield in the following seasons seems to justify such expenses. The combined effect of reduction of water loss with temperature control as well as a higher soil porosity closer to the surface, stimulates root growth (Pinamonti et al., 1995). Soil water is conserved by reducing evaporation to a third (Baxter, 1970).

In response to reports of crop residue mulches increasing crop yields in West Africa, field trials by Buerkert et al. (2000) showed an increase in cereal total dry matter of up to 73% with mulching in the semi-arid Sahel region. The mulching also improved spatial phosphorous availability due to increases in root length density. The increased root development due to mulching can be attributed to a combination of reduced fluctuations of soil moisture, less resistance to root elongation and hormonal feedback that stimulates root growth through larger bacterial populations in the soil. Mulch applications also reduced surface crust formations.

5.2 Irrigation schedules

In response to increasing worldwide water scarcity, it is becoming more important for irrigation scheduling to be applied (Šircelj et al., 2007). Irrigation in the agricultural sector is seen as either highly inefficient in using water, or irrigation is emphasised as essential to food security and a sufficient supply needs to be maintained to meet increasing demands caused by global population growth (Fereres and Soriano, 2007).

Irrigation scheduling is based directly on sensing drought stress in plants (Šircelj et al., 2007). This is done by evaluating biochemical and physiological stress indicators. Studies on drought responses in apples have focused largely on physiological responses like stomatal reactions, photosynthesis or osmotic adjustments and less on biochemical responses. Biochemical responses to water stress which have been studied are carbohydrates, hormonal responses including abscisic acid, volatile compound emissions from leaves as well as active oxygen species and antioxidant responses. The most consistent biochemical drought markers are the glutathione disulphide (GSSG) and zeaxanthin concentrations and could be used to develop "highly targeted irrigation schedules" in apple tree orchards (Šircelj et al., 2007).

Regulated deficit irrigation (RDI) is when water is applied below levels that are required to satisfy the crop's evapotranspiration during certain periods in the season (Ruiz Sánchez et al., 2010). It is used to increase water use efficiency in fruit crop production (Fereres and Soriano, 2007; Jones, 2004). Precision irrigation methods like drip irrigation have by and large reduced the amount of water that is required for agricultural crops and created a need to develop methods to accurately schedule and control irrigation (Jones, 2004). A slight plant water deficit results in an increased sink of carbohydrates to fruit as well as a reduction of excessive vegetative growth in pear fruit (Chalmers et al., 1986). For apples, deficit irrigation reduce mean fruit weight, but has no significant effect on gross yield (Mpelasoka et al., 2001). However, this was not the case for several other studies and RDI was deemed inappropriate for fruit crop production (Volschenk and Gindaba, 2014). Deficit irrigation during periods of vegetative growth up until 40 days after full bloom (dafb) or fruit ripening until harvest resulted in a restriction of photosynthesis and according to Volschenk and Gindaba (2014) resulted in yield and fruit quality reduction in 'Golden Delicious' apples.

The use of deficit irrigation in fruit crop production does however seem to be warranted late in the season (i.e. after harvest). This is because post-harvest stomatal control is related to atmospheric evaporative demand and VPD of the surrounding atmosphere of the tree rather than to a soil moisture stress. An increased VPD sensitivity of stomata is also not apparent when soil moisture deficit increases late in the season (Pretorius and Wand, 2003). Greater soil water depletion is therefore possible after harvest, especially in winter rainfall areas, where soil profiles are replenished promptly.

In a review of deficit irrigation in different fruit trees, Ruiz Sánchez et al. (2010) concluded various important considerations for efficient RDI strategies. Firstly, emphasis should be placed on understanding the stages of when plants are sensitive to water deficits, to ensure a minimal impact on yield and fruit quality. Secondly, RDI allows excessive vegetative growth to be inhibited while not affecting fruit growth, which is why, for RDI to be applied successfully, vegetative vs. reproductive phases need to be clearly differentiated. Thirdly, a successful RDI strategy is apparent when fruit growth rate is not negatively affected and plant water status is restored prior to critical phenotypical stages. Fourthly, cultivar specific adaptations need to be made to the deficit irrigation, ensuring that fruit load of the return bloom is not limited. Interaction between fruit load and water stress also needs to be considered to ensure fruit thinning and pruning practises do not restrain yields.

The long-term effects of RDI need to be considered in conjunction with climatic conditions, as the response thereto might differ than short-term effects. WUE in terms of harvestable fruit per unit of irrigated water has consistently been shown to be highest for RDI when compared to full irrigation or continuous deficit irrigation.Regulated deficit irrigation therefore seems to be a successful strategy that can be adopted in water-limited areas in response to water limitations due to climatic and population growth factors.

6. Sunburn ameliorating strategies for apples

In a water-scarce South Africa, increasing temperatures and worsening rainfall patterns are predicted to affect various sectors. The agricultural sector is predicted to be affected the most (Benhin, 2008). With these changing conditions, as well as continually rising standards of visual appearance which bring higher prices in the international market, sunburn is predicted to become more prevalent and problematic. This is due to tree management practices being geared towards improved fruit colour development, which expose fruit to sunburn conditions (Wünsche et al., 2001).

Recent sunburn studies on ‘Cripps’ Pink’ apples showed a significant increase of sunburn in response to water stress (Makeredza et al., 2013). Both half and no irrigation treatments had a significantly higher sunburn incidence and a linear relationship was determined between irrigation and sunburn incidence. Fruit on water-stressed trees had higher fruit surface temperatures and thus higher sunburn incidence and severity.

Previous studies have also reported increased sunburn development in response to water stress (Barber and Sharpe, 1971). Reducing water stress conditions in apple is therefore important as it facilitates evaporative cooling in the tree, reducing fruit surface temperatures and therefore sunburn incidence (Van den Ende, 1999). Sufficient irrigation could, as such, be seen as a preventative measure for increasing sunburn incidences with rising global temperatures.

To ensure good fruit with a high standard of visual appearance for international markets, various sunburn control strategies have been developed. Such strategies are successfully implemented when they reduce both solar radiation intensity as well as fruit surface temperatures below critical threshold levels.

6.1 Evaporative cooling

Evaporative cooling (EC) systems utilise overhead irrigation to make the tree canopy wet when temperatures exceed a certain threshold temperature. This cools the fruit and reduces the surface temperature, which results in reduction of moderate sunburn injury (Evans et al., 1995). EC systems have been used commercially in South Africa for many years (Wand et al., 2002).

In a study that quantified a reducing sunburn injury by evaporative cooling, a reduction of about 9.3% and 15.8% for two seasons was observed in ‘Jonagold’ apples

(Parchomchuk and Meheriuk, 1996). A pulse application of water was activated by sensors that trigger a cooling cycle of two minutes on and four minutes off when a temperature threshold of 32˚C was reached. This was due to a reduction of about 8.1˚C (Parchomchuk and Meheriuk, 1996). Overhead water application every 15 and 30 minutes can reduce fruit surface temperature by up to 8˚C (Wünsche et al., 2001). Amelioration of fruit temperature was also observed in a study on various apple cultivars with five minutes open and 15 minutes closed overhead sprinkling at 30˚C (Gindaba and Wand, 2005).

6.2 Light-reflecting material

In comparison to evaporative cooling, reflective materials are more conservative in terms of water use, but also more cost-effective (Parchomchuk and Meheriuk, 1996). Reflective materials reduce both FST as well as UV radiation, due to highly reflective characteristics of the light-reflecting material (Glenn et al., 2002).

Surround® WP, a crop protectant that consists of kaolin clay is used to protect plants against insect infestation by forming a protective mineral barrier (Puterka et al., 2003). Additionally it has been used as a reflective particle film in various studies (Gindaba and Wand, 2005; Glenn et al., 2002; Le Grange et al., 2004). Glenn et al., (2002) found that FST on 'Stayman' apples was reduced to temperatures close to ambient conditions after application of Surround® WP. This seemed to demonstrate the ability of the particle film to reflect direct radiant thermal IR radiation, but also that particle film emissivity was equal to that of the plant material. A reduction of UV-B also occurs, specifically a reduction of UV penetration to the fruit surface, however with no reduction in solar injury (Glenn et al., 2002). A hundred percent kaolin film reflective material was able to reduce sunburn on 'Royal Gala', 'Fuji' and 'Granny Smith' apples (Le Grange et al., 2004). In terms of FST reductions during specific parts of the day, kaolin particle film significantly reduces FST during late morning and early afternoon, but not midday (Gindaba and Wand, 2005). This results in effective reduction in sunburn severity, an advantage resulting from reflection of short wavelengths which cause damage to fruit skin.

A negative impact of these particles in some cases, is a reduction of red colour development in cultivars like 'Fuji' and 'Honeycrisp', however this is not always the case (Gindaba and Wand, 2005). Another disadvantage is that binding of such particles is not very strong and numerous applications have to be done to have an effect, especially after rainfall (Racsko and Schrader, 2012). At harvest it is then in turn difficult to remove these products from the crevasses in the fruit, being the calyx and stem-end areas (Le Grange et al., 2004).

In addition to light-reflecting material, are sunscreen suppressants like RAYNOX®, which contain compounds that absorb high-intensity UV radiation and enter higher energy states (Racsko and Schrader, 2012). Raynox® which is a lipophilic spray that binds to the cuticular waxes on fruits and unlike clay-containing particle films, does not wash off from rain or overhead irrigation. It is also transparent and able to reduce solar radiation transmitted to the fruit, to some extent (Schrader et al., 2008). This product consistently reduced sunburn incidence by 50% in Washington State, USA over a seven year period.

6.3 Shade nets

Reduction of direct solar radiation and thus FST can be achieved by using high-density polyethylene nets over tree canopies, therefore reducing sunburn incidence (Gindaba and Wand, 2005; Racsko and Schrader, 2012). Indirect factors that influence sunburn incidence are also affected, like a reduction in wind velocity, which increases humidity (Gindaba and Wand, 2005).

In a study by (Smit, 2007) shade nets reduced sunburn for both seasons of a trial on 'Fuji' and 'Braeburn' apples and one season for 'Royal Gala'. The 20% shade net that was used for the trials did not have a sunburn ameliorating effect on 'Cripps' Pink'. A complete eradication of sunburn was achieved (compared to 20% sunburn if no shade nets were used) on 'Granny Smith' apples (Smit et al., 2008). This is substantial, since 'Granny Smith' is a cultivar that is highly susceptible to sunburn damage (Racsko and Schrader, 2012). A reduction of FST by over 5˚C on hot days can be achieved using shade nets, which in turn reduces the sunburn incidence as less fruit reach the threshold temperatures at which sunburn occurs. Smit et al. (2008) recorded significant reductions in fruit temperatures however this was not the case for the air temperature under the nets. The ameliorating effect of shade nets on sunburn incidence in apple orchards might therefore be due to a reduced incidence of direct sunlight, specifically UV radiation, reaching the fruit.

In a study by Stampar et al. (2002) shade nets did not reduce apple fruit quality or quantity, however, 'Jonagold' seemed to have a reduced blush area under black nets. A negative effect of using shade nets is that red colour development on the shaded side of fruit is often poor (Jakopic et al., 2007). This is due to the prevalent use of black nets, which reduce incident solar radiation (Stampar et al., 2002). This was true for 'Fuji' and 'Braeburn' cultivars, but 'Cripps' Pink' and 'Royal Gala' were not affected in a study by Smit et al. (2008). The two unaffected cultivars in this study, were however affected by shade nets in a study by Gindaba and Wand (2005), where a lower blush colour was observed.

6.4 Cultural Practices

Good light penetration is important for good colouration and high quality apples. To achieve this, trees are planted closely and trellised, trained and pruned correctly (Van den Ende, 1999). With proper thinning, nutrition and irrigation such orchards should be able to withstand heatwaves, with good leaf coverage to protect fruits against excessive radiation.

In the 1990s, apple growers in Washington State and elsewhere adopted high-density plantings on size-controlling rootstocks, which considerably enhanced direct sun exposure of the fruit; as a result the incidence of sunburn increased substantially (Racsko and Schrader, 2012). The direct sun-exposure also benefits fruit colour development, making the fruit more marketable to ever-rising visual standards for export (Wünsche et al., 2001). This goes hand in hand with the choice of rootstock, where dwarfing rootstocks have trees with less dense canopies. This results in higher light exposure to fruits and therefore a higher susceptibility to sunburn (Wünsche et al., 2000). Pruning methods should therefore be done with emphasis on providing fruit with filtered light, as well as allowing fruit to acclimatize by removing water shoots regularly (Van den Ende, 1999). It is also important to prevent heavily cropped branches from bending over as this exposes fruits to high radiation. This can be done by effective thinning practices as well as propping or tying up branches to maintain them in their original positions (Van den Ende, 1999).

In terms of cultivar variation, ‘Granny Smith’, ‘Mutsu’, ‘Fuji’, ‘Braeburn’ and ‘Jonagold’ are more susceptible to sunburn than ‘Golden Delicious’ or ‘Gala’. This variation is also very much dependent on tree age, vigour and crop load. The more susceptible basal cultivars, which have less foliage towards the tops, like ‘Fuji’, ‘Braeburn’ and ‘Delicious’, can be trained and supported when still young to avoid this problem (Van den Ende, 1999).

6.5 Chemical Protectants

Chemical protectants are naturally occurring metabolites that protect fruit from attaining high FST and excessive sunlight damage (Racsko and Schrader, 2012). Certain metabolites are increased in the fruit, which improves its ability to avoid damage from sunburn. Photo-oxidative stress of sunburnt tissues is due to excessive reactive oxygen species (Yuri et al., 2010). In response to this, enzymatic and non-enzymatic responses are triggered (Chen et al., 2008); like carotenoids, ascorbic acid and phenols like quercetin.

Ascorbic acid, a key component of the central antioxidant system that protects against photo-oxidative injury, correlates positively to susceptibility of fruit to sunburn (Andrews et al., 1999). Exogenous applications of ascorbic acid resulted in a reduction of sunburn in ‘Fuji’ apples, but not in ‘Granny Smith’. A 4% concentration reduced sunburn more than lower concentrations; however, signs of phytotoxicity were observed in ‘Fuji’ apples. Together with this and the cost of this product, as well the required frequency of applications that is required, this product is commercially unviable and unpractical (Racsko and Schrader, 2012).

Application of abscisic acid (ABA), a plant hormone, significantly reduced sunburn necrosis on ‘Tsugaru’ apples and sunburn browning in ‘Senshu’ apples (Iamsub et al., 2009), both red bi-colour cultivars. Application of ABA was associated with reduced lipid peroxidation, increased total antioxidant capacity, phenolics, ascorbic acid, anthocyanin and chlorophyll content in the apple peel; in addition to the reduced sunburn. The results show that ABA mediates specific mechanisms which are needed for normal cell functioning under severe FST and high solar radiation.

6.6 Foliar calcium and boron applications

A first report of the possible role of foliar B application in combination with Ca was done by Lötze and Hoffman (2014). A significant reduction in sunburn incidence was observed on 'Golden Delicious' apples after foliar applications of a combination of Ca and B. Even though the exact mode of action is unknown, it can be hypothesised that the ameliorating effect can be attributed to the role of Ca and B in cell wall strengthening and plasma membrane integrity (Bush, 1995; De Freitas et al., 2010; Figueroa et al., 2012; Hirschi, 2004). Alternatively, Ca and B could play roles in secondary messenger signalling in response to abiotic stress like severe temperatures or light intensity (Bolaños et al., 2004; Matoh and Kobayashi, 1998; Peryea and Drake, 1991; Sanders et al., 2002).

7. Conclusion

In conclusion, fruit quality should remain an area of importance in research. This seems to become more important, with rising threats to fruit quality caused by changing climates, along with stricter export regulations. More research needs to be done in terms of heat tolerant and water stress resistant crop varieties, as well as improving farming technologies (Benhin, 2008). Farming technologies that conserve water and provide efficient water use are imperative as South Africa's vulnerability to climate change rises. In an increasing water scarce environment, the plant's response to water stress and its water use need to be understood sufficiently to make informed decisions concerning water conserving farming technologies. This will become more important as insufficient supply of water becomes the norm rather than the exception in the future (Fereres and Soriano, 2007).

Sunburn incidence on apples, which increases in response to water stress (Makeredza et al., 2013) remains an area of concern. This is especially so due to the profitability of unblemished, high quality apples in the international markets. Even though shade nets are successful at eradicating sunburn completely, alternatives like foliar application of Ca and B seem promising, due to their ease of application as well as low infrastructural expenses, which are not the case for shade nets.

8. References

Adger, W.N., 2006. Vulnerability. Glob. Environ. Chang. 16, 268–281.doi:10.1016/j.gloenvcha.2006.02.006

Afzal, M., Battilani, A., Solimando, D., Ragab, R., 2016. Improving water resources management using different irrigation strategies and water qualities: Field and modelling study. Agric. Water Manag. 176, 40–54. doi:10.1016/j.agwat.2016.05.005

Andrews, P.K., Johnson, J.R., Fahy, D., Gish, N., 1999. Sunburn protection in apples with ascorbic acid. Le Fruit Belge 481, 157–161.

Barber, H.N., Sharpe, P.J.H., 1971. Genetics and physiology of sunscald of fruits. Agric. Meteorol. 8, 175–191. doi:10.1016/0002-1571(71)90107-5

Baxter, P., 1970. Effect of a weed-free or straw mulched strip on the growth and yield of young fruit trees. Aust. J. Exp. Agric. Anim. Husb. 10, 467–473.

Bengough, A.G., McKenzie, B.M., Hallett, P.D., Valentine, T.A., 2011. Root elongation, water stress, and mechanical impedance: A review of limiting stresses and beneficial root tip traits. J. Exp. Bot. 62, 59–68. doi:10.1093/jxb/erq350

Benhin, J.K.A., 2008. South African crop farming and climate change: An economic assessment of impacts. Glob. Environ. Chang. 18, 666–678. doi:10.1016/j.gloenvcha.2008.06.003

Bolaños, L., Lukaszewski, K., Bonilla, I., Blevins, D., 2004. Why boron? Plant Physiol. Biochem. 42, 907–912. doi:10.1016/j.plaphy.2004.11.002

Boo, Y.C., Jung, J., 1999. Water Deficit — Induced oxidative stress and antioxidative defenses in rice plants. J. Plant Physiol. 155, 255–261. doi:10.1016/S0176-1617(99)80016-9

Buerkert, A., Bationo, A., Dossa, K., 2000. Mechanisms of residue mulch-induced cereal growth increases in West Africa. Soil Sci. Soc. Am. J. 64, 346. doi:10.2136/sssaj2000.641346x

Bush, D.S., 1995. Calcium regulation in plant cells and its role in signaling. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46, 95–122.

Chalmers, D.J., Burge, G., Jerie, P.H., Mitchell, P.D., 1986. The mechanism of regulation of Bartlett pear fruit and vegetative growth by irrigation withholding and regulated deficit irrigation. J. Am. Soc. Hortic. Sci. 111, 904–907.

Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to drought - From genes to the whole plant. Funct. Plant Biol. 30, 239–264. doi:10.1071/FP02076

Chen, L.S., Li, P., Cheng, L., 2008. Effects of high temperature coupled with high light on the balance between photooxidation and photoprotection in the sun-exposed peel of apple. Planta 228, 745–756. doi:10.1007/s00425-008-0776-3

De Freitas, S.T., Amarante, C.V.T. do, Labavitch, J.M., Mitcham, E.J., 2010. Cellular approach to understand bitter pit development in apple fruit. Postharvest Biol. Technol. 57, 6–13. doi:10.1016/j.postharvbio.2010.02.006

Department of Water and Sanitation, 2009. Water for Growth and Development in South Africa.

Dzikiti, S., Gush, M., Lötze, E., Schmeisser, M., Midgley, S., Taylor, N., Volschenk, T., 2014. Knowledge review on water use, water productivity and fruit quality in high performing apple orchards.

Eakin, H., Luers, A.L., 2006. Assessing the vulnerability of social-environmental systems. Annu. Rev. Environ. Resour. 31, 365–394. doi:10.1146/annurev.energy.30.050504.144352

Evans, R.G., Kroeger, M.W., Mahan, M.O., 1995. Evaporative cooling of apples by overtree sprinkling. Appl. Eng. Agric. 11, 93–99.

Failla, O., Treccani, C.P., Mignani, I., 1990. Water status, growth and calcium nutrition of apple trees in relation to bitter pit. Sci. Hortic. (Amsterdam). 42, 55–64. doi:10.1016/0304-4238(90)90147-7.

Fares, A., Alva, A.K., 2000. Evaluation of capacitance probes for optimal irrigation of citrus through soil moisture monitoring in an entisol profile. Irrig. Sci. 19, 57–64. doi:10.1007/s002710050001

Faust, M., 1989. Physiology of temperate zone fruit trees. John Wiley & Sons, Inc., New York.

Felicetti, D.A., Schrader. 2008. Photooxidative sunburn of apples: Characterization of a third type of apple sunburn. Intl. J. Fruit Sci. 8:160-172. http://dx.doi.org/10.1080/15538360802526472

Fereres, E., Soriano, M.A., 2007. Deficit irrigation for reducing agricultural water use. J. Exp. Bot. 58, 147–159. doi:10.1093/jxb/erl165

Figueroa, C.R., Opazo, M.C., Vera, P., Arriagada, O., Díaz, M., Moya-León, M.A., 2012. Effect of postharvest treatment of calcium and auxin on cell wall composition and expression of cell wall-modifying genes in the Chilean strawberry (*Fragaria chiloensis*) fruit. Food Chem. 132, 2014–2022. doi:10.1016/j.foodchem.2011.12.041

Fischer, G., Shah, M., Tubiello, F.N., van Velthuizen, H., 2005. Socio-economic and climate change impacts on agriculture: an integrated assessment, 1990-2080. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 360, 2067–2083. doi:10.1098/rstb.2005.1744

Gindaba, J., Wand, S.J.E., 2005. Comparative effects of evaporative cooling, kaolin particle film, and shade net on sunburn and fruit quality in apples. HortSci. 40, 592–596.

Glenn, D.M., Prado, E., Erez, A., McFerson, J., Puterka, G.J., 2002. A reflective, processed-kaolin particle film affects fruit temperature, radiation reflection, and solar injury in apple. J. Am. Soc. Hortic. Sci. 127, 188–193.

Gong, D., Kang, S., Yao, L., Zhang, L., 2007. Estimation of evapotranspiration and its components from an apple orchard in northwest China using sap flow and water balance methods. Hydrol. Process. 21, 931–938. doi:10.1002/hyp

Gonzalez-Rodriguez, D., Cournède, P.-H., de Langre, E., 2016. Turgidity-dependent petiole flexibility enables efficient water use by a tree subjected to water stress. J. Theor. Biol. 398, 20–31. doi:10.1016/j.jtbi.2016.03.011

Green, S.R., Vogeler, I., Clothier, B.E., Mills, T.M., Van Den Dijssel, C., 2003. Modelling water uptake by a mature apple tree. Aust. J. Soil Res. 41, 365–380. doi:10.1071/SR02129

Gush, M.B., Dzikiti, S., Clulow, A.D., Mengistu, M.G., Jarmain, C., Taylor, N.J., Everson, C., 2014. The water use of selected fruit tree orchards (Volume 2): Technical report on measurements and modelling. Pretoria, RSA.

Hirschi, K.D., 2004. The calcium conundrum. Both versatile nutrient and specific signal. Plant Physiol. 136, 2438–2442. doi:10.1104/pp.104.046490.2438

Hsiao, T.C., 1973. Plant responses to water stress. Annu. Rev. Plant Physiol. 24, 519–570. doi:DOI: 10.1146/annurev.pp.24.060173.002511

Hsiao, T.C., Acevedo, E., Fereres, E., Henderson, D.W., 1976. Water stress, growth, and osmotic adjustment. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 290, 57–69.

Huang, Z., Xu, Z., Blumfield, T.J., Bubb, K., 2008. Effects of mulching on growth, foliar photosynthetic nitrogen and water use efficiency of hardwood plantations in subtropical Australia. For. Ecol. Manage. 255, 3447–3454. doi:10.1016/j.foreco.2008.02.038

Iamsub, K., Sekozawa, Y., Sugaya, S., Gemma, H., Kamuro, Y., 2009. Alleviating sunburn injury in apple fruit using natural and fertilizer forms of S-abscisic acid and its underlying mechanism. J. Food, Agric. Environ. 7, 446–452.

IPCC, 2007. Climate Change 2007: impacts, adaptation and vulnerability: contribution of Working Group II to the fourth assessment report of the Intergovernmental Panel, Genebra, Suíça. Cambridge. doi:10.1256/004316502320517344

Jakopic, J., Veberic, R., Stampar, F., 2007. The effect of reflective foil and hail nets on the lighting, color and anthocyanins of 'Fuji' apple. Sci. Hortic. (Amsterdam). 115, 40–46. doi:10.1016/j.scienta.2007.07.014

Jerie, P.H., van den Ende, B., Dann, I.R., 1989. Managing tree vigour and fruitfulness in deciduous orchards. Acta Hort. 240, 127–134. doi:10.17660

Ji, H., Liu, L., Li, K., Xie, Q., Wang, Z., Zhao, X., Li, X., 2014. PEG-mediated osmotic stress induces premature differentiation of the root apical meristem and outgrowth of lateral roots in wheat. J. Exp. Bot. 65, 4863–4872. doi:10.1093/jxb/eru255

Jones, H.G., 2004. Irrigation scheduling: Advantages and pitfalls of plant-based methods. J. Exp. Bot. 55, 2427–2436. doi:10.1093/jxb/erh213

Jones, H.G., Lakso, A.N., Syvertsen, J.P., 1985. Physiological control of water status in temperate and subtropical fruit trees. Hortic. Rev. (Am. Soc. Hortic. Sci). 7, 301–344.

