
**A STUDY OF THE DYNAMICS OF SUNBURN REDUCTION IN APPLE
(*Malus domestica*) USING FOLIAR APPLICATIONS OF A COMBINATION
OF BORON AND CALCIUM**

By

Anthony Mwije

Dissertation presented for the degree of Doctor of Philosophy at Stellenbosch University



Promoter: Dr. E. Lötze

Department of Horticultural Science, Stellenbosch University

Co-Promoter: Dr. E. W. Hoffman

Department of Horticultural Science, Stellenbosch University

March 2020

SUMMARY

Apple fruit sunburn browning (SBB) is largely a climate-related disorder that reduces fruit quality leading to serious financial losses. The aim of this research was to unravel the mode-of-action by which post-full-bloom foliar boron plus calcium (B+Ca) suppress fruit SBB incidence in apple orchards. Scientific elucidation of such mode-of-action is essential for successful recommendation and adoption of this low-cost approach at farm level. Therefore, in this study, four varying foliar B+Ca formulations were applied post-full-bloom in orchards of a bi-colour ('Cripps Pink') and two green ('Golden Delicious' and 'Granny Smith') apple cultivars. The experimental sites / orchards were located in Elgin and Stellenbosch in Western Cape Province, South Africa, and the three cultivars chosen for this study sharply contrast in SBB susceptibility. A preceding and related study found that peel tissue anatomical differences and mineral concentrations did not to explain the SBB incidence reduction, hence this study focused on investigating the possibility of a biochemical-based mode-of-action. Thus, peel tissue biochemicals (phenolics, chlorophyll, carotenoid and total peroxides) were determined, and compared to SBB incidence at harvest. In 2014/15 season, fruit peel was sampled at four early maturity stages / days after full bloom (DAFB). In 2015/16, samples were collected at four bi-weekly intervals towards fruit harvest maturity window. Results showed that treatments influenced peel biochemical levels in varying degrees and with cases of significant ($p < 0.05$) interaction effects with fruit maturity stages. Significant SBB suppression occurred for 'Cripps Pink' (2015/16) and 'Golden Delicious' experiments, where the control (no B+Ca) had highest incidence. In 'Granny Smith', the SBB differences within treatments did not differ significantly. No treatment-induced changes in any peel biochemical occurred in patterns that could yield the sought mode-of-action; for instance, all treatments reduced SBB considerably in 'Cripps Pink', whereas it was the high B treatments (0.08 and 0.17 g.l⁻¹) in 'Golden Delicious'. This highlighted the influence of cultivar and treatment formulation in respective SBB suppression outcomes and corresponding in peel biochemical levels. With treatment formulation, inclusion of zinc (Zn) significantly lowered photosynthetic pigment attributes, and associated with SBB incidence that was on par with the control (no B+Ca). Overall, this study concluded that different cultivars require different foliar B+Ca formulations to suppress SBB, and generally, Zn should not be included. Further results from a multivariate analysis indicated that the sought mode-of-action could be under an additive-metabolite-action phenomenon, involving contributory roles of several peel biochemicals as influenced by particular

foliar B+Ca treatments. However, further studies are required to establish the physiological mechanism underlying this postulated mode-of-action.

OPSOMMING

Sonbrandverbruining in appels is grootliks 'n klimaatsgebonde defek wat vrugkwaliteit verlaag en lei tot ernstige finansiële verliese. Die doel van die navorsing was om die metode-van-werking van die boor plus kalsium (B+Ca) vooroesblaarspuitte, wat sonbrandverbruining (SBB) voorkoms in appelboorde verminder, te ontrafel. Die wetenskaplike toeligting van die metode-van-werking is essensieël vir die suksesvolle aanbeveling en implementering van die lae-koste benadering op plaas vlak. Dus is vier B+Ca formulasies na volblom toegedien op 'n twee-kleur ('Cripps Pink') en twee, groen ('Golden Delicious' en 'Granny Smith') appel kultivars. Die proefpersele/ boorde was geleë in Elgin en Stellenbosch in die Wes-Kaap provinsie, Suid-Afrika en die drie kultivars wat vir die studie gekies is, verteenwoordig ook skerp kontraste in SBB voorkoms. 'n Voorafgaande en verwante studie het gevind dat die skilweefselanatomie en minerale voedingselement-konsentrasies nie SBB voorkoms verlaging verklaar nie, daarom het hierdie studie gefokus op die moontlike biochemies – gebasseerde metode-van-werking. Dus is skilweefsel biochemiese komponente (fenole, chlorofil, karotenoïede en totale peroksiede) bepaal en vergelyk met die SBB voorkoms by oes. In die 2014/15 seisoen, is vrugskille gemonster op vier vroeë volwassenheid stadia/ dae na volblom (DNVB). In 2015/16 is monsters ingesamel op vier twee-weeklikse intervalle van die oesrypheidsvenster van vrugte. Resultate het getoon dat behandelings die vrug biochemiese vlakke in variërende grade beïnvloed het, asook met betekenisvolle ($p < 0.02$) interaksie effekte met vrugvolwasenheid stadia. Betekenisvolle SBB vermindering het voorgekom net in 'Cripps Pink' (2015/16) en 'Golden Delicious' proewe, waar die kontrole (geen B+Ca) die hoogste voorkoms gehad het. In 'Granny Smith', het geen betekenisvolle verskille in SBB tussen behandelings voorgekom nie. Geen behandelings-geïnduseerde veranderinge in die skilbiochemie het voorgekom in patrone wat die verlangde metode-van-werking kon verklaar nie; byvoorbeeld het al die behandeling SBB aansienlik verlaag in 'Cripps Pink' (2015/16), tenoor slegs behandelings met verhoogde B vlakke (0.08 en 0.17 g.l⁻¹) in 'Golden Delicious'. By formulasies vir die behandelings, het die insluit van sink (Zn) alle fotosintetiese eienskappe oor alle kultivars verlaag en was dit geassosieer met SBBB voorkoms wat soortgelyk was as die kontrole (geen B +Ca). Oorkoepelend het die studie bepaal dat verskillend kultivars verskillende behandelings vir effektiewe SBB-onderdrukking benodig, en dat Zn normaalweg nie ingesluit moet word nie. Verdere resultate van 'n veelvuldige analise het aangedui dat die verlangde metode-van-werking onder 'n fenomeen van bykomende-metaboliet-aksie kan funksioneer, wat die bydraende rolle van verskeie skil biochemiese komponente behels van spesifieke blaar B+Ca behandelings. Nietemin

is verdere studies nodig om die onderliggende fisiologiese meganisme van die gepostuleerde metode-van-werking te bepaal.

TABLE OF CONTENTS

Summary	i
Opsomming.....	iii
Table of contents.....	v
Conference presentations and publications.....	vi
Acknowledgements.....	vii
Dedication	viii
Declaration.....	ix
Note	x
General introduction	1
References	4
Literature review.....	6
1. Introduction.....	6
2. Current approaches used to mitigate apple fruit sunburn	7
3. Need for alternative approaches in mitigating apple fruit sunburn.....	9
4. Apple fruit and/or tree systemic approaches in mitigating fruit sunburn	10
5. Prospects of mitigating apple fruit sunburn using boron plus calcium.....	11
6. Possible approaches to unravel the boron plus calcium mode-of-action	13
7. Conclusion	19
References	21
PAPER I: Boron plus calcium induced changes in apple peel phenolic levels	39
PAPER II: Boron plus calcium induced changes in apple peel photosynthetic pigment levels ...	73
PAPER III: Boron plus calcium induced changes in apple peel oxidative stress levels.....	106
PAPER IV: Relating foliar boron plus calcium induced peel biochemical changes to suppression of sunburn browning incidence in ‘Golden Delicious’ apples	129
1. General discussion	152
2. General conclusion.....	157
References	159
Appendixes	167

CONFERENCE PRESENTATIONS AND PUBLICATIONS

a. Conference presentations

Mwije, A., Hoffman, E.W. and Lötze, E. 2016. Foliar nutrition regimes of combined boron and calcium minerals ameliorate photo-thermal stress tolerance in apple fruits. RUFORUM Biennial Conference, Cape Town South Africa, October 2016 (**Oral**)

Mwije, A., Hoffman, E.W. and Lötze, E. 2016. Foliar nutrition regimes of combined boron and calcium minerals ameliorate photo-thermal stress tolerance in apple fruits. RUFORUM Biennial Conference, Cape Town South Africa, October 2016 (**Poster**)

Mwije, A., Hoffman, E.W. and Lötze, E. 2018. Investigating mode-of-action by which boron plus calcium foliar applications reduce apple sunburn. African Combined Congress, Cape Town, South Africa, January 2018. (**Oral**)

Mwije, A., Hoffman, E.W. and Lötze, E. 2018. Sunburn incidence and polyphenol patterns as influenced by boron plus calcium foliar applications in ‘Cripps Pink’ apple. African Combined Congress, Cape Town, South Africa, January 2018. (**Oral**)

b. Publications

- i. **Mwije, A.** 2017. Pioneering a new paradigm of sunburn mitigation in apples. In: Bringing Science to Communities: Voices from the Field (Issue 1) (Ed. Withers, J). A RUFORUM Publication. Kampala, Uganda.
- ii. **Mwije, A.**, Hoffman, E.W. and Lötze, E. 2020a. Peel biochemical changes after foliar application of combined boron and calcium in ‘Cripps Pink’, ‘Golden Delicious’ and ‘Granny Smith’ apples. I. Phenolics and selected physico-chemical attributes. *Submitted for peer review*
- iii. **Mwije, A.**, Hoffman, E.W. and Lötze, E. 2020b. Peel biochemical changes after foliar application of combined boron and calcium in ‘Cripps Pink’, ‘Golden Delicious’ and ‘Granny Smith’ apples. II. Photosynthetic pigments, total peroxides and photochemical efficiency attributes. *Submitted for peer review*

ACKNOWLEDGEMENTS

I sincerely acknowledge Drs. E. Lötze and E.W. Hoffman for accepting me in their research team, and I am also very grateful to the previous Vice Chancellor of Makerere University, Professor John Ddumba-Ssentamu, and his then Deputy (Finance and Administration) Professor Barnabas Nawangwe (current Vice Chancellor), for accepting to fund my studies under the modalities of Staff Training and Development. I thank Professors Denis Mpairwe, Johnny Mugisha, and Bernard Bashaasha of College of Agricultural and Environmental Sciences, for recommending and supporting me during my studies. I am also very thankful to RUFORUM's Executive Secretary, Professor Adipala Ekwamu, Dr. Moses Osiru (then Deputy Executive Secretary), and Dr. Paul Nampala (then Grants Manager) for the doctoral study grant sourced from Carnegie Corporation of New York. I also thank former staff of RUFORUM Drs. Solange Uwituze and Sylvia Mkadawire for supporting me at early stages of this study program. I also thank Professor Leopoldt van Huyssteen (then Acting Rector Stellenbosch University), for accepting my post-graduate (Ph.D.) application in the Department of Horticultural Science at Stellenbosch University under the RUFORUM-GTA arrangement.

Everyone, regardless of achievements, begins and ends within family, as thus with heartfelt gratitude, I extend my very sincere acknowledgements to my wife Joanita, for keeping the family and our very young children in good shape and spirits whilst I was away for this long study period, and as well for reading through my manuscripts and keeping all the backups! To you Joanita, and our children Catherine, Giovanni and Jourdan, I am so glad that you kept strong all through the long period of my absence. I promise that I will always be there for all of you, especially the times when you need the best of my support!

Lastly, I ask God almighty to bless all of you, and as well as many not mentioned here. A limitation of writing-space is inevitable for me, as I have made many friends along this study journey. You will always be in my prayers for the valuable roles and assistances you rendered to me which have enabled me attain this significant career and academic milestone.

DEDICATION

To you, Nduhukire Meres, despite the enormous domestic challenges,
You kept a very confident attitude, becoming a beacon of immense strength in our home!
Your gifted hands worked so hard, perfectly negating the impasses about our daily bread!
My Mother! I Pray God Blesses You Abundantly With His Very Choicest Blessings!



DECLARATION

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

Date: March 2020

NOTE

This dissertation mainly consists of four chapters structured as independent research papers, therefore, slight repetition, particularly in the materials and methods was inevitable.

GENERAL INTRODUCTION

The Western Cape Province is the major production region of apple fruit in South Africa (DAFF, 2016). This region experiences a Mediterranean-type climate characterized by winter rainfall and high solar irradiance and temperature in the summer, a time when apple fruit is at advanced maturity. As thus, sunburn becomes a serious threat to production of quality apple fruit in this region (DAFF, 2016). Apple fruit sunburn is a physiological disorder where the peel tissue is only affected and is induced by solar irradiance and/or heat (temperature) stresses (Wünsche et al., 2004; Schrader et al. 2008; Racskó & Schrader, 2012).

Post-full-bloom foliar applications of combined boron plus calcium (B+Ca) are reportedly a potential alternative approach to reduce high apple fruit sunburn incidence (Lötze & Hoffman, 2014; Daiber, 2017; Lötze et al. 2017; 2018). In studies under South African conditions, involving ‘Cripps Pink’, ‘Golden Delicious’ and ‘Granny Smith’ apple cultivars, the foliar B+Ca treatments albeit varying in formulation reportedly had no negative effects on both the tree or fruit, and generally reduced sunburn browning (SBB) (most economically important type) (Daiber, 2017; Lötze et al., 2017; 2018). The main obvious advantage with this new approach is that it is less expensive compared to traditional strategies like orchard shade netting and overhead cooling. In addition, the foliar B+Ca approach has a low water footprint, as for instance there would be reduced water costs to clean and/or wash inert chemical stains as would be required with kaolin products. Thus, the foliar B+Ca approach is a potential approach to promote among commercial apple fruit growers to reduce fruit SBB phenomenon.

The post-full-bloom foliar B+Ca approach is novel in that it has no component to shield, reflect or absorb SBB inducing factors as observed in comparable existing methods (Schrader, 2011; Racskó & Schrader, 2012; Sotiropoulos et al., 2016). Neither can this new foliar B+Ca approach emulate evaporative cooling as detailed in previous studies of Gindaba & Wand (2005), Van Den Dool (2006) and Schrader et al. (2008). Thus, the mode-of-action by which post-full-bloom foliar B+Ca reduces apple fruit SBB incidence at harvest maturity is unknown. This presents a serious challenge to formulate this new approach for a commercial scale purpose or to refine existing protocols to accommodate possible unique cultivar requirements, as indicated by variable sunburn incidence reductions that emerged with different foliar B+Ca formulations (Daiber, 2017). Understanding the respective mode-of-action is thus critical for the promotion, refining and exploitation of this new

foliar B+Ca approach among commercial growers as observed with previous comparable developments of Schrader (2011).

Therefore, the main objective of the research described in four studies of this dissertation was to unravel the mode-of-action by which post-full-bloom foliar applications of B+Ca reduce apple fruit SBB incidence at harvest maturity. These studies focused on determining whether foliar B+Ca reduces SBB incidence by enhancing relevant protection metabolites in the fruit peel tissues, thus protecting against effects of excessive solar irradiance and temperature that induce and/or cause sunburn. The absence of apple fruit anatomical changes amongst foliar B+Ca treatments particularly with lower sunburn classes (Daiber, 2017), motivated this research approach, as well as the fact that SBB type (class 1 and 2) is most economically important. In addition, an earlier study by Schrader (2001) revealed no significant differences amongst cell membrane integrity in apple fruit with and without SBB. Further still, changes in apple fruit peel tissue biochemicals and/or metabolites are known to strongly associate with SBB induction and severity development (Felicetti & Schrader, 2008; 2009a, b; Yuri et al., 2010; Racskó & Schrader, 2012; Zupan et al., 2014).

In the studies described in this dissertation, experiments were conducted over two seasons (2014/15 and 2015/16). Apple fruit peel was sampled consecutively during the season following post-full-bloom foliar applications of varying foliar B+Ca treatments in one bi-colour ('Cripps Pink') and two green ('Golden Delicious' and 'Granny Smith') apples. In paper 1, the investigation aimed at determining possible foliar B+Ca induced changes in fruit peel phenolics and flavonoid levels at four selected early maturity stages as days after full bloom (DAFB), as well as at four maturity stages towards the harvest maturity window of respective cultivars. In this study, the three phenolic parameters considered were (i) total phenolics, (ii) total flavonoids and (iii), total flavonoids to total phenolics ratio. A possible mode-of-action for the foliar B+Ca mediated SBB incidence reduction was sought from relating variability and dynamics and/or trends of the three phenolic parameters at the selected fruit maturity stages to SBB incidence reduction outcomes recorded at harvest.

In paper 2, the objective of the study was to determine possible foliar B+Ca induced changes in apple fruit peel chlorophyll pigments and total carotenoids levels. The variability and trends of six parameters (chlorophyll *a*, *b*, *a* to *b* ratio, total chlorophyll (TCHL), total carotenoids (TCAR) and TCHL to TCAR ratio) among treatments were related to SBB incidence reduction outcomes recorded at harvest.

In paper 3, the effect of foliar B+Ca treatments on oxidative stress (total peroxides) levels of apple fruit peel tissue was studied. Again, the prospect of a possible mode-of-action for the foliar B+Ca treatments mediated apple fruit SBB incidence reduction was sought from relating variability and trends of total peroxides and SBB incidence reduction recorded outcomes at harvest.

As no single peel metabolite changes could explain the reduction of class 1 and 2 SBB in ‘Golden Delicious’ and ‘Cripps Pink’ apples satisfactorily, an alternative multivariate data analysis to yield a possible mode-of-action, was investigated in paper 4. The objective in this paper was to determine if seasonal metabolite dynamics in apple fruit peel, as induced by the varying foliar B+Ca treatments, had a significant relationship with the SBB incidence reduction for SBB classes 1 and 2. This involved using canonical correlation analysis (CCorA) for only ‘Golden Delicious’ apple peel biochemical data as ‘Cripps Pink’ data could not raise adequate sample size requirements for the CCorA.

Three types of CCorA were performed, involving: (i) full experiment observations, (ii) observations from treatments that were associated with least SBB incidence reduction and (iii), observations from treatments associated with best SBB incidence reduction. In these three CCorA scenarios, relationships were determined between two data sets that included nine apple peel metabolites and four sunburn incidence variables. Statistics and outputs from the respective CCorA indicated the strength or importance of the data components to the respective relationships between the data sets. Examining CCorA outputs from scenario (ii) and (iii), the involvement of several biochemical aspects in the significant model of scenario (ii) led to the proposition of an additive-metabolite-action (AMA) phenomenon as a postulate to the sought mode-of-action.

References

- DAFF. 2016. A profile of the South African apple market value chain. Directorate Marketing, Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa.
- Daiber, S.H. 2017. Quantifying changes in tree physiology after amelioration to reduce sunburn on apples. MSc Thesis. Department of Horticultural Science, Stellenbosch University.
- Felicetti, D.A. & Schrader, L.E. 2008. Changes in pigment concentrations associated with the degree of sunburn browning of 'Fuji' apple. *Journal of the American Society of Horticultural Science* 133:27-34.
- Felicetti, D.A. & Schrader, L.E. 2009a. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. II. Phenolics. *Plant Science* 176:84-89.
- Felicetti, D.A. & Schrader, L.E. 2009b. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. I. Chlorophylls and Carotenoids. *Plant Science* 176:78-83.
- 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. *HortScience* 40:592-596.
- Lötze, E. & Hoffman, E.W. 2014. Foliar application of calcium plus boron reduces the incidence of sunburn in 'Golden Delicious' apple. *Journal of Horticultural Science and Biotechnology* 89:607-612.
- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2017. Boron in combination with calcium reduces sunburn in apple fruit. *Boron* 2:123-127.
- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2018. Evaluating the efficacy of a preharvest combination of calcium and boron as foliar application to reduce sunburn on 'Cripps Pink' apples. *Acta Horticulture* 1217:61-68.
- Racskó, J. & Schrader, L.E. 2012. Sunburn of apple fruit: Historical background, recent advances and future perspectives. *Critical Reviews in Plant Sciences* 31:455-504.
- Schrader, L.E. 2011. Scientific basis of a unique formulation for reducing sunburn of fruits. *HortScience* 46:6-11.
- Schrader, L. E., Zhang, J., and Duplaga, W. K. 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Online. *Plant Health Progress* doi:10.1094/PHP-2001-1004-01-RS

- Schrader, L., Sun, J., Zhang, J., Felicetti, D. & Tian, J. 2008. Heat and light-induced apple skin disorders: Causes and prevention. *Acta Horticulturae* 772:51-58.
- Sotiropoulos, T., Petridis, A., Koukourikou-Petridou, M. & Koundouras, S. 2016. Evaluation of 'Sun Protect' in protecting apples (*Malus × domestica* Borkh.) against sunburn. *Horticultural Science (Prague)* 43:175-180.
- Van Den Dool, K. 2006. Evaporative Cooling of apple and pear orchards. MSc Thesis. Department of Horticultural Science, Stellenbosch University.
- Wünsche, J.N., Bowen, J., Ferguson, I., Woolf, A. & Mcghie, T. 2004. Sunburn on apples - Causes and control mechanisms. *Acta Horticulturae* 636:631-636.
- 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. *Journal of Food, Agriculture and Environment* 8:920-925.
- Zupan, A., Mikulic-Petkovsek, M., Slatnar, A., Stampar, F. & Veberic, R. 2014. Individual phenolic response and peroxidase activity in peel of differently sun-exposed apples in the period favorable for sunburn occurrence. *Journal of Plant Physiology* 171:1706-1712.

LITERATURE REVIEW

The importance of apple fruit sunburn, shortcomings of existing mitigation options and prospective approaches to investigate the mode-of-action by which foliar B plus Ca reduce incidence of this disorder at harvest maturity

1. Introduction

The apple (*Malus domestica*, Borkh) is an important crop for human diet requirements and is currently the fourth most consumed fruit in the world after citrus, grapes and bananas (Konarska, 2013; Delgado-Pelayo et al., 2014). Certainly, such consumer driving apple production in non-traditional growing regions (Turyomurugyendo et al., 2004; Griesbach, 2007; Ashebir et al., 2010; Ntakyo et al., 2013). However, in these new apple growing areas, the relatively higher solar irradiance and temperatures (Baroniya et al., 2014) reverberates concerns about increased risk and revenue losses of potential orchard incomes due to fruit sunburn (Wünsche et al., 2001; Piskolczi et al., 2004; Wünsche et al., 2004a, b). Due to climate change effects, particularly frequent droughts, high temperature and shortages in irrigation water, the risk of apple fruit sunburn is also increasing in the traditional (temperate) apple growing areas (Rouault & Richard, 2003; Midgley & Lötze, 2011; Botai et al., 2017; Mwije, 2017). Further still, the risk of apple fruit sunburn and subsequent revenue losses is likely to exacerbate by the increasing trend of adopting intensive orchard systems (Wünsche et al., 2004b).

Schrader et al. (2001), Felicetti & Schrader (2008a) and Racskó & Schrader (2012), described the three known disorders and/or types (necrosis, photooxidative and browning) of apple fruit sunburn. Sunburn browning (SBB) is most economically important, and is manifested as golden-yellow to bronze blemishes on the apple peel, which is categorized depending on severity as class 1, 2 and 3 (Schrader et al., 2001; 2008; Racskó & Schrader, 2012). Induction of SBB occurs when the temperature on the apple fruit surface reaches 45 to 49 °C in the presence of excess or normal photosynthetic active radiation (PAR) (Schrader et al., 2001; 2003; Wünsche et al., 2001; 2004a; Chen et al., 2008). To date, three main approaches (shade nets, overhead cooling and particle films) are used to reduce apple fruit SBB incidence,

however, these deliver varying results, have expensive implementation costs, and negative effects on both tree and fruit have been reported (Gindaba & Wand, 2005; 2007a; Wand & Gindaba, 2005; Lolicato, 2011).

The phenomenon of sunburn is a serious constraint to premium quality and marketability of the resultant apple fruit (Hamadziripi, 2012; Racskó & Schrader, 2012). In South Africa, apple fruit growers primarily target the fresh and/or export market because of the higher associated income compared to processing secondary products such as juices (DAFF, 2016). However, sales in this lucrative market niche are reliant on the fruit external appearance and colour as these factors greatly influence consumer choices (Gamble et al., 2006; Peneau et al., 2006; Hamadziripi, 2012). Thus, unappealing fruit skin characteristics associated with SBB lead to significant percentage of prospective first-grade South African apples to be culled and/or downgraded to juice making or sold in low-end markets, depending on damage severity (Hamadziripi, 2012), leading to revenue losses in potential orchard income.

Earlier studies indicated that as high as 50 % of South African apples are lost at farm gate and/or pack houses due to sunburn disorders (Bergh et al., 1980). Recent indications show that up to 27, 20 and 40 to 56 % of South African produced ‘Cripps Pink’, ‘Golden Delicious’ and ‘Granny Smith’ apples respectively cannot be exported because of sunburn disorders (Wand et al., 2006; Gindaba & Wand, 2007b; Lötze & Hoffman, 2014). Since these cultivars contribute a very significant percentage of South African apple fruit exports (DAFF, 2016), therefore sunburn is responsible for huge losses of potential orchard revenues in this production region. Economic burden of apple fruit sunburn further manifests in increased production costs as the currently common mitigating approaches are expensive to implement (Dussi et al., 2005; Lolicato, 2011).

2. Current approaches used to mitigate apple fruit sunburn

Elevated oxidative stress levels and subsequent accumulation of reactive oxygen species (ROS) in the apple fruit peel tissue due to high temperature effect of solar irradiance in presence of excess or normal photosynthetic active radiation (PAR) is the major inducer and cause of apple fruit SBB (Chen et al., 2008; Zhang et al., 2015). Apple fruit from the outer tree canopy is most at risk of SBB damage (Wünsche, 2004a), and this risk increases as fruit maturity progresses (Wand & Gindaba, 2005). However, peel tissue of these outer canopy apple fruit is acclimated to high light and temperature conditions by upregulation of metabolites associated with photoprotection and antioxidant mechanisms (Ma & Cheng, 2003; Li et al., 2013; Yuri et al.,

2014). These innate secondary plant product up regulations aim to protect the photosynthetic apparatus and to detoxify the harmful oxidative stress species (Racskó & Schrader, 2012). However, under field conditions of continuous or elevated levels of relevant SBB causative stresses, these protective plant mechanisms cannot aptly avert apple fruit SBB induction and subsequent severity development (Merzlyak et al., 2002; Felicetti & Schrader, 2008b; 2009a, b; Yuri et al., 2010; Racskó & Schrader, 2012). Thus, there is a mandatory requirement for adequate and timely deployment of sunburn incidence suppressing interventions by apple fruit growers prior to harvest maturity, lest huge revenue losses will occur due to the compromised quality of their produce (Wünsche et al., 2004a, b).

Sunburn induction in apple fruit is associated with both direct and indirect factors (Racskó & Schrader, 2012). The current common practices used in apple orchards mainly aim to shield or reduce exposure of apple fruit from the direct SBB causative factors of high solar light and heat. Practices that target the direct factors include (i) orchard shade netting (Arthurs et al., 2013; McCaskill et al., 2016; Kalcsits et al., 2017) and (ii), fruit bagging (Feng et al., 2014a; Sharma et al., 2014). In addition, (iii) kaolin or non-kaolin foliar applications is used to either reflect or absorb SBB causative factors (Glenn et al., 2002; 2009; Schrader, 2011; Sotiropoulos et al., 2016). Orchard overhead cooling similarly reduces the apple fruit SBB incidence by targeting the direct causative factors by lowering apple fruit surface heat load (Parchomchuk & Meheriuk, 1996; Green et al., 2014). These practices that target direct SBB causative factors are considered orchard climate ameliorating strategies (Gindaba & Wand, 2007b).

The strategies that target indirect apple fruit sunburn causative factors are those of cultural practices, and they vary widely among production regions worldwide (Racskó & Schrader, 2012). They include mulching, fertilizer/nutrient management, cover cropping, judicious pruning or thinning as a crop load manipulation in addition to irrigation timing as well as manipulation or choice of tree training systems and rootstocks (Racskó & Schrader, 2012; Barasu, 2017). These cultural practices influence several aspects in relation to apple fruit SBB development, for instance, through reducing tree water stress and providing appreciable canopy cover shielding apple fruit (Racskó & Schrader, 2012; Torres et al., 2013; 2016). Tree canopy structure also influences the occurrence of moderate moving air currents (wind) within the orchard trees and this can as well decrease heat load on the apple fruit surface (Kocsisne et al., 2013). Nutrient deficiencies may exacerbate photo-damaging processes in plants (Agati et al., 2013), and since SBB induction in apple fruit is centred around chlorophyll pigment destruction

processes (Wünsche et al, 2001), it is plausible to presume that nutrient status of apple fruit peel may be important in providing resistance to SBB induction.

3. Need for alternative approaches in mitigating apple fruit sunburn

In almost all apple growing regions worldwide, fruit SBB is quite common (Wünsche et al., 2001; 2004a; Wand & Gindaba, 2005; Gindaba & Wand, 2006; Yuri et al., 2010; Torres et al., 2016 a, b). Amidst the current increasing risk of apple fruit sunburn, due to factors alluded to earlier, the cost of existing mitigation approaches and coupled with their failure to yield consistent results, it is therefore inevitable to develop novel mitigation approaches that should preferably be environmentally friendly, especially with regard to low water footprint, sustainable as well as cost effective. In principle, the currently available major apple sunburn mitigation approaches focus on the shielding or reduction of exposure of the apple fruit to high solar irradiance and temperature (Racskó & Schrader, 2012). The apple fruit sunburn phenomenon is characterised by an interaction of a wide array of indirect and a couple of direct factors (Racskó & Schrader, 2012). Perhaps, better mitigation strategies would include or involve approaches that offer multiple benefits to fruit resistance mechanisms like as illustrated in the case of ‘Sun Protect’ product in the study of Sotiropoulos et al. (2016).