Kiker, G. A, 1999. Synthesis report for the vulnerability and adaptation assessment section of the South African country study on climate change.

Kramer, P., Boyer, J., 1995. Water Relations of Plants and Soils. Academic Press, New York.

Lakso, A.N., 1979. Seasonal changes in stomatal response to leaf water potential in apple. J. Am. Soc. Hort. 104, 58–60.

Le Grange, M., Wand, S.J.E., Theron, K.I., 2004. Effect of kaolin applications on apple fruit quality and gas exchange of apple leaves. Acta Hort. 636, 545–550.

Lötze, E., Hoffman, E.W., 2014. Foliar application of calcium plus boron reduces the incidence of sunburn in 'Golden Delicious' apple. J. Hort. Sci. Biotechnol. 89, 607–612. doi:10.1080/14620316.2014.11513127

Makeredza, B., Schmeisser, M., Lötze, E., Steyn, W.J., 2013. Water stress increases sunburn in 'Cripps' Pink’ apple. Hort Sci. 48, 444–447.

Makeredza, B., 2011. Studies Towards understanding Sunburn in apple. MSc Agric Thesis. Faculty of AgriScience. University of Stellenbosch. Stellenbosch.

Matoh, T., Kobayashi, M., 1998. Boron and calcium, essential inorganic constituents of pectic polysaccharides in higher plant cell walls. J. Plant Res. 111, 179–190. doi:10.1007/BF02507164

McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G., Yepez, E.A., 2008. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? New Phytol. 178, 719–739. doi:10.1111/j.1469-8137.2008.02436.x

Midgley, G.F., Chapman, R. a, Hewitson, B., Johnston, P., De Wit, M., Ziervogel, G., Mukheibir, P., Van Niekerk, L., Tadross, M., Van Wilgen, B.W., Kgope, B., Morant, P.D., Theron, A.K., Scholes, R.J., Forsyth, G.G., 2005. A Status Quo , vulnerability and adaptation assessment of the physical and socio-economic effects of climate change in the Western Cape, analysis.

Mpelasoka, B.S., Behboudian, M.H., Green, S.R., 2001. Water use, yield and fruit quality of lysimeter-grown apple trees: Responses to deficit irrigation and to crop load. Irrig. Sci. 20, 107–113. doi:10.1007/s002710100041

Müller, C., Cramer, W., Hare, W.L., Lotze-Campen, H., 2011. Climate change risks for African agriculture. doi:10.1073/pnas.1015078108

Munné-Bosch, S., Peñuelas, J., 2004. Drought-induced oxidative stress in strawberry tree (Arbutus unedo L.) growing in Mediterranean field conditions. Plant Sci. 166, 1105–1110. doi:10.1016/j.plantsci.2003.12.034

NDA, 2013. Abstract of Agricultural Statistics 2013.

Parchomchuk, P., Meheriuk, M., 1996. Orchard cooling with pulsed overtree irrigation to prevent solar injury and improve fruit quality of “Jonagold” apples. Hort Sci. 31, 802–804.

Perring, M.A., 1986. Incidence of bitter pit in relation to the calcium content of apples: Problems and Paradoxes, a Review Journal of the Science of Food and Agriculture, 37(7), 591-606.

Peryea, F.J., Drake, S.R., 1991. Influence of mid-summer boron sprays on boron content and quality indices of “Delicious” apple. J. Plant Nutr. 14, 825–840.

Pinamonti, F., Zorzi, G., Gasperi, F., Silvestri, S., Stringari, G., 1995. Growth and nutritional status of apple trees and grapevines in municipal solid-waste-amended soil. Acta Hort. 38, 313-322.

Pretorius, J.J.B., Wand, S.J.E., 2003. Late-season stomatal sensitivity to microclimate is influenced by sink strength and soil moisture stress in 'Braestar' apple trees in South Africa. Sci. Hort. (Amsterdam). 98, 157–171. doi:10.1016/S0304-4238(02)00209-1

Psarras, G., Merwin, I.A., 2000. Water stress affects rhizosphere respiration rates and root morphology of young “Mutsu” apple trees on M.9 and MM.111 rootstocks. J. Am. Soc. Hort. Sci. 125, 588–595.

Puterka, G.J., Reinke, M., Luvisi, D., Ciomperik, M.A., Bartels, M.A., Wendel, L., Glenn, D.M., 2003. Particle film , Surround WP , effects on Glassy-winged Sharpshooter Behavior and Its utility as a barrier to Sharpshooter infestations in Grape. Plant Heal. Prog. 1–8. doi:10.1094/PHP-2003-0321-01-RS.Abstract

Racsko, J., Schrader, L.E., 2012. Sunburn of Apple Fruit: Historical Background, Recent Advances and Future Perspectives. CRC. Crit. Rev. Plant Sci. 31, 455–504. doi:10.1080/07352689.2012.696453

Ramos, C., Intrigliolo, D.S., Thompson, R.B., 2011. Global change challenges for horticultural systems, in: Crop Stress Management & Global Climate Change. pp. 58–84.

Rana, G., Katerji, N., 2000. Measurement and estimation of actual evapotranspiration in the field under Mediterranean climate: A review. Eur. J. Agron. 13, 125–153. doi:10.1016/S1161-0301(00)00070-8

Reddy, A.R., Chaitanya, K.V., Vivekanandan, M., 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol. 161, 1189–1202. doi:10.1016/j.jplph.2004.01.013

Rijsberman, F.R., 2006. Water scarcity: Fact or fiction? Agric. Water Manag. 80, 5–

22. doi:10.1016/j.agwat.2005.07.001

Ruiz Sánchez, M.C., Domingo Miguel, R., Castel Sánchez, J.R., 2010. Deficit irrigation in fruit trees and vines in Spain. Spanish J. Agric. Res. 8, 5–20.

Sade, N., Gebremedhin, A., Moshelion, M., 2012. Risk-taking plants. Plant Signal. Behav. 7, 767–770. doi:10.4161/psb.20505

Salau, O.A., Opara-Nadi, O.A., Swennen, R., 1992. Effects of mulching on soil properties, growth and yield of plantain on a tropical ultisol in southeastern Nigeria. Soil Tillage Res. 23, 73–93. doi:10.1016/0167-1987(92)90006-W

Sanders, D., Pelloux, J., Brownlee, C., Harperc, J.F., 2002. Calcium at the Crossroads of Signaling. Plant Cell 14, 401–417.

Saure, M.C., 2005. Calcium translocation to fleshy fruit: its mechanism and endogenous control. Sci. Hort. 105, 65–89. doi:10.1016/j.scienta.2004.10.003

Schrader, L., Sun, J., Felicetti, D., Seo, J.-H., Jedlow, L., Zhang, J., 2003a. Stress-induced disorders: Effects on apple fruit quality. Washingt. Tree Fruit Postharvest Conf. 1–7.

Schrader, L., Sun, J., Zhang, J., Felicetti, D., Tian, J., 2008. Heat and light-induced apple skin disorders: Causes and prevention. Acta Hort. 51–58. doi:10.17660/ActaHortic.2008.772.5

Schrader, L., Zhang, J., Sun, J., 2003. Environmental stresses that cause sunburn of apple. Acta Hort. 618, 397–405.

Sharp, R.E., Davies, W.J., 1989. Regulation of growth and development of plants growing with restricted supply of water, in: Plants under stress. p. 71.

Šircelj, H., Tausz, M., Grill, D., Batič, F., 2007. Detecting different levels of drought stress in apple trees (*Malus domestica* Borkh.) with selected biochemical and physiological parameters. Sci. Hort. 113, 362–369. doi:10.1016/ j.scienta.2007.04.012

Šircelj, H., Tausz, M., Grill, D., Batič, F., 2005. Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. J. Plant Physiol. 162, 1308–1318. doi:10.1016/j.jplph.2005.01.018

Smirnoff, B.Y.N., 1993. The role of active oxygen in the response of plants to water deficit and desiccation. Rev. Lit. Arts Am. 125, 27–58. doi:10.1111/j.1469-8137.1993.tb03863.

Smit, A., 2007. Apple tree and fruit responses to shade netting. MSc Agric. thesis. Faculty of AgriScience. Stellenbosch University. Stellenbosch.

Smit, A., Steyn, W.J., Wand, S.J.E., 2008. Effects of shade netting on gas exchange of blushed apple cultivars, In XXVII International Horticultural Congress-IHC2006: International Symposium on Enhancing Economic and Environmental 772 ,73–80.

Stampar, F., Veberic, R., Zadravec, P., Hudina, M., Usenik, V., Solar, A., Osterc, G., 2002a. Yield and fruit quality of apples cv. “Jonagold” under hail protection nets. Gartenbauwissenschaft 67, 205–210.

Steudle, E., 2000. Water uptake by roots: effects of water deficit. J. Exp. Bot. 51, 1531–1542. doi:10.1093/jexbot/51.350.1531

Taiz, L., Zeiger, E., 2010. Plant Physiology. Sinauer Associates, Sunderland, MA, USA.

Taleisnik, E., Peyrano, G., Cordoba, a., Arias, C., 1999. Water retention capacity in root segments differing in the degree of exodermis development. Ann. Bot. 83, 19–27. doi:10.1006/anbo.1998.0781

Taylor, N.J., Gush, M.B., 2014. The water use of selected fruit tree orchards (Volume 1): Review of available knowledge.

Turner, N.C., 1979. Drought resistance and adaptation to water deficits in crop plants, in: Mussel, H., Staples, R.C. (Eds.), Stress Physiology in Crop Plants. Wiley, New york, pp. 343–372.

Van den Ende, B., 1999. Sunburn Management. Compact Fruit Tree 32, 13–14. doi:10.1017/CBO9781107415324.004

Volschenk, T., Gindaba, J., 2014. The effect of reduced soil water status on “Golden Delicious” apple trees, In X International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems 1058, 229-235.

Wand, S.J.E., Steyn, W.J., Mdluli, M.J., Marais, S.J.S., Jacobs, G., 2002. Overtree evaporative cooling for fruit quality enhancement. SA Fr J. 1, 8-21.

Wang, Z., Stutte, G.W., 1992. The role of carbohydrates in active osmotic adjustment in apple under water stress. J. Am. Soc. Hort. Sci. 117, 816–823.

Westgate, M.E., Boyer, J.S., 1985. Osmotic adjustment and the inhibition of leaf, root, stem and silk growth at low water. Planta 164, 540–549.

Wiid, N., Ziervogel, G., 2012. Adapting to climate change in South Africa: commercial farmers’ perception of and response to changing climate. South African Geogr. J. 94, 152–173. doi:10.1080/03736245.2012.742783

Woolf, A.B., Ferguson, I.B., 2000. Postharvest responses to high fruit temperatures in the field. Postharvest Biol. Technol. 21, 7–20. doi:10.1016/S0925-5214(00)00161-7

Wullschleger, S.D., Meinzer, F.C., Vertessy, R.A., 1998. A review of whole-plant water use studies in tree. Tree Physiol. 18, 499–512. doi:10.1093/treephys/18.8-9.499

Wünsche, J., Lakso, A N, 2000. Apple tree physiology: Implications for orchard and tree management. Compact Fruit Tree 33, 82–88.

Wünsche, J.N., Greer, D.H., Palmer, J.W., Lang, A., McGhie, T., 2001. Sunburn- The cost of a high light environment. Acta Hort. 349–356. doi:10.17660/ActaHortic.2001.557.46

Wünsche, J.N., Palmer, J.W., Greer, D.H., 2000. Effects of crop load on fruiting and gas-exchange characteristics of 'Braeburn'/M.26 apple trees at full canopy. J. Am. Soc. Hort. Sci. 125, 93–99.

Yang, H., Reichert, P., Abbaspour, K.C., Zehnder, A.J.B., 2003. A water resources threshold and its implications for food security. Environ. Sci. Technol. 37, 3048–3054. doi:10.1021/es0263689

Yuncong, Y., Jie, Z., Streeter, J.G., Ferree, D.C., 2010. Influence of soil drought stress on photosynthesis, carbohydrates and the nitrogen and phophorus absorb in different section of leaves and stem of Fugi/M.9EML, a young apple seedling. African J. Biotechnol. 9, 5320–5325. doi:10.5897/AJB10.092

Yuri, J.A., Neira, A., Quilodran, A., Razmilic, I., Motomura, Y., Torres, C., Palomo, I., 2010. Sunburn on apples is associated with increases in phenolic compounds and antioxidant activity as a function of the cultivar and areas of the fruit. J. Food, Agric. Environ. 8, 920–925.

Zagdańska, B., Wiśniewski, K., 1996. Changes in the thiol/disulfide redox potential in wheat leaves upon water deficit. J. Plant Physiol. 149, 462–465. doi:10.1016/S0176-1617(96)80149-0

Zhang, J., Kirkham, M.B., 1996. Antioxidant Responses to Drought in Sunflower and Sorghum Seedlings. New Phytol. , 132, 361–373.

Paper 1

Evaluating the effect of combined calcium and boron formulations as pre-harvest foliar application on the reduction of sunburn in 'Cripps' Pink', 'Granny Smith' and 'Golden Delicious' apples

1. Introduction

Total apple production in South Africa amounted to almost a million tonnes for 2014/2015 (DAFF/PPECB, 2015; Hortgro, 2015). Of this, 23% was sold on the local market, 45 % was exported and 32 % was processed. The net realisation of exported fruit per ton was R10 689 for the 2014/2015 season. For fruit sold on the local market, a ton of apples only achieved 50 % of this amount and processed fruit realised a mere R1 142 per ton (DAFF/PPECB, 2015; Hortgro, 2015).

Sunburn negatively affects the appearance of apples, which is one of the main factors in consumer preference (Gamble et al., 2006). The United Kingdom market tolerates no sunburn on apple cultivars such as 'Granny Smith' and 'Golden Delicious' (Jeanne-Mari Burger, Fruitways (Pty) Limited, Somerset West, South Africa, personal communication, January 2015). Therefore, to maintain profitability, sunburn incidence needs to be minimised to ensure a high percentage exportable fruit.

Exportable fruit numbers can be negatively affected by up to 50 % under local South African conditions, due to the incidence of sunburn (Makeredza et al., 2013). Sunburn is a physiological disorder caused by high flux densities of solar radiation, either directly or indirectly (Racsko and Schrader, 2012). Directly, as solar radiation, causing the formation of Reactive Oxygen Species (ROS), this in turn is responsible for oxidative damage to lipids, proteins and DNA (Felicetti and Schrader, 2008). Sunburn is also induced indirectly, by an increase in radiant heating that causes sunburn (Felicetti and Schrader, 2008; Racsko and Schrader, 2012; Schrader et al., 2001).

Sunburn types

Sunburn browning, sunburn necrosis and photo-oxidative sunburn are the three types of sunburn that can occur in apples (Schrader et al. 2001). Sunburn browning is caused by a combination of high temperatures and high solar radiation (Schrader et al., 2001). The minimum fruit surface temperature (FST) needed to induce sunburn browning, as well as the threshold temperature varies between cultivars from 46˚C to 49˚C, with 'Cripps Pink' having the highest threshold temperature (Schrader et al., 2001). This threshold temperature needs to be maintained for 60 minutes for damage to occur. These observations were made in Washington State (USA) and it is possible that, in the Southern Hemisphere at lower latitudes, with higher UV-B radiation, lower threshold temperatures may already induce sunburn (Racsko and Schrader, 2012).

Sunburn browning is the most prevalent of the three types, specifically on sun-exposed apples (Racsko and Schrader, 2012). The sunburn progresses from a yellow spot on the sun-exposed side of the fruit, to brown and bronze and finally, a dark-tan spot in very severe cases. The browning is due to the fact that the concentrations of anthocyanins and chlorophylls decrease, whereas carotenoids and glycosides increase in the peel (Felicetti and Schrader, 2008; Racsko and Schrader, 2012; Schrader et al., 2003).

Sunburn necrosis is categorised as a black or dark brown necrotic spot on the exposed side of the fruit. It occurs under conditions where the FST reaches 52˚C for as little as 10 minutes, at which point membrane integrity is destroyed (Racsko and Schrader, 2012; Schrader et al., 2001). Solar radiation does not play a role in inducing sunburn necrosis and only the high FST causes thermal death (Schrader et al., 2003). If the conducive conditions for sunburn necrosis persist for one to four days, the necrotic spot will often collapse (Racsko and Schrader, 2012). Photo-oxidative sunburn occurs when "shaded (non-acclimated) apples suddenly exposed to sunlight became photobleached and eventually necrotic" (Felicetti and Schrader, 2008). Initial symptoms include the appearance of a white spot after sudden exposure to solar radiation, on areas of the apple peel not previously exposed to full sunlight. These areas would most likely have been covered by other fruit or foliage, or the fruit found inside the canopy. Summer pruning or shifting of branches under increasing fruit loads would have exposed these areas on apples (Racsko and Schrader, 2012). Experiments by Felicetti and Schrader (2008), suggested that photobleaching or photo-oxidative sunburn is due to visible solar radiation and not primarily due to UV-A, UV-B or high FST. Photo-oxidative sunburn can result in the development of sunburn necrosis (Felicetti and Schrader, 2008).

Sunburn reduction strategies

Sunburn reducing strategies are required, in response to an increased sunburn incidence, with the adoption of high-density plantings on dwarfing rootstocks in the last two decades of about 1500 plants per hectare. This is done to achieve higher yields at an earlier stage of orchard growth. In response to this, sunburn management strategies have been developed to reduce the incidence and severity of the symptoms as fruit exposure to sun increased with the new training systems (Racsko and Schrader, 2012). The costs of these management practices need to be weighed up against the increased profitability of class 1 exportable fruit.

Evaporative cooling, by means of overhead irrigation systems, results in a reduction in fruit surface temperature as water evaporates from the fruit surface and latent heat is lost (Gindaba and Wand, 2007, 2005; Iglesias and Alegre, 2006; Marais, 2005). It is an effective sunburn reducing strategy, however in a comparative study by Gindaba and Wand (2005), it was far less effective than kaolin particle film application and shade nets, which completely eradicated sunburn.

Shade nets, also used as a farming practice to reduce hail storm damage in certain areas (Basile et al., 2012; Bogo et al., 2012; Hunsche et al., 2010; Iglesias and Alegre, 2006; Jakopic et al., 2007), reduce sunburn damage on apples (Bogo et al., 2012; Hunsche et al., 2010) due to the reduced FST under shade nets by 3-6˚C (Solomakhin and Blanke, 2010). Local studies have also observed significant reductions of up to 10 ˚C (Gindaba and Wand, 2005; Smit et al., 2008). Shade nets also increase the relative humidity and reduce the wind speed under the nets, thereby reducing transpiration rates from leaves and decreasing irrigation requirements (Warner, 1997). Shade nets furthermore reduce the amount of UV-B and visible solar radiation reaching the fruit, which in turn decreases incidence of sunburn browning. A drawback of this strategy is that it has a negative impact on blush colour development as well as high costs for infrastructure, making it less viable for commercial farms (Gindaba and Wand, 2005).

Light-reflective material or particle films like "Surround® WP" or "RAYNOX®" can be used to protect fruit from sunburn (Erez and Glenn, 2004; Glenn et al., 2002; Le Grange et al., 2004; Schrader et al., 2001) by reflecting the solar radiation (Felicetti and Schrader, 2008). More specifically, the UV-B radiation is reduced and therefore reflected by the product. This is only effective in reducing sunburn types that require solar radiation, i.e. sunburn browning. "Surround® WP", which is a kaolin-based particle film spray has been proven to be effective on 'Fuji', 'Royal Gala' and 'Granny Smith' (Gindaba and Wand, 2005; Le Grange et al., 2004). It was ineffective on 'Cripps' Pink' apples in a study by Erez and Glenn (2004), but very effective in a study by Gindaba and Wand (2005). It was able to reduce the fruit surface temperatures to below the FST thresholds (Schrader et al., 2001). 'RAYNOX®', a lipid soluble spray was found to reflect solar radiation, specifically UV-B rays and to some extent UV-A and photosynthetically active radiation, thereby decreasing sunburn by up to 50 % in Washington State, USA, over a seven year period (Schrader et al., 2008). A drawback of this sunburn reducing strategy is that a reduction of red colour development was observed in 'Cripps' Pink' and 'Royal Gala' (Gindaba and Wand, 2005).

An alternative approach to existing management practices was reported by Lötze and Hoffman (2014), who were able to reduce sunburn using a foliar application of mineral nutrients. A significant reduction of sunburn incidence was achieved with eight foliar applications of a Manni-Plex® Ca Manni-Plex® B formulation, early in the season on 'Golden Delicious'. The appeal of this alternative versus those mentioned above is its low cost due to no infrastructural expenses and its simplicity of use.

In response to increasing sunburn-conducive conditions in future as predicted for local apple production areas by IPCC (2007), the objective of this study was to further assess the efficacy of the foliar application of calcium and boron combinations in decreasing sunburn by i) extending the cultivar range to include 'Granny Smith' and 'Cripps' Pink' apples in addition to 'Golden Delicious' and ii), by evaluating alternative formulations of combined Ca and B.

2. Materials and Methods

Experiment 1

Plant Material

This experiment was performed over two consecutive seasons, 2014/2015 and 2015/2016 on bearing 'Cripps' Pink' trees on rootstock M793 on the Welgevallen Experimental Farm, University of Stellenbosch, Stellenbosch, South Africa (33°56'52.5"S, 18°52'19.9"E). The trees were planted in 1998 at a spacing of 1.5 m x 4.0 m in south-west by north-east row orientation. Trees used in this experiment are low yielding. Trees were managed as a commercial unit according to standard practices. Irrigation was supplied via micro-jets.

Statistical Design and treatments

The experiment was laid out as a Randomised Complete Block Design (RCBD) with single tree replicates. In the 2014/2015 season, full bloom occurred on 13th October 2014, six treatments replicated five times (Table 1), were applied from approx. 40 dafb on a weekly basis for 6 weeks. This protocol differed from the protocol used by Lötze and Hoffman (2014), omitting the first two applications to determine whether it is possible to reduce the number of applications without reducing the efficacy of the treatment. The dates of the applications are provided in Table 2. The active ingredients in the treatments are presented in Table 3.

Table 3 All solutions/powders/granules (excl. the control) were mixed in 10 litres of water. In the 2015/2016 season, treatments were adjusted after reflection on the first season's results on 'Cripps' Pink'.

In the second season (2015/2016), the same trees were used and six sprays were applied from approx. 28 dafb (Table 1). Full bloom occurred on 12th October 2015. During this season, the last two sprays were omitted as compared to the first two applications that were omitted in 2014/15, based on total of eight application dates of Lötze and Hoffman (2014).

Motorized Stihl® knap sack sprayers were used to apply the treatments early during the morning to reduce possible leaf burn with Ca. Approximately 2 $L.tree^{-1}$ of the solution was applied until run-off on both sides of the tree, which amounted to a high volume application.

Measurements

Two weeks after the last foliar application, six fruitlets of similar size from the outside of the canopy on both sides of the tree were sampled for mineral analyses carried out by Bemlab (Pty) Ltd (Strand, South Africa). Only the peel was analysed for mineral content. The standard mineral analysis included the macro nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) and the micronutrients sodium (Na), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and boron (B).

Fruit quality parameters were recorded at harvest for both seasons on ‘Cripps Pink’ apples. Ten fruit of similar size were harvested randomly from each replicate. The green background fruit colour was measured using the Green colour chart (Unifruco Research Services, Bellville, South Africa). The ‘Pink Lady’ red colour chart was only used in the second season to record blush colour. Flesh firmness was measured on both sides of the fruit, after removing peel disks from the fruit, using a flesh texture analyser (Guss electronic model GS 20, Strand, South Africa). Percentage starch conversion was measured by cutting the fruit in half; applying an iodine solution and evaluating the starch break down after 1 minute using the starch conversion chart (Unifruco Research Services, Bellville, South Africa). Total soluble solids (TSS) was measured by juicing the apple flesh (excluding core with seeds), that was pooled from the 10 fruit per tree. A handheld refractometer (Model N1, Atago, Tokyo, Japan) was used for measuring total soluble solids (TSS).

Harvest Protocol

In the first season (2014/2015), all the apples were harvested from the experimental trees according to predetermined quadrants as depicted in Figure 1, to enable mapping the differences in sunburn severity on the tree, according to bearing position and exposure to direct sunlight based on orientation. After harvest, fruit were individually classified according to sunburn severity.

In the second season (2015/2016), only a subsample of approximately 17 kg fruit was harvested from quadrant A and B respectively (Figure 1) to elucidate the greatest difference in sunburn incidence between treatments, as the previous season's results confirmed the highest sunburn incidence in the upper quadrants.