Existing approaches (shade netting, overhead cooling and particle films) to mitigate apple fruit SBB are quite costly and have quite serious challenges on both the tree and fruit physiology (Lolicato, 2011). For instance, by limiting apple fruit light exposure, adequate accumulation of primary and secondary metabolites are compromised, as thus also respective fruit colour and quality (Chen et al., 2012; Feng et al., 2014b; Meng et al., 2015). Shielding the fruit with chemically inert and easily washable particle films of the reflective kaolin has been used widely (Wünsche et al., 2004b), but this ‘easily washable’ unavoidably requires more use of water at the farm and in the respective fruit pack houses. In addition, kaolin negatively affects apple tree photosynthesis and fruit colour (Rosati, 2007). Raynox® is a waxing product that reduces apple fruit sunburn incidence, without leaving visible deposits on fruit (Schrader, 2011). However, products like Raynox® require adequate monitoring of weather elements to be applied at a time when sunburn-induction is expected (Lolicato, 2011). This presents a challenge to already resource constrained producers that are increasingly exposed to variability in weather. To meet these challenges the need to develop alternatives as either stand-alone or complementing existing approaches is quite very clear.

One prospective area to explore when aiming for an alternative and novel approach to suppress sunburn incidence in apple fruit orchards is to, deliberately, seek to ‘*systemically*’ induce and/or reinforce the capacity of the apple peel tissue to resist SBB. And, again one such novel potential systemic approach and without known negative effects on both fruit and tree physiology, is a foliar B+Ca approach identified by Lötze & Hoffman (2014) in South Africa. In the period 2014/17, successful fruit SBB reduction in three distinct apple cultivars (‘Cripps Pink’, ‘Golden Delicious’ and ‘Granny Smith’) was generally achieved using post-full-bloom foliar B+Ca used in South Africa as per the studies of Daiber (2017) and Lötze et al. (2017; 2018). This foliar B+Ca approach appears as a truly systemic approach with yet to be elucidated underlying physiological mechanisms that causes the associated suppression of SBB because these foliar treatments do not have ingredients that ‘shield’ the fruit as observed in for instance kaolin or wax products.

4. Apple fruit and/or tree systemic approaches in mitigating fruit sunburn

A review of currently available literature on sunburn in pome fruit reveals that efforts to boost innate apple fruit mechanisms to resist SBB, either singly or with other strategies is less researched, with only a few studies being reported (Johnson et al., 1999; Iams et al., 2008; 2009; Mupambi et al., 2014; Sotiropoulos et al., 2016). Systemic approaches and particularly delivered by foliar means in orchards are beneficial in that they are associated with low costs to implement, have low water print across the fruit value chain, contain extra minerals that could cater for fruit quality and are easy to adopt as well as implement in other important crops. It is with these immense benefits that such systemic approaches are sought, especially to deploy among commercial apple fruit growers. Notably, foliar applications with intent to systemically reduce apple fruit sunburn were first reported two decades ago by Johnson et al. (1999) using ascorbic acid (AsA) foliar sprays, but this translated into a high cost which became prohibitive and impractical to deploy amongst apple fruit commercial growers (Racskó & Schrader, 2012).

Again, almost a decade ago in Japan another ‘systemic’ approach using S-Abcisic acid (S-ABA) based formulations was reported to have reduced apple fruit SBB (Iams et al., 2008; 2009), but these S-ABA treatments did not yield tangible results under South African conditions (Mupambi et al., 2014; 2018). In Greece, Sotiropoulos et al. (2016) reported successfully SBB incidence reduction in ‘Granny Smith’ apples using a boron (B) based and/or containing commercial foliar product ‘Sun Protect’. This ‘Sun Protect’ appears as a ‘*quasi-systemic*’ since as according to the study of Sotiropoulos et al. (2016), this product contains

ingredients that shield the fruit and those that potentially influence the fruit physiology to resist SBB development.

Importantly, the converging results from both different researchers and regions (South Africa and Greece), with both distinct ('Cripps Pink' and 'Golden Delicious') and similar ('Granny Smith') cultivars strongly suggest the potential of B based and/or containing formulations as new systemic and alternative approaches to suppress SBB incidence in apple fruit orchards. However, between the two foliar B formulations approaches mentioned above, the foliar B+Ca approach is a better alternative as the Ca can prevent other endemic compromises to apple fruit quality, especially bitter pit disorders (Saure, 1996; Lötze & Theron, 2007). The mode-of-action associated with the foliar B+Ca approach in reducing apple fruit SBB incidence is however not understood (Lötze et al., 2017), but with 'Sun Protect', increased antioxidant capacity and shielding fruit from causative agents was articulated by Sotiropoulos et al. (2016). From this perspective, the novel foliar B+Ca approach introduced by Lötze & Hoffman (2014) is of significant research interest, as its mode-of-action requires elucidation in order to enable full exploitation of its envisaged potential in suppressing fruit sunburn cost effectively in apple orchards. The absence of knowledge about the foliar B+Ca treatments mode-of-action is a serious challenge to its further development, refinement and/or optimisation to cater for possible differing cultivar needs.

5. Prospects of mitigating apple fruit sunburn using boron plus calcium

Recent research by Lötze & Hoffman (2014), Daiber (2017) and Lötze et al. (2017) provide a new and systemic paradigm to apple fruit sunburn mitigation as emphasised by Mwijje (2017). Obviously, this new and systemic paradigm is in sharp contrast to the conventional approach of 'shielding' the apple fruit from sunburn inducing agents, as it intently aims at promoting the fruit innate mechanisms to resist development of SBB, especially the class 1 type, which is the mildest indication of sunburn browning (Appendix 1), but yet most economically important. Lötze & Hoffman (2014) reported up to 10 percent higher sunburn incidence in the control (untreated fruit) compared to foliar B+Ca treated 'Golden Delicious' fruit. Studies conducted in Greece by Sotiropoulos et al. (2016), using the boron-containing 'Sun Protect', reported that in 'Granny Smith' two-thirds of greener (class 1) fruit were associated with the treatment, whereas the majority of the control fruit exhibited sunburn symptoms typical of class 2 fruit. Daiber (2017) and Lotze et al. (2017; 2018) reported a significant difference in class 1 sunburn incidence, ranging between 5 – 8 %, between foliar B+Ca treatments and the control for 'Cripps

Pink' apples. Daiber (2017) and Lotze et al. (2017; 2018) further reported some foliar B+Ca treatments resulted in up to 13 % more class 0 fruit than could be achieved for control, while a reduction of up to 11 % in class 1 sunburn fruit could be obtained by some foliar B+Ca treatments in comparison to the control in 'Golden Delicious' apples.

The use of foliar B+Ca to mitigate apple fruit sunburn has several envisaged benefits, in line with afore mentioned merits of systemic remedies to suppress sunburn in apple fruit orchards. For instance, reducing shade netting and overhead cooling costs and lowering the water footprint across the production and marketing chain through reduced water usage, with respect to both irrigation and fruit washing. In addition, the foliar B+Ca treatment approach also promote production of quality apple fruit in two ways, first, the fruit can mature in adequate sunlight which supports quality through good colour development and accumulation of health-promoting secondary metabolites (Gindaba & Wand, 2005; Wand et al., 2006; Feng et al., 2014b). Secondly, this foliar B+Ca approach contributes towards control of other common apple fruit disorders like bitter pit by possibly increasing fruit Ca content (Saure, 1996; De Freitas & Mitcham, 2012; De Freitas et al., 2013). In addition, this foliar B+Ca approach can be regarded as a strong adaptive treatment with the potential of being adopted for other horticultural crops that are similarly affected by sunburn.

Envisaging the realization of this new foliar B+Ca approach is largely a congruence of three aspects: first, the concept of phytochemical farming, which is well established, and relates to judicious use of foliar nutrition practices to reduce abiotic stresses in perennial crops like fruit trees (Treutter, 2010; Murtic et al., 2012). Secondly, since foliar nutrition is already a regular practice in apple orchards (Porro et al., 2002; Tojanko & Ternar, 2002), the suggested use of foliar B+Ca with the intent to reduce apple fruit SBB incidence can easily be adopted by commercial growers, especially after elucidation of its mode-of-action and subsequent refinement. Thirdly, foliar nutrition with B, Ca or their various combinations and formulations have been shown to reduced SBB incidence in apple fruit of distinct cultivars and production regions.

Despite the sound forecasts of the possible impact of foliar B+Ca treatments may have to promote quality apple fruit production and as well in the industry at large, there is a considerable challenge in realising these benefits due to a lack of understanding of its mode-of-action (Lötze et al., 2017). This must be resolved to effectively realise and exploit this new approach in horticulture, as it holds the key to its possible refinement that may be necessary for the different apple cultivars as indicated by the variable sunburn reduction in results from

Daiber (2017). Previous apple fruit sunburn mitigation innovations have been accompanied by scientific explanations of the mode-of-action (Iams et al., 2009; Schrader, 2011; Mupambi et al., 2014; 2018; Sotiropoulos et al., 2016), and this fostered their evaluation through research and/or at farm level, resulting in their adoption or non-adoption by growers.

6. Possible approaches to unravel the boron plus calcium mode-of-action

6.1 Effect on levels of apple fruit peel phenolics

Phenolic compounds synthesis in plants utilizes up to 20% of the photosynthetic fixed carbon (Bravo, 1998; Weisshaar & Jenkins, 1998; Vogt, 2010). This investment is indicative of their important role in providing resistance or tolerance to abiotic and biotic stresses (Dixon & Paiva, 1995; Petkovs et al., 2008; Lombardo et al., 2009; Schovankova & Opatova, 2011). Apple fruit sunburn strongly associates with changes in levels of peel phenolics, possibly as an innate resistance mechanism to this abiotic stress (Felicetti & Schrader, 2008b; 2009b; Yuri et al., 2010; Zupan et al., 2014). Weather elements synonymous with inducing apple fruit sunburn influence accumulation of phenolic compounds in the peel (Hagen et al., 2007; Yuri et al., 2010; Li et al., 2013). Such increase in peel antioxidative phenolics reportedly prevented photodamage and subsequent sunburn in ‘Braeburn’ apple (Solovchenko & Schmitz-Eiberger, 2003). In ‘Cameo’ apple, upregulation of phenolic metabolites and respective genes reduced levels of skin burning (Harb et al., 2013). In addition, the antioxidant capacity of apple peel is positively correlated to its phenolic content (Boyer & Liu, 2004; Tsao et al., 2005), and notably, the phenolic flavonoid type are very powerful antioxidants (Agati et al., 2012; 2013). Therefore, phenolic flavonoids can potentially curtail oxidative stress in apple peel and the associated sunburn development (Solovchenko & Schmitz-Eiberger, 2003).

Plant B and Ca nutrition status also associates with the dynamics of phenolic compounds in the tissues, and ultimately thus affects respective total antioxidant capacity levels (Cakmak & Romheld, 1997; Ruiz et al., 1998; Hajiboland & Farhanghi, 2010; Eichholz et al., 2011; Waraich et al., 2012; Hajiboland et al., 2013). For instance, application of kaolin (containing significant amount of calcium oxide) increased phenolic levels in *Vitis vinifera* L. grape berries through a positive effect on the phenylpropanoid and flavonoid pathways (Conde et al., 2016). Calcium nutrition levels and phenolic contents of anthocyanin and flavonoid types had positive correlations in ‘Elstar’ apple peel (Awad & De Jager, 2002). In addition, two applications of a Phostrade-Ca formulation (which contains phosphorus and very low amounts of Ca and

nitrogen), starting at five weeks before harvest and at 14-day interval also increased phenolic levels of anthocyanins and flavanols in 'Braeburn' apple peel (Bizjak et al., 2013).

Thus, foliar application of B and Ca minerals through post-full-bloom foliar B+Ca treatments may upregulate phenolic biochemicals and/or metabolites in the apple fruit peel tissues, and possibly resulting in offering resistance to SBB development. These relationships alluded to above between B, Ca, phenolic dynamics and apple SBB development provided a strong motivation to examine apple peel phenolic levels among varying foliar B+Ca treatments in an attempt to unravel the mode-of-action by which apple fruit SBB incidence is reduced.

6.2 Effect on levels of apple fruit peel photosynthetic pigments

The photosynthetic efficiency and functionality of plant photosystems decline with increasing UV-B and high solar irradiance, in a process known as photodamage, photo-inactivation or photoinhibition (Iwanzik et al., 1983; Barber & Andersson, 1992; Aro et al., 1993; 2005; Demming-Adams et al., 1997; Murata et al., 2012). Photodamage is reversible whereas photo-oxidation is not (Murata et al., 2007; Takahashi & Badger, 2011). Heat induced photodamage impairs the Calvin cycle and oxygen evolving complex (Smillie, 1992; Yamane et al., 1998), while light induced photodamage arises with the resulting oxidative stress damage to photosystems (Aro et al., 1993). Since apple fruit SBB is associated with excess heat and light, the two scenarios of photo-damage occurring simultaneously are possible.

Photodamage causes destruction of chlorophyll signalled by the conversion of chlorophyll *b* (CHLb) to chlorophyll *a* (CHLa) (Kim et al., 2009; Hortensteiner & Krautler, 2011; Tanaka & Tanaka, 2011). Chlorophyll pigment destruction is principal in apple fruit sunburn development phenomenon (Wünsche et al., 2001; Felicetti & Schrader, 2008b; 2009a). UV-B radiation exacerbates photodamage in plants (Jansen et al., 1996; Klem et al., 2015), as it increases the magnitude of the CHLa:CHLb ratio, with a significant reduction in CHLb levels as opposed to that of CHLa (Hollosy, 2002). Increasing severity of apple fruit sunburn is associated with high peel CHLa:CHLb ratio supposedly from very low CHLb (Felicetti & Schrader, 2008b). In general, apple fruit SBB development results in a reduction of total chlorophyll and the associated increase in carotenoid or xanthophyll pigments in the peel (Ma & Cheng, 2003; Felicetti & Schrader, 2008b; 2009a).

The photodamage of Photosystem (PS) II may also occur within normal PAR ranges where the D1 protein reportedly degrades, but the D2 protein remains stable. However, there is rapid

degradation of both proteins in the presence of UV-B radiation (Jansen et al., 1996; Edelman & Mattoo, 2008; Hideg et al., 2013; Klem et al., 2015). Harmful levels of photodamage occur when the rate of D1 repair is compromised by the ensuing oxidative stress species (Nishiyama et al., 2005; Murata et al., 2007; 2012; Takahashi & Murata, 2008; Takahashi & Badger, 2011), that possibly enhancing D1 protein repair in apple peel under the relevant causative stresses can compromise SBB induction and development.

Foliar Ca enhances photoprotection and reduces photo-damage in plant tissues under heat and light stresses supposedly through enhancing D1 protein repair, increasing antioxidant capacity and through reduction of oxidative stress (Jiang & Huang, 2001a, b; Yang et al., 2015; Sakhonwasee & Phingkanan, 2017). In addition, limited B supply reportedly increased photodamage and oxidative stress, while being associated with reduced leaf chlorophyll levels (Hajiboland & Farhanghi, 2010). Ganie et al. (2013) also reported that a full bloom foliar B application in sweet cherry resulted in increased chlorophyll and carotenoid pigments, whereas, Jiang and Huang (2001a, b) reported increased chlorophyll with foliar Ca application in two grass species exposed to heat stress. These findings in various species of plants suggest that possibly foliar B+Ca application may be able to reduce photodamage processes in apple peel tissues exposed to high light and heat stress, and possibly therefore compromise or reduce sunburn incidence in apple fruit orchards.

In relation to investigating a foliar B+Ca treatments mode-of-action of apple fruit sunburn incidence reduction, two important observations are, first, dynamics or levels of chlorophyll and carotenoid pigments associate with apple fruit sunburn (Wünsche et al., 2001; Felicetti & Schrader, 2008b; 2009a). Secondly, foliar applications of B and Ca mineral nutrients in plants associates with an enhancement of chlorophyll and carotenoid levels (Jiang & Huang, 2001a; b; Hajiboland & Farhanghi, 2010; Ganie et al., 2013; Yang et al., 2015; Sakhonwasee & Phingkanan, 2017). Therefore, a possible relationship between foliar B+Ca application, chlorophyll, carotenoids and apple fruit sunburn phenomenon requires investigation in effort to elucidate the sought mode-of-action by which foliar B+Ca suppresses SBB in in apple fruit orchards.

6.3 Effect on levels of apple fruit peel oxidative stress

Light, temperature and UV-B stresses lead to severe oxidative stress in plants, characterized by elevated levels of ROS such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) leading to a compromise in functional cell physiology (Smillie, 1992;

Cakmak, 1994; Noctor & Foyer, 1998; Asada, 1999; Wahid et al., 2007; Fahad et al., 2013; Nawkar et al., 2013). Such oxidative stress damages cellular phospholipid membranes, proteins, nucleic acids as well as photosystems (Aro et al., 1993; Noctor & Foyer, 1998; Murata et al., 2007; Wahid et al., 2007), and also such oxidative stress in fruit peel is heavily associated with the induction and severity development of the apple SBB disorder (Wünsche et al., 2004b; Zupan et al., 2014).

In plant cells, B and Ca are reportedly associated with maintaining integrity, proper functioning and structure of the membrane (Kobayashi et al., 1999; Camacho-Cristobal et al., 2008; Herrera-Rodriguez et al., 2010; Ranade-Malvi, 2011). Proper functioning and structure of plant cell membranes and walls is important in withstanding abiotic stresses (Blevins & Lukaszewski, 1998; Bolanos et al., 2004; Imran & Gurmani, 2011). This suggests that B and Ca have important abiotic stress resistance functions, as they may be involved in the prevention of membrane destruction, in particular lipid peroxidation processes in the event of elevated oxidative stress. This supposition is supported by the evidence that foliar B or Ca applications have been associated with enhanced antioxidant capacity, in different plant species, also in particular in fruit tissues (Jiang & Huang, 2001b; Verlag et al., 2002; Agarwal et al., 2005; Gunes et al., 2006; Thurzo et al., 2010; Singh et al., 2012).

In addition, calcium, through calmodulin has specific roles in maintaining metabolic activities of plants under abiotic stress, and is a known secondary messenger in the regulation of core plant physiological functions (Chen & Li, 2001; Aghdam et al., 2012; Batisti & Kudla, 2012; Waraich et al., 2012). Although antioxidant enzymes and capacity in plants were elevated with insufficient B, this did not prevent cellular oxidative stress and damage (Cakmak & Romheld, 1997; Hajiboland & Farhanghi, 2010), suggesting that plant antioxidant mechanisms function optimally with sufficient B.

According to Sotiropoulos et al. (2016) and product manufactures of 'Sun Protect' (www.compo-expert.com), the ingredients α -tocopherol, boron (2 %), phenolic acids and the Ultraviolet (UV) absorbers reportedly offer protection against sunburn of vegetables and fruits based on biologically active boron and tocopherol that eliminate ROS and stabilises cell membranes while phenolic acids and UV absorbers protect from radiation. This suggests that the formulation 'Sun protect' operates by improving the antioxidant capacity to prevent lipid peroxidation, as well as provide 'physical shielding' to fruit against harmful radiation. Thus, the findings of Sotiropoulos et al. (2016) suggest that in apple fruit SBB incidence mitigation, the cell membrane and antioxidant capacity of the peel are key components in the amelioration

mechanism. The reported antioxidant role of B and Ca in plants and association of oxidative stress with apple sunburn strongly validates evaluation of possible changes in peel tissue oxidative stress in an effort to trace the mode-of-action by which foliar B+Ca mediates apple fruit SBB incidence reduction. Oxidative stress in apple peel can be evaluated with assaying lipid peroxidation or peroxidative activity (Du & Bramlage, 1992; 1995; Rao et al., 1998; Blokhina et al., 2003).

6.4 Influence of environmental factors on the apple fruit peel biochemical profile

Climatic factors and conditions of apple peel physiology that induce and precipitate severity of sunburn have been adequately established (Schrader et al., 2001; Wünsche et al., 2001; 2004a; Chen et al., 2008; Felicetti & Schrader, 2008a; Racskó & Schrader, 2012). Hence, the mere sighting of apple fruit sunburn provides evidence that a particular threshold of fruit surface temperature occurred, inducing oxidative stress to be followed by upregulation of phenolics and carotenoids coupled with destruction of chlorophyll (Felicetti & Schrader, 2008a; 2009a, b), and hence the visual yellow-brown sunburn blemishes which are symptomatic of SBB (Racskó & Schrader, 2012). Apple peel biochemistry is strongly influenced by sunburn and its causative agents together with orchard production practices and seasonal (cropping year) differences (Ma & Cheng, 2003; 2004; Merzlyak et al., 2005; Chen et al., 2009; Yuri et al., 2010; Lin-Wang et al., 2011; Feng et al., 2013; 2014b; Glenn & Yuri, 2013; Wang et al., 2015; Jing et al., 2016; Barasu, 2017). Apple fruit peel biochemistry is in addition to the above factors further influenced by orchard practices like fertilization, pesticide application and pathogen attack as well as fruit harvest-date decisions (Schovankova & Opatova, 2011; Le Bourvellec et al., 2015; Conde et al., 2016).

Therefore, from the above, experiments or studies aimed at unravelling the sought mode-of-action by which foliar B+Ca suppress SBB incidences in apple fruit orchards should importantly take into account (i) environment differences among experiment sites and (ii) ensure uniformity in orchard practices. Using multi-environments within in a single season can show stable treatment formulations in reaction to different environments, greatly aiding the foliar B+Ca approach protocol refinement and/or optimisation needs.

6.5 Influence of genotype and rootstock on apple fruit peel biochemical profile

The genetic control and functioning of cellular mechanisms to resist abiotic or biotic stresses are highly conserved across plant species (Felix et al., 1999; Palma et al., 2007; Giri et al., 2013; You & Chan, 2015; Zhang, 2015). Similarly is the common shikimate / phenylpropanoid pathway (Fraser & Chapple, 2011; Tohge et al., 2013; Mondal & Roy, 2017), which yields flavonoids and phenols that are associated with presumed apple fruit innate resistance to SBB (Felicetti & Schrader, 2008b; 2009b; Chen et al., 2009; Yuri et al., 2010). Apple cultivars (for instance ‘Cripps Pink’, ‘Golden Delicious’ and ‘Granny Smith’ which are popular apple cultivars in South Africa) show variability of fruit peel phenolic content and composition (Wolfe et al., 2003; Khanizadeh et al., 2008; Volz & McGhie, 2011; Jakobek et al., 2013; Savikin et al., 2014; De Paepe et al., 2015). Perhaps, amongst other factors, this peel biochemical variability partly explains why apple cultivars are also known to vary in susceptibility to sunburn as reported by Lolicato (2011). This suggests that involving distinct and genetically related cultivars in the investigation to determine a mode-of-action by which foliar B+Ca treatments mediate apple fruit sunburn incidence reduction at harvest maturity can yield information that can shed more light on the response of different cultivars and ultimately benefit foliar B+Ca protocol refinement efforts.

In Western Cape, South Africa, ‘Cripps Pink’, ‘Golden Delicious’ and ‘Granny Smith’ apples blossom at the same time, around mid-October, but harvest maturity is reached at different times. ‘Granny Smith’ and ‘Cripps Pink’ are both late maturing cultivars that are harvested in March and April respectively, while ‘Golden Delicious’ is harvested end of February or early March. ‘Granny Smith’ apple is known to be very susceptible to SBB disorders (Lolicato, 2011; Sotiropoulos et al., 2016), partially due to the deep chlorophyll-green peel that easily displays the SBB associated golden-yellow to bronze blemishes. In ‘Cripps Pink’ however, the characteristic red blush that increases with fruit maturity and masks the relevant SBB symptoms (Makaredza et al., 2015) and can therefore heavily influence SBB incidence assessment in this particular cultivar. In addition, red apple fruit attain high temperature more readily in comparison to the green ones (Ferguson et al., 1998). ‘Granny Smith’ and ‘Golden Delicious’ apples are genetically distant, being both chance seedlings from Australia and the United States of America (USA) respectively. ‘Cripps Pink’ apple is a cross of ‘Lady Williams’ and ‘Golden Delicious’ (Cripps et al., 1993). Therefore, these three cultivars with their variable responses to sunburn inducing conditions, and distinct genotypic differences, make them a perfect choice for foliar B+Ca apple fruit sunburn reduction investigations and/or studies.

Apple rootstocks are known to influence the fruit peel biochemical profile, perhaps because of their variable effects on canopy structure along with the drought tolerance and water relations of the tree (Mainla et al., 2011; Ceymann, 2013; Kviklys et al., 2014; Jemric et al., 2016; Samuoliene et al., 2016; Tworkoski et al., 2016). Canopy and drought dynamics relate strongly to apple fruit sunburn incidence (Hamadziripi, 2012; Makedredza et al., 2013; Barasu, 2017), suggesting that rootstocks play an important role in apple fruit SBB development. Therefore, this infers that experiments relating to investigations aimed at unravelling the mode-of-action by which post-full-bloom foliar applications of foliar B+Ca treatments mediate apple fruit SBB incidence reduction should either keep uniformity or create variability in rootstocks, in combination of formulation treatments, as per particular aims of the study. Consideration of rootstock as well as genetic differences in such experiments can provide adequate information for enabling appropriate recommendations for foliar B+Ca treatments protocol refinement in order to accurately and reliably promote effective variants of this technology on a commercial scale as per cultivar and/or rootstock requirements.

7. Conclusion

This review emphasizes the increasing importance, albeit negative sided, of the fruit sunburn phenomenon in commercial apple production amidst climate changes, conversions in orchard management to intensive production systems along with the shortcomings of existing or traditional methods to reduce sunburn disorders. Furthermore, this review emphasizes the need to develop novel alternative fruit sunburn incidence mitigation methods, particularly, harnessing the recently introduced foliar B+Ca treatments approach for commercial scale use, focusing on South African apple farming systems. Such research efforts are essential to maintain profitability and ensure continued quality produce in the apple fruit industry, enabling South African producers to retain and expand their firm current position in the competitive and lucrative export market. This review further highlights that the main causative agents of apple SBB (excess light and temperature) also influence the biochemical profile of the fruit peel, and explores the envisaged potential or ability of B and Ca to also impact on these apple peel metabolites. Thus, reverberating on the necessity to focus on peel pigment content and composition, on a seasonal basis, when investigating the mode-of-action of the foliar B+Ca mediated apple fruit SBB reduction. Existing literature further suggests that a viable mode-of-action may be deductible from conducting experiments at experimental sites that vary in climatic conditions, using different and/or related cultivars (red/blushing and green fruit). In addition, the variability as contributed by rootstocks and orchard practices like shade netting

and irrigation, among other factors, is recognized to influence the biochemical profile of the apple peel and should be put in consideration as per aims of a given study in this particular research area. Overall, this review justifies the strong merit for investigating changes within the fruit peel biochemical domain to unravel the mode-of-action by which the foliar B+Ca treatments suppress apple fruit SBB incidence in apple orchards.

References

- Agarwal, S., Sairam, R.K., Srivastava, G.C., Tyagi, A. & Meena, R.C. 2005. Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. *Plant Science* 169:559-570.
- Agati, G., Azzarello, E., Pollastri, S. & Tattini, M. 2012. Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science* 196:67-76.
- Agati, G., Brunetti, C., Ferdinando, M.D., Ferrini, F., Pollastri, S. & Tattini, M. 2013. Functional roles of flavonoids in photoprotection: New evidence, lessons from the past. *Plant Physiology and Biochemistry* 72:35-45.
- Aghdam, M.S., Hassanpouraghdam, M.B., Paliyath, G. & Farmani, B. 2012. The language of calcium in postharvest life of fruits, vegetables and flowers. *Scientia Horticulturae* 144:102-115.
- Aro, E.M., Virgin, I. & Andersson, B. 1993. Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. *Biochimica et Biophysica Acta* 1143:113-134.
- Aro, E.M., Suorsa, M., Rokka, A., Allahverdiyeva, Y., Paakkarinen, V., Saleem, A., Battchikova, N. & Rintamaki, E. 2005. Dynamics of photosystem II: A proteomic approach to thylakoid protein complexes. *Journal of Experimental Botany* 56:347-356.
- Arthurs, S.P., Stamps, R.H. & Giglia, F.F. 2013. Environmental modification inside photoselective shadehouses. *HortScience* 48:975-979.
- Asada, K. 1999. The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology* 50:601-639.
- Ashebir, D., Deckers, T., Nyssen, J., Bihon, W., Tsegay, A., Tekie, H., Poesen, J., Haile, M., Wondumagegnehu, F, Raes, D, Behailu, M & Deckers, J. 2010. Growing apple (*Malus domestica*) under tropical mountain climate conditions in Northern Ethiopia. *Experimental Agriculture* 46:53-65.
- Awad, M.A. & De Jager, A. 2002. Relationships between fruit nutrients and concentrations of flavonoids and chlorogenic acid in 'Elstar' apple skin. *Scientia Horticulturae* 92:265-276.
- Barasu, P.D. 2017. Acclimation of apple peel to light and temperature and the effect thereof on red colour development and tolerance to sunburn. MSc Thesis. Department of

Horticultural Science, Stellenbosch University.