Sunburn incidence and severity

All apples were graded into six sunburn classes, as well as Class 0 (no sunburn) using the adjusted sunburn incidence classification guide (Figure 2**Figure 2**). The adjusted classification guide was based on the one created by Felicetti and Schrader (2008) on 'Fuji' apples, with the addition of sunburn necrosis (Class 5) and photo-oxidative sunburn (Class 6), for 'Cripps' Pink'. Classes 1 to 4 represented increasing grades of sunburn browning, Class 5 represented sunburn necrosis and Class 6 represented photo-oxidative bleaching.

As this classification is subjective, error may arise with the involvement of various evaluators and this may influence results for small sample sizes. In the first season, classification was performed by different people. However, to minimise variation due to subjectivity, one person was used before final classification was recorded to make final judgement of classification of sunburn by peers. In the second season, only one quadrant was harvested and only one person classified the sunburn incidence, removing possible subjective variation.

Experiment 2

This experiment was performed for one season only (2015/16) to assess cultivar differences ('Golden Delicious' and 'Granny Smith') in response to the treatments of combined calcium and boron formulations.

Plant Material

The experiment was carried out on a commercial farm, Applethwaite Farm (Pty) Limited, Elgin Valley, South Africa (34°12'08.0"S 18°59'16.5"E), using 'Granny Smith' and 'Golden Delicious' trees that were planted in 1990 in alternating rows, in a north-west by south-east orientation, on M793 rootstock.

Statistical Design and Treatments

The experiment was laid out as a RCBD with single tree replicates. Seven treatments were repeated seven times (Table 1). Full bloom occurred on 16th October 2015, and six weekly foliar applications starting from approx. 40 dafb were applied as per the spraying dates presented in Table 2. The different treatments are given in Table 1, all solutions/powders/granules (excl. the control) were mixed with 10 litres of water before application. Active ingredients for the treatments are presented in Table 3.

Motorized Stihl® knap sack sprayers were used to apply the treatments early during the morning to reduce possible leaf burn with Ca and approx. 2 $L.tree^{-1}$ was applied until run-off on both sides of the tree, which amounted to a high volume application.

Measurements

Fruit sampling for mineral analyses was done according to procedures in experiment 1. As in experiment 1 only the fruit peels were analysed in experiment 2. Fruit quality parameters were recorded at harvest on 10 fruit of similar size, harvested randomly from both sides of the tree, per replicate. Analyses were performed according to procedures in experiment 1.

Harvest Protocol

Approximately 17 kg fruit was harvested per tree, just before commercial harvest, from quadrant A (Figure 1) that corresponded with the western side of the tree and accordingly showed the highest sunburn incidence. The harvest date for 'Golden Delicious' was on 26th February 2016 and for 'Granny Smith', 17th March 2016. Fruit were transported to the laboratory at the Department of Horticultural Science, Stellenbosch University for further evaluation.

Sunburn incidence and severity

Individual fruit of ‘Granny Smith’ (Figure 3) and ‘Golden Delicious’ (Figure 3), were graded into five sunburn classes, as well as Class 0, representing no sunburn, using an adapted version of the ‘Cripps’ Pink’ sunburn chart in experiment 1 (Figure 2).

Statistical Analysis

SAS Enterprise Guide 5.1 software (SAS Institute Inc. 2003, Cary, USA) was used to analyse the data. An analysis of variance (ANOVA) was performed using a General Linear Method. Means were separated by Least Significant Differences (LSD) when significant differences occurred at a 5% confidence level ($p \leq 0.05$).

3. Results

Experiment 1

Mineral aAnalysis

In December 2014, ‘Cripps Pink’, showed no significant differences between treatments for N, K or Ca concentrations of the fruit peel (Table 4). There were, however, significant differences between treatments for B concentrations. The treatments that contained Calcinit™ ($Ca(NO_3)_2$) showed significantly higher B concentrations in the apple peel than those that did not contain Calcinit™, including the control (Table 4).

At harvest, in May 2015, there were once again no significant differences in mineral concentrations between treatments for N ($p=0.3424$), K ($p=0.6498$) and Ca ($p=0.8042$) (Table 5).There were, however, significant differences between treatments in B concentrations in the peels ($p=0.0136$). Spraybor® Calcinit™ had significantly higher B concentrations in the peel than Spraybor® Manni-Plex® Ca as well as the control

(Table 5).

The following season, there were no significant differences between treatments for K (p=0.4320) or Ca (p=0.1893), but significant differences for N concentrations (p=0.021) occurred. The treatment 0.5 $g.L^{-1}$ Spraybor®Calsol® had a significantly higher N concentration compared to the treatment Manni-Plex® B Calsol (Tabel 6). There was also a significantly higher B concentration (p=0.0308) for treatment 1.0 $g.L^{-1}$ Spraybor® Calsol® than the control (Table 6).

At harvest, there were no significant differences between treatments for N (p=0.9434), K (p=0.7751) and Ca (p=0.4099) concentrations in the peel (Table 7). A significant difference between treatments in B concentrations in the peel (p=0.0062), with Manni-Plex® B Calsol® being higher than both the control and Manni-Plex® B Manni-Plex® Cal-Zn treatment (Table 7).

Sunburn Incidence and Severity

In the first season (2014/2015), no significant differences were observed between treatments for any of the sunburn classes or total sunburn incidence (Table 8).

In the second season (2015/2016), significant differences were only observed between treatments for Class 1 sunburn (p=0.0001). The control had the highest incidence of Class 1 fruit compared to all other treatments, with a sunburn percentage that was up to 8% higher than that of the spray treatments (Table 9).

Fruit Quality and Maturity

No significant differences between treatments in terms of fruit quality and maturity parameters were recorded in either of the two seasons (Table 10 and 11).

Experiment 2

Mineral analysis in apple fruits after application of combined calcium and boron formulations

Mineral analysis of 'Granny Smith' apple peels two weeks after final foliar application showed no significant differences between treatments for N (p=0.270), P (p=0.781), K (p=0.469, Ca (p=0.348) or Mg (0.784) concentrations. There was, however, a significant difference (p=0.021) in B concentrations between treatments. The treatment 1.0 $g.L^{-1}$ Spraybor® Calsol® had a significantly higher B concentration than the treatment Manni-Plex® B Manni-Plex® Cal-Zn (Table 12). None of the Ca B spray treatments showed significant differences in peel B concentration compared to the control treatment.

Mineral analysis of 'Golden Delicious' apple peels similarly showed no significant differences between treatments for N (p=0.481), P (p=0.193), K (p=0.079) and Mg (p=0.238) concentrations (Table 13). A significant difference between treatments was found for Ca (p=0.037) and B (p<0.0001) concentrations (Table 13). The treatment 0.75 $g.L^{-1}$ Spraybor® Calsol® had a significantly higher Ca concentration than the treatment Manni-Plex® B Manni-Plex® Cal-Zn. None of the Ca B spray treatments showed significant differences in peel Ca concentration compared to the control treatment. The 0.75 $g.L^{-1}$ Spraybor® Calsol® combination also had a significantly higher B concentration than the Manni-Plex® B Manni-Plex® Cal-Zn treatment as well as the control treatment. The 1.0 $g.L^{-1}$ Spraybor® Calsol® combination had a significantly higher B concentration than the Manni-Plex® B Manni-Plex® Cal-Zn treatment (Table 13).

Sunburn Incidence and Severity

There were no significant differences between treatments for 'Granny Smith' apples in terms of sunburn classes and total sunburn incidence (p=0.5064) (Table 14).

For 'Golden Delicious' apples, there were no significant differences between treatments for total sunburn incidence (p=0.1753). There were however significant differences between treatments for Class 0 (p=0.0087) and Class 1 (p=0.0164). The treatment combination of 0.5 g.L^{-1} Spraybor® Calsol® had the highest percentage of fruit with no sunburn (Class 0) and differed significantly from both control and Manni-Plex® B Manni-Plex® Cal-Zn treatments. For Class 1 sunburn, the 0.5 g.L^{-1} Spraybor® Calsol® treatment had significantly less fruit in the Class 1 category than the control and the Manni-Plex® B Manni-Plex® Cal-Zn treatments (Table 15).

Fruit Quality and Maturity

There were no significant differences between treatments for all fruit quality and maturity parameters for 'Granny Smith' apples (Table 16). Significant differences between treatments were observed in 'Golden Delicious" apples for TSS (p=0.0122) and starch breakdown (p=0.0323). The TSS in the 'No-burn' / Calcimax® treatment was significantly higher than all other treatments. However, none of the spray treatments differed significantly from the control treatment. The starch breakdown was significantly more advanced in the 1.0 g.L^{-1} Spraybor® Calsol® treatment compared to the control treatment (Table 17).

4. Discussion

To receive the highest income for apples in the international market, growers in South Africa must meet continually rising standards of visual appearance when exporting fruit (Wünsche et al., 2001). This implies that sunburn incidence be reduced to Class 1 or 0 sunburn severity (Fig 2 - 4). 'Granny Smith' apples classified as Class 1 sunburn are not accepted for export to the United Kingdom market, whereas 'Golden Delicious' with Class 1 sunburn is still accepted for export to the European Union market (Jeanne-Mari Burger, Fruitways (Pty) Ltd, Somerset West, South Africa, personal communication, January 2015). In 2015, the UK received 25 % of South African export apples and the EU 7%; together these regions make up the largest market segment of exported fruit (Hortgro, 2015). It is therefore economically critical to focus research endeavours on reducing all classes of sunburn, thereby increasing Class 0 apples for export. Increasing Class 0 apples by decreasing Class 1 and 2 sunburnt apples would seem easier than decreasing severe necrotic sunburn.

A significant amelioration of sunburn on 'Golden Delicious' apples, as reported by

Lötze and Hoffman (2014), was a first recording of a reduction of sunburn incidence after applying foliar B in combination with Ca. Therefore, further research was done to determine the viability on other cultivars, to possibly obtain comparable results and to study the effect of formulation in reducing sunburn incidence. In response to the study by Lötze and Hoffman (2014) various physiological changes in response to foliar Ca and B applications were studied so as to correlate this to sunburn incidence and a possible mode of action. In this paper, the mineral analysis, fruit quality evaluation and sunburn incidence were evaluated for three different cultivars, following treatment with B and Ca in 6 different formulations of two seasons. The mineral analysis was done on the fruit peel to determine effective uptake and presence of these elements in the concerned areas were sunburn occurs (i.e. the peel). Fruit quality parameters were also evaluated to determine if the treatments had a negative effect, for example like shade nets kaolin-particle films, on red colour development.

In 2014/2015, no significant differences between treatments were found in 'Cripps' Pink' despite of a relatively high average sunburn percentage of approx. 22 %, with the highest sunburn incidence in Class 1 (approx. 8 %) followed by Class 2 (approx. 5%). This may have been due to the fact that harvesting was postponed in an attempt to exacerbate sunburn to elucidate any possible treatment effect, which unfortunately resulted in masking of sunburn (Makeredza et al., 2015) due to the bicolour nature of the 'Cripps' Pink' apple. The masking of the lower classes of sunburn by the pink colour development of 'Cripps' Pink' therefore seems to have influenced the visual sunburn classification process. The further the season progresses the less solar energy is reflected due to wax layer changes as fruit matures and experiences colour change (Chen et al., 2009; Racsko and Schrader, 2012). This will have a negative effect on sunburn development towards the end of the season, as a later harvest date, puts fruit at risk of sunburn damage due to a lower reflectance of solar energy.

In the 2015/2016 season, 'Cripps' Pink' fruit was harvested at an earlier maturity to avoid severe masking due to the pink colour development which would have prevented accurate visual grading of sunburn. This resulted in a very high sunburn incidence of approx. 49.1 %. All treatments reduced sunburn incidence for class 1 fruit significantly (8.0 – 11 %) compared to the control (15.9 %), confirming results from Lötze and Hoffman (2014) for 'Golden Delicious'. The same formulations of Ca and B were however not used in this study, and this did not seem to affect the results for sunburn reduction in 'Cripps' Pink'.

No significant differences between treatments were observed in 'Granny Smith' for either total sunburn (approx. 26.9 %) or class 1 sunburn (approx. 13.5 %). However, this cultivar was harvested pre-optimum, confirmed by the low starch breakdown percentages (approx. 10 %). This was in response to the desire by Applethwaite (Pty) limited to harvest apples in the block where the trial was being carried out. An earlier harvest reduces the risk of possible sunburn-conducive conditions that might have occurred later in the season, and hence possible sunburn incidence and occurrence due treatment effects in 'Granny Smith' may have been missed by harvesting the fruits early. No literature was found that indicate an increased susceptibility to sunburn in apples, however fruit maturity is influenced by sunburn. It is also possible that the lack of results was due to a lack of response of 'Granny Smith' to this treatment, indicating variation between cultivars.

For 'Golden Delicious', the average sunburn incidence was approx. 26.0 %. The 0.5 $g.L^{-1}$ Spraybor® Calsol® treatment was the only treatment that had significantly lower (5.65 %) Class 1 fruit than the control (16.4 %) and Manni-Plex® B Manni-Plex® Cal-Zn treatments (17.4 %). The B and Ca sources used in these trials that resulted in a reduction of sunburn (class 1) were Spraybor® (Sodium Borate, Nulandis) and Calsol® (Aquasol, 15.5 % N + 19 % Ca, Agrocom, Southern Africa) in calcium nitrate form. This differed from the B and Ca sources used in the study by Lötze and Hoffmann (2014), which reduced total sunburn significantly compared to the control. This may indicate that a combination with alternative formulations than the Manni-Plex formulations reported by Lötze and Hoffman (2014) can reduce sunburn incidence significantly in at least 'Cripps' Pink' and 'Golden Delicious' fruit. The lack of a significant reduction of sunburn incidence in 'Cripps' Pink' (2014/2015), 'Golden Delicious' and 'Granny Smith' with the Manni-Plex® Cal-Zn Manni-Plex® B treatment was unexpected and differed from previous findings (Lötze and Hoffman, 2014). This may be partly due to the change in the Manni-Plex Ca formulation in 2015/2016 with the addition of Zinc. For 'Cripps' Pink' apples, the role of formulation did not seem to be the primary factor contributing to the reduction in Class 1 apples, as all B and Ca formulations were able to significantly reduce sunburn compared to the control in

2015/2016.

In 2014/2015 at harvest, ‘Cripps’ Pink’ apple peel showed a significant difference in B concentration between the control (lowest) and Spraybor® Manni-Plex® Ca treatments, and the Spraybor® Calcinit™ treatment. A trend from December 2014 to harvest in May 2015 showed that, regardless of the B formulation of the treatment, treatments containing $Ca(NO_3)_2$ (Calcinit™) as Ca source, always had the highest B concentration (although not consistently significant).

In 2015/2016, at harvest for ‘Cripps’ Pink’ the Manni-Plex® B Calsol® treatment had a significantly higher B concentration than the Manni-Plex® B Manni-Plex® Cal-Zn treatment and the control. The 1.0 $g.L^{-1}$ Spraybor® Calsol® treatment showed a similar trend in comparison to the control from December until harvest in April (although not significant at harvest). These results confirm the previous season’s results with respect to the efficacy of combinations including calcium nitrate. According to this data, B uptake is improved with the addition of Calsol®, specifically in ‘Cripps’ Pink’ apple peels. However, contrary to expectations, no Ca increase was found in any of the treatments where Ca was applied in either of the two seasons.

In ‘Golden Delicious’, Ca peel concentrations differed significantly between treatments after application (Table 13). The lowest Ca concentration was found in the Manni-Plex® B Manni-Plex® Cal-Zn treatment which differed significantly from the 0.75 $g.L^{-1}$ Spraybor® Calsol® treatment with the highest Ca concentration. B concentrations in the peel differed significantly between the control and Manni-Plex® B Manni-Plex® Cal-Zn treatment, which were lower than the highest B concentration in the 0.75 $g.L^{-1}$ Spraybor® Calsol® treatment. This confirmed the trends with regards to high B concentrations in the peel of ‘Granny Smith’ and ‘Cripps’ Pink’ when a calcium nitrate as Ca source was used in the foliar application. Wojcik et al. (2008) found no significant effect on fruit B concentration at the end of the season on ‘Jonagold’ apples after a foliar application of B. However, this may be different from peel concentrations and in this study B was not applied in combination with calcium nitrate. At this stage, the actual concentration of B or Ca in the fruit peel at harvest does not seem to be correlated to the reduction of sunburn incidence in class 1 fruit and this experiment and study data cannot explain this observation.

There were no significant differences between treatments in terms of fruit quality parameters for ‘Cripps’ Pink’ in 2014/2015. However, during the first season the ‘Cripps’ Pink’ apples were harvested post optimum. This could explain the results for sunburn incidence in the first season, where no significant differences were observed between treatments. The bicolour nature of ‘Cripps’ Pink’ apples resulted in masking of lower classes of sunburn. It was in these classes where in the consecutive season, significant differences between treatments were observed on all cultivars.

The earlier harvest maturity in ‘Cripps’ Pink’ in 2015/2016, resulted in fruit with less co-pigmentation, confirmed by the relatively low pink colour development observed at harvest, ranging from 1.52 to 2.92 between treatments (a scale from 1 (no colour) to 12 (highest colour). Therefore, fruit quality was not significantly affected by the foliar applications.

For ‘Golden Delicious’ apples, a significant difference was observed in TSS values between the ‘No-burn’/Calcimax®’ treatment and both the Manni-Plex® B Calsol® and the 1.0 $g.L^{-1}$ Spraybor® Calsol® treatments, the former having a higher value. A higher TSS value is said to predispose apples to sunburn (Makeredza, 2011), making this treatment unfavourable for sunburn reduction from this perspective. Starch breakdown also differed significantly between treatments, whereby the 1.0 $g.L^{-1}$ Spraybor® Calsol® treatment had approx. 10 % greater starch breakdown than the control hence indicating that this fruit was more mature.

No phytotoxicity symptoms were observed in response to foliar applications on ‘Cripps’ Pink’ and ‘Golden Delicious’ apples. However, the ‘No-burn’/Calcimax® treatment showed signs of fruit damage on all replications. A brown discolouration and in many cases an indentation at the calyx end of the fruit were observed, perhaps due to the treatment forming drops at the bottom of the fruit after application.

Conclusion

Even though the foliar applications were not able to reduce the incidence of all sunburn classes in all three cultivars significantly, a reduction of class 1 sunburn incidence in ‘Golden Delicious’ and ‘Cripps’ Pink ‘ can already impact significantly on returns on farm level for exports to the UK and Europe. This makes this sunburn ameliorating strategy cost-effective. The exact concentration of B or Ca in the fruit peel at harvest does not explain the results of a reduction in class 1 sunburn. Further research needs to be done on Ca and B concentrations in the peel as well as the whole fruit, to correlate it with a reduction of sunburn. Alternatively, an appropriate concentration of these minerals within the peel needs to be elucidated at which sunburn reduction occurs.

Fruit maturity plays some role in the expression of the sunburn incidence – either contributing towards masking sunburn in very mature fruit (‘Cripps’ Pink’) or reducing susceptibility to sunburn of immature fruit (‘Granny Smith’) and may influence the results of the expression of the foliar combination of B and Ca foliar treatments.

The mode of action by which the combination of Ca and B foliar applications reduce sunburn could not be determined any further in the scope of this study. Further research needs to be conducted to determine the mode of action by looking at biochemical and/or metabolic effects of these treatments on apple fruit.

Acknowledgments

Funding for this trial was obtained from Nulandis (Pty) Limited, South Africa.

5. References

Basile, B., Giaccone, M., Cirillo, C., Ritieni, A., Graziani, G., Shahak, Y., Forlani, M., 2012. Photo-selective hail nets affect fruit size and quality in Hayward kiwifruit. Sci. Hortic. (Amsterdam). 141, 91–97. doi:10.1016/j.scienta.2012.04.022

Bogo, A., Casa, R.T., Agostineto, L., Gonçalves, M.J., Rufato, L., 2012. Effect of hail protection nets on apple scab in "Royal Gala" and "Fuji" apple cultivars. Crop Prot. 38, 49–52. doi:10.1016/j.cropro.2012.03.020

Chen, L.-S., Li, P., Cheng, L., 2009. Comparison of thermotolerance of sun-exposed peel and shaded peel of "Fuji" apple. Environ. Exp. Bot. 66, 110–116. doi:10.1016/j.envexpbot.2008.12.017

DAFF/PPECB, 2015. Annual Report 2014-2015. http://www.daff.gov.za/.

Erez, A., Glenn, D.M., 2004. The effect of particle film technology on yield and fruit quality. Acta Horticullturae 636, 505–508.

Felicetti, D.A., Schrader, L.E., 2008. Photooxidative Sunburn of Apples: Characterization of a Third Type of Apple Sunburn. Int. J. Fruit Sci. 8, 160–172. doi:10.1080/15538360802526472

Gamble, J., Jaeger, S.R., Harker, F.R., 2006. Preferences in pear appearance and response to novelty among Australian and New Zealand consumers. Postharvest Biol. Technol. 41, 38–47. doi:10.1016/j.postharvbio.2006.01.019

Gindaba, J., Wand, S.J.E., 2007. Do fruit sunburn control measures affect leaf photosynthetic rate and stomatal conductance in "Royal Gala" apple? Environ. Exp. Bot. 59, 160–165. doi:10.1016/j.envexpbot.2005.11.001

Gindaba, J., Wand, S.J.E., 2005. Comparative effects of evaporative cooling, kaolin particle film, and shade net on sunburn and fruit quality in apples. HortSci. 40, 592–596.

Glenn, D.M., Prado, E., Erez, A., McFerson, J., Puterka, G.J., 2002. A reflective, processed-kaolin particle film affects fruit temperature, radiation reflection, and solar injury in apple. J. Am. Soc. Hortic. Sci. 127, 188–193.

Hortgro, 2015. Key Deciduous Fruit Statistics. www.hortgro.co.za.

Hunsche, M., Blanke, M.M., Noga, G., 2010. Does the microclimate under hail nets influence micromorphological characteristics of apple leaves and cuticles? J. Plant Physiol. 167, 974–80. doi:10.1016/j.jplph.2010.02.007

Iglesias, I., Alegre, S., 2006. The effect of anti-hail nets on fruit protection, radiation, temperature, quality and profi tability of “ Mondial Gala” apples 8, 91–100.

IPCC, 2007. Climate Change 2007: impacts, adaptation and vulnerability: contribution of Working Group II to the fourth assessment report of the Intergovernmental Panel, Genebra, Suíça. Cambridge. doi:10.1256/004316502320517344

Jakopic, J., Veberic, R., Stampar, F., 2007. The effect of reflective foil and hail nets on the lighting, color and anthocyanins of “Fuji” apple. Sci. Hortic. (Amsterdam). 115, 40–46. doi:10.1016/j.scienta.2007.07.014

Korkmaz, N., Askin, M.A., 2015. Effects of calcium and boron foliar application on Pomegranate (*Punica granatum* L.) fruit quality, yield and seasonal changes of leaf mineral nutrition. Acta Hort. 1089, 413–422.

Le Grange, M., Wand, S.J.E., Theron, K.I., 2004. Effect of kaolin applications on apple fruit quality and gas exchange of apple leaves. Acta Hort. 636, 545–550.

Lötze, E., Hoffman, E.W., 2014. Foliar application of calcium plus boron reduces the incidence of sunburn in “Golden Delicious” apple. J. Hortic. Sci. Biotechnol. 89, 607–612. doi:10.1080/14620316.2014.11513127

Makeredza, B., Marais, H., Schmeisser, M., Lötze, E., Steyn, W.J., 2015. Ripening associated red color development masks sunburn browning in apple peel. HortSci. 50, 814–818.

Makeredza, B., Schmeisser, M., Lötze, E., Steyn, W.J., 2013. Water stress increases sunburn in 'Cripps' Pink’ apple. HortSci. 48, 444–447.

Makeredza, B., 2011. Studies towards understanding sunburn in apple. MSc Agric thesis. Faculty of AgriScience. University of Stellenbosch. Stellenbosch.

Marais, S., 2005. Sunburn control in apple fruit. MSc Agric thesis. Faculty of AgriScience. University of Stellenbosch. Stellenbosch.

Racsko, J., Schrader, L.E., 2012. Sunburn of Apple Fruit: Historical Background, Recent Advances and Future Perspectives. CRC. Crit. Rev. Plant Sci. 31, 455–504. doi:10.1080/07352689.2012.696453

Schrader, L., Sun, J., Felicetti, D., Seo, J.-H., Jedlow, L., Zhang, J., 2003. Stress-induced disorders: Effects on apple fruit quality. Washington. Tree Fruit Postharvest Conf. 1–7.

Schrader, L., Sun, J., Zhang, J., Felicetti, D., Tian, J., 2008. Heat and Light-Induced Apple Skin Disorders: Causes and Prevention. Acta Hort. 51–58. doi:10.17660/ActaHortic.2008.772.5

Schrader, L.E., Zhang, J., Duplaga, W.K., 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Plant Heal. Prog. 10, 1-5 doi:10.1094/PHP-2001-1004-01-RS

Smit, A., Steyn, W.J., Wand, S.J.E., 2008. Effects of Shade Netting on Gas Exchange of Blushed Apple Cultivars Acta Hort. 772, 73–80.