- Barber, J. & Andersson, B. 1992. Too much of a good thing: light can be bad for photosynthesis. *Trends in Biochemical Sciences* 17:61-66.
- Baroniya, S.S., Kataria, S., Pandey, G.P. & Guruprasad, K.N. 2014. Growth, photosynthesis and nitrogen metabolism in soybean varieties after exclusion of the UV-B and UV-A/B components of solar radiation. *Crop Journal* 2:388-397.
- Batisti, O. & Kudla, J. 2012. Analysis of calcium signaling pathways in plants. *Biochimica et Biophysica Acta* 1820:1283-1293.
- Bergh, O., Franken, J., Van Zyl, E.J., Kloppers, F. & Dempers, A. 1980. Sunburn on apples: Preliminary results of an investigation conducted during the 1978-79 season. *Deciduous Fruit Grower* 30:8-22.
- Bizjak, J., Weber, N., Mikulic-Petkovsek, M., Slatnar, A., Stampar, F., Alam, Z., Stich, K., Halbwirth, H., Veberic, R. 2013. Influence of phosphate Ca on color development and anthocyanin content of 'Braeburn' apple (*Malus domestica* Borkh.). *HortScience* 48:193-199.
- Blevins, D.G. & Lukaszewski, K.M. 1998. Boron in plant structure and function. *Annual Review of Plant Physiology and Plant Molecular Biology* 49:481-500.
- Blokhina, O., Virolainen, E. & Fagerstedt, K. V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals of Botany* 91:179-194.
- Bolanos, L., Lukaszewski, K., Bonilla, I. & Blevins, D. 2004. Why boron? *Plant Physiology and Biochemistry* 42:907-912.
- Botai, C., Botai, J., De Wit, J.P., Ncongwane, K.P. & Adeola, A.M. 2017. Drought Characteristics over the Western Cape Province, South Africa. *Water* 9:876.
- Boyer, J. & Liu, R.H. 2004. Apple phytochemicals and their health benefits. *Nutrition Journal* 3:5.
- Bravo, L. 1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*. 56:317-333.
- Cakmak, I. 1994. Activity of ascorbate-dependent H₂O₂-scavenging enzymes and leaf chlorosis are enhanced in magnesium- and potassium-deficient leaves, but not in phosphorus-deficient leaves. *Journal of Experimental Botany* 45:1259-1266.

- Cakmak, I. & Romheld, V. 1997. Boron deficiency-induced impairments of cellular functions in plants. *Plant and Soil* 193:71-83.
- Camacho-Cristobal, J.J., Herrera-Rodriguez, M.B., Beato, V.M., Rexach, J., Navarro-Gochicoa, M.T., Maldonado, J.M. & Gonzalez-Fontes, A. 2008. The expression of several cell wall-related genes in *Arabidopsis* roots is down-regulated under boron deficiency. *Environmental and Experimental Botany* 63:351-358.
- Ceymann, M. 2013. Polyphenol content and profile in apples and its potential relevance to human health. PhD Dissertation, Bayerische Julius-Maximilians-Universität Würzburg, Germany.
- Chen, W.P. & Li, P.H. 2001. Chilling-induced Ca^{2+} overload enhances production of active oxygen species in maize (*Zea mays* L.) cultured cells: The effect of abscisic acid treatment. *Plant, Cell and Environment* 24:791-800.
- Chen, C.S., Zhang, D., Wang, Y.Q., Li, P.M. & Ma, F.W. 2012. Effects of fruit bagging on the contents of phenolic compounds in the peel and flesh of 'Golden Delicious', 'Red Delicious', and 'Royal Gala' apples. *Scientia Horticulturae* 142:68-73.
- 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.
- Chen, L.S., Li, P. & Cheng, L. 2009. Comparison of thermotolerance of sun-exposed peel and shaded peel of 'Fuji' apple. *Environmental and Experimental Botany* 66:110-116.
- Conde, A., Pimentel, D., Neves, A., Dinis, L.T., Bernardo, S., Correia, C.M., Geros, H. & Moutinho-Pereira, J. 2016. Kaolin foliar application has a stimulatory effect on phenylpropanoid and flavonoid pathways in grape berries. *Frontiers in Plant Science* 7:1150.
- Cripps, J.E.L., Richards, L.A. & Mairata, A.M. 1993. 'Pink Lady' apple. *HortScience* 28:1057.
- DAFF. 2016. A profile of the South African apple market value chain. Directorate Marketing, Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa.
- Daiber, S.H. 2017. Quantifying changes in tree physiology after amelioration to reduce sunburn on apples. MSc Thesis. Department of Horticultural Science, Stellenbosch University.
- De Freitas, S.T. & Mitcham, E.J. 2012. Factors involved in fruit calcium deficiency disorders.

Horticultural Reviews 40:107-146.

De Freitas, S.T., Do Amarante, C.V.T., Dandekar, A.M. & Mitcham, E.J. 2013. Shading affects flesh calcium uptake and concentration, bitter pit incidence and other fruit traits in 'Greensleeves' apple. *Scientia Horticulturae* 161:266-272.

De Paepe, D., Valkenborg, D., Noten, B., Servaes, K., Diels, L., De Loose, M., Van Droogenbroeck, B. & Voorspoels, S. 2015. Variability of the phenolic profiles in the fruits from old, recent and new apple cultivars cultivated in Belgium. *Metabolomics* 11:739-752.

Delgado-Pelayo, R., Gallardo-Guerrero, L. & Hornero-Mendez, D. 2014. Chlorophyll and carotenoid pigments in the peel and flesh of commercial apple fruit varieties. *Food Research International* 65:272-281.

Demming-Adams, B., Adams III, W.W., Grace, S.C. 1997. Physiology of light tolerance in plants. *Horticultural Reviews* 18: 215-246.

Dixon, R.A. & Paiva, N.L. 1995. Stress-Induced Phenylpropanoid Metabolism. *The Plant Cell* 7:1085-1097.

Du, Z. & Bramlage, W.J. 1992. Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. *Journal of Agricultural and Food Chemistry* 40:1566-1570.

Du, Z. & Bramlage, W.J. 1995. Peroxidative activity of apple peel in relation to development of poststorage disorders. *HortScience* 30:205-209.

Dussi, M.C., Giardina, G. & Reeb, P. 2005. Shade nets effect on canopy light distribution and quality of fruit and spur leaf on apple cv. Fuji. *Spanish Journal of Agricultural Research* 3:253-260.

Edelman, M. & Mattoo, A.K. 2008. D1-protein dynamics in photosystem II: The lingering enigma. *Photosynthesis Research* 98:609-620.

Eichholz, I., Huyskens-Keil, S., Kroh, L.W. & Rohn, S. 2011. Phenolic compounds, pectin and antioxidant activity in blueberries (*Vaccinium corymbosum* L.) influenced by boron and mulch cover. *Journal of Applied Botany and Food Quality* 84:26-32.

Fahad, S., Chen, Y., Saud, S., Wang, K., Xiong, D., Chen, C., Wu, C., Shah, F., Nie, L. & Huang, J. 2013. Ultraviolet radiation effect on photosynthetic pigments, biochemical attributes, antioxidant enzyme activity and hormonal contents of wheat. *Journal of Food,*

- Agriculture and Environment 11:1635-1641.
- Felicetti, D.A. & Schrader, L.E. 2008a. Photooxidative sunburn of apples: Characterization of a third type of apple sunburn. *International Journal of Fruit Science* 8:160-172.
- Felicetti, D.A. & Schrader, L.E. 2008b. Changes in pigment concentrations associated with the degree of sunburn browning of 'Fuji' apple. *Journal of the American Society of Horticultural Science* 133:27-34.
- Felicetti, D.A. & Schrader, L.E. 2009a. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. II. Phenolics. *Plant Science* 176:84-89.
- Felicetti, D.A. & Schrader, L.E. 2009b. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. I. Chlorophylls and Carotenoids. *Plant Science* 176:78-83.
- Felicetti, D.A. & Schrader, L.E. 2010. Postharvest changes in pigment concentrations in 'Fuji' apples with 'Fuji' stain. *Scientia Horticulturae* 125:283-288.
- Felix, G., Duran, J.D., Volko, S. & Boller, T. 1999. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant Journal*. 18:265-276.
- Feng, F., Li, M., Ma, F. & Cheng, L. 2013. Phenylpropanoid metabolites and expression of key genes involved in anthocyanin biosynthesis in the shaded peel of apple fruit in response to sun exposure. *Plant Physiology and Biochemistry* 69:54-61.
- Feng, F., Li, M., Ma, F. & Cheng, L. 2014a. The effects of bagging and debagging on external fruit quality, metabolites, and the expression of anthocyanin biosynthetic genes in 'Jonagold' apple (*Malus domestica* Borkh.). *Scientia Horticulturae* 165:123-131.
- Feng, F., Li, M., Ma, F. & Cheng, L. 2014b. Effects of location within the tree canopy on carbohydrates, organic acids, amino acids and phenolic compounds in the fruit peel and flesh from three apple (*Malus × domestica*) cultivars. *Horticulture Research* 1:14019.
- Ferguson, I.B., Snelgar, W., Lay-Yee, M., Watkins, C.B. & Bowen, J.H. 1998. Expression of heat shock protein genes in apple fruit in the field. *Australian Journal of Plant Physiology* 25:155-163.
- Fraser, C.M. & Chapple, C. 2011. The phenylpropanoid pathway in arabidopsis. *The Arabidopsis Book* 10:1199.
- Gamble, J., Jaeger, S.R. & Harker, F.R. 2006. Preferences in pear appearance and response to

- novelty among Australian and New Zealand consumers. *Postharvest Biology and Technology* 41:38-47.
- Ganie, M.A., Akhter, F., Bhat, M.A., Malik, A.R., Junaid, J.M., Shah, M.A., Bhat, A.H. & Bhat, T.A. 2013. Boron - a critical nutrient element for plant growth and productivity with reference to temperate fruits. *Current Science* 104:76-85.
- 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. *HortScience* 40:592-596.
- Gindaba, J. & Wand, S.J.E. 2007a. Do fruit sunburn control measures affect leaf photosynthetic rate and stomatal conductance in 'Royal Gala' apple? *Environmental and Experimental Botany* 59:160-165.
- Gindaba, J. & Wand, S.J.E. 2007b. Climate-ameliorating measures influence photosynthetic gas exchange of apple leaves. *Annals of Applied Biology* 150:75-80.
- Giri, J., Dansana, P.K., Kothari, K.S., Sharma, G., Vij, S. & Tyagi, A.K. 2013. SAPs as novel regulators of abiotic stress response in plants. *BioEssays* 35:639-648.
- Glenn, D.M. 2009. Particle film mechanisms of action that reduce the effect of environmental stress in 'Empire' apple. *Scientia Horticulturae* 134:314-321.
- Glenn, D.M. & Yuri, J.A. 2013. Photosynthetically active radiation (PAR) × ultraviolet radiation (UV) interact to initiate solar injury in apple. *Scientia Horticulturae* 162:117-124.
- 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. *Journal of the American Society for Horticultural Science* 127:188-193.
- Green, S.R., Goodwin, I. & Cornwall, D. 2014. Evaporative cooling effects on tree transpiration. *Acta Horticulturae* 1038:401-406.
- Gregan, S.M., Wargent, J.J., Liu, L., Shinkle, J., Hofmann, R., Winefield, C., Trought, M. & Jordan, B. 2012. Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon Blanc grapes. *Australian Journal of Grape and Wine Research* 18:227-238.
- Griesbach, J. 2007. *Growing Temperate Fruit Trees in Kenya*. World Agroforestry Centre (ICRAF). Nairobi, Kenya.

- Gunes, A., Soylemezoglu, G., Inal, A., Bagci, E.G., Coban, S. & Sahin, O. 2006. Antioxidant and stomatal responses of grapevine (*Vitis vinifera* L.) to boron toxicity. *Scientia Horticulturae* 110:279-284.
- Hagen, S.F., Borge, G.I.A., Bengtsson, G.B., Bilger, W., Berge, A., Haffner, K. & Solhaug, K.A. 2007. Phenolic contents and other health and sensory related properties of apple fruit (*Malus domestica* Borkh., cv. Aroma): Effect of postharvest UV-B irradiation. *Postharvest Biology and Technology* 45:1-10.
- Hajiboland, R. & Farhanghi, F. 2010. Remobilization of boron, photosynthesis, phenolic metabolism and anti-oxidant defense capacity in boron-deficient turnip (*Brassica rapa* L.) plants. *Soil Science and Plant Nutrition* 56:427-437.
- Hajiboland, R., Bahrami-Rad, S. & Bastani, S. 2013. Phenolics metabolism in boron- deficient tea [*Camellia sinensis* (L.) O. Kuntze] plants. *Acta Biologica Hungarica* 64:196-206.
- Hamadziripi, E.T. 2012. The effect of canopy position on the fruit quality and consumer preference of apples. MSc Thesis. Department of Horticultural Science, Stellenbosch University.
- Harb, J., Saleh, O., Kitemann, D., Neuwald, D.A., Frank, W. & Reski, R. 2013. Upregulation of polyphenol-related genes prevents 'skin burning' of well-colored 'Cameo' apples stored under stressful controlled atmosphere conditions. *Postharvest Biology and Technology* 77:121-127.
- Hernandez, I., Alegre, L., Breusegem, F. V & Munne-Bosch, S. 2009. How relevant are flavonoids as antioxidants in plants? *Trends in Plant Science* 14:125-132.
- Herrera-Rodriguez, M.B., Gonzalez-Fontes, A., Rexach, J., Camacho-Cristobal, J.J., Maldonado, J.J. & Navarro-Gochicoa, M.T. 2010. Role of boron in vascular plants and response mechanisms to boron stresses. *Plant Stress* 4:115-122.
- Hideg, E., Jansen, M.A.K. & Strid, A. 2013. UV-B exposure, ROS, and stress: Inseparable companions or loosely linked associates? *Trends in Plant Science* 18:107-115.
- Hollosy, F. 2002. Effects of ultraviolet radiation on plant cells. *Micron*. 33:179-197.
- Hortensteiner, S. & Krautler, B. 2011. Chlorophyll breakdown in higher plants. *Biochimica et Biophysica Acta* 1807:977-988.
- Huovinen, P., Gomez, I. & Lovengreen, C. 2006. A five-year study of solar ultraviolet radiation in southern Chile (39°S): Potential impact on physiology of coastal marine algae?

Photochemistry and Photobiology 82:515-522.