Solomakhin, A., Blanke, M., 2010. The microclimate under coloured hailnets affects leaf and fruit temperature, leaf anatomy, vegetative and reproductive growth as well as fruit colouration in apple. Ann. Appl. Biol. 156, 121–136. doi:10.1111/j.1744-7348.2009.00372.x

Warner, G., 1997. Sunburn is a hot topic in orchards of Washington. Good Fruit Grow. 22–23.

Wojcik, P., Wojcik, M., Klamkowski, K., 2008. Response of apple trees to boron fertilization under conditions of low soil boron availability. Sci. Hortic. (Amsterdam). 116, 58–64. doi:10.1016/j.scienta.2007.10.032

Wünsche, J.N., Greer, D.H., Palmer, J.W., Lang, A., McGhie, T., 2001. Sunburn- the cost of a high light environment. Acta Hort. 557, 349–356. doi:10.17660/ActaHortic.2001.557.46

6. Tables and Figures

Table 1: Treatment ingredients for both seasons and cultivars.

No.	2014/2015 Season		2015/2016 Season	
1	Control	-	Control	-
2	0.6ml** Manni-Plex B****	1ml Manni-Plex Ca	0.6ml Manni-Plex B	1ml Manni-Plex Cal-Zn
3	0.6ml Manni-Plex B	6.8 ml Calcinit	0.6ml Manni-Plex B	6.5g Calsol
4	1g Spraybor	1ml Manni-Plex Ca	0.5g Spraybor	6.5g Calsol
5	1g Spraybor	6.8ml Calcinit	0.75g Spraybor	6.5g Calsol
6	0.6ml Manni-Plex B	-	1g Spraybor	6.5g Calsol
7*	-	-	4.5ml No-burn***	6.0g Calcimax***

*Treatment 7 was only applied on Granny Smith and Golden Delicious.
**Quantities given were mixed with 1 litre of water.
***The No-burn and Calcimax treatments were alternated weekly and not applied together.
**** Manni-Plex products supplied by Elim Kunsmis Pty Ltd (2014/15), Nexus (2015/16) and all other products supplied by Nulandis

Table 2: Spray dates for both cultivars of both seasons.

Cultivar	1	2	3	4	5	6
Cripps' Pink (2014/2015)	11-Nov	18-Nov	25-Nov	1-Dec	8-Dec	17-Dec
Cripps' Pink (2015/2016)	5-Nov	12-Nov	19-Nov	27-Nov	3-Dec	10-Dec
Granny Smith (2015/2016)	25-Nov	2-Dec	9-Dec	15-Dec	22-Dec	29-Dec
Golden Delicious (2015/2016	25-Nov	2-Dec	9-Dec	15-Dec	22-Dec	29-Dec

Table 3: Active Ingredients of treatments for both seasons.

Component of Treatments	Active Ingredients
Manni-Plex Ca	100 $g.kg^{-1}$ Ca
	80 $g.kg^{-1}$ N
Manni-Plex Cal-Zn	60 $g.kg^{-1}$ Ca
	60 $g.kg^{-1}$ N
	30 $g.kg^{-1}$ Zn
Manni-Plex B	33 $g.kg^{-1}$ B
	50 $g.kg^{-1}$ N
Calcinit $Ca(NO_3)_2$	190 $g.kg^{-1}$ Ca
	155 $g.kg^{-1}$ N
Spraybor $Na_2B_4O_7{\cdot}10H_2O$	205 $g.kg^{-1}$ B
Calsol $Ca(NO_3)_2$	190 $g.kg^{-1}$ Ca
	155 $g.kg^{-1}$ N
Spraybor $Na_2B_4O_7{\cdot}10H_2O$	165 $g.kg^{-1}$ B
Calcimax	78.5 $g.kg^{-1}$ Ca
	5 $g.kg^{-1}$ B

Table 4: Mineral analysis of 'Cripps' Pink' apple peel two weeks after final foliar application in December 2014 on Welgevallen Experimental farm (Experiment 1).

Treatment	N (mg/100g)	K (mg/100g)	Ca (mg/100g)	B (mg/kg)
Control	326[ns]	191[ns]	17.52[ns]	6.62[b*]
Manni-Plex B & Manni-Plex Ca	331	205	18.80	8.10[b]
Manni-Plex B & Calcinit	299	193	18.46	11.8[a]
Spraybor & Manni-Plex Ca	309	184	16.48	7.30[b]
Spraybor & Calcinit	350	187	18.60	11.1[a]
Manni-Plex B	309	195	17.88	7.46[b]
P	*0.0953*	*0.1133*	*0.7376*	*<0.0010*

*letters indicating significant differences for $P < 0.05$

Table 5: Mineral Analysis of 'Cripps' Pink' apple peel at harvest in May 2015 on Welgevallen Experimental Farm (Experiment 1).

Treatment	N (mg/100g)	K (mg/100g)	Ca (mg/100g)	B (mg/kg)
Control	73.8^{ns}	101^{ns}	16.0^{ns}	4.56^{b*}
Manni-Plex B & Manni-Plex Ca	78.0	111	15.7	6.04^{ab}
Manni-Plex B & Calcinit	72.0	105	16.7	6.46^{ab}
Spraybor & Manni-Plex Ca	69.0	109	16.6	4.48^{b}
Spraybor & Calcinit	85.4	126	16.0	6.82^{a}
Manni-Plex B	73.6	112	14.4	5.40^{ab}
P	*0.3424*	*0.6498*	*0.8042*	*0.0136*

*letters indicating significant differences for P < 0.05

Table 6: Mineral Analysis of 'Cripps' Pink' apple peel two weeks after final foliar application in December 2015 on Welgevallen Experimental Farm (Experiment 1).

Treatment	N (mg/100g)	K (mg/100g)	Ca (mg/100g)	B (mg/kg)
Control	128.6^{ba}	44.0^{ns}	4.63^{ns}	1.37^{b*}
Manni-Plex B & Manni-Plex Cal-Zn	142.6^{ba}	47.6	4.96	1.64^{ab}
Manni-Plex B and Calsol	121.8^{b}	40.7	5.27	2.20^{ab}
0.5 g.L^{-1} Spraybor & Calsol	175.6^{a}	52.0	6.60	1.97^{ab}
0.75 g.L^{-1} Spraybor & Calsol	151.4^{ba}	42.0	4.70	1.78^{ab}
1.0 g.L^{-1} Spraybor & Calsol	149.4^{ba}	45.5	5.48	2.35^{a}
P	*0.0210*	*0.4320*	*0.1893*	*0.0308*

*letters indicating significant differences for P < 0.05

Table 7: Mineral Analysis of 'Cripps' Pink' apple peel at harvest in April 2016 on Welgevallen Experimental Farm (Experiment 1).

Treatment	N (mg/100g)	K (mg/100g)	Ca (mg/100g)	B (mg/kg)
Control	160.8[ns]	130.2[ns]	14.88[ns]	5.50[b*]
Manni-Plex B & Manni-Plex Cal-Zn	157.4	122.4	15.08	5.70[b]
Manni-Plex B and Calsol	160.8	130.6	15.82	8.18[a]
0.5 $g.L^{-1}$ Spraybor & Calsol	164.8	131.4	15.56	6.24[ba]
0.75 $g.L^{-1}$ Spraybor & Calsol	165.8	128.8	17.44	7.06[ba]
1.0 $g.L^{-1}$ Spraybor & Calsol	158.2	125.4	17.56	7.36[ba]
P	0.9434	0.7751	0.4099	0.0062

*letters indicating significant differences for $P < 0.05$

Table 8: Sunburn classification data for 2014/2015 for 'Cripps' Pink' apples at Welgevallen Experimental farm.

Treatment	Total Sunburn (%)	Class 0 (%)	Class 1 (%)	Class 2 (%)	Class 3 (%)	Class 4 (%)	Class 5 (%)	Class 6 (%)	Class 1&2 (%)
Control	20.46^{ns}	79.54^{ns}	7.00^{ns}	4.31^{ns}	3.00^{ns}	2.45^{ns}	3.17^{ns}	0.46^{ns}	11.31^{ns}
Manni-Plex B & Manni-Plex Ca	25.85	74.15	8.07	5.24	3.03	3.19	4.12	1.30	13.31
Manni-Plex B & Calcinit	18.19	81.81	6.75	4.21	2.35	1.93	2.69	1.41	10.96
Spraybor & Manni-Plex Ca	25.55	74.45	8.61	6.59	4.29	2.44	2.69	1.03	15.20
Spraybor & Calcinit	23.81	76.19	8.43	4.64	2.53	3.06	3.26	1.68	13.07
Manni-Plex B	22.07	77.93	8.47	3.67	1.80	2.98	4.11	1.68	12.14
P	*0.6097*	*0.6097*	*0.8841*	*0.5014*	*0.2510*	*0.858*	*0.7483*	*0.3263*	*0.6981*

Table 9: Sunburn classification data for 2015/2016 for 'Cripps' Pink' apples at Welgevallen Experimental farm.

Treatment	Total Sunburn	Class 0	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 1&2
Control	56.0[ns]	43.9[ns]	15.9[a*]	19.9[ns]	9.25[ns]	1.26[ns]	9.12[ns]	0.67[ns]	35.8[ns]
Manni-Plex B & Manni-Plex Cal-Zn	48.6	51.4	9.26[b]	16.2	6.76	1.05	14.0	1.34	25.4
Manni-Plex B & Calcinit	46.7	53.3	8.92[b]	14.4	8.60	2.69	10.9	1.23	23.3
0.5 $g.L^{-1}$ Spraybor & Calsol	46.7	53.3	8.02[b]	15.2	6.74	2.03	14.1	0.68	23.2
0.75 $g.L^{-1}$ Spraybor & Calsol	44.4	55.6	10.2[b]	14.1	8.04	1.89	9.59	0.53	24.3
1.0 $g.L^{-1}$ Spraybor & Calsol	52.1	47.9	11.0[b]	20.7	9.11	2.31	8.14	0.83	31.7
P	*0.456*	*0.4564*	*0.0001*	*0.5842*	*0.7285*	*0.5291*	*0.359*	*0.6623*	*0.1014*

*letters indicating significant differences for P < 0.05

Table 10: Fruit quality parameters of 'Cripps' Pink' at Welgevallen Experimental farm at harvest, May 2015.

Treatment	Firmness (kg)	Diameter (mm)	Mass (g)	TSS (˚Brix)	Green back ground colour	Starch Break down (%)
Control	8.31^{ns}	54.9^{ns}	102.8^{ns}	15.7^{ns}	3.51^{ns}	70.6^{ns}
Manni-Plex B & Manni-Plex Ca	8.27	54.5	98.1	15.5	3.33	74.8
Manni-Plex B & Calcinit	8.22	54.8	100.3	15.1	3.45	78.1
Spraybor & Manni-Plex Ca	8.28	54.7	100.2	15.0	3.37	73.8
Spraybor & Calcinit	8.53	53.7	89.0	15.9	3.28	76.8
Manni-Plex B	8.52	53.5	90.5	16.1	3.32	71.7
P	*0.5109*	*0.0814*	*0.1419*	*0.4282*	*0.5394*	*0.0552*

Table 11: Fruit quality parameters of 'Cripps' Pink' at Welgevallen Experimental farm at harvest, April 2016.

Treatment	Firmness (kg)	Diameter (mm)	Mass (g)	TSS (˚Brix)	Green Back ground colour	Red over colour	Starch Breakdown (%)
Control	9.70^{ns}	62.31^{ns}	87.20^{ns}	14.40^{ns}	2.72^{ns}	2.34^{ns}	18.9^{ns}
Manni-Plex B & Manni-Plex Cal-Zn	9.66	61.64	84.64	13.84	2.47	1.70	16.3
Manni-Plex B & Calsol	9.54	63.27	89.78	14.30	2.37	2.46	32.3
0.5 g.L^{-1} Spraybor & Calsol	9.61	62.94	92.00	13.80	2.49	1.52	17.0
0.75 g.L^{-1} Spraybor & Calsol	10.00	61.06	80.10	14.68	2.6	2.92	24.3
1.0 g.L^{-1} Spraybor & Calsol	9.55	61.45	83.12	14.76	2.45	2.16	27.4
P	*0.5216*	*0.7466*	*0.7526*	*0.2015*	*0.6737*	*0.1534*	*0.1595*

Table 12: Mineral analysis of 'Granny Smith' apple peel two weeks after final foliar application in December 2015, Applethwaite Farm (Experiment 2).

Treatment	N (mg/100g)	P (mg/100g)	K (mg/100g)	Ca (mg/100g)	Mg (mg/100g)	B (mg/kg)
Control	107[ns]	23.7[ns]	229[ns]	14.85[ns]	12.2[ns]	11.8[ba*]
Manni-Plex B & Manni-Plex Cal-Zn	102	24.2	221	14.88	12.3	11.2[b]
Manni-Plex B & Calsol	122	26.8	252	15.32	12.9	12.1[ba]
0.5 $g.L^{-1}$ Spraybor & Calsol	115	24.7	239	16.48	12.9	14.5[ba]
0.75 $g.L^{-1}$ Spraybor & Calsol	117	25.4	227	17.38	12.9	13.9[ba]
1.0 $g.L^{-1}$ Spraybor & Calsol	105	20.6	217	16.32	11.5	15.9[a]
No-burn alt. Calcimax	114	27.1	248	14.84	12.8	14.0[ba]
P	*0.2695*	*0.7812*	*0.4687*	*0.3483*	*0.7840*	*0.0208*

*letters indicating significant differences for $P < 0.05$

Table 13: Mineral analysis of 'Golden Delicious' apple peel two weeks after final foliar application in December 2015, Applethwaite Farm (Experiment 2).

Treatment	N (mg/100g)	P (mg/100g)	K (mg/100g)	Ca (mg/100g)	Mg mg/100g)	B (mg/kg)
Control	89^{ns}	14.3^{ns}	173^{ns}	10.57^{ba*}	12.1^{ns}	7.42^{bc}
Manni-Plex B & Manni-Plex Cal-Zn	84	10.6	123	8.65^{b}	9.4	5.60^{c}
Manni-Plex B & Calsol	85	12.8	154	12.38^{ba}	10.9	7.78^{bac}
0.5 $g.L^{-1}$ Spraybor & Calsol	90	13.7	163	12.73^{ba}	11.1	9.17^{bac}
0.75 $g.L^{-1}$ Spraybor & Calsol	94	13.1	167	16.88^{a}	12.5	11.8^{a}
1.0 $g.L^{-1}$ Spraybor & Calsol	99	14.5	185	12.60^{ba}	12.6	11.6^{ba}
No-burn alt. Calcimax	90	14.8	170	11.84^{ba}	11.5	9.34^{bac}
P	*0.4814*	*0.1934*	*0.0793*	*0.0376*	*0.2383*	*0.0003*

*letters indicating significant differences for P < 0.05

Table 14: Sunburn classification of ‘Granny Smith’ apples at Applethwaite farm, Elgin Valley, 2016.

Treatment	Total Sunburn (%)	Class 0 (%)	Class 1 (%)	Class 2 (%)	Class 3 (%)	Class 4 (%)	Class 5 (%)	Class 6 (%)	Class 1- 2 (%)
Control	31.8^{ns}	68.9^{ns}	15.8^{ns}	6.81^{ns}	4.83^{ns}	1.61^{ns}	1.51^{ns}	0.56^{ns}	22.6^{ns}
Manni-Plex B & Manni-Plex Cal-Zn	28.6	71.0	12.4	8.15	4.92	1.85	1.53	0.14	20.6
Manni-Plex B & Calsol	25.6	75.1	14.7	5.52	2.46	0.67	1.59	0.00	20.2
0.5 $g.L^{-1}$ Spraybor & Calsol	28.5	73.1	14.8	5.63	4.42	1.32	1.46	0.00	19.7
0.75 $g.L^{-1}$ Spraybor & Calsol	24.5	75.5	11.2	7.23	2.78	1.68	1.31	0.23	18.5
1.0 $g.L^{-1}$ Spraybor & Calsol	23.0	76.6	12.5	4.52	3.19	1.09	1.71	0.45	17.0
No-burn alt. Calcimax	26.6	73.0	13.3	5.46	2.97	2.54	2.22	0.55	18.8
P	*0.5064*	*0.5064*	*0.2702*	*0.5853*	*0.4411*	*0.6659*	*0.987*	*0.1762*	*0.7196*

Table 15: Sunburn classification of 'Golden Delicious' apples at Applethwaite farm, Elgin Valley, 2016

Treatment	Total Sunburn (%)	Class 0 (%)	Class 1 (%)	Class 2 (%)	Class 3 (%)	Class 4 (%)	Class 5 (%)	Class 6 (%)	Class 1-2 (%)	Class 3-5 (%)
Control	35.8^{ns}	64.2^{b}	16.4^{a}	10.6^{a}	5.1^{ns}	2.3^{ns}	0.75^{ns}	0.6^{ns}	27.0^{a}	8.2^{ns}
**M B & M Cal-Zn	33.0	67.0^{b}	17.4^{a}	6.9^{ba}	4.2	2.4	1.57	0.6	24.3^{a}	8.1
M B & Calsol	23.9	76.1^{ba}	10.2^{ba}	5.7^{ba}	4.6	2.6	0.46	0.3	15.9^{ba}	7.7
0.5 g.L^{-1} Spraybor & Calsol	12.5	87.5^{a}	5.65^{b}	3.3^{b}	1.8	0.8	0.23	0.7	9.0^{b}	2.8
0.75 g.L^{-1} Spraybor & Calsol	27.3	72.7^{ba}	11.4^{ba}	8.7^{ba}	4.4	2.4	0.23	0.2	20.1^{ba}	7.0
1.0 g.L^{-1} Spraybor & Calsol	19.7	80.3^{ba}	10.7^{ba}	4.5^{ba}	2.6	1.2	0.32	0.5	15.2^{ba}	4.1
No-burn alt. Calcimax	29.9	70.1^{ba}	14.2^{ba}	8.2^{ba}	4.7	1.4	1.16	0.2	22.4^{a}	7.3
P	*0.1753*	0.0087	*0.0164*	*0.0642*	*0.1893*	*0.3412*	*0.1413*	*0.8207*	*0.0088*	*0.0593*

*letters indicating significant differences for P < 0.05

**M is Manni-Plex

Table 16: Fruit quality and maturity of 'Granny Smith' at Applethwaite, Elgin Valley at harvest, March 2016.

Treatment	Firmness (kg)	Diameter (mm)	Mass (g)	TSS (˚Brix)	Green back ground colour	Starch Break down (%)
Control	8.95^{ns*}	67.33^{ns}	135.74^{ns}	10.86^{ns}	1.09^{ns}	8.57^{ns}
M B & M Cal-Zn	8.84	67.56	136.26	10.83	1.02	10.29
M B & Calsol	8.81	67.41	135.37	10.83	1.04	11.36
0.5 g.L^{-1} Spraybor & Calsol	8.98	66.89	133.41	10.41	1.01	10.43
0.75 g.L^{-1} Spraybor & Calsol	8.74	68.26	140.01	10.63	1.04	11.57
1.0 g.L^{-1} Spraybor & Calsol	8.73	68.19	140.66	10.47	1.04	10.21
No-burn / Calcimax	8.79	68.29	141.07	10.76	1.07	13.50
P	*0.6647*	*0.7283*	*0.6752*	*0.8268*	*0.7782*	*0.5184*

*letters indicating significant differences for P < 0.05

**M is Manni-Plex

Table 17: Fruit quality and maturity of 'Golden Delicious' at Applethwaite, Elgin Valley at harvest, February 2016.

Treatment	Firmness (kg)	Diameter (mm)	Mass (g)	TSS (˚Brix)	Green back ground colour	Starch Break down (%)
Control	7.84[ns]	69.47[ns]	151.26[ns]	12.03[ba]	1.80[ns]	17.29[b]
**M B & M Cal-Zn	7.97	69.25	146.96	12.01[ba]	1.86	21.21[ba]
M B & Calsol	8.00	68.51	146.27	11.81[b]	1.75	23.00[ba]
0.5 $g.L^{-1}$ Spraybor & Calsol	7.89	69.41	150.09	12.11[ba]	1.83	32.14[ba]
0.75 $g.L^{-1}$ Spraybor & Calsol	7.84	68.94	147.61	11.94[ba]	1.83	31.07[ba]
1.0 $g.L^{-1}$ Spraybor & Calsol	7.78	69.77	152.46	11.90[b]	1.86	39.21[a]
No-burn / Calcimax	8.13	67.56	138.70	12.63[a]	1.91	28.36[ba]
P	*0.3834*	*0.3605*	*0.3124*	*0.0122*	*0.6961*	*0.0323*

*letters indicating significant differences for $P < 0.05$

**M is Manni-Plex

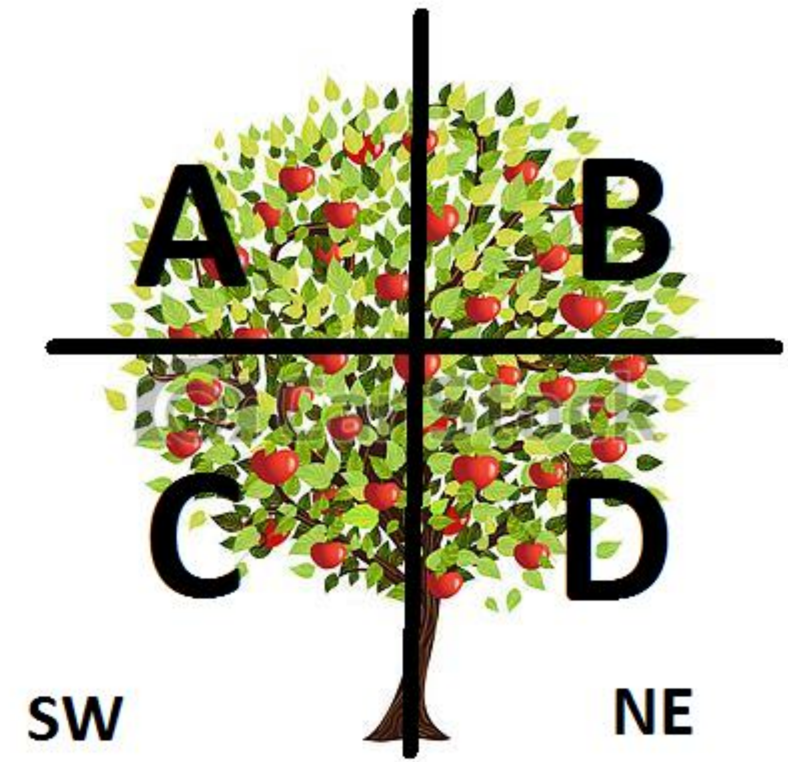

Figure 1: A picture of the four quadrants trees were split into. Fruit were harvested according to these quadrants, as viewed down the rows. The horizontal line was 1.5 m from ground level.

Figure 2: 'Cripps' Pink' sunburn classification guide. Class 0 being no sunburn, class 1-4 (progression of sunburn browning), class 5 (sunburn necrosis) and class 6 (photo-oxidative sunburn). This classification guide was based on the classification guide of 'Fuji' apples by Felicetti and Schrader (2008).

Figure 3: 'Granny Smith' sunburn classification guide. Class 0 being no sunburn, class 1-4 (progression of sunburn browning) and class 5 (sunburn necrosis). This classification guide was based on the classification guide of 'Fuji' apples

by Felicetti and Schrader (2008).

Figure 4: ‘Golden Delicious’ sunburn classification guide. Class 0 being no sunburn, class 1-4 (progression of sunburn browning) and class 5 (sunburn necrosis). This classification guide was based on the classification guide of ‘Fuji’ apples by Felicetti and Schrader (2008).

Paper 2

Quantification of physical changes in the apple peel of 'Cripps' Pink' after foliar applications of combinations of Ca and B to reduce sunburn incidence.

1. Introduction

Both calcium (Ca) and boron (B) are essential plant nutrients (Bolaños et al., 2004; White and Broadley, 2003). They play very important roles in various structural, metabolic and biochemical processes and functions. A deficiency of these nutrients results in a plethora of symptoms and abnormalities, like cork spot, bitter pit or water core. It has therefore been difficult to determine the primary function of specifically B in plants (Brown et al., 2002).

Calcium plays various roles in the plasma membrane and cell walls of plant cells (Bush, 1995; Figueroa et al., 2012; Hirschi, 2004). Apoplastic Ca in association with the cell wall has the highest proportion of total Ca, but a proportion is exchangeable on the plasma membrane (Hirschi, 2004). When Ca binds to the membrane, the catabolism of phospholipids and mono-galactosyl-glycerol becomes delayed and membrane integrity is preserved (De Freitas et al., 2010). This increases membrane restructuring processes and decreases senescence-related membrane lipid changes (Picchioni et al.,1998). Additionally, Ca is exchanged with other cations in the external solution, like H^+ or Na^+ in response to stress, to regulate selectivity of ion uptake. For example, an increase in Ca concentration ameliorates growth inhibition caused by salinity stress. Ca is also involved in cell elongation and division and is a regulatory ion in carbohydrate translocation in the plant (Hirschi, 2004).

Calcium is also a fundamental component of signalling in eukaryotes and plays a role in triggering a response to normalise cellular activity (Hirschi, 2004). Signal transduction and the role Ca plays as a secondary messenger therein, has only been introduced in the nineties (Bush, 1995; Matoh and Kobayashi, 1998). A change in free cytosolic Ca occurs in response to a wide variety of abiotic and biotic signals. Abiotic stimuli range from light (including UV/B radiation), temperature, hyperosmotic stress and oxidative stress (Sanders et al., 2002). Ca fluctuations around the endoplasmic reticulum, vacuole and plasma membrane play an important role in plant growth and environmental stress adaptation (Hirschi, 2004). According to Logan and Knight (2003), Ca spikes in and around other endo-membranes and the mitochondria are possibly also important for certain cellular responses. Recent studies have highlighted the role of Ca signalling in the chloroplast, with first evidence by Stael et al. (2012) showing calcium-dependent protein phosphorylation at the thylakoid membrane. Ca needs to be taken up by the thylakoid lumen so that the oxygen evolving complex (or water splitting complex) is supplied with a necessary cofactor (Cinco et al., 2004). Calcium-dependent protein phosphorylation is required for production of protease in the thylakoid, which is responsible for "protein turnover of photo-damaged D1 subunits of photosystem two". To prevent photo-inhibition, this D1 subunit needs to be constantly recycled by protease (Stael et al., 2012).

Boron is an essential trace element that maintains optimal fruit quality and yield (Peryea and Drake, 1991). It is involved in three main processes: maintaining both cell wall and membrane structure and functioning as well as supporting metabolic activities (Bolaños et al., 2004). In terms of membrane structure, B is associated with polyhydroxyl compounds of membranes and affects pectin substance formation (Baker et al., 1956). It was also hypothesised by Bolaños et al. (2004) that B binds to glycoproteins and glycolipids. The association of B with these membrane constituents explains its importance in maintaining structural membrane integrity. According to Cakmak and Römheld (1997), B seems to protect cells against toxic oxygen species in the plasma membrane to prevent peroxidative damage. Also, high fruit B can cause earlier colour development, increased occurrences of decay and a reduction of firmness (Peryea and Drake, 1991).

Boron is also involved in signal transduction and gene expression and was found to be essential for bacteria-plant signalling (Bolaños et al., 2004). In response to the effect that Ca has on boron-responsive genes in alfalfa, Bolaños et al. (2004) speculated a "boron-calcium interplay" in signalling.

Ca and B are important in the functioning and maintenance of membrane structures. A mode of action of these minerals in ameliorating sunburn can therefore be linked to the prevention of the loss of membrane integrity associated with sunburnt apples (Schrader et al., 2001). Apple peels with photo-oxidative sunburn show signs of loss in membrane integrity, with significant electrolyte leakage occurring (Felicetti and Schrader, 2008). Reactive oxygen species, formed by photo-inhibition when shaded fruit are suddenly exposed to light, are responsible for this loss in membrane integrity, causing damage to the photosynthetic apparatus. Membrane leakage occurs not only with photo-oxidative sunburn, but also with sunburn necrosis. Sunburn necrosis develops in response to "heat stress-induced biophysical reactions", like that of a membrane phase transition, denaturing of proteins and injury to metabolic apparatus (Racsko and Schrader, 2012). The whole photosynthetic apparatus becomes damaged and chlorophyll is completely degraded, membranes are damaged and electrolytes leak freely (Schrader et al., 2001).

Unlike sunburn necrosis and bleaching, sunburn browning is a "sub-lethal event" and cell membrane integrity is therefore not destroyed (Schrader et al., 2001). However it was observed by Havaux et al. (1996) that increased thylakoid membrane leakiness occurred as a result of moderate stress due to sunburn browning. This deformation of the thylakoid membrane can be countered by inducing zeaxanthin synthesis, which alters the physical state of the thylakoid membrane. This theory was confirmed by Chen et al. (2008), who showed an increase in zeaxanthin which was needed to protect thylakoid membranes under stress conditions. The importance of Ca here is that it is a cofactor for photosynthetic oxygen evolution and is part of the oxygen-evolving complex which catalyses water into dioxygen, protons and electrons (Cinco et al., 2004). The percentage dry mass of fruit also increases as sunburn incidence increases. This is possibly due to a water loss as the fruit cells perish (Racskó et al., 2005). Severe sunscald damage results in severe changes in the cuticle, epidermal and sub-epidermal cells (Schrader et al., 2001). Racskó et al. (2005) also observed a cell wall thickening in response to sunburn damage. Higher dry matter content resulting in an increase in firmness was also observed by Makeredza et al. (2011).

In response to research by Lötze and Hoffman (2014), which showed a significant reduction in sunburn incidence on ‘Golden Delicious’ apples after foliar applications of a combination of Ca and B, the mode of action required further investigation. To determine the role of Ca and B in the amelioration of sunburn in apples, concentrations of these minerals in the fruit peel were quantified and correlated to the cell wall thickness of individual cells in the peel (epidermis), just below the cuticle. An established direct effect of Ca application to fruit is that cell walls are reinforced through calcium bridges between polymers and a partial reduction of cell wall-modifying enzyme activity (Ortiz et al., 2011). It is hypothesised that an increase in cell wall thickness due to supplementation of Ca and B through foliar application is correlated with a reduction in sunburn.

2. Materials and Methods

2.1 Plant Material

Bearing ‘Cripps’ Pink’ trees on M793 rootstocks on the Welgevallen Experimental Farm, University of Stellenbosch, Stellenbosch, South Africa (33°56'52.5"S, 18°52'19.9"E) were used for the trial. The trees were planted in 1998 at a spacing of 1.5 m x 4.0 m in south-west by north-east row orientation. Trees were managed as a commercial unit according to standard practices. Irrigation was supplied via micro-jets.

2.2 Statistical Design and treatments

The experiment was laid out as a Randomised Complete Block Design (RCBD) with single tree replicates. Six treatments were replicated five times (Table 1). The treatments were applied from approx. 28 days after full bloom (dafb) on a weekly basis for 6 weeks (Table 2). This differed from the protocol used by Lötze and Hoffman (2014), by omitting the last two application dates, to determine whether it is possible to reduce the number of applications without reducing the efficacy of the treatment. All solutions/powders/granules (excl. the control) were mixed in 10 litres of water.

Motorized Stihl® knap sack sprayers were used to apply the treatments early in the morning to reduce possible leaf burn with Ca. Approximately 2L.tree^{-1} of the solution was applied until run-off, which amounted to a high volume application.

2.3 Harvest Protocol

All fruit on the tree were harvested on 27 April 2015. Fruit were individually classified according to sunburn severity using the sunburn chart for 'Fuji' developed by Felicetti and Schrader (2008), with the addition of sunburn necrosis (class 5) for 'Cripps' Pink'.

To create a contrast between sunburn incidences for subsequent peel analyses, only fruit from class 0 (no sunburn), class 3 (visual sunburn browning) and Class 5 (necrosis) were subsampled. Approximately five apples were peeled to obtain 15g fresh peel per replicate (6 per treatment) for further analyses. Similarly, single fruit from each of the selected classes were sampled per treatment, for scanning electron microscopy analysis of the cell wall and cuticle thickness of the fruit peel cells.

2.4 Measurements

2.4.1 SEM measurements of Cell wall and cuticle thickness in the 'Cripps Pink' apple peels of selected sunburn classes in the different combined Ca plus B foliar treatments

At harvest, apple peel pieces of about 2 mm x 8 mm x 3 mm section were cut from the apple peel, using a Minora® blade – resulting in exposing mainly the exocarp and a thin section of the mesocarp (pulp). These peel sample sections were then placed in 100% ethanol solution to be freeze dried. Freeze drying was done at the Centre for Imaging and Analysis (University of Cape Town, South Africa) using Balzers® EM CPD020 critical point dryer (Leica Microsystems, Wetzler, Germany). The drying was done with 100% ethanol solution at 80°C .

SEM analysis was performed at the Centre for Analytical Facilities (CAF), University of Stellenbosch, using the "EVO" SEM. The freeze dried samples were placed on double sided adhesive carbon tape. These were then placed in the gold coater (Edwards® S150A Sputter Coater, UK) for 3 min ensuring that the samples are full coated with gold so that the sample surfaces are electrically conductive for SEM analysis. The samples were then placed in the scanning electron microscope (Carl ZEISS EVO® MA15VP, Carl Zeiss Microscopy GmbH, Jena, Germany) for imaging. The beam conditions for analysis were set at extra high tension (EHT) voltage level of 7 kV; with working distance (WD) between beam tip and sample at 10 mm. Images were taken on two positions on the exocarp as shown in Figure 5.

In this study, the cell wall thickness was determined manually from SEM images. Both, the thickness of the cuticles and cell walls were determined from the SEM images using NIS Elements® D microscope imaging software (Nikon Instruments Inc., Melville, USA). The software was used to calibrate the measurements and cell walls of six different cells per image were performed to quantify average cell wall thickness. Similarly, six measurements across the cuticle were performed to determine the average cuticle thickness.

2.4.2 Dry mass of selective apple peels

Percentage dry mass of peels was determined for fruit in each sunburn class (Class 0, Class 3 and Class 5). Approximately five fruit were peeled on the sun-exposed side resulting in 15g fresh mass sample, which was placed in brown paper bags, oven dried at 50°C and the dry mass percentage calculated using the formula below.

$$Percentage\ dry\ mass = \frac{dry\ mass}{(dry\ mass + wet\ mass)} \times 100$$

.

2.4.3 Mineral analysis of the peel

Mineral analysis of the peel from six non-blemished fruit (Class 0) per tree, for each treatment, was determined at harvest by Bemlab (Pty) Ltd, Strand, South Africa. Only calcium (Ca), boron (B), potassium (K) and nitrogen (N) minerals were analysed in the peel samples.

2.4.5 Statistical Analysis

SAS Enterprise Guide 5.1 software (SAS Institute Inc. 2003, Cary, USA) was used to analyse the peel mass data. An analysis of variance (ANOVA) was performed using a General Linear Method. Means were separated by Least Significant Differences (LSD) when significant differences occurred at a 5% confidence level ($p \leq 0.05$). For the cell wall thickness data, standard errors were provided by statistical analysis done by NIS Element® D software (Nikon Instruments Inc., Melville, USA).

3. Results

3.1 Percentage dry mass of peels

There were no significant differences between treatments for dry mass percentages of peels (Table 20). There were, however, significant differences in the percentage dry mass of peels between sunburn severities, with Class 5 sunburn having a significantly lower dry mass percentage than Class 0 and Class 3.

3.2 Cell wall and cuticle thickness

Peels from the control had the thinnest cell walls in all three classes compared to the other treatments (Table 22: Cell wall thickness of cells (µm) below cuticle of apple peel for the different Ca/B foliar sprays during 2014/15 for 'Cripps' Pink' fruit with in Class 0, Class 3 and Class 5 sunburn.

Treatment	**Cell wall 0 (µm)**	**Cell wall 3 (µm)**	**Cell wall 5 (µm)**	**Ca (mg/100g)**	**B (mg/kg)**	**Class 0 (%)**	**Class 3 (%)**	**Class 5 (%)**
Control	1.21±0.25	1.31±0.61	1.47±0.17	17.52ns	6.62b*	79.54ns	3.00ns	3.17ns

Manni-Plex B & Manni-Plex Ca	1.26±0.55	2.42±0.73	-	18.80	8.10b	74.15	3.03	4.12
Manni-Plex B & Calcinit	1.65±0.38	3.24±0.52	2.77±0.46	18.46	11.8a	81.81	2.35	2.69
Spraybor & Manni-Plex Ca	2.17±0.43	3.48±0.73	-	16.48	7.30b	74.45	4.29	2.69
Spraybor & Calcinit	2.58±0.62	2.60±0.58	2.86±0.56	18.60	11.1a	76.19	2.53	3.26
Manni-Plex B	2.20±0.52	2.15±0.45	3.54±0.81	17.88	7.46b	77.93	1.80	4.11
P	-	-	-	0.7376	<0.001	0.6097	0.2510	0.7483

*** Mineral analysis and sunburn percentage was used from results presented in Chapter 1.**

Table 23: Cuticle thickness of apple peel (µm) for the different Ca/B foliar sprays during 2014/15 for fruit with of Class 0, Class 3 and Class 5 sunburn.

Treatment	**Cuticle 0 (µm)**	**Cuticle 3 (µm)**	**Cuticle 5 (µm)**	**Ca (mg/100g)***	**B (mg/kg)***	**Class 0 (%)***	**Class 3 (%)***	**Class 5 (%)***
Control	7.33±0.97	9.08±1.52	4.21±0.66	17.52ns	6.62b*	79.54ns	3.00ns	3.17ns
Manni-Plex B & Manni-Plex Ca	14.32±1.96	9.96±2.11	-	18.8	8.10b	74.15	3.03	4.12
Manni-Plex B & Calcinit	3.20±1.04	7.32±0.87	11.96±1.62	18.46	11.8a	81.81	2.35	2.69

Spraybor & Manni-Plex Ca	8.36±1.43	10.02±1.20	-	16.48	7.30b	74.45	4.29	2.69
Spraybor & Calcinit	7.74±1.63	7.45±1.89	11.22±1.71	18.6	11.1a	76.19	2.53	3.26
Manni-Plex B	16.53±1.90	15.37±3.49	19.00±5.00	17.88	7.46b	77.93	1.8	4.11
P	-	-	-	0.7376	<0.001	0.6097	0.2510	0.7483

* Mineral analysis and Sunburn percentage was used from results presented in Chapter 1.

). The thickest cell walls were found for the Spraybor® Calcinit™ treatment for 0 (no sunburn), the Spraybor® Manni-Plex® Ca treatment for Class 3 sunburn and the Manni-Plex® B treatment for Class 5 sunburn. The thinnest cuticle (3.20 µm) was recorded for the Manni-Plex® B Calcinit™ treatment that had no sunburn (Class 0). The thickest cuticle (19.00 µm) was observed for the Manni-Plex B® treatment for Class 5 sunburn (**Error! Reference source not found.**).

Figure 6 shows a SEM image of a peel of the control which had the thinnest cell walls for all sunburn severities, but with gradual increases in thickness as sunburn severity increased as can be seen in Figure 7 and Figure 8. Figure 9 shows SEM images of control Class 3 apple peel which had the thinnest cell walls, and those treated with Spraybor® Manni-Plex® Ca treatment, which had the thickest cell walls. Figure 10 shows SEM images of control Class 0 apple peel and Spraybor® Calcinit™ treatment depicting cuticle thickness differences.

4. Discussion

In terms of dry mass, there were no significant differences in mean dry mass between the treatments for all three sunburn classes evaluated. However, significant differences were observed between sunburn severities. Apples with Class 5 sunburn had the lowest percentage peel dry mass compared to Class 0 and Class 3. This could be due to the severe electrolyte leakage and plasma membrane breakdown that occurs with sunburn necrosis (Andrews and Johnson, 1996; Schrader et al., 2001). A definite progressive drying out of the peel could be observed as sunburn severity increases.

The importance of Ca and B for cell wall functioning and maintenance has been well documented over the years (Baker et al., 1956; Bolaños et al., 2004; Cakmak and Römheld, 1997; de Freitas et al., 2013; Hirschi, 2004; Picchioni et al., 1998). However, a correlation between cell wall thickening and Ca and B has not yet been documented. Racskó et al. (2005) claimed that cell walls in apple fruits thicken with severe sunburn damage. Cell wall thickening of the first and second cell layers below the cuticle occurs in response to sunburn browning (Racsko and Schrader, 2012). This thickening and lignification of cell walls was visible prior to sunburn symptom appearance (Andrews and Johnson, 1996). In response to foliar Ca sprays, cell wall structures were reinforced by establishing Ca bridges between polymers. A secondary effect of these sprays was that cell wall-modifying enzymes were inhibited resulting in polymer solubilisation delays in cell walls (Andrews and Johnson, 1996).

From the SEM images of the apple peel, a trend points towards thicker cell walls of cells in the epidermis, just below the cuticle, in response to early foliar applications of Ca and B combinations. This was noticed in the peels from all three sunburn classes 0, 3 and 5), where the control consistently had the thinnest cell walls. The foliar applications of combinations of Ca and B did increase cell wall thickness, with the Spraybor® Calcinit™ treatment having the thickest cell walls for class 0 apples. This was perhaps due to the role of Ca and B in maintaining plasma membranes and binding to cell wall constituents according to Bolaños et al. (2004), De Freitas et al. (2010) and Hirschi (2004). Cell wall thickening was observed on sunburn Class 5 apple peels compared to Class 0 (no sunburn) fruit. In other words, a progression of cell wall thickening occurred as sunburn severity increased, resulting in the thickest cell walls with the most severe sunburn incidence in Class 5. This was observed on the control fruits and confirmed results by Hao and Huang (2004), where a cell wall thickening was observed on 'Fuji' apples with sunburn necrosis (Class 5 sunburn). This trend was not very strongly observed in the control, where cell thickness increased with less than a micrometre in length in response to sunburn necrosis. This was perhaps due to a lack of available Ca and B in the apoplast that can associate with cell wall constituents, reflecting a restrained thickening of the cell wall in comparison with cell wall increases as sunburn severity increased.

There was a relationship between Ca and B peel concentration and cell wall thickness in class 0 apples, where the lowest cell wall thickness was observed on the untreated peels (1.21 µm) versus those that were treated such as Spraybor® Calcinit™ (2.58 µm). Overall results indicated that there is a tendency towards an effect of foliar applications of a combination of Ca and B on cell wall thickness.

In terms of cuticle thickness, a formulation effect was observed, but could not be justifiably termed significant. More research is needed to show significant improvements in cell wall thickness in response to Ca and B applications. The cuticle thickness on the epidermis of the peel was thinner (50 %) in sunburn Class 5 apples than both Class 3 and Class 0 sunburnt apples of the control. This confirms results by Andrews and Johnson (1996) who observed a thinning of the wax cuticle with sunburn incidence.

A direct effect of cell wall properties and sunburn incidence could not be confirmed. Even though the results appeared promising with regards to an effect of the foliar applications on changes in the peel, the foliar applications of Ca and B did not directly reduce sunburn via physical changes in the peel. The results therefore do not explain the mode-of-action satisfactorily.

Future research could be expanded to include SEM analyses of fruit before foliar Ca and B applications (40 dafb), as well as after the final application (72 dafb) along with an increase in sample size to quantify the altering effects of these mineral elements on cell wall and cuticle thickening.

Acknowledgements

I would like to acknowledge Nulandis (Pty) Ltd, South Africa for funding this project as well as Angelique Laurie and Madeleine Franzenburg at the SEM for their help.

5. References

Andrews, P.K., Johnson, J.R., 1996. Physiology of sunburn development in apples. Good Fruit Grow. 47, 33–36.

Baker, J.E., Gauch, H.G., Dugger, W.M., 1956. Effects of boron on the water relations of higher plants. Ann. Rev. Plant Physiol. 31, 89–94.

Bolaños, L., Lukaszewski, K., Bonilla, I., Blevins, D., 2004. Why boron? Plant Physiol. Biochem. 42, 907–912. doi:10.1016/j.plaphy.2004.11.002

Brown, P.H., Bellaloui, N., Wimmer, M.A., Bassil, E.S., Ruiz, J., Hu, H., Pfeffer, H., Dannel, F., Römheld, V., 2002. Boron in plant biology. Plant Biol. 4, 205–223. doi:10.1055/s-2002-25740

Bush, D.S., 1995. Calcium regulation in plant cells and its role in signaling. Ann. Rev. Plant Physiol. Plant Mol. Biol. 46, 95–122.

Cakmak, I., Römheld, V., 1997. Boron deficiency-induced impairments of cellular functions in plants, in: Plant Soil 193, 71-83. doi:10.1023/A:1004259808322.

Chen, L.S., Li, P., Cheng, L., 2008. Effects of high temperature coupled with high light on the balance between photooxidation and photoprotection in the sun-exposed peel of apple. Planta 228, 745–756. doi:10.1007/s00425-008-0776-3

Cinco, R.M., Robblee, J.H., Messinger, J., Fernandez, C., McFarlane Holman, K.L., Sauer, K., Yachandra, V.K., 2004. Orientation of calcium in the Mn_4Ca cluster of the oxygen-evolving complex determined using polarized strontium EXAFS of photosystem II membranes. Biochem. 43, 13271–13282. doi:10.1021/ bi036308v

De Freitas, S.T., Amarante, C.V.T. do, Dandekar, A.M., Mitcham, E.J., 2013. Shading affects flesh calcium uptake and concentration, bitter pit incidence and other fruit traits in “Greensleeves” apple. Sci. Hort. 161, 266–272. doi:10.1016/ j.scienta.2013.07.019

De Freitas, S.T., Amarante, C.V.T. do, Labavitch, J.M., Mitcham, E.J., 2010. Cellular approach to understand bitter pit development in apple fruit. Post. Biol. Technol. 57, 6–13. doi:10.1016/j.postharvbio.2010.02.006

Felicetti, D., Schrader, L., 2008. Changes in pigment concentrations associated with the degree of sunburn browning of "Fuji" apple. J. Am. Soc. Hortic. Sci. 133, 27–34. doi:10.1016/j.plantsci.2008.09.013

Figueroa, C.R., Opazo, M.C., Vera, P., Arriagada, O., Díaz, M., Moya-León, M.A., 2012. Effect of postharvest treatment of calcium and auxin on cell wall composition and expression of cell wall-modifying genes in the Chilean strawberry (*Fragaria chiloensis*) fruit. Food Chem. 132, 2014–2022. doi:10.1016/j.foodchem.2011.12.041

Hao, Y.Y., Huang, W.D., 2004. Changes in antioxidative system and cell ultrastructure in the fruit peels of apple during sunburn development. J. Plant Physiol. Mol. Biol. 30, 19–26.

Havaux, M., Tardy, F., Ravenel, J., Chanu, D., Parot, P., 1996. Thylakoid membrane stability to heat stress studied by flash spectroscopic measurements of the electrochromic shift in intact potato leaves: influence of the xanthophyll content. Plant. Cell Environ. 19, 1359–1368. doi:10.1111/j.1365-3040.1996.tb00014.x

Hirschi, K.D., 2004. The calcium conundrum. Both versatile nutrient and specific signal. Plant Physiol. 136, 2438–2442. doi:10.1104/pp.104.046490.2438

Logan, D.C., Knight, M.R., 2003. Mitochondrial and cytosolic calcium dynamics are differentially regulated in plants. Plant Physiol. 133, 21–24. doi:10.1104/pp.103.026047.Plant

Lötze, E., Hoffman, E.W., 2014. Foliar application of calcium plus boron reduces the incidence of sunburn in "Golden Delicious" apple. J. Hort. Sci. Biotechnol. 89, 607–612. doi:10.1080/14620316.2014.11513127

Makeredza, B., 2011. Studies towards understanding sunburn in apple. MSc Agric thesis, Department of Horticultural Science. Faculty of AgriScience. University of Stellenbosch.

Matoh, T., Kobayashi, M., 1998. Boron and calcium, essential inorganic constituents of pectic polysaccharides in higher plant cell walls. J. Plant Res. 111, 179–190. doi:10.1007/BF02507164

Ortiz, A., Graell, J., Lara, I., 2011. Preharvest calcium applications inhibit some cell wall-modifying enzyme activities and delay cell wall disassembly at commercial harvest of “Fuji Kiku-8” apples. Post. Biol. Technol. 62, 161–167. doi:10.1016/j.postharvbio.2011.04.014

Peryea, F.J., Drake, S.R., 1991. Influence of mid-summer boron sprays on boron content and quality indices of “delicious” apple. J. Pl. Nutr. 14, 825–840.

Picchioni, G.A., Watada, A.E., Conway, W.S., Whitaker, B.D., Sams, C.E., 1998. Postharvest calcium infiltration delays membrane lipid catabolism in apple fruit. J. Agric. Food Chem., 46, 2452–2457. doi:10.1021/jf971083e

Racsko, J., Schrader, L.E., 2012. Sunburn of Apple Fruit: Historical Background, Recent Advances and Future Perspectives. CRC. Crit. Rev. Plant Sci. 31, 455–504. doi:10.1080/07352689.2012.696453

Racskó, J., Szabó, Z., Nyéki, J., 2005. Importance of the supraoptimal radiance supply and sunburn effects on apple fruit quality. Acta Biol. Szeged. 49, 111–114. doi:10.1007/s00425-008-0776-3

Sanders, D., Pelloux, J., Brownlee, C., Harperc, J.F., 2002. Calcium at the Crossroads of Signaling. Plant Cell 14, 401–417.

Schrader, L.E., Zhang, J., Duplaga, W.K., 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Plant Heal. Prog., *10*, 1-5. doi:10.1094/PHP-2001-1004-01-RS

Stael, S., Rocha, A.G., Wimberger, T., Anrather, D., Vothknecht, U.C., Teige, M., 2012. Cross-talk between calcium signalling and protein phosphorylation at the thylakoid. J. Exp. Bot. 63, 1725–1733. doi:10.1093/jxb/err403

White, P.J., Broadley, M.R., 2003. Calcium in plants. Ann. Bot. 92, 487–511. doi:10.1093/aob/mcg164

6. Tables and Figures

Table 18: Treatments applied on 'Cripps' Pink' at Welgevallen Experimental Farm during 2014/15.

Treatment	2014/2015	
1	Control	
2	6ml* Manni-Plex B	10 ml Manni-Plex Ca
3	6 ml Manni-Plex B	68 ml Calcinit
4	10g Spraybor	10 ml Manni-Plex Ca
5	10g Spraybor	68 ml Calcinit
6	6 ml Manni-Plex B	

*Concentrations for 10 L water.