- Iamsub, K., Sekozawa, Y., Sugaya, S., Gemma, H. & Kamuro, Y. 2008. Improvement of fruit quality by S-ABA and the fertilizer formulated K, P, Mg, Bo, Mn containing S-ABA as pre-harvest application on peaches and apples. *Acta Horticulturae* 804:219-224.
- 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. *Journal of Food, Agriculture and Environment* 7:446-452.
- Imran, M. & Gurmani, Z.A. 2011. Role of macro and micro nutrients in the plant growth and development. *Science, Technology & Development* 30:36-40.
- Iwanzik, W., Tevini, M., Dohnt, G., Voss, M., Weiss, W., Graber, P. & Renger, G. 1983. Action of UV-B radiation on photosynthetic primary reactions in spinach chloroplasts. *Physiologia Plantarum* 58:401-407.
- Jakobek, L., Garcia-Villalba, R. & Tomas-Barberan, F.A. 2013. Polyphenolic characterisation of old local apple varieties from Southeastern European region. *Journal of Food Composition and Analysis* 31:199-211.
- Jansen, M.A.K., Gaba, V., Greenberg, B.M., Mattoo, A.K., Edelman, M. & Dagan, B. 1996. Low threshold levels of ultraviolet-B in a background of photosynthetically active radiation trigger rapid degradation of the D2 protein of photosystem-II. *The Plant Journal* 9:693-699.
- Jemric, T., Fruk, I., Fruk, M., Radman, S., Sinkovic, L. & Fruk, G. 2016. Bitter pit in apples: Pre- and postharvest factors: A review. *Spanish Journal of Agricultural Research* 14:1-12.
- Jiang, Y. & Huang, B. 2001a. Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. *Crop Science* 41:436-442.
- Jiang, Y. & Huang, B. 2001b. Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. *Journal of Experimental Botany* 52:341-349.
- Jing, C., Ma, C., Zhang, J., Jing, S., Jiang, X., Yang, Y. & Zhao, Z. 2016. Effect of debagging time on pigment patterns in the peel and sugar and organic acid contents in the pulp of 'Golden Delicious' and 'Qinguan' apple fruit at mid and late stages of development. *PLoS ONE* 11:10.
- Johnson, J.R., Fahy, D., Gish, N., Andrews, P.K. 1999. Influence of ascorbic acid sparys on

- apple sunburn. *Good Fruit Grower* 50 (13): 81-83.
- Kalcsits, L., Musacchi, S., Layne, D.R., Schmidt, T., Mupambi, G., Serra, S., Mendoza, M., Asteggiano, L., Jarolmasjed, S., Sankaran, S., Khot, L.R. & Espinoza, C.Z. 2017. Above and below-ground environmental changes associated with the use of photosensitive protective netting to reduce sunburn in apple. *Agricultural and Forest Meteorology* 237-238:9-17.
- Khanizadeh, S., Tsao, R., Rekika, D., Yang, R., Charles, M.T. & Rupasinghe, H.P. V. 2008. Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. *Journal of Food Composition and Analysis* 21:396-401.
- Kim, E.H., Li, X.P., Razeghifard, R., Anderson, J.M., Niyogi, K.K., Pogson, B.J. & Chow, W.S. 2009. The multiple roles of light-harvesting chlorophyll a/b -protein complexes define structure and optimize function of Arabidopsis chloroplasts: A study using two chlorophyll b-less mutants. *Biochimica et Biophysica Acta* 1787:973-984.
- Klem, K., Holub, P., Stoch, M., Nezval, J., Spunda, V., Triska, J., Jansen, M.A.K., Robson, T.M., Urban, O. 2015. Ultraviolet and photosynthetically active radiation can both induce photoprotective capacity allowing barley to overcome high radiation stress. *Plant Physiology and Biochemistry* 93:74-83.
- Kobayashi, M., Nakagawa, H., Asaka, T. & Match, T. 1999. Borate-rhamnogalacturonan II bonding reinforced by Ca²⁺ retains pectic polysaccharides in higher-plant cell walls. *Plant Physiology* 119:199-204.
- Kocsisne, G.M., Kocsis, L., Van Maurik, D., Nyeki, J., Szabo, Z. & Soltesz, M. 2013. Influence of black hail nets on canopy size, reproductive potential, and fruit characteristics of apple trees. *Acta Horticulturae* 984:157-162.
- Konarska, A. 2013. The structure of the fruit peel in two varieties of *Malus domestica* Borkh. (Rosaceae) before and after storage. *Protoplasma* 250:701-714.
- Kviklys, D., Liaudanskas, M., Janulis, V., Viskelis, P., Rubinskiene, M., Lanauskas, J. & Uselis, N. 2014. Rootstock genotype determines phenol content in apple fruits. *Plant, Soil and Environment* 60:234-240.
- Le Bourvellec, C., Bureau, S., Renard, C.M.G.C., Plenet, D., Gautier, H., Touloumet, L., Girard, T. & Simon, S. 2015. Cultivar and year rather than agricultural practices affect primary and secondary metabolites in apple fruit. *PLoS ONE* 10:11.

- Li, P., Ma, F. & Cheng, L. 2013. Primary and secondary metabolism in the sun-exposed peel and the shaded peel of apple fruit. *Physiologia Plantarum* 148:9-24.
- Lin-Wang, K., Micheletti, D., Palmer, J., Volz, R., Lozano, L., Espley, R., Hellens, R.P., Chagne, D., Rowan, D.D., Troglio, M., Iglesias, I. & Allan, A.C. 2011. High temperature reduces apple fruit colour via modulation of the anthocyanin regulatory complex. *Plant, Cell and Environment* 34:1176-1190.
- Lolicato, S. 2011. Sun Protection for Fruit - A practical manual for protecting sunburn on fruit. Department of Primary Industries, Farm Services Victoria Division, Victoria, Australia.
- Lombardo, S., Pandino, G., Mauro, R. & Mauromicale, G. 2009. Variation of phenolic content in globe artichoke in relation to biological, technical and environmental factors. *Italian Journal of Agronomy* 4:181-189.
- Lötze, E. & Hoffman, E.W. 2014. Foliar application of calcium plus boron reduces the incidence of sunburn in ‘Golden Delicious’ apple. *Journal of Horticultural Science and Biotechnology* 89:607-612.
- Lötze, E. & Theron, K.I. 2007. Evaluating the effectiveness of pre-harvest calcium applications for bitter pit control in ‘Golden Delicious’ apples under South African conditions. *Journal of Plant Nutrition* 30:471-485.
- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2017. Boron in combination with calcium reduces sunburn in apple fruit. *Boron* 2:123-127.
- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2018. Evaluating the efficacy of a preharvest combination of calcium and boron as foliar application to reduce sunburn on ‘Cripps Pink’ apples. *Acta Horticulture* 1217:61-68.
- Ma, F. & Cheng, L. 2003. The sun-exposed peel of apple fruit has higher xanthophyll cycle-dependent thermal dissipation and antioxidants of the ascorbate-glutathione pathway than the shaded peel. *Plant Science* 165:819-827.
- Ma, F. & Cheng, L. 2004. Exposure of the shaded side of apple fruit to full sun leads to up-regulation of both the xanthophyll cycle and the ascorbate-glutathione cycle. *Plant Science* 166:1479-1486.
- Mainla, L., Moor, U., Karp, K. & Pussa, T. 2011. The effect of genotype and rootstock on polyphenol composition of selected apple cultivars in Estonia. *Zemdirbyste-Agriculture* 98:63-70.

- Makaredza, B., Schmeisser, M., Lötze, E. & Steyn, W.J. 2013. Water stress increases sunburn in 'Cripps' Pink' apple. *HortScience* 48:444-447.
- Makaredza, B., Marais, H., Schmeisser, M., Lötze, E. & Steyn, W.J. 2015. Ripening associated red color development masks sunburn browning in apple peel. *HortScience* 50:814-818.
- McCaskill, M.R., McClymont, L., Goodwin, I., Green, S. & Partington, D.L. 2016. How hail netting reduces apple fruit surface temperature: A microclimate and modelling study. *Agricultural and Forest Meteorology* 226 -227:148-160.
- McKenzie, R., Bodeker, G., Scott, G., Lantz, K. & Slusser, J. 2006. Geographical differences in erythemally-weighted UV measured at mid-latitude USDA sites. *Photochemical & Photobiological Sciences* 5:343-352.
- Meng, R., Qu, D., Liu, Y., Gao, Z., Yang, H., Shi, X. & Zhao, Z. 2015. Anthocyanin accumulation and related gene family expression in the skin of dark-grown red and non-red apples (*Malus domestica* Borkh.) in response to sunlight. *Scientia Horticulturae* 189:66-73.
- Merzlyak, M.N. & Solovchenko, A.E. 2002. Photostability of pigments in ripening apple fruit: A possible photoprotective role of carotenoids during plant senescence. *Plant Science* 163:881-888.
- Merzlyak, M.N., Solovchenko, A.E. & Chivkunova, O.B. 2002. Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. *Plant Physiology and Biochemistry* 40:679-684.
- Merzlyak, M.N., Solovchenko, A.E., Smagin, A.I. & Gitelson, A.A. 2005. Apple flavonols during fruit adaptation to solar radiation: Spectral features and technique for non-destructive assessment. *Journal of Plant Physiology* 162:151-160.
- Midgley, S.J.E. & Lötze, E. 2011. Climate change in the Western Cape of South Africa: Trends, projections and implications for chill unit accumulation. *Acta Horticulturae* 903:1127-1134.
- Mondal, S.K. & Roy, S. 2017. Genome-wide sequential, evolutionary, organizational and expression analyses of phenylpropanoid biosynthesis associated MYB domain transcription factors in Arabidopsis. *Journal of Biomolecular Structure and Dynamics* 10:1080.
- Munne-Bosch, S., Penuelas, J. & Barcelona, A.D. 2003. Photo- and antioxidative protection

- during summer leaf senescence in *Pistacia lentiscus* L. grown under mediterranean field conditions. *Annals of Botany* 92:385-391.
- Mupambi, G., Reynolds, J.S. & Steyn, W.J. 2014. Foliar S-ABA application does not reduce sunburn in 'Granny Smith' apple. *Acta Horticulturae* 1042:303-309.
- Mupambi, G., Schmeisser, M., Dzikiti, S., Reynolds, S., Steyn, W.J. 2018. Ineffectiveness of foliar S-ABA application as an apple sunburn suppressant explained through effects on peel biochemistry and leaf ecophysiology. *Scientia Horticulturae* 232: 256-263.
- Murata, N., Takahashi, S., Nishiyama, Y. & Allakhverdiev, S.I. 2007. Photoinhibition of photosystem II under environmental stress. *Biochimica et Biophysica Acta* 1767:414-421.
- Murata, N., Allakhverdiev, S.I. & Nishiyama, Y. 2012. The mechanism of photoinhibition in vivo: Re-evaluation of the roles of catalase, α -tocopherol, non-photochemical quenching, and electron transport. *Biochimica et Biophysica Acta* 1817:1127-1133.
- Murtic, S., Civic, H., Duric, M., Sekularac, G., Kojovic, R., Kulina, M. & Krsmanovic, M. 2012. Foliar nutrition in apple production. *African Journal of Biotechnology* 11:10462-10468.
- Mwije, A. 2017. Pioneering a new paradigm of sunburn mitigation in apples. In *Bringing Science to Communities: Voices from the Field (Issue 1)* (Ed. Withers, J). A RUFORUM Publication. Kampala, Uganda.
- Nawkar, G.M., Maibam, P., Park, J.H., Sahi, V.P., Lee, S.Y. & Kang, C.H. 2013. UV-induced cell death in plants. *International Journal of Molecular Sciences* 14:1608-1628.
- Nishiyama, Y., Allakhverdiev, S.I., Takahashi, S., Higashi, S., Watanabe, M., Nishiyama, Y. & Murata, N. 2005. Two-step mechanism of photodamage to photosystem II: Step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center. *Biochemistry* 44:8494-8499.
- Noctor, G. & Foyer, C.H. 1998. Ascorbate and Glutathione: Keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* 49:249-279.
- Ntakyo, P.R., Mugisha, J. & Elepu, G. 2013. Socio-economic factors affecting apple production in South-Western Uganda. *African Crop Science Journal* 21:311-321.
- Palma, K., Zhao, Q., Cheng, Y.T., Bi, D., Monaghan, J., Cheng, W., Zhang, Y. & Li, X. 2007. Regulation of plant innate immunity by three proteins in a complex conserved across the

- plant and animal kingdoms. *Genes & Development* 21:1484-1493.
- Parchomchuk, P. & Meheriuk, M. 1996. Orchard cooling with pulsed overtree irrigation to prevent solar injury and improve fruit quality of 'Jonagold' apples. *HortScience* 31:802-804.
- Peneau, S., Hoehn, E., Roth, H.R., Escher, F. & Nuessli, J. 2006. Importance and consumer perception of freshness of apples. *Food Quality and Preference* 17:9-19.
- Petkovs, M.M., Stampar, F. & Veberic, R. 2008. Increased phenolic content in apple leaves infected with the apple scab pathogen. *Journal of Plant Pathology* 90:49-55.
- Piskolczi, M., Varga, C. & Racskó, J. 2004. A review of the meteorological causes of sunburn injury on the surface of apple fruit (*Malus domestica* Borkh). *Journal of Fruit and Ornamental Plant Research* 12:245-252.
- Porro, D., Dorigatti, C. & Ramponi, C. 2002. Can foliar application modify nutritional status and improve fruit quality? Results on apple in Northeastern Italy. *Acta Horticulturae* 594:521-526.
- Racskó, J. & Schrader, L.E. 2012. Sunburn of apple fruit: Historical background, recent advances and future perspectives. *Critical Reviews in Plant Sciences* 31:455-504.
- Ranade-Malvi, U. 2011. Interaction of micronutrients with major nutrients with special reference to potassium. *Karnataka Journal of Agricultural Science* 24:106-109.
- Rao, M. V, Watkins, C.B., Brown, S.K. & Weeden, N.F. 1998. Active oxygen species metabolism in 'White Angel' x 'Rome Beauty' apple selections resistant and susceptible to superficial scald. *Journal of the American Society for Horticultural Science* 123:299-304.
- Rosati, A. 2007. Physiological effects of kaolin particle film technology: A Review. *Functional Plant Science and Biotechnology* 1:100-105.
- Rouault, M. & Richard, Y. 2003. Intensity and spatial extension of drought in South Africa at different time scales. *Water SA* 29:489-500.
- Ruiz, J.M., Bretones, G., Baghour, M., Ragala, L., Belakbir, A. & Romero, L. 1998. Relationship between boron and phenolic metabolism in tobacco leaves. *Phytochemistry* 48:269-272.
- Sakhonwasee, S. & Phingkanan, W. 2017. Effects of the foliar application of calcium on

- photosynthesis, reactive oxygen species production, and changes in water relations in tomato seedlings under heat stress. *Horticulture Environment and Biotechnology* 58:119-126.
- Samuoliene, G., Viskeliene, A., Sirtautas, R. & Kviklys, D. 2016. Relationships between apple tree rootstock, crop-load, plant nutritional status and yield. *Scientia Horticulturae* 211:167-173.
- Saure, M.C. 1996. Reassessment of the role of calcium in development of bitter pit in apple. *Australian Journal of Plant Physiology* 23:237-243.
- Savikin, K., Zivkovic, J., Zdunic, G., Godevac, D., Dordevic, B., Dojcinovic, B. & Dordevic, N. 2014. Phenolic and mineral profiles of four Balkan indigenous apple cultivars monitored at two different maturity stages. *Journal of Food Composition and Analysis* 35:101-111.
- Schovankova, J. & Opatova, H. 2011. Defensive reactions of apple cultivars Angold and HL 1834 after fungal infection. *Horticultural Science* 38:87-95.
- Schrader, L.E. 2011. Scientific basis of a unique formulation for reducing sunburn of fruits. *HortScience* 46:6-11.
- Schrader, L., Zhang, J. & Sun, J. 2003. Environmental stresses that cause sunburn of apple. *Acta Horticulturae* 618:397-405.
- Schrader, L., Sun, J., Zhang, J., Felicetti, D. & Tian, J. 2008. Heat and light-induced apple skin disorders: Causes and prevention. *Acta Horticulturae* 772:51-58.
- Schrader, L. E., Zhang, J., and Duplaga, W. K. 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Online. *Plant Health Progress* doi:10.1094/PHP-2001-1004-01-RS.
- Singh, D.P., Beloy, J., Mcinerney, J.K. & Day, L. 2012. Impact of boron, calcium and genetic factors on vitamin C, carotenoids, phenolic acids, anthocyanins and antioxidant capacity of carrots (*Daucus carota*). *Food Chemistry* 132:1161-1170.
- Smillie, R.M. 1992. Calvin cycle activity in fruit and the effect of heat stress. *Scientia Horticulturae* 51:83-95.
- Smillie, R.M. & Hetherington, S.E. 1999. Photoabatement by anthocyanin shields photosynthetic systems from light stress. *Photosynthetica* 36:451-463.