Table 19: Spraying dates of weekly foliar applications.

Cultivar Cripps' Pink	Number of foliar applications					
	1	**2**	**3**	**4**	**5**	**6**
2014/2015	11-Nov	18-Nov	25-Nov	1-Dec	8-Dec	17-Dec
Days after full bloom	28	35	42	49	56	65

Table 20: Mean dry mass percentages of 15 g fresh mass 'Cripps' Pink' peel at harvest for Class 0, Class 3 and Class 5 sunburn.

Treatment	Class 0 (%)	Class 3 (%)	Class 5 (%)
Control	22.7[ns]	22.7[ns]	10.7[ns]
Manni-Plex B & Manni-Plex Ca	20.6	21.1	6.7
Manni-Plex B & Calcinit	21.3	23.3	11.3
Spraybor & Manni-Plex Ca	23.3	22.5	13.3
Spraybor & Calcinit	21.3	20.7	10.0
Manni-Plex B	22.0	22.0	13.3
P	0.8915	0.7550	0.5732

Table 21: Mean dry mass percentages (%) of peels at harvest between sunburn classes of 15g off 6 fruit per treatment and sunburn class at harvest.

Sunburn Class	Dry mass (%)
0	21.78[a]
3	22.00[a]
5	11.05[b]

P	<0.0001

Table 22: Cell wall thickness of cells (µm) below cuticle of apple peel for the different Ca/B foliar sprays during 2014/15 for 'Cripps' Pink' fruit with in Class 0, Class 3 and Class 5 sunburn.

Treatment	Cell wall 0 (µm)	Cell wall 3 (µm)	Cell wall 5 (µm)	Ca (mg/100g)	B (mg/kg)	Class 0 (%)	Class 3 (%)	Class 5 (%)
Control	1.21±0.25	1.31±0.61	1.47±0.17	17.52[ns]	6.62[b*]	79.54[ns]	3.00[ns]	3.17[ns]
Manni-Plex B & Manni-Plex Ca	1.26±0.55	2.42±0.73	-	18.80	8.10[b]	74.15	3.03	4.12
Manni-Plex B & Calcinit	1.65±0.38	3.24±0.52	2.77±0.46	18.46	11.8[a]	81.81	2.35	2.69
Spraybor & Manni-Plex Ca	2.17±0.43	3.48±0.73	-	16.48	7.30[b]	74.45	4.29	2.69
Spraybor & Calcinit	2.58±0.62	2.60±0.58	2.86±0.56	18.60	11.1[a]	76.19	2.53	3.26
Manni-Plex B	2.20±0.52	2.15±0.45	3.54±0.81	17.88	7.46[b]	77.93	1.80	4.11
P	-	-	-	0.7376	<0.001	0.6097	0.2510	0.7483

*** Mineral analysis and sunburn percentage was used from results presented in Chapter 1.**

Table 23: Cuticle thickness of apple peel (µm) for the different Ca/B foliar sprays during 2014/15 for fruit with of Class 0, Class 3 and Class 5 sunburn.

Treatment	Cuticle 0 (µm)	Cuticle 3 (µm)	Cuticle 5 (µm)	Ca (mg/100g)*	B (mg/kg)*	Class 0 (%)*	Class 3 (%)*	Class 5 (%)*
Control	7.33±0.97	9.08±1.52	4.21±0.66	17.52[ns]	6.62[b*]	79.54[ns]	3.00[ns]	3.17[ns]
Manni-Plex B & Manni-Plex Ca	14.32±1.96	9.96±2.11	-	18.8	8.10[b]	74.15	3.03	4.12
Manni-Plex B & Calcinit	3.20±1.04	7.32±0.87	11.96±1.62	18.46	11.8[a]	81.81	2.35	2.69
Spraybor & Manni-Plex Ca	8.36±1.43	10.02±1.20	-	16.48	7.30[b]	74.45	4.29	2.69
Spraybor & Calcinit	7.74±1.63	7.45±1.89	11.22±1.71	18.6	11.1[a]	76.19	2.53	3.26
Manni-Plex B	16.53±1.90	15.37±3.49	19.00±5.00	17.88	7.46[b]	77.93	1.8	4.11
P	-	-	-	0.7376	<0.001	0.6097	0.2510	0.7483

* Mineral analysis and Sunburn percentage was used from results presented in Chapter 1.

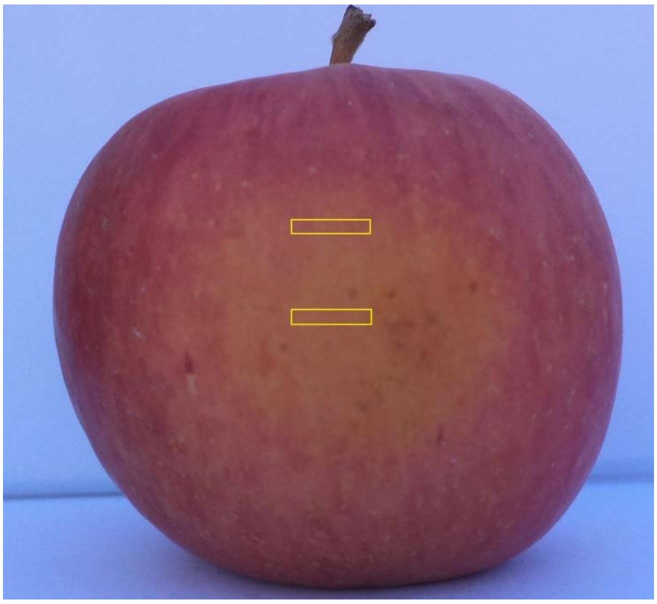

Figure 5: Position of exocarp removed from apple fruit peel samples using a Minora® razor blade. Yellow areas indicate the peel area that was removed for the SEM analysis work.

Figure 6: SEM image of apple fruit peel sample from the control treatment with no sunburn (left) and a typical source of the sample,class 0 sunburn on 'Cripps Pink' apple fruit (right)

Figure 7: SEM image of apple fruit peel sample from the control treatment with class 3 sunburn browning (left) and a typical source of the sample, class 3 sunburn on 'Cripps Pink' apple fruit (right).

Figure 8: SEM image of apple peel fruit peel sample from the control treatment with class 5 / sunburn necrosis (left) and a typical source of the sample, class 5 sunburn on 'Cripps Pink apple fruit (right).

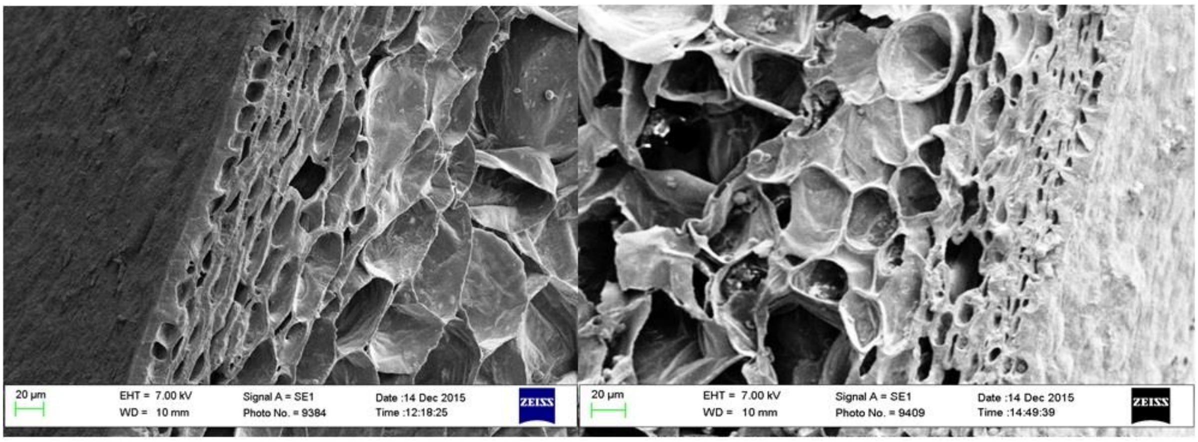

Figure 9: SEM images of apple peel samples of class 3 sunburn depicting cell wall thickness differences.The images are from control treatment (left) and Spraybor® plus Manni-Plex® Ca (right)

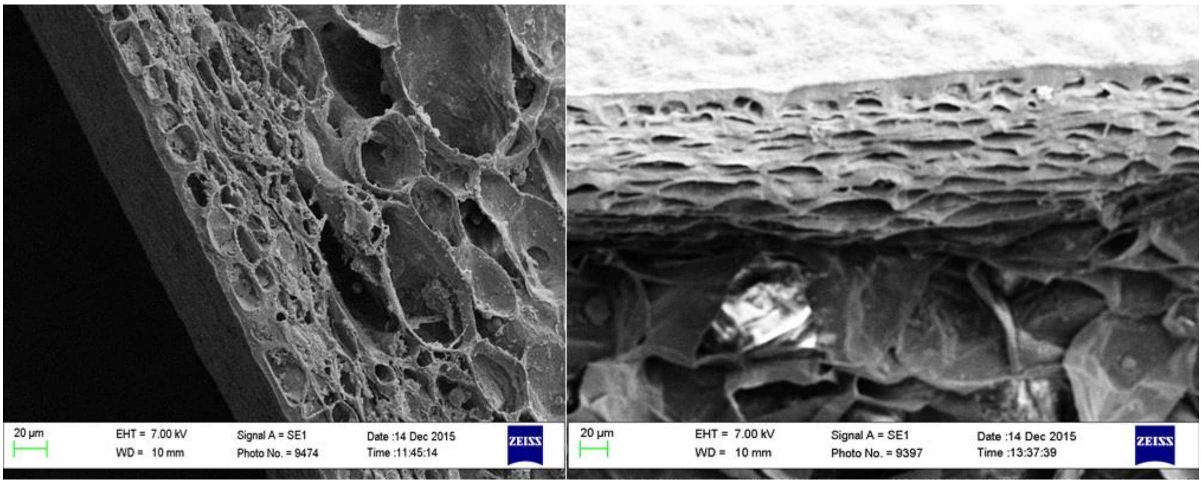

Figure 10: SEM images of apple peel samples of class 0 depicting cuticle thickness differences. The images are from control treatment (left) and Spraybor® plus Calcinit™ treatment (right)

Paper 3

Quantification of physiological changes in ‘Cripps’ Pink’ apple peel in response to foliar applications of a combination of calcium and boron to reduce sunburn in apples

1. Introduction

The main climatic constraints to apple production faced in South African deciduous fruit growing regions is excess heat and light (Jackson, 2003). Sunburn on apples is therefore a major concern for South African producers and will become an even more important research area in the future (Racsko and Schrader, 2012). Sunburn is caused by a high fruit surface temperature (FST) which causes sunburn necrosis (SN) or sunburn browning (SB) depending on the temperature threshold that is reached. It is a response to a combination of direct factors, including heat and irradiance (Yuri et al., 2010), which induce sunburn; as well as indirect factors that influence the severity of an already induced degree of sunburn.

Irradiance, or more specifically, exposure to sunlight, has been reported as the most important factor causing sunburn in apples (Racsko and Schrader, 2012; Van den Ende, 1999). According to Racsko and Schrader (2012), three components of light play a role once the fruit surface is exposed to direct sunlight: 1) high energy ultraviolet spectrum (280nm-400nm), 2) visible light (PAR) (400nm-700nm) and 3) near infrared radiation (NIR) (>740nm). Of these three components, UV-B photons in the high energy spectrum have the highest energy (Jenkins, 2009; Rozema et al., 1997; Solovchenko and Schmitz-Eiberger, 2003). UV-B photons can cause damage to macromolecules like DNA, generate reactive oxygen species (ROS) and impair cellular processes like DNA replication (Jenkins, 2009). Solar radiation causes two of the three sunburn types, being sunburn browning (SB) and photo oxidative sunburn (PS). The UV-B radiation is essential for SB to occur (Racsko and Schrader, 2012; Schrader et al., 2003a).

The direct effect of irradiance and more specifically ROS, which damage the photosystem, was quantified with fluorescence measurements. These measurements of maximal photochemical efficiency (Fv/Fm) were used to indicate stress to chlorophyll in previous studies related to sunburn amelioration (Chen et al., 2008; Hengari et al., 2014). A reduction in Fv/Fm values was recorded after exposure to high temperatures and radiation. Such measurements are more important in full red or blushed cultivars, where sunburn incidence could be underestimated due to masking (Makeredza et al., 2015). Even though masking of less severe sunburn browning can be observed, a significant reduction of Fv/Fm was recorded in both 'Cripps' Pink' and 'Fuji' cultivars (Makeredza et al., 2015), which are both blushing cultivars. In the study by Hengari et al. (2014), irreversible damage was recorded when fruit were exposed to temperatures between 45 and 50˚C. Irreversible damage, in response to severe stress, is quantified when Fv/Fm values fall below 0.6. The normal range for Fv/Fm is between 0.7 and 0.8 (Ritchie, 2006). In a study by Steyn et al. (2009), on 'Forelle' pears, a reduction of Fv/Fm was observed with increasing temperature and light levels, resulting in irreversible damage below 0.6. Excessive light that cannot be sequestered by the photosynthetic apparatus may result in oxidative damage (Steyn et al., 2009).

Alongside irradiance, high temperature (ambient and/or FST) is the other direct factor responsible for the formation of sunburn on apple fruit (Gindaba and Wand, 2005; Iglesias and Alegre, 2006; Makeredza, 2011; Parchomchuk and Meheriuk, 1996; Racsko and Schrader, 2012). High temperature is a cause for both sunburn necrosis and sunburn browning, with the later also requiring irradiance (Gindaba and Wand, 2005; Makeredza, 2011; Marais, 2005; Schrader et al., 2003b, 2009).

Schrader et al. (2001) were the first to determine the FST at which SB and SN is induced in apples. For SB, this was about 46˚C as the lowest threshold temperature on a cultivar like 'Honeycrisp' and about 49˚C as the highest threshold temperature on 'Cripps' Pink' apples observed by Schrader et al. (2008) under Washington State (USA) conditions. SN occurs at a fruit threshold temperature of about 52˚C (Racsko and Schrader, 2012). The heat exchanged between the fruit and the atmosphere, termed the heat balance, is what affects fruit surface temperature (Racsko and Schrader, 2012; Schrader et al., 2003b). The FST will rise when the heat loss is smaller than the "incoming heat load" (Racsko and Schrader, 2012). At these threshold FSTs, thermal death of cells in the peel occur (Schrader et al., 2003b), membrane integrity is lost and electrolytes are leaked from the cells (Schrader et al., 2008). A thermal imaging device was also used to measure maximum FST of fruit, to quantify the physiology of the direct effect of temperature on sunburn incidence. A treatment effect on FST could then be elucidated. The same device was also used in a study by Moffat (2013) on grape bunch temperatures.

The objective of this study was to determine if the combination of Ca and B foliar applications early in the season directly affected fruit physiology (Fv/Fm) and/or FST at selected times during the season, and if these changes can be correlated directly to the reduction in sunburn incidence at harvest.

2. Materials and Methods

2.1 Experimental layout

The experiment was performed over two consecutive seasons, 2014/15 and 2015/16 as described in detail in paper 1. Bearing 'Cripps' Pink' trees on M793 rootstock were selected for this trial on the Welgevallen Experimental Farm, University of Stellenbosch, Stellenbosch, South Africa. The experiment was laid out as a randomised complete block design (RCBD) with single tree replicates. The foliar applications (**Error! Reference source not found.**) were applied weekly for six weeks at approximately 28 days after full bloom (dafb). Approximately 2 $L.tree^{-1}$ was applied.

2.2 Measurements

2.2.1 Sunburn incidence

Sunburn incidence data from results from chapter 1 was used. Unlike in chapter 1, in some cases, sunburn was classified as necrotic or as sunburn without necrosis, by combining sunburn class 1 to class 4, which are all sunburn browning. In other cases, the total sunburn was differentiated between the eastern and the western side of the trees, as fruit were harvested separately in the first season.

2.2.2 Thermal images of the apple fruits

The thermal images of the 'Cripps Pink' apple fruits were captured using a RAZ-IR® NANO Thermal Camera System (Sierra Pacific Innovations Corp, USA). Thermal images were taken during peak solar radiation hours, from 12:00 to 14:00 hours on four fruits (two each eastern and western sides of the tree) that were previously labelled on each of the 30 experimental apple trees. On The emissivity of the thermal imaging camera was set to 0.98, which is suitable for apples (Dr. Albert Strever, Stellenbosch University, personal communication, January 2015). The thermal imaging camera was kept at a constant distance of about 25cm from the fruit peel surface when an image of the exposed side of the fruit was captured. Fruit on the north-eastern side were captured first, down the row from tree 1 to 30, and then fruit on the south-western side were captured back up the row from tree 30 to 1. Then, Guide IrAnalyser® software (Wuhan Guide Infrared Co., 2010) was used to extract the temperature data from the thermal images. The relevant area of the image was encircled; giving a maximum fruit surface temperature output as shown in Figure 11. This output was then multiplied by the emissivity value (0.98) to estimate the fruit surface temperature. During the 2014/2015 season, measurements were taken on the 30th January, 20th February, 30th March and 24th April 2015 while during the following season (2015/2016) measurements were taken on 25th March and 3rd April 2016.

2.2.3 Infrared measurement of the apple fruit surface temperature

Fruit surface temperature (FST) measurements were taken on the same labelled fruit as those used for the thermal imaging, using a handheld infrared thermometer (Raynger® MX4, Raytek Co., Santa Cruz, USA). The thermometer was held at a standard distance of 30 cm from the fruit and the exposed side was always measured. The measurements were performed on average 16 times per fruit. In 2014/2015, measurements were taken once a month during hotter hours of the day from 12:00 to 14:00. This was done on the following days: 26th November, 27th November, 2nd December and 8th December 2014, as well as on 30th January, 20th February and 24th April 2015 In 2015/2016 measurements were taken throughout the day at 11:00, 13:00 and 15:00 on 10th March and 8th April 2016.

2.2.4 Chlorophyll Fluorescence

Maximum quantum yield of fluorescence (Fv/Fm) was measured by harvesting six fruit per tree (three per eastern and western side) and marking the exposed side of the fruit as either east- or west-facing. The fruit were placed in cooler boxes and kept in complete darkness in order to dark adapt them for at least 30 minutes. In 2014/2015, fruit were harvested early in the morning. However, in the second season they were harvested during the late afternoon. This was done to ensure that the fruit which had experienced photo-oxidative damage throughout the day did not have the opportunity to recover overnight. Fv/Fm was measured in a dark room using a pulse modulated fluorimeter (FMS2, Hansatech Instruments Ltd., King's Lynn, Norfolk, England) on both the exposed and shaded sides of each fruit. For the 2014/2015 season, measurements were taken on 1st December, 8th December, 15th December, 22nd December 2014 and 25th April 2015 respectively. The following season (2015/2016), measurements were taken on 19th January, 23rd February, 25th March and 19th April 2016 respectively.

2.2.5 Leaf Gas exchange

Transpiration rate and light-saturated net CO_2 assimilation rate were measured on two healthy leaves on each side of the tree, under ambient temperature conditions from 09:00 using a LI-6400 infrared gas analyser (Li-Cor, Lincoln, Nebraska, USA). The 2x3 OPAQUE LED was used in a closed chamber system and the cuvette CO_2 concentration was set at 380µmol.mol^{-1}. The photosynthetic photon flux density was set at 1500 µmol.m^{-2}.s^{-1}. In the first season, spot measurements were made on 2nd December 2014 and 27th March 2015, both from 09:00 onwards. In the second season (2015/2016), measurements were taken only on 30th January 2016. Two exposed leaves were measured per tree on the northeast-facing side.

2.2.6 Weather data

The weather data over the seasons was obtained from the Sonbesie weather station at Stellenbosch University. Ambient temperature (˚C) was in some cases used to reflect the temperature while measurements were made; in other cases, the maximum temperature of that day was used (Figure 12 and Figure 13). Solar radiation ($W.m^{-2}$) was also used to indicate the amount of radiation the fruit received during measurements, but maximum daily solar radiation was also shown over the season, where the highest recorded solar radiation is reflected. Relative humidity (%) was also given for the periods at which measurements were made, but also the lowest daily (minimum) relative humidity was also shown. Wind velocity ($m.s^{-1}$) data was also shown to portray the wind conditions during hours at which measurements were made. According to Schrader et al. (2003b), these parameters all correlate with FST and may directly or indirectly affect sunburn incidence (Racsko and Schrader, 2012).

The temperatures at which sunburn browning and necrosis would occur were calculated using a formula developed by Schrader et al. (2003b) that correlated maximum air temperature with fruit surface temperature. According to this formula, sunburn browning would occur at maximum temperatures of 34˚C and sunburn necrosis at maximum temperatures of 35˚C. Since this formula has not been tested for conditions in the Western Cape, South Africa which are different to those in the Washington State, USA; these temperatures are relative.

2.2.7 Pink colour development

The thermal imaging camera was used to take pictures simultaneously with the thermal images in the 2015/2016 season. These were used to track pink colour blushing of the 'Cripps' Pink' apples using the 'Pink Lady' colour chart developed by TopFruit (Pty) Ltd, South Africa. The values are averages of values given to the amount of pink colouration on fruit according to the colour chart.

2.3 Statistical Analysis

SAS Enterprise Guide 5.1 software (SAS Institute Inc. 2003, Cary, USA) was used to analyse the data. An analysis of variance (ANOVA) was performed using a General Linear Method. Means were separated by Least Significant Differences (LSD) when significant differences occurred at a 5% confidence level ($p \leq 0.05$).

3. Results

3.1 Thermal Imaging

In the first season, there were no significant differences between treatments with regards to fruit surface temperature data from thermal imaging in February, March or April 2015 (Table 25). In January 2015, there was a significant difference between the Manni-Plex® B treatment and the Spraybor® Manni-Plex® Cal-Zn combination, but no foliar treatment was significantly different to the control. In the second season, there were once again no significant differences between treatments in March and April.

3.2 Infrared measurement of fruit surface temperature

In the first season (2014/2015), measurements in November and December were taken on the eastern side. Significant differences were observed only on 26th November 2014, with the Manni-Plex® B Calcinit™ treatment having a significantly higher FST than the control (Table 26). From January to April, measurements were taken on both the eastern and the western side of trees (Table 27). There were no significant differences between treatments, except for the eastern side on 20th February 2015. The Manni-Plex® B Calcinit™ combination had a significantly lower fruit surface temperature (FST) than the Manni-Plex® B treatment. No foliar treatment was significantly different to the control.

In the second season (2015/2016), significant differences were observed for FST measurements taken at 10:00 on 10th March (Table 28). The 0.5 $g.L^{-1}$ Spraybor® Calsol® treatment had a significantly higher FST than the 0.75 $g.L^{-1}$ Spraybor® Calsol® combination, Manni-Plex® B Manni-Plex® Cal-Zn combination and control treatments. In March, the FST measurements taken at 13:00 and 15:00 showed no significant differences between treatments. However, there were significant differences in FST measurements taken at 15:00 on 8th April. The 0.75 $g.L^{-1}$ Spraybor® Calsol® combination had a significantly higher fruit surface temperature than those under the Manni-Plex® B Calsol® treatment combination. There were no significant differences between treatments for FST measurements taken at the 10:00 and 13:00 (Table 29).

3.3 Chlorophyll fluorescence (Fv/Fm) measurements

In the first season, there were no significant differences between treatments on 8th December and 15th December (Table 30). There was however a significant difference between treatments on 12th December, where the control treatment had a lower Fv/Fm than the Manni-Plex® B Manni-Plex® Ca combination as well as the Manni-Plex® B Calcinit™ combination. The Fv/Fm values were however still above 0.850, indicating no stress to the photosystem. At the end of the season (April 2015), no significant differences were observed among all the treatments.

In the second season (2015/2016), there were no significant differences in December and January 2016 measurements (Table 31). There were, however, significant differences between treatments in February and March 2016. In February 2016, Fv/Fm values of fruit from the Manni-Plex® B Calsol® treatment and the 1.0 $g.L^{-1}$ Spraybor® Calsol® treatment had a significantly higher Fv/Fm than the control and the Manni-Plex® B Manni-Plex® Cal-Zn treatment. The later treatment also had a significantly lower Fv/Fm than the 0.5 $g.L^{-1}$ Spraybor® Calsol® treatment. In March 2016, the 1.0 $g.L^{-1}$ Spraybor® Calsol® treatment again had a significantly higher Fv/Fm than the control and the Manni-Plex B® Manni-Plex® Cal-Zn treatment. In January 2016, shaded fruit from the western side of the apple trees treated with Manni-Plex® B Manni-Plex® Cal-Zn had significantly higher Fv/Fm values than the 1.0 $g.L^{-1}$ Spraybor® Calsol® treatment, but neither differed significantly from the other treatments. The exposed side of the fruit also had significant differences, with the Manni-Plex® B Manni-Plex® Cal-Zn and the 0.5 $g.L^{-1}$ Spraybor® Calsol® treatments having a higher Fv/Fm value than the Manni-Plex® B Calsol® and the 1.0 $g.L^{-1}$ Spraybor® Calsol® treatments (Table 32). In March 2016, exposed fruit from the eastern side of the apple tree showed significant differences between treatments. The 1.0 $g.L^{-1}$ Spraybor®

Calsol® and 0.5 g.L^{-1} Spraybor® Calsol® treatments had a significantly higher Fv/Fm values than the control treatment (**Error! Reference source not found.**). The exposed fruit at the western side of the apple tree showed the highest photo-oxidative damage in the 2015/2016 season, with consistently lower Fv/Fm values compared to exposed fruits on the eastern side and shaded fruit from both sides of the apple tree.

3.4 Leaf Gas exchange

In the first season, there were no significant differences between treatments for net CO_2 assimilation rate (A) or transpiration rate (E) in December 2014. In March 2015, there were significant differences between treatments for both A and E values (Table 33). In both cases the Manni-Plex® B Calcinit™ treatment had higher A and E values than the Spraybor® Calcinit™ treatment, and also a higher A value compared to the control and Manni-Plex® B Manni-Plex® Ca treatments (**Error! Reference source not found.**). In the second season, there were no significant differences between treatments for A or E in February 2016.

3.5 Pink Colour development

There were no significant differences between treatments from January to March 2016 in terms of pink blush development. However, a significant difference was observed in April 2016, where fruit treated with Manni-Plex® B Calcinit™, showed significantly more blushed fruit than the Spraybor® Calcinit™ treatment (**Error! Reference source not found.**).

3.6 Weather data

In season 1, 2014/2015 (Figure 12), the temperatures in the Stellenbosch area only reached ambient maximum temperatures that can induce sunburn browning on five days, with only one day reaching maximum temperatures above 38 ˚C for sunburn necrosis to occur. Maximum daily air temperature is highly correlated with FST (Schrader et al., 2003b), which can be predicted using the formula y= 1.17x + 6.21

(where y =FST and x = maximum air temperature), for weather conditions in Washington State, USA. Using this formula, the maximum daily temperature at which sunburn would occur could therefore be determined as 34 ˚C for SB and 38 ˚C for SN (Figures 2 and 3). These temperatures are however relative with other factors like wind velocity and humidity also greatly contributing to FST. Both seasons had one day that had ambient temperatures that would allow FST to reach above 51˚C and cause SN. However, in the second season, temperatures were higher than those in the first season, of which 17 days had temperatures reaching the threshold for inducing sunburn browning versus the first season (2014/2015) which had only five such days of temperatures to induce sunburn browning.

4. Discussion

In this trial, some physiological effects of foliar combinations of Ca and B mineral applications on sunburn reduction were studied. The mode of action behind the amelioration of sunburn damage recorded in the study by Lötze and Hoffman(2014) is still unknown. Since sunburn is a physiological disorder with physical repercussions (Racsko and Schrader, 2012), this study looked into the possibility that Ca and B applications produced a physiological effect that results in the reduction of sunburn.

The hypothesised effect the treatments would have on the physiology was not realised in terms of a lower FST that would result in a lower sunburn incidence. There were no significant differences between treatments in terms of FST for months February to April 2015. In January 2015, a difference was observed between FST for the Manni-Plex® B treatment, which had the lowest FST, and the Spraybor® Manni-Plex® Ca treatment, which had the highest FST between all the treatments. The ambient temperature while these temperatures were measured was 26.13˚C. According to the formula from Schrader et al. (2003b), the FST in such ambient conditions should be about 11˚C higher. Only the Manni-Plex® B treatment reduced the temperature difference below the 11˚C threshold.

Nevertheless, other factors could also have influence the FST such as relative humidity, solar radiation and wind velocity (Schrader et al., 2003b). An increase of wind velocity from 0.5 to 3.5m.s^{-1} reduces FST by about 5˚C, due to the disruption of the boundary layer. During our recording of FST, wind velocity was 4.25 $m.s^{-1}$. In fact, wind velocity fell within the range determined by the regression equation of Schrader et al. (2003b) for all thermal imaging recording dates. However, wind velocity is largely influenced by row orientation, canopy conditions as well as the presence of wind breaks in the orchard which were present in this trial. The values mentioned in the tables are therefore subject to these factors and are assumed considerably lower in the orchard. The higher FST than expected (above 11˚C from ambient temperature) in January 2015 can also be attributed to the high solar radiation during data recording. According to Schrader et al. (2003b), maximum FST can be estimated with solar radiation from: maximum FST = 0.03(mean solar radiation) + 22.83. In January 2015, the expected FST would therefore be 52.8˚C. This was not the case as ambient temperature was relatively low, relative humidity was high and wind velocity is assumed to have reduced the FST.

The second season was warmer with more days where fruit were exposed to temperatures at which they are at greater risk to sunburn. Maximum daily air temperature is highly correlated with FST (Schrader et al., 2003b), which could be predicted using the formula y=1.17x + 6.21 (where y =FST and x = maximum air temperature). This formula is however based on weather conditions which exist in Washington State, USA and has not been verified for South African conditions. Between the seasons, the FST was higher than ambient temperature in the second season, in some treatments more than 20˚C higher (3rd April 2016). This also shows that the second year, more severe conditions were observed which also resulted in a higher sunburn incidence. The second season was considerably warmer, with more days that had temperatures conducive to sunburn. This resulted in a higher visible sunburn incidence in the second season compared to the first season, in all sunburn classes (paper 1).

In addition to the maximum temperature determined by the thermal imaging camera, the laser gun was used to determine the average FST on the exposed side of fruit. For the first season, significant differences were observed on 26th November, which was two days after the first foliar application. The control treatment had a significantly lower

FST than the Manni-Plex® B Calcinit™ treatment, which was perhaps due to a lower reflection of radiation by cuticular wax (Racsko and Schrader, 2012), in response to some of the applied minerals of this formulation still being present on the fruit surface. This would however need to be substantiated with applicable measurements.

In 2015, fruit surface temperatures were measured on fruits from both the east and west sides of the apple trees. Fruit surface temperatures on the western side were higher than those of the eastern side, which would substantiate findings that fruit borne on north-western sides on trees in the southern hemisphere are more susceptible to sunburn (Gindaba and Wand, 2007; Racsko and Schrader, 2012), due to increased FST. Significant differences in fruit surface temperatures between Manni-Plex® B and Manni-Plex® B Calcinit™ treatments were observed on the fruits from the eastern side of the apple trees in February 2015. It seemed that the addition of Calcinit™ (a calcium nitrate), instead of Manni-Plex® Ca (a calcium chelate) in combination with Manni-Plex® B significantly reduced FST by almost 5˚C. In the second season, significant differences were observed for the 10:00 measurement in March 2016 where the control, Manni-Plex® B Manni-Plex® Cal-Zn and 0.75 g Spraybor® Calsol® treatment combinations had significantly lower FST than the 0.5 g Spraybor® Calsol® treatment. This indicated that the foliar applications do not decrease FST directly as hypothesised, especially at such a late time of the season. In April 2016, for the 15:00 measurement, the Manni-Plex® B Calsol® treatment had a significantly lower FST than the 0.75 g Spraybor® Calsol®. In both instances, this did not correlate with the sunburn incidence quantified for each foliar treatment as the observed reduced FST values did not relate or result into reduced sunburn incidence in the treated apple fruits.

In all treatments, colour development was slow in January and February 2015, with exponential increases in March and April 2015 on all labelled fruit. No relationship was found between colour development and FST. However, the Manni-Plex® B Calcinit™ treatment showed significantly better colour development than the Spraybor® Calcinit™ treatment. Furthermore, the first season's (2014/2015) data that was affected by the red-blush colour masking of sunburn phenomenon, perhaps resulting in non-significant differences between treatments for class 1. This changed in the second season when fruit was harvested earlier and resulted in significant differences in sunburn incidence between treatments.

When considering FST data from both the thermal imaging and the infrared temperature measurement, it can be concluded that there is no relationship between FST as influenced by the different treatments and sunburn incidence. However, with regards to FST only, neither season indicated heat stress conditions that would induce sunburn. In fact, the daily maximum temperatures recorded during the season, showed only a few days of heat stress in the first season. Sunburn did occur nevertheless indicating the contribution of the other factors discussed before. The orchard used for this trial on the Welgevallen Experimental Farm, Stellenbosch, is highly prone to sunburn, which could be factor that contributed to sunburn incidence.

Sunburn masking by pigmentation did not affect chlorophyll fluorescence measurements (Fv/Fm), which indicated the maximum quantum efficiency of photosystem II, show stress tolerance of the apples if to excessive light and FST, for values below 0.6. Thus, these measurements were used to monitor the development of stress damage over the season and the onset of sunburn in this bi-colour apple cultivar. According to Wünsche et al. (2001), chlorophyll fluorescence can be correlated directly with visual sunburn score. This is however more difficult with blushed or red colour cultivars, due to masking of sunburn browning (Makeredza et al., 2015). Conversely, in this trial where sunburn occurred, there were no significant incidences where permanent stress occurred. Measurements in the first season were performed on fruit harvested early in the morning, which gave Fv/Fm values of photosystems that had repaired themselves over night. In the second season, fruit were harvested late afternoon, to show some photo-oxidative damage that would have occurred during the day. The Fv/Fm values were therefore slightly lower.

Nevertheless, for both seasons, no significant permanent stress could be identified based on the threshold value of 0.6. However, as can be seen in **Error! Reference source not found.**, the exposed side of fruits on the western side of trees (i.e. fruit that received afternoon sun) had Fv/Fm values below 0.8 throughout the season, compared to values above 0.8 in the shaded fruit. The east-west differentiation of the fluorescence measurements in the second season clearly shows that west-facing trees were exposed to higher stress conditions than those facing the east. This correlates with FST measurements which also showed higher FST on the west-facing side.

The control treatment continuously had the lowest Fv/Fm values (not significant) which was also associated with a significantly higher Class 1 sunburn percentage at harvest compared to the Ca and B treatments formulation, in the second season. Thus, although Fv/Fm of fruit in this trial did not reach the low values usually associated with stress, the lower values recorded in the control showed a trend that associated with a higher incidence of class 1 sunburn than the Ca and B treatments with a slightly higher Fv/Fm. The reduction of Fv/Fm in fruit could therefore not be the mode of action by which the foliar applications of the Ca and B combinations, contrary to the hypothesis of this particular experiment. In the study by Makeredza et al. (2015), the lowest Fv/Fm values were measured on fruit with sunburn browning at harvest compared to fruit that had no sunburn. A lower Fv/Fm value can be indicative of sunburn damage, especially if it falls below the 0.6 threshold, which indicated permanent damage. A higher Fv/Fm value could indicate that the fruit is more prone to become sunburnt; however, it is still able to recover.

In the first season (2014/2015), the Manni-Plex® B Calcinit™ treatment had a significant positive effect on photosynthesis as well as transpiration when compared to the control and other treatments. If the tree is photosynthesising well and transpiration is high, it indicates that the tree is not stressed. Since water stress increases sunburn incidence, one could relate a high transpiration rate to a reduced risk of sunburn for fruit on such trees. It was however not possible to relate this effect to a significant reduction of sunburn.

Conclusions

In conclusion, although there were significant differences between treatments with respect to all physiological parameters quantified in this study at some point, none of the effects could be related directly to a reduction in sunburn incidence, neither could the physiological changes as a result of the treatments explain the mode of action of the significant reduction of sunburn incidence observed. Thus, continued research into the mode of action of the foliar combination of Ca and B early during the season needs to be conducted in alternative fields such as biochemical and/or metabolic reactions. Additionally, formula should be developed similar to those developed for Washington State (USA) condition to predict sunburn incidence based on various environmental parameters.

Acknowledgements

Funding for these trials was provided for by Nulandis (Pty) Limited, South Africa.

5. References

Chen, L.S., Li, P., Cheng, L., 2008. Effects of high temperature coupled with high light on the balance between photooxidation and photoprotection in the sun-exposed peel of apple. Planta 228, 745–756. doi:10.1007/s00425-008-0776-3

Gindaba, J., Wand, S.J.E., 2007. Do fruit sunburn control measures affect leaf photosynthetic rate and stomatal conductance in “Royal Gala” apple? Environ. Exp. Bot. 59, 160–165. doi:10.1016/j.envexpbot.2005.11.001

Gindaba, J., Wand, S.J.E., 2005. Comparative effects of evaporative cooling, kaolin particle film, and shade net on sunburn and fruit quality in apples. HortSci. 40, 592–596.

Hengari, S., Theron, K.I., Midgley, S.J.E., Steyn, W.J., 2014. Response of apple (*Malus domestica* Borkh.) fruit peel photosystems to heat stress coupled with moderate photosynthetic active radiation at different fruit developmental stages. Sci. Hortic. 178, 154–162. doi:10.1016/j.scienta.2014.08.019

Iglesias, I., Alegre, S., 2006. The effect of anti-hail nets on fruit protection, radiation, temperature, quality and profitability of “Mondial Gala” apples. Journal of Applied Horticulture 8, 91–100.

Jackson, J.E., 2003. Biology of apples and pears. Cambridge Univ. Press, 501. doi:10.1017/CBO9780511542657

Jenkins, G.I., 2009. Signal transduction in responses to UV-B radiation. Annu. Rev. Plant Biol. 60, 407–431. doi:10.1146/annurev.arplant.59.032607.092953

Lötze, E., Hoffman, E.W., 2014. Foliar application of calcium plus boron reduces the incidence of sunburn in “Golden Delicious” apple. J. Hortic. Sci. Biotechnol. 89, 607–612. doi:10.1080/14620316.2014.11513127

Makeredza, B., Marais, H., Schmeisser, M., Lötze, E., Steyn, W.J., 2015. Ripening associated red color development masks sunburn browning in apple pee. Hort Sci. 50, 814–818.

Makeredza, B., 2011. Studies Towards understanding Sunburn in apple. MSc Thesis, Faculty of AgriScience, University of Stellenbosch, Stellenbosch.

Marais, S., 2005. Sunburn control in apple fruit. MSc Agric thesis. Faculty of AgriScience. University of Stellenbosch. Stellenbosch.

Moffat, T.F. 2013. Sensor technology to assess grape bunch temperature variability in *Vitis vinifera L.* cv. Shiraz. MSc thesis. University of Stellenbosch.

Parchomchuk, P., Meheriuk, M., 1996. Orchard cooling with pulsed overtree irrigation to prevent solar injury and improve fruit quality of “Jonagold” apples. HortSci. 31, 802–804.

Racsko, J., Schrader, L.E., 2012. Sunburn of apple fruit: Historical background, recent advances and future perspectives. CRC. Crit. Rev. Plant Sci. 31, 455–504. doi:10.1080/07352689.2012.696453

Ritchie, G. a, 2006. Chlorophyll Fluorescence : What is it and what do the numbers mean? USDA For. Serv 43. Proc. 34–43.

Rozema, J., van de Staaij, J., Björn, L.O., Caldwell, M., 1997. UV-B as an environmental factor in plant life: stress and regulation. Trends Ecol. Evol. 12, 22–28. doi:10.1016/S0169-5347(96)10062-8

Schrader, L., Sun, J., Felicetti, D., Seo, J.-H., Jedlow, L., Zhang, J., 2003a. Stress-induced disorders: Effects on apple fruit quality. Washington. Tree Fruit Postharvest Conf. 1–7.

Schrader, L., Sun, J., Zhang, J., Felicetti, D., Tian, J., 2008. Heat and light-induced Apple Skin Disorders: Causes and Prevention. Acta Hort. 51–58. doi:10.17660/ActaHortic.2008.772.5

Schrader, L., Zhang, J., Sun, J., 2003b. Environmental stresses that cause sunburn of apple. Acta Hort. 618, 397–405.

Schrader, L.E., Kahn, C., Elfving, D.C., 2009. Sunburn browning decreases at-harvest internal fruit quality of apples (*Malus domestica* Borkh.). Int. J. Fruit Sci. 9, 425–437. doi:10.1080/15538360903378781

Schrader, L.E., Zhang, J., Duplaga, W.K., 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Plant Heal. Prog. doi:10.1094/PHP-2001-1004-01-RS

Solovchenko, A., Schmitz-Eiberger, M., 2003. Significance of skin flavonoids for UV-B-protection in apple fruits. J. Exp. Bot. 54, 1977–1984. doi:10.1093/jxb/erg199

Steyn, W.J., Wand, S.J.E., Jacobs, G., Rosecrance, R.C., Roberts, S.C., 2009. Evidence for a photoprotective function of low-temperature-induced anthocyanin accumulation in apple and pear peel. Physiol. Plant. 136, 461–472. doi:10.1111/j.1399-3054.2009.01246.x

Van den Ende, B., 1999. Sunburn Management. Compact Fruit tree 32, 13–14. doi:10.1017/CBO9781107415324.004

Wünsche, J.N., Greer, D.H., Palmer, J.W., Lang, A., McGhie, T., 2001. Sunburn- the cost of a high light environment. VII International Symposium on Orchard and Plantation Systems *557,* 349–356.

Yang, S.A., Endo, K., 1994. Acclimation temperature affects activities of 5’-nucleotidase and acid phosphatase and lipid and fatty acid composition in carp muscle microsomes. J. Food Sci. 59, 1009–1012. doi:10.1111/j.1365-2621.1994.tb08178.x

Yuri, J.A., Neira, A., Quilodran, A., Razmilic, I., Motomura, Y., Torres, C., Palomo, I., 2010. Sunburn on apples is associated with increases in phenolic compounds and antioxidant activity as a function of the cultivar and areas of the fruit. J. Food Agric. Environ. 8, 920–925.

Zhang, J., Di, B., Li, Y., Zhang, J., Sun, J., Liu, Y., 2007. Effect of microenvironments and exogenous substance application on 5???-nucleotidase activities in apple peel. Front. Agric. China 1, 67–71. doi:10.1007/s11703-007-0012-0

6. Tables and Figures

7. Table 24: Treatment composition for both seasons applied as foliar application to 'Cripps' Pink' at Welgevallen Experimental farm, Stellenbosch.

2014/2015 Season		2015/2016 Season	
Control	-	Control	-
0.6ml* Manni-Plex B	1ml Manni-Plex Ca	0.6ml Manni-Plex B	1ml Manni-Plex Cal-Zn
0.6ml Manni-Plex B	6.8 ml Calcinit	0.6ml Manni-Plex B	6.5g Calsol
1g Spraybor	1ml Manni-Plex Ca	0.5g Spraybor	6.5g Calsol
1g Spraybor	6,8ml Calcinit	0.75g Spraybor	6.5g Calsol
0.6ml Manni-Plex B	-	1g Spraybor	6.5g Calsol

*Quantities given were mixed with 1 litre of water.

Table 25: Maximum FST (°C) (by thermal imaging) of 'Cripps' Pink' apples at Welgevallen Experimental Farm, Stellenbosch, during the season (2014/2015) between 12:00 and 14:00, in response to foliar applications of calcium and boron, with mean weather conditions presented for time of measurement (12:00 to 14:00).

Weather parameters/ Treatments	**Temp. 30-Jan**	**Temp. difference**	**Temp. 20-Feb**	**Temp. difference**	**Temp. 30-Mar 2015**	**Temp. difference**	**Temp. 24-Apr 2015**	**Temp. difference**	**Total Sunburn without Necrosis (%)****	**Necrosis (%)****
Ambient temperature (˚C)	26.1	-	26.3	-	26.5	-	25.4	-	-	-
Relative Humidity (%)	57.3	-	47.9	-	45.7	-	33.2	-	-	-
Solar Radiation ($W.m^{-2}$)	998.0	-	929.3	-	752.0	-	646.0	-	-	-
Wind Velocity ($m.s^{-1}$)	4.3	-	2.8		1.9		1.3	-	-	-
Control	37.3 ^{ab*}	11.1	36.4 ^{ns}	10.1	34.4 ^{ns}	7.9	40.0 ^{ns}	14.6	17.2 ^{ns}	3.2 ^{ns}
***M B & M Ca	38.6^{ab}	12.5	35.3	9.1	34.0	7.5	39.4	14.0	20.8	4.1
M B & Calcinit	38.4 ^{ab}	12.2	35.8	9.5	33.6	7.1	38.9	13.5	15.8	2.6
Spraybor & M Ca	41.4 ^{a}	15.3	34.8	8.6	35.0	8.5	39.7	14.3	23.0	2.7
Spraybor & Calcinit	37.8 ^{ab}	11.7	36.1	9.9	36.1	9.5	39.3	13.9	20.3	3.3
M B	36.8 ^{b}	10.7	39.8	13.5	35.0	8.4	40.7	15.3	18.6	4.1
P	*0.0226*	-	*0.1269*	-	*0.5973*	-	*0.7566*	-	*0.1970*	0.4668

*letters indicating significant differences for $P < 0.05$
**Data represented from Chapter 1
*** M is an abbreviation for Manni-Plex

Table 26: Maximum FST (°C) of 'Cripps' Pink' apples at Welgevallen Experimental Farm, Stellenbosch, towards the end of the second season (2016), in response to foliar applications of calcium and boron, with mean weather conditions during time of measurement (12:00 to 14:00).

Weather parameters/ Treatments	25-Mar 2016	Temp. difference	3-Apr 2016	Temp. difference	Total Sunburn without necrosis (%)**	Necrosis (%)**
Ambient temperature (˚C)	25.4	-	23.4	-	-	-
Relative Humidity (%)	53.0	-	39.1	-	-	-
Solar Radiation ($W.m^{-2}$)	460.0	-	832.0	-	-	-
Wind Velocity ($m.s^{-1}$)	2.8	-	1.6	-	-	-
Control	39.7 ^{ns}	14.3	41.1 ^{ns}	17.8	47.0 ^{ns}	9.1 ^{ns}
***M B & M Cal-Zn	37.4	12.0	43.8	20.4	34.6	14.0
M B & Calsol	38.8	13.4	41.0	17.7	35.8	10.9
0.5 $g.L^{-1}$ Spraybor & Calsol	40.3	14.9	43.1	19.7	32.6	14.1
0.75 $g.L^{-1}$ Spraybor & Calsol	39.4	14.1	40.2	16.8	34.8	9.6
1.0 $g.L^{-1}$ Spraybor & Calsol	37.6	12.3	41.3	18.0	44.0	8.1
P	0.4286		0.4748		0.1212	0.3586

*letters indicating significant differences for P < 0.05
**Data represented from Chapter 1
*** M is an abbreviation for Manni-Plex

Table 27: FST (°C) of 'Cripps' Pink' apples at Welgevallen Experimental Farm, Stellenbosch, during the 2014/2015 season, in response to foliar applications of calcium and boron, as measured with an infrared thermometer, with mean weather conditions during time of measurement.

Weather parameters/ Treatments	26-Nov	27-Nov	2-Dec	8-Dec	30-Jan	20-Feb	24-Apr
Mean Temperature (˚C)	19.9	20.4	21.0	n/a	26.4	21.7	25.3
Solar Radiation ($W.m^{-2}$)	389.6	542.1	1112.6	n/a	1028.0	821.2	667.6
Relative Humidity (%)	63.4	65.7	59.3	n/a	54.9	44.7	33.2
Wind Velocity ($m.s^{-1}$)	4.1	4.0	7.2	n/a	3.8	1.9	1.3
Control	22.5 ^{b*}	18.6 ^{ns}	27.9 ^{ns}	34.6 ^{ns}	31.0 ^{ns}	29.6 ^{ns}	34.2 ^{ns}
**M B & M Ca	23.0 ^{ab}	18.4	29.1	34.0	31.5	28.9	34.2
M B & Calcinit	24.0 ^{a}	18.6	28.4	33.4	31.5	28.5	33.4
Spraybor & M B	23.7 ^{ab}	18.8	29.9	34.2	31.9	30.4	35.0
Spraybor & Calcinit	22.8 ^{ab}	18.5	28.7	33.5	32.2	28.8	35.0
M B	23.3 ^{ab}	18.8	28.1	34.4	32.2	30.2	33.8
P	*0.0349*	*0.2698*	*0.6937*	*0.9128*	*0.4950*	*0.2514*	*0.7787*

*letters indicating significant differences for P < 0.05
** M is an abbreviation for Manni-Plex

Table 28: FST (°C) of 'Cripps' Pink' apples at Welgevallen Experimental Farm, Stellenbosch, as measured with an infrared thermometer from January to April, in response to foliar applications of calcium and boron, with east-west differentiation for 2014/2015.

Treatment	30-Jan (˚C)		20-Feb (˚C)		24-Apr (˚C)		Total Sunburn (%)**	
	West	**East**	**West**	**East**	**West**	**East**	**West**	**East**
Control	30.7 ns	31.3 ns	28.5 ns	30.7 ba*	35.3 ns	33.1 ns	22.2 ns	18.7 ns
**M B & M Ca	30.9	32.0	28.1	29.8 ba	35.7	32.8	30.8	20.9
M B & Calcinit	31.5	31.6	28.5	28.5 b	35.2	31.6	21.1	15.3
Spraybor & M B	31.5	32.2	30.1	30.6 ba	36.5	33.4	27.5	23.6
Spraybor & Calcinit	32.4	32.1	28.4	29.3 ba	37.6	32.5	25.8	21.8
M B	31.3	33.1	27.2	33.2 a	33.2	34.3	25.0	19.1
P	0.6852	0.5035	0.1717	0.0344	0.3641	0.5931	0.3288	0.5283

*letters indicating significant differences for $P < 0.05$
**Data represented from Chapter 1
*** M is an abbreviation for Manni-Plex

Table 29: FST (°C) of 'Cripps' Pink' apples at Welgevallen Experimental Farm, Stellenbosch, as measured with an infrared thermometer from January to April, in response to foliar applications of calcium and boron, with east-west differentiation for 2014/2015.