- Solovchenko, A. & Schmitz-Eiberger, M. 2003. Significance of skin flavonoids for UV-B-protection in apple fruits. *Journal of Experimental Botany* 54:1977-1984.
- Solovchenko, A.E. & Merzlyak, M.N. 2008. Screening of visible and UV radiation as a photoprotective mechanism in plants. *Russian Journal of Plant Physiology* 55:719-737.
- Sotiropoulos, T., Petridis, A., Koukourikou-Petridou, M. & Koundouras, S. 2016. Evaluation of 'Sun Protect' in protecting apples (*Malus × domestica* Borkh.) against sunburn. *Horticultural Science (Prague)* 43:175-180.
- Takahashi, S. & Badger, M.R. 2011. Photoprotection in plants: A new light on photosystem II damage. *Trends in Plant Science* 16:53-60.
- Takahashi, S. & Murata, N. 2008. How do environmental stresses accelerate photoinhibition? *Trends in Plant Science* 13:178-182.
- Tanaka, R. & Tanaka, A. 2011. Chlorophyll cycle regulates the construction and destruction of the light-harvesting complexes. *Biochimica et Biophysica Acta* 1807:968-976.
- Thurzo, S., Szabo, Z., Nyeki, J., Nagy, P.T., Silva, A.P. & Goncalves, B. 2010. Effect of boron and calcium sprays on photosynthetic pigments, total phenols and flavonoid content of sweet cherry (*Prunus avium* L.). *Acta Horticulturae* 868:457-462.
- Tohge, T., Watanabe, M., Hoefgen, R. & Fernie, A.R. 2013. Shikimate and phenylalanine biosynthesis in the green lineage. *Frontiers in Plant Science* 4:62.
- Tojnko, S., Ternar, T. & Cmelik, Z. 2002. Effect of foliar application and fertigation with some nutrients on fruit mineral content of young 'Golden Delicious' apple trees. *Acta Horticulturae* 594:185-189.
- Torres, C.A., Sepulveda, A., Gonzalez-Talice, J., Yuri, J.A. & Razmilic, I. 2013. Fruit water relations and osmoregulation on apples (*Malus domestica* Borkh.) with different sun exposures and sun-injury levels on the tree. *Scientia Horticulturae* 161:143-152.
- Torres, C.A., Sepulveda, A., Leon, L. & Yuri, J.A. 2016. Early detection of sun injury on apples (*Malus domestica* Borkh.) through the use of crop water stress index and chlorophyll fluorescence. *Scientia Horticulturae* 211:336-342.
- Torres, C.A., Leon, L. & Sanchez-Contreras, J. 2016. Spectral fingerprints during sun injury development on the tree in Granny Smith apples: A potential non-destructive prediction tool during the growing season. *Scientia Horticulturae* 209:165-172.
- Treutter, D. 2010. Managing phenol contents in crop plants by phytochemical farming and

- breeding-visions and constraints. *International Journal of Molecular Sciences* 11:807-857.
- Tsao, R., Yang, R., Xie, S., Sockovie, E. & Khanizadeh, S. 2005. Which polyphenolic compounds contribute to the total antioxidant activities of apple? *Journal of Agricultural and Food Chemistry* 53:4989-4995.
- Turyomurugyendo, L., Boffa, J.M. & Hakiza, J.J. 2004. Introduction of deciduous fruit tree growing in the tropical highlands of Kabale, Uganda. *Uganda Journal of Agricultural Sciences* 9:470-479.
- Twoorkoski, T., Fazio, G. & Glenn, D.M. 2016. Apple rootstock resistance to drought. *Scientia Horticulturae* 204:70-78.
- Verlag, F., Schmitz-Eiberger, M., Haefs, R., Noga, G., Schmitz-Eiberger, M., Haefs, R. & Noga, G. 2002. Calcium deficiency - Influence on the antioxidative defense system in tomato plants. *Journal of Plant Physiology* 159:733-742.
- Vogt, T. 2010. Phenylpropanoid biosynthesis. *Molecular Plant* 3:2-20.
- Volz, R.K. & McGhie, T.K. 2011. Genetic variability in apple fruit polyphenol composition in *Malus × domestica* and *Malus sieversii* germplasm grown in New Zealand. *Journal of Agricultural and Food Chemistry* 59:11509-11521.
- Wahid, A., Gelani, S., Ashraf, M. & Foolad, M.R. 2007. Heat tolerance in plants: An overview. *Environmental and Experimental Botany* 61:199-223.
- Wand, S.J.E. & Gindaba, J. 2005. Controlling sunburn: What are the options? *South African Fruit Journal* 4:24-26.
- 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. *Scientia Horticulturae* 107:271-276.
- Wang, H., Arakawa, O. & Motomura, Y. 2000. Influence of maturity and bagging on the relationship between anthocyanin accumulation and phenylalanine ammonia-lyase (PAL) activity in 'Jonathan' apples. *Postharvest Biology and Technology* 19:123-128.
- Wang, X., Wei, Z. & Ma, F. 2015. The effects of fruit bagging on levels of phenolic compounds and expression by anthocyanin biosynthetic and regulatory genes in red-fleshed apples. *Process Biochemistry* 50:1774-1782.
- Waraich, E.A., Ahmad, R., Halim, A. & Aziz, T. 2012. Alleviation of temperature stress by nutrient management in crop plants: A review. *Journal of Soil Science & Plant Nutrition*

12:221-244.

- Weisshaar, B. & Jenkins, G.I. 1998. Phenylpropanoid biosynthesis and its regulation. *Current Opinion in Plant Biology* 1:251-257.
- Wolfe, K., Wu, X. & Liu, R.H. 2003. Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry* 51:609-614.
- Wünsche, J.N., Greer, D.H., Palmer, J.W., Lang, A. & McGhie, T. 2001. Sunburn - The cost of a high light environment. *Acta Horticulturae* 557:349-356.
- Wünsche, J.N., Bowen, J., Ferguson, I., Woolf, A. & McGhie, T. 2004a. Sunburn on apples - Causes and control mechanisms. *Acta Horticulturae* 636:631-636.
- Wünsche, J.N., Lombardini, L. & Greer, D.H. 2004b. 'Surround' particle film applications - Effects on whole canopy physiology of apple. *Acta Horticulturae* 636:565-571.
- Yamane, Y., Kashino, Y., Koike, H. & Satoh, K. 1998. Effects of high temperatures on the photosynthetic systems in spinach: Oxygen-evolving activities, fluorescence characteristics and the denaturation process. *Photosynthesis Research* 57:51-59.
- Yang, S., Wang, F., Guo, F., Meng, J.J., Li, X.G. & Wan, S.B. 2015. Calcium contributes to photoprotection and repair of photosystem II in peanut leaves during heat and high irradiance. *Journal of Integrative Plant Biology* 57:486-495.
- You, J. & Chan, Z. 2015. ROS regulation during abiotic stress responses in crop plants. *Frontiers in Plant Science* 6:1092.
- 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. *Journal of Food, Agriculture and Environment* 8:920-925.
- Yuri, J.A., Neira, A., Maldonado, F., Quilodran, A., Simeone, D., Razmilic, I. & Palomo, I. 2014. Total phenol and quercetin content and antioxidant activity in apples in response to thermal, light stress and to organic management. *Journal of Applied Botany, Food Quality* 87:131-138.
- Zhang, B. 2015. MicroRNA: A new target for improving plant tolerance to abiotic stress. *Journal of Experimental Botany* 66:1749-1761.
- Zhang, J., Niu, J., Duan, Y., Zhang, M., Liu, J., Li, P. & Ma, F. 2015. Photoprotection

mechanism in the 'Fuji' apple peel at different levels of photooxidative sunburn. *Physiologia Plantarum* 154:54-65.

Zupan, A., Mikulic-Petkovsek, M., Slatnar, A., Stampar, F. & Veberic, R. 2014. Individual phenolic response and peroxidase activity in peel of differently sun-exposed apples in the period favorable for sunburn occurrence. *Journal of Plant Physiology* 171:1706-1712.

INVESTIGATING THE MODE-OF-ACTION BY WHICH POST-FULL-BLOOM FOLIAR APPLICATIONS OF A COMBINATION OF BORON AND CALCIUM IMPACT ON APPLE FRUIT SUNBURN BROWNING INCIDENCE AT HARVEST MATURITY

I. Boron plus calcium induced changes in apple peel phenolic levels

Abstract

This study investigated apple peel phenolic levels and related them with sunburn browning (SBB) incidence at fruit harvest, following post-full-bloom foliar applications of boron plus calcium (B+Ca) treatments. The aim was to determine if there are treatment-induced phenolic level changes that offer explanation (mode-of-action) for SBB incidence suppression. Four foliar B+Ca treatments and control (no B+Ca) were applied to ‘Cripps Pink’ (CP), ‘Golden Delicious’ (GD) and ‘Granny Smith’ (GS) bearing trees during 2014/15 (CP) and 2015/16 (all cultivars). Fruit for the peel samples was randomly sampled at selected maturity stages (days after full bloom (DAFB)), for the phenolic assays, and results were compared to SBB incidence suppression outcomes. In 2014/15, treatment effects, fruit position and orientation and respective interaction effects in CP were insignificant, except total flavonoids (TF) which had significant treatment effects with fruit orientation at 184 DAFB, and fruit position (inward or outward of tree canopy) which significantly differed at 214 DAFB. Therefore, results from 2014/15 revealed that potential significant treatment phenolic level changes could occur towards harvest maturity and not early in season. Therefore, in 2015/16, all peel was sampled from outer canopy, and towards fruit harvest maturity. The results from 2015/16 showed that total phenolics (TP) and TF decreased with progression of DAFB, and treatments and DAFB jointly influenced phenolic levels as manifested by significant interaction effects on TP (GD and GS) and TF in all cultivars. The highest SBB incidence (classes 1 and 2) and lowest sunburn (class 0 / no sunburn) was associated with control, but significant differences were only reported for CP and GD. However, variability in phenolics could not explain foliar B+Ca treatment effects on SBB incidence reduction as to yield the sought mode-of-action. This study concludes that the effect of the studied foliar B+Ca treatments on apple peel phenolic levels does not offer a mode-of-action for sunburn incidence reduction that occurred in CP and GD cultivars.

Keywords: ‘Cripps Pink’, ‘Golden Delicious’, ‘Granny Smith’, flavonoids to phenolics ratio

1. Introduction

The phenolic levels in apple peel increases during sunburn browning (SBB) development (Chen et al., 2008; 2009, Felicetti & Schrader, 2008; 2009; Yuri et al., 2010), and is a facet of an innate and/or innate fruit mechanism to resist SBB (Merzlyak et al., 2002; Yuri et al., 2010). Flavonoids, the major apple fruit peel phenolic compounds present in apple fruit peel tissues could potentially give protection against SBB development through their powerful antioxidant capacity (Pietta, 2000; Wolfe et al., 2003; Tsao et al., 2005). In addition, flavonoids offer photoprotection capacity to the photosynthetic systems of the apple peel tissue from excessive and harmful solar radiation (Tsao et al., 2003; 2005; Solovchenko & Schmitz-Eiberger, 2003; Khanizadeh et al., 2008; Falcone Ferreyra et al., 2012). Further still, incidence of high solar irradiance (light) and temperature, which are synonymous with apple fruit SBB induction also elevate synthesis of flavonoids in the apple peel (Yuri et al., 2014). Thus, overall, phenolic levels in apple peel positively correlate with the total antioxidant capacity (Lee et al., 2003; Wolfe et al., 2003; Tsao et al., 2005), which makes such phenolic metabolite aspects very important in the dynamics of apple fruit SBB phenomenon.

Post-full-bloom foliar applications of combined B and Ca (B+Ca) could be an alternative approach to suppress fruit SBB incidence in apple orchards (Lötze & Hoffman, 2014; Daiber, 2017; Lötze et al., 2017; 2018). However, its mode-of-action is unknown, which impedes full exploitation of this envisaged low-cost approach to mitigating apple fruit SBB, particularly among commercial growers. Thus, there is a need to understand the role of phenolics of the apple peel tissue, particularly as pertaining to understanding how particular foliar B+Ca formulations induce resistance to SBB development. The possible resistance roles of phenolic compounds to fruit SBB development motivated this study with the hypothesis that the foliar B+Ca treatments enhance the abundance of the peel photoprotective and antioxidative phenolic metabolites resulting in protecting the fruit from SBB.

The study aimed at determining whether differences occur in apple peel phenolic levels in three important South African produced apples following the foliar B+Ca treatments. The impact of various foliar B+Ca formulations on phenolic levels was determined and whether if such differences correlate with apple fruit SBB incidence suppression outcomes to therefore present a possible mode-of-action by which respective foliar B+Ca treatments suppress the sunburn.

2. Materials and methods

a. Experimental layout

The experiments were conducted using with 20-year-old trees of ‘Cripps Pink’ and 28-year-old trees of both ‘Golden Delicious’ and ‘Granny Smith’. The experiments were located in two orchards with a history of fruit sunburn (Hortec Pty Ltd, 5 Old Paardevlei Rd, Somerset West). The ‘Golden Delicious’ and ‘Granny Smith’ apple experiments were carried out at Applethwaite (Pty) farm (34°12'08.0'S; 18°59'16.5'E) located in Elgin, where the trees were planted in a North-West to South-East orientation. The ‘Cripps Pink’ experiments were carried out at Welgevallen Experimental Farm of Stellenbosch University (33°56'52.5'S; 18°52'19.9'E) located in Stellenbosch, with trees planted in a South-West to North-East row orientation. The trees at both experimental sites were trained as central leaders on M793 rootstocks and received fertilizer application, pest and disease control, thinning as well as irrigation according to commercial practice (Daiber, 2017).