Treatment	30-Jan (˚C)		20-Feb (˚C)		24-Apr (˚C)		Total Sunburn (%)**	
	West	**East**	**West**	**East**	**West**	**East**	**West**	**East**
Control	30.7 ns	31.3 ns	28.5 ns	30.7 ba*	35.3 ns	33.1 ns	22.2 ns	18.7 ns
**M B & M Ca	30.9	32.0	28.1	29.8 ba	35.7	32.8	30.8	20.9
M B & Calcinit	31.5	31.6	28.5	28.5 b	35.2	31.6	21.1	15.3
Spraybor & M B	31.5	32.2	30.1	30.6 ba	36.5	33.4	27.5	23.6
Spraybor & Calcinit	32.4	32.1	28.4	29.3 ba	37.6	32.5	25.8	21.8
M B	31.3	33.1	27.2	33.2 a	33.2	34.3	25.0	19.1
P	0.6852	0.5035	0.1717	0.0344	0.3641	0.5931	0.3288	0.5283

*letters indicating significant differences for P < 0.05
**Data represented from Chapter 1
*** M is an abbreviation for Manni-Plex

Table 30: FST (°C) of 'Cripps' Pink' apples at Welgevallen Experimental Farm, Stellenbosch, as measured with an infrared thermometer from 10:00 to 15:00 in March and April 2016, with mean weather conditions during time of measurement (10:00 to 15:00).

	10 March 2016			8 April 2016		
Weather parameters/ Treatments	**10am**	**13pm**	**15pm**	**10am**	**13pm**	**15pm**
Mean Temp (˚C)	20.6	22.7	23.5	23.3	28.4	28.9
Solar Radiation ($W.m^{-2}$)	545.8	934.9	844.5	462.7	776.1	686.5
Relative Humidity (%)	64.9	55.7	48.6	52.0	34.7	31.9
Wind Velocity ($m.s^{-1}$)	0.9	4.3	5.0	1.5	3.0	3.8
Control	26.5 [b*]	31.8 [ns]	27.9 [ns]	21.1 [ns]	31.9 [ns]	29.4 [ab]
**M B & M Cal-Zn	26.2 [b]	29	28.7	21.1	29.8	29.5 [ab]
M B & Calsol	28.0 [ab]	29.6	27.9	23.3	30.1	28.2 [b]
0.5 g.L-1 Spraybor & Calsol	31.1 [a]	31.2	28.8	23.3	30.4	28.6 [ab]
0.75 g.L-1 Spraybor & Calsol	26.4 [b]	30.5	29.3	22	29.8	32.1 [a]
1.0 $g.L^{-1}$ Spraybor & Calsol	28.9 [ab]	31	29.2	21.9	32.5	30.6 [ab]
P	*0.0017*	*0.3529*	*0.6952*	*0.4833*	*0.4798*	*0.0329*

*letters indicating significant differences for P < 0.05.
** M is an abbreviation for Manni-Plex

Table 31: Chlorophyll fluorescence (Fv/Fm) of 'Cripps' Pink' apple surfaces at Welgevallen Experimental Farm, Stellenbosch, after foliar application of calcium and boron during 2014/2015. Weather conditions on the day of measurement are also presented.

Weather parameters/ Treatments	**8-Dec-14**	**12-Dec-14**	**15-Dec-14**	**24-Apr-15**
Mean Temp (˚C)	31.5	25.4	23.1	23.9
Solar Radiation ($W.m^{-2}$)	n/a	n/a	n/a	610.7
Relative Humidity (%)	40.0	55.0	49.7	35.6
Wind Velocity ($m.s^{-1}$)	9.9	4.2	4.8	1.53
Control	0.864 ^{ns}	0.874 ^{b*}	0.838 ^{ns}	0.820 ^{ns}
**M B & M Ca	0.863	0.903 ^{a}	0.841	0.801
M B & Calcinit	0.858	0.900 ^{a}	0.846	0.829
Spraybor & M B	0.864	0.898 ^{ab}	0.853	0.812
Spraybor & Calcinit	0.850	0.884 ^{ab}	0.839	0.822
M B	0.850	0.878 ^{ab}	0.841	0.810
P	*0.3082*	*0.0012*	*0.2677*	*0.4096*

*letters indicating significant differences for P < 0.05
** M is an abbreviation for Manni-Plex

Table 32: Chlorophyll fluorescence (Fv/Fm) of 'Cripps' Pink' apple surfaces at Welgevallen Experimental Farm, Stellenbosch, after foliar application of calcium and boron during 2015/2016. Weather conditions on the day of measurement are also presented.

Weather parameters/ Treatments	**18 December 2015**	**19 January 2016**	**23 February 2016**	**25 March 2016**	**Total Sunburn (%)**	**Class 1 (%)**
Ambient Temp (˚C)	25.0	34.4	24.3	26.2	-	-
Radiation ($W.m^{-2}$)	1024.0	912.7	921.0	511.7	-	-
Humidity (%)	51.8	33.6	56.2	49.4	-	-
Wind speed (m/s^{-1})	3.1	2.9	2.6	2.4	-	-
Control	0.821^{ns}	0.795^{ns}	0.774 ^{bc*}	0.812 ^{b}	56.0 ^{ns}	15.9 ^{a}
***M B & M Cal-Zn	0.834	0.829	0.770 ^{c}	0.818 ^{b}	48.6	9.3 ^{b}
M B & Calsol	0.829	0.803	0.810 ^{a}	0.845 ^{ab}	46.7	8.9 ^{b}
0.5 $g.L^{-1}$ Spraybor & Calsol	0.836	0.836	0.801 ^{ab}	0.829 ^{ab}	46.7	8.0 ^{b}
0.7 5$g.L^{-1}$ Spraybor & Calsol	0.838	0.804	0.787 ^{abc}	0.828 ^{ab}	44.4	10.2 ^{b}
1.0 $g.L^{-1}$ Spraybor & Calsol	0.832	0.803	0.809 ^{a}	0.855 ^{a}	52.1	11.0 ^{b}
P	0.2098	0.0560	0.0247	0.0026	0.4561	0.0001

*letters indicating significant differences for $P < 0.05$

**Data represented from Chapter 1

*** M is an abbreviation for Manni-Plex

Table 33: Chlorophyll fluorescence of 'Cripps' Pink' apple surfaces at Welgevallen Experimental Farm, Stellenbosch, over the 2015/2016 season, after foliar application with calcium and boron. Measurements differentiate between the east and west side of the tree as well as the exposed and shaded side of fruits.

	19 December 2015				19 January 2016				23 February 2016				25 March 2016			
	West		East		West		East		West		East		West		East	
Treatment	**Exposed**	**Shaded**	**Exposed**	**Shaded**	**Exposed**	**Shaded**	**Exposed**	**Shaded**	**Exposed**	**Shaded**	**Exposed**	**Shaded**	**Exposed**	**Shaded**	**Exposed**	**Shaded**
Control	0.745^{ns}	0.870^{ns}	0.791^{ns}	0.875^{ns}	0.673^{ba}	0.871^{ba}*	0.783^{ns}	0.854^{ns}	0.648^{ns}	0.856^{ns}	0.725^{ns}	0.867^{ns}	0.737^{ns}	0.875^{ns}	0.778^{b}	0.858^{ns}
**M B & M Cal-Zn	0.788	0.873	0.803	0.871	0.766^{a}	0.874^{a}	0.808	0.868	0.652	0.856	0.740	0.833	0.718	0.874	0.812^{ba}	0.869
M B & Calsol	0.770	0.870	0.808	0.869	0.654^{b}	0.867^{ba}	0.828	0.861	0.713	0.867	0.800	0.858	0.799	0.880	0.827^{ba}	0.875
0.5 g.L^{-1} Spraybor & Calsol	0.784	0.871	0.813	0.875	0.799^{a}	0.873^{ba}	0.797	0.876	0.705	0.863	0.793	0.842	0.752	0.861	0.840^{a}	0.862
0.75 g.L^{-1} Spraybor & Calsol	0.792	0.866	0.822	0.873	0.699^{ba}	0.870^{ba}	0.784	0.862	0.697	0.864	0.731	0.855	0.767	0.870	0.822^{ba}	0.852
1.0 g.L^{-1} Spraybor & Calsol	0.774	0.866	0.819	0.870	0.655^{b}	0.854^{b}	0.829	0.874	0.731	0.872	0.781	0.853	0.816	0.879	0.850^{a}	0.874
P	*0.2792*	*0.6750*	*0.5049*	*0.5879*	*0.0229*	*0.0291*	*0.2992*	*0.4736*	*0.1668*	*0.2649*	*0.2512*	*0.4634*	*0.0508*	*0.8190*	*0.0080*	*0.7066*

*letters indicating significant differences for $P < 0.05$

** M is an abbreviation for Manni-Plex

Table 34: Leaf net CO_2 assimilation rate (A) and transpiration rate (E) of 'Cripps' Pink' apple trees at Welgevallen Experimental Farm, Stellenbosch, during both seasons, in response to foliar applications of calcium and boron.

Treatment	2 December 2014		27 March 2015		4 February 2016	
	A ($\mu mol.m^{-2}.s^{-1}$)	E (mmol)	A ($\mu mol.m^{-2}.s^{-1}$)	E (mmol)	A ($\mu mol.m^{-2}.s^{-1}$)	E (mmol)
Control	12.8[ns]	3.8[ns]	9.6[b*]	3.0[ab]	9.6[ns]	1.7[ns]
**M B & M Ca	12.5	3.0	8.8[b]	3.0[ab]		
M B & Calcinit	14.1	4.2	12.5[a]	4.1[a]	8.4	1.7
Spraybor & M B	11.8	3.3	10.8[ab]	3.9[ab]	-	-
Spraybor & Calcinit	12.3	3.3	9.1[b]	2.8[b]	-	-
M B	11.6	3.0	10.6[ab]	3.7[ab]	-	-
MB &M Cal-Zn					7.5	1.4
0.5 $g.L^{-1}$ Spraybor & Calsol	-	-	-	-	7.8	1.6
0.75 $g.L^{-1}$ Spraybor & Calsol	-	-	-	-	10.7	2.6
1.0 $g.L^{-1}$ Spraybor & Calsol	-	-	-	-	8.5	1.6
P	*0.4127*	*0.0860*	*0.0423*	*0.0087*	*0.2239*	*0.0604*

*letters indicating significant differences for $P < 0.05$
** M is an abbreviation for Manni-Plex

Table 35: Pink colour development of 'Cripps' Pink' apples at Welgevallen Experimental farm, Stellenbosch, in relation to maximum FST during the 2014/2015 season, following foliar applications of calcium and boron.

	30 Jan 2015		20 Feb 2015		30 Mar 2015		24 Apr 2015	
Treatment	**Pink colour**	**Max FST (˚C)**	**Pink colour**	**Max FST (˚C)**	**Pink colour**	**Max FST (˚C)**	**Pink colour**	**Max FST (˚C)**
Control	0.0^{ns}	37.3^{ab*}	0.9^{ns}	36.4^{ns}	3.2^{ns}	34.4^{ns}	7.8^{ab}	40.0^{ns}
**M B & M Ca	0.0	38.6^{ab}	0.4	35.3	2.0	34.0	6.8^{ab}	39.4
M B & Calcinit	0.0	38.4^{ab}	0.6	35.8	3.2	33.6	8.2^{a}	38.9
Spraybor & M Ca	0.1	41.4^{a}	0.5	34.8	2.7	35.0	7.0^{ab}	39.7
Spraybor & Calcinit	0.2	37.8^{ab}	0.2	36.1	1.7	36.1	5.4^{b}	39.3
M B	0.1	36.8^{b}	0.8	39.8	2.0	34.9	7.8^{ab}	40.7
P	0.1123	0.0226	0.2470	0.1269	0.1683	0.5973	0.0356	0.7566

*letters indicating significant differences for P < 0.
** M is an abbreviation for Manni-Plex

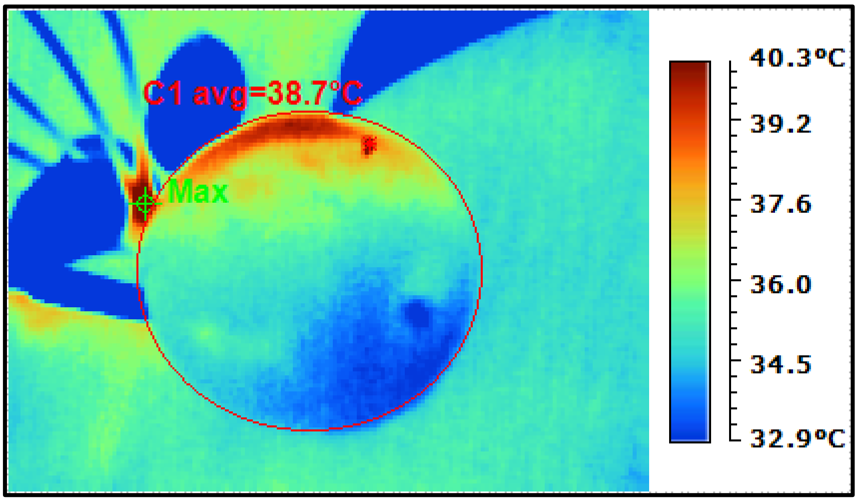

Figure 11: Thermal image of a ‘Cripps Pink’ fruit taken with a thermal imaging camera (RAZ-IR® NANO thermal camera), showing the encircling procedure to give maximum temperature on the fruit.

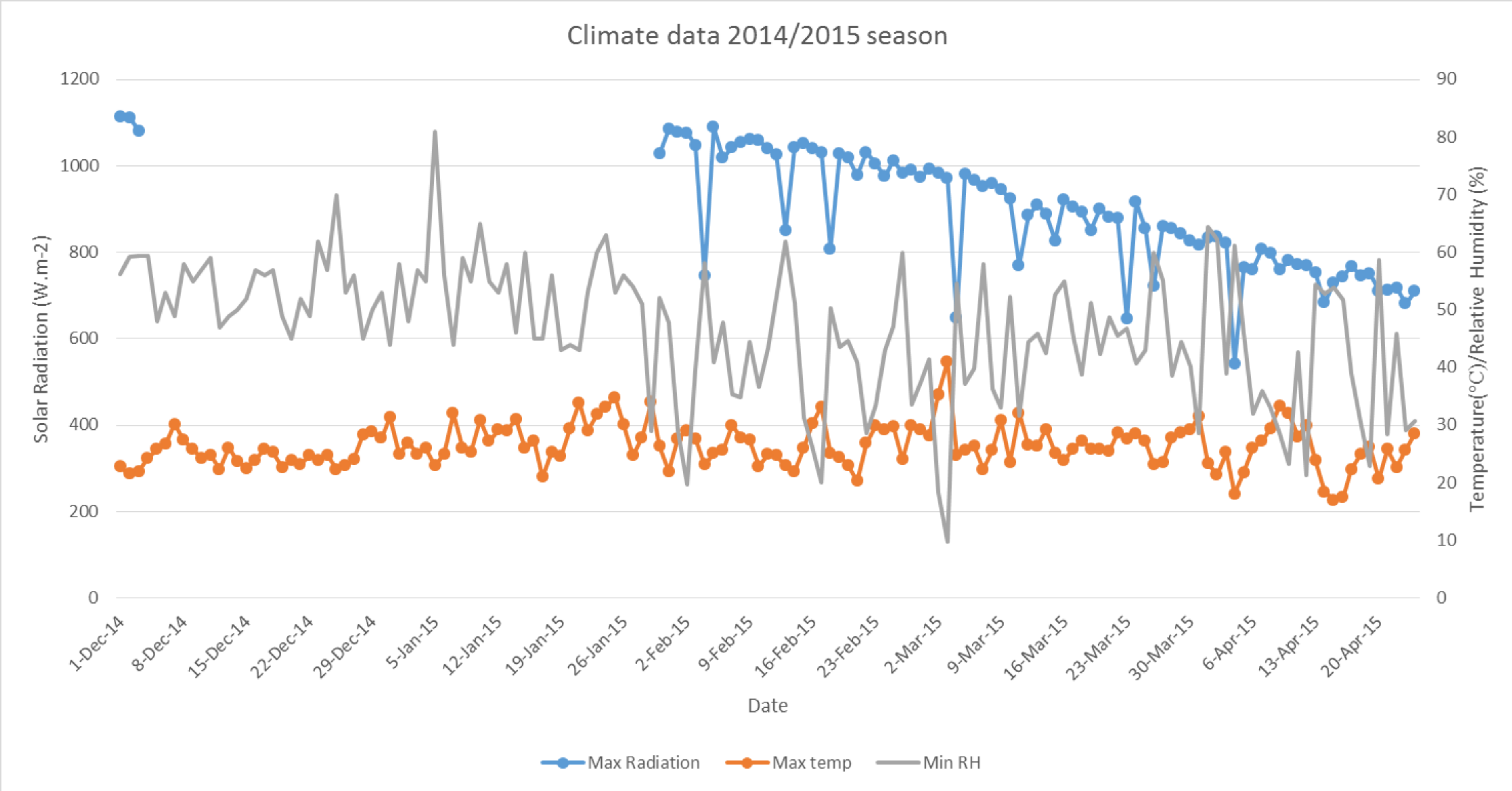

Figure 12: Daily weather data for 2014/2015 at Sonbesie weather station, Stellenbosch, with daily maximum solar radiation (W.m^{-2}) on the primary y-axis and daily maximum temperature (˚C) and daily minimum relative humidity (%) on the secondary y-axis.

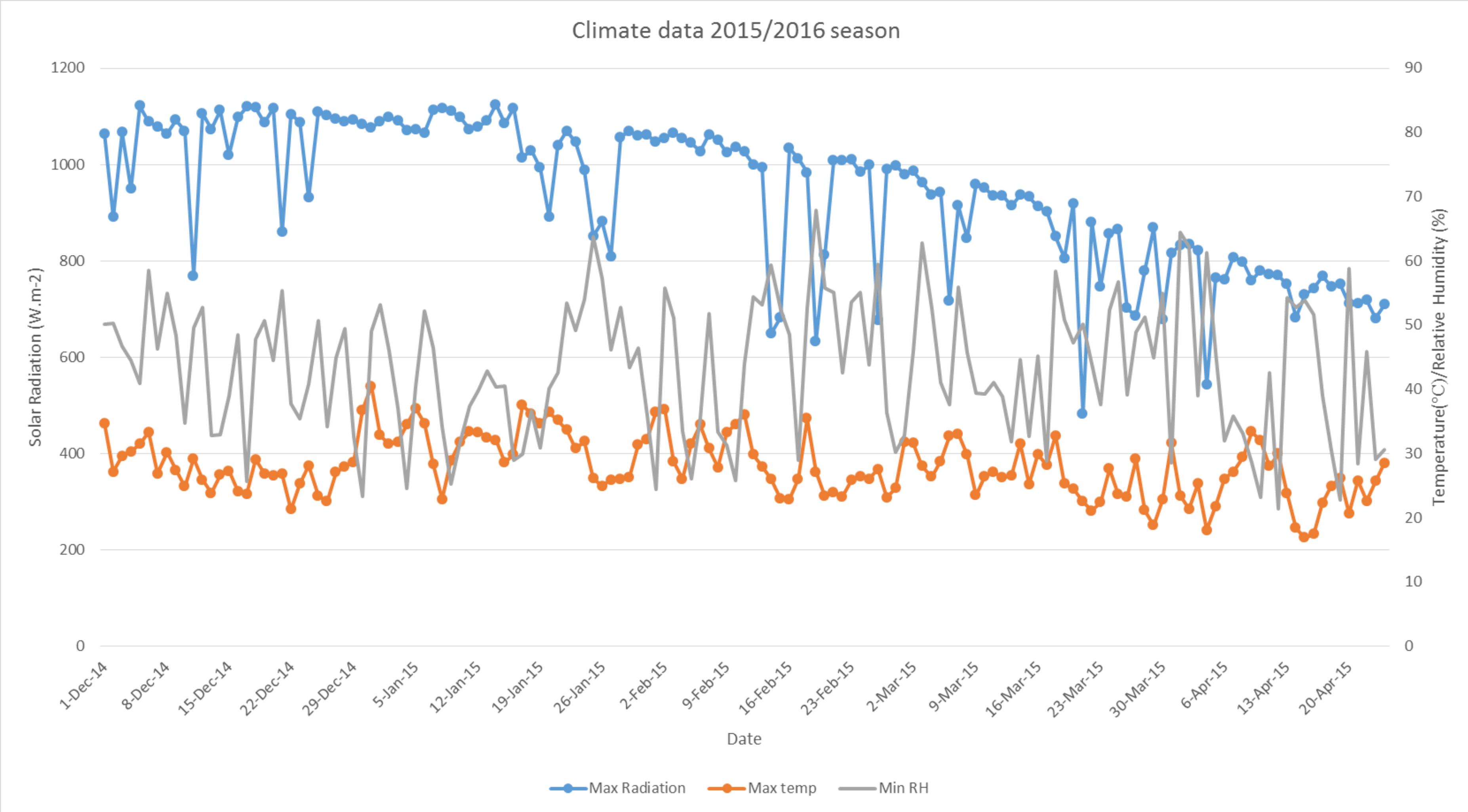

Figure 13: Daily climate data for 2015/16 at Sonbesie weather station, Stellenbosch with daily solar radiation (W.m^{-2}) on the primary y-axis and daily max temperature (˚C) and daily minimum relative humidity (%) on the secondary y-axis.

General Conclusion

As part of the main objective of this thesis, a mode of action for the reduction of sunburn from the application of foliar Ca and B, needed to be elucidated. This was done in response to initial findings of a reduction in sunburn on 'Golden Delicious' apples in response to the application of Ca and B. This objective was not achieved,however, the efficacy of similar Ca and B foliar treatments was confirmed on 'Cripps' Pink' apples and successful reduction on sunburn on 'Golden Delicious' apples could be achieved using an alternative treatment to the Manni-Plex® Ca Manni-Plex® B that was reported previously by Lötze and Hoffman (2014).

Sunburn incidence in class 1 sunburn category was reduced in 'Cripps' Pink' and 'Golden Delicious' apples in this study. With the former, the formulation did not play a role in terms of significant sunburn reduction of sunburn in class 1. All treatments had a significantly lower class 1 sunburn percentage than the control. Underestimation of sunburn severity of bicolour cultivars can easily occur in research, but can also be beneficial when marketing fruit, since visual appearance plays a big role in determining consumer acceptance. For 'Golden Delicious' apples, the 0.5 $g.L^{-1}$ Spraybor® Calsol® treatment was the only treatment that had significantly lower class 1 sunburn than the control and the Manni-Plex® Cal-Zn Manni-Plex® B treatment. A change in the Manni-Plex® Ca formulation from 2011-13 to 2015 seasons is presumed to have negatively affected the efficacy of this particular treatment formulation, which was able to reduce sunburn significantly in initial studies as reported by Lötze and Hoffman (2014). This was perhaps due to of the presence of zinc or the reduced content of calcium as an active ingredient. No significant reduction in sunburn was recorded on 'Granny Smith' apples, perhaps due to it being harvested prematurely or a lack of response of this cultivar to the treatments indicating variation in cultivar response.

A direct effect of cell wall and cuticle thickness properties and sunburn incidence could not be confirmed. However, the results did show trends of an effect of the foliar applications on changes in the peel cells with regards to cuticle thickness as well as cell wall thickness. Nevertheless, foliar applications of Ca and B did not directly reduce sunburn via physical changes in the peel. The results therefore do not explain the mode-of-action satisfactorily. Future research could be expanded to include SEM analyses of fruit before foliar Ca and B applications, as well as after the final application (72 dafb). A larger sample size to quantify the positive effects of these elements on cell wall and cuticle thickening is also important for future research.

In terms of physiological parameters quantified in this study, there were significant differences between treatments on a physiological level for both chlorophyll fluorescence and FST. However, none of the effects could be related directly to a reduction in sunburn incidence, neither could the physiological changes as a result of the treatments explain the mode of action of the significant reduction of sunburn incidence that was observed. Continued research into the mode of action of these foliar applications needs to be done, perhaps on a biochemical and/or metabolic level. Further research into the physiology of sunburn under Western Cape conditions would require the anticipation of weather conditions that are conducive to sunburn damage when doing physiological measurements on fruits. This was reflected in the inconclusive results on FST measurements throughout the season.

Fruit quality research remains important, with continued high demands on its standard to the international export market. Rising threats to fruit quality caused by changing climates further motivate research in this field. With this in mind, more research needs to be done with regards to heat and water stress in crops. Farming technologies that conserve water and provide efficient water use are imperative as South Africa's vulnerability to climate change rises. Insufficient water supply will become the norm rather than the exception in the future.

Sunburn incidence on apples, which increases in response to water stress, remains an area of concern. Even though shade nets are successful at eradicating sunburn completely, alternatives like foliar application of Ca and B seem promising, due to its ease of application as well as low infrastructural expenses. Further research into the mode of action therefore needs to be done, to harness this sunburn reducing strategy to its optimum potential.