Daiber (2017) determined the efficacy of various combinations of foliar B+Ca to decrease sunburn in apple, with experiments were laid out in a randomised complete block design, and using single trees as experimental units, within 10 blocks for ‘Cripps Pink’ and seven blocks for both ‘Golden Delicious’ and ‘Granny Smith’. The research reported here utilised five replications of Daiber’s (2017) experiment sites for the more detailed, apple peel biochemical assessments. The ‘Cripps Pink’ experiment was consecutively conducted in 2014/15 and 2015/16 seasons, whereas ‘Golden Delicious’ and ‘Granny Smith’ experiments were only conducted in 2015/16. The 2014/15 experiment was also exploratory including fruit position and orientation factors and findings generally informed the experimental practice in 2015/16. In addition, the 2014/15 ‘Cripps Pink’ experiment was harvested late on 15 May 2015 (214 DAFB) instead of optimum maturity at 15 April 2015 (184 DAFB), with the intention to increase SBB incidence so as to better contrast the treatments in reducing sunburn incidence. However, in 2015/16, ‘Cripps Pink’ experiment was harvested at optimum maturity to prevent heavy masking effect of pink colour that was observed in 2014/15 (Daiber, 2017), that actually prevented an effective evaluation of SBB incidence differences among the foliar B+Ca treatments. The ‘Golden Delicious’ apple fruit were also harvested at optimum maturity on 29 February 2016 (136 DAFB), while ‘Granny Smith’ apple fruit were harvested pre-optimum on 14 March 2016 (150 DAFB) by request of producer, thus sample collection ended at 136 DAFB, instead of the planned 150 DAFB. Five replicates were analysed in the laboratory for the phenolic levels per foliar B+Ca treatment and per fruit maturity stage (DAFB).

b. Treatments

The choice of the foliar B+Ca treatment formulations for this study followed previous findings in ‘Golden Delicious’ apples (Lötze & Hoffman, 2014), and alternative formulations were included (Tables 1 and 2). In 2014/15, three treatments were applied, six times, at weekly intervals, starting at approximately 28 DAFB (Table 3). These treatments were, (i) 0.02 g.l⁻¹ B plus 0.10 g.l⁻¹ Ca (B`0.02+Ca`0.10), (ii) 0.02 g.l⁻¹ B combined with 1.29 g.l⁻¹ Ca (B`0.02+Ca`1.29), and (iii), control (B`0.00+Ca`0.00) where no additional foliar B+Ca was provided. The treatments were applied until run-off (high volume), using a motorized Stihl® backpack sprayer.

In the 2015/16 experiments, Daiber (2017) included alternative formulations of the foliar B+Ca combination to investigate their impact on apple fruit sunburn mitigation. However, due to the different aims of this study, only five of Daiber’s treatments were utilised in this study (Tables 1 and 2). The choices were based on previous findings in ‘Golden Delicious’ apples that indicated the possible role of the concentration of Ca and/or B on SBB incidence reduction (Lötze & Hoffman, 2014). These treatments were, (i) 0.02 g.l⁻¹ B plus 0.06 g.l⁻¹ Ca (B`0.02+znCa`0.06) which was introduced as a low Ca treatment, (ii) 0.02 g.l⁻¹ B plus 1.24 g.l⁻¹ Ca (B`0.02+Ca`1.24), (iii) 0.08 g.l⁻¹ B plus 1.24 g.l⁻¹ Ca (B`0.08+Ca`1.24). In addition, (iv) 0.17 g.l⁻¹ B plus 1.24 g.l⁻¹ Ca (B`0.17+Ca`1.24) as well as (v), control (B`0.00+Ca`0.00) with no added foliar B+Ca. The post-full-bloom foliar B+Ca treatments were applied once a week and for six consecutive weeks, from approximately 30 DAFB in ‘Cripps Pink’ and 39 DAFB in both ‘Golden Delicious’ and ‘Granny Smith’ (Table 3).

c. Fruit sampling and biochemical analyses

Fruit sampling for the laboratory analyses was carried out in the morning hours between 06:00 and 07:00 for consistency purposes. Six fruit, without any visual sunburn, were randomly picked from the outer canopy positions, three fruit from each side of the tree. Thereafter, fruit were transported in a well-insulated cool box within two hours to the laboratory at Department of Horticultural Science, Stellenbosch University where whole fruitlets were peeled and the peels were combined to form a respective foliar B+Ca treatment replicate for a given cultivar and fruit maturity stage (DAFB). The number of fruit sampled was reduced to four fruit later in the season (two from each respective side of the trees), when more peel per fruit could be recovered.

In 2014/15, apple fruit sampling was done at four (35, 49, 56 and 70 DAFB) early maturity stages, and approximately at weekly intervals. In addition, fruit were sampled at optimum (184 DAFB) and late (214) harvest dates. At the four early maturity stages, fruit were randomly sampled in outer

canopy position on both sides of the row, whereas as on optimum and late harvest dates, fruit were sampled including fruit position (inner and outer canopy) and orientation (East or West) aspects. Because of findings from 2014/15, a bi-weekly interval was adopted in 2015/16, both sides of the trees, and at four selected fruit maturity stages towards harvest maturity as per each cultivar. In ‘Cripps Pink’, fruit was sampled at 122, 136, 150 and 164 DAFB, in ‘Golden Delicious’ at 80, 94, 108 and 122 DAFB, whereas in ‘Granny Smith’ samples were collected at 94, 108, 122 and 136 DAFB. The sampling procedure was planned to be concluded 2 weeks before the 2015/16 seasons’ determined optimum maturity date for each cultivar. The fruit samples of ‘Golden Delicious’ and ‘Cripps Pink’ apples were peeled immediately upon arrival in the laboratory, while ‘Granny Smith’ apple fruit samples were held for one day at - 0.5 °C and peeled the next day. This was due to the limitation in the skilled personnel to process and handle all the fruit samples from the three cultivars in a single day. The apple fruit peel samples were milled in liquid nitrogen, and thereafter the milled samples were stored at - 80 °C until performance of the respective phenolic analyses.

d. Extraction and determination of apple peel total phenolics and flavonoids

Analytical grade solvents and chemicals sourced from Sigma Aldrich, South Africa were used in the quantitative determination of total phenolics and flavonoids in apple peel samples. These were; Ethanol (96 %), Folin-Ciocalteu phenol reagent, Gallic acid, Catechin, Aluminium chloride, Sodium nitrite, Sodium hydroxide, and Sodium carbonate. The Milli-Q system (Millipore, Bedford, MA, USA), was the source of the double distilled water required. The total phenolics (TP) was extracted and determined using a slightly modified Folin-Ciocalteu (FC) method of Slinkard and Singleton (1977). TP extraction occurred by stirring 100 mg frozen apple peel sample with 10 ml of 80 % ethanol in a 50 ml centrifuge tube, at 4 °C, for 1 hour in the dark. This was followed by centrifugation at 4 °C using the Eppendorf™ 5810R cooling centrifuge for 15 minutes at 3220 g. Then, 2 ml of the supernatant was centrifuged at 20000 g at 4 °C using Eppendorf™ 5417R cooling centrifuge for 15 minutes, from which 1 ml of the resultant supernatant was collected and stored at - 20 °C until TP quantification.

The TP was quantified by adding 450 µl of the FC reagent (1:9 dilution with double distilled water) to 50 µl of the sample or blank (80 % ethanol) in a Lasec® (Cape Town, South Africa) plastic cuvette, followed by vortexing, and was left to stand for 5 minutes. Thereafter 500 µl of 5.6 % sodium carbonate was added, vortexed and then left to stand for 90 minutes at room temperature. After 90 minutes, absorbance of the contents in each cuvette was measured immediately at 750 nm using a UV-visible light spectrophotometer (Cary 50 Bio, Varian, Australia (Pty) Ltd, Melbourne, Australia).

The TP was expressed in Gallic acid equivalents (GAE) as mg GAE. 100 g⁻¹ of apple peel fresh weight (FW). These GAE units were obtained from standard curve prepared with Gallic acid ranging from 0.05 to 0.25 mg. ml⁻¹.

Total flavonoids (TF) was extracted and determined following a slightly modified Aluminium Chloride colorimetric method of Wolfe et al. (2003). TF extraction occurred by stirring 100 mg of frozen apple peel sample with 10 ml of 80 % ethanol in a 50 ml centrifuge tube, at 4 °C, for 1 hour in the dark, followed by centrifugation with Eppendorf™ 5810R at 4 °C and 3220 g, for 15 minutes. Thereafter, 2 ml of the supernatant was centrifuged further at 20000 g using Eppendorf™ 5417R at 4 °C for 15 minutes, where after 1 ml of the supernatant was kept at - 20 °C until TF quantification. To a 250 µl volume of sample or blank (80 % ethanol) was added 1250 µl of double distilled water, followed by the addition of 75 µl of 5 % sodium nitrite solution, where after the mixture was left to stand for 5 minutes. Thereafter, 150 µl of 10 % Aluminium Chloride was added and left to stand for 6 minutes, before 500 µl of 1M sodium hydroxide and 275 µl of double distilled water was added to the mixture. Following each step where sample and/or the reagent was added, the mixture in the reaction test tube was vortexed. Then, the test tube content were poured in Lasec® (Cape Town, South Africa) plastic cuvettes where after absorbance was immediately measured at 510 nm with a UV-visible light spectrophotometer (Cary 50 Bio, Varian, Australia (Pty) Ltd, Melbourne, Australia). The TF was expressed in Catechin Equivalents (CE) as mg CE. 100 g⁻¹ of apple peel fresh weight (FW). These CE units were obtained from a standard curve prepared using Catechin ranging from 0.05 to 0.25 mg. ml⁻¹.

e. Sunburn incidence

Daiber (2017) assessed sunburn incidence in all the experiment sites, and the conclusions from this study showed that the highest sunburn occurred in classes 1 (16%) and 2 (11%) in the control (no B+Ca) and the highest efficiency of the foliar B+Ca treatment in suppressing sunburn browning was also observed in these classes. Furthermore, despite SBB being the most economically important, bleaching and sunburn necrosis are other forms and/or different kind of sunburn whose mitigation has not previously or is generally not approached with systemic or quasi-systemic methods. Therefore, in this study only the statistics of total sunburn, class 0 (no sunburn), class 1 and 2 browning types are presented in this dissertation alongside the peel biochemical data of respective foliar B+Ca treatments. Appendixes 1 and 2 show the weather data at the experimental sites as well as the charts that were used to evaluate sunburn incidence in the experiments.

f. Data analyses

The TP and TF data was used to generate another response variable, the TF:TP ratio and was expressed as a percentage. Thereafter, variability of these three apple peel phenolic (TP, TF and TF:TP) parameters was analysed using analysis of variance techniques with Statistica software. Differences were significant for $p < 0.05$ and the means across foliar B+Ca treatments and fruit maturity (DAFB) levels were separated with the Fisher's LSD posthoc test. The respective means plus standard errors of each phenolic parameter among the foliar B+Ca treatments with progression of fruit maturity or DAFB were illustrated in trends with using XLSTAT software (Appendixes 3, 4 and 5). These curves were further smoothed with two-point moving average technique to enable easier comparison of seasonal trends (not statistical) among treatments. In the experiments where SBB incidence suppression occurred, the respective phenolic parameter trends were evaluated and contrasted amongst varying foliar B+Ca treatments, using statistical characteristics of respective trend gradient and/or slope (b) obtained with XLSTAT software. The statistical analyses results and behaviour of pigments in their respective trend curves were related to the SBB incidence recorded at harvest maturity, in order to identify foliar B+Ca treatments that influenced apple peel phenolic parameters, whilst associating with significant SBB incidence reduction.

3. Results

a. 'Cripps Pink'

i. 2014/15 four early DAFB stages – phenolics, flavonoids and flavonoids to phenolics ratio

There was no significant interaction between treatments and DAFB for peel TP and TF levels. The total phenolics (TP) and total flavonoids (TF) levels of the immature 'Cripps Pink' apple fruit peel did not differ significantly amongst the foliar boron plus calcium (B+Ca) treatments, but did within the early fruit (35, 49, 56 and 70) DAFB periods (Table 4). The fruit peel TP level at developmental stages 35 and 70 DAFB was lowest and comparable, whereas the fruit peel TP level recorded at both 49 and 56 DAFB was the highest and comparable, but jointly these differed significantly. The fruit peel TF level increased significantly from each maturity stage to the next from 35 to 56 DAFB (Table 4), but then TF decreased sharply from 56 to 70 DAFB to reach a similar level as at 35 DAFB.

There was no significant interaction between treatments and DAFB for peel total flavonoids to total phenolics ratio (TF:TP) as well (Table 4). And, the TF:TP levels of the maturing 'Cripps Pink' apple fruit peel did not differ significantly amongst the boron plus calcium (B+Ca) treatments, but only did so amongst DAFB periods (Table 4), with fruit peel TF:TP level at 56 DAFB highest and significantly

different from values recorded at 35, 49 and 70 DAFB. Differences in TF:TP values amongst 35, 49 and 70 DAFB were not significant.

The trends showed that peel TP (Figure 1.1) and TF (Figure 1.2) increased from 49 to 56 DAFB in all treatments and the control, followed by a decline towards 70 DAFB, and generally the control (no B+Ca) had higher TP and TF trends compared to the foliar B+Ca treatments from 56 to 70 DAFB. The moving mean trends showed that peel TF:TP increased from 49 to 56 DAFB in all treatments, and increased further or stayed relatively constant towards 70 DAFB with exception of treatment B^{0.02}+Ca^{0.10} (Figure 1.3), and on 56 DAFB, the TF:TP trend was the highest in treatment B^{0.02}+Ca^{1.29}.

ii. 2014/15, optimum harvest maturity date (184 DAFB)

Interaction between treatment and fruit orientation was not significant for TP and TF (Table 5). The differences in these phenolic parameters were all not significant amongst the B+Ca treatments at 184 DAFB (Table 5). Differences within TP were also not significant within the two fruit orientations of East and West sides of the trees. However, TF levels were significantly different between East and West, where the East oriented fruit peel showed higher TF than the West oriented fruit. A significant interaction effect between treatments and fruit orientation was recorded for TF:TP (Table 6). This interaction effect as presented in Table 6, showed that the West oriented fruit had lower apple peel TF:TP than the East for B^{0.00}+Ca^{0.00} (control) and treatment B^{0.02}+Ca^{1.29}. Only in treatment B^{0.02}+Ca^{0.10} was the TF:TP higher in the West than in the East. For West fruit, TF:TP was higher in B^{0.02}+Ca^{0.10} compared to the other two treatments, while in East fruit, TF:TP was higher in B^{0.02}+Ca^{1.29} compared to the control.

iii. 2014/15 late harvest date (214 DAFB)

There were no significant interaction effects between treatments, position and orientation. The differences in apple peel phenolic parameters (TP, TF and TF:TP) were also all not significant amongst the foliar B+Ca treatments at 214 DAFB (Table 7). However, differences within TP, TF and TF:TP were all significant within the two fruit positions (inward or outward) of the trees, and no significant differences were recorded for the fruit orientation at this late maturity stage. The outward canopy positioned fruit recorded high and significant TP and TF levels from the inward positioned fruit, and the reverse was true for the TF:TP levels.

iv. 2015/16, phenolics, flavonoids to phenolics ratio and flavonoids

Treatment and fruit maturity did not interact significantly for TP and TF:TP in 2015/16 (Table 8). Total phenolics (TP) levels did not differ significantly among the foliar B+Ca treatments, but was different between fruit maturity stages (Table 8). At 122 DAFB, the highest fruit peel TP level was recorded and it was significantly different from that at 136 and 150 DAFB (which did not differ significantly) as well as that at 164 DAFB. Fruit peel TP generally decreased significantly with progress of DAFB. The TF:TP was also not significantly different amongst foliar B+Ca treatments, but was within the maturity stages (DAFB). The TF:TP at 122 and 150 DAFB was the lowest and highest, respectively, and these were significantly different from each other, as well as to the TF:TP levels recorded at both 136 and 164 DAFB. In addition, the TF:TP at 136 DAFB was higher and significantly different from that recorded at 164 DAFB. Generally, fruit peel TF:TP increased from 122 to 150 DAFB with significant differences at each maturity stage and the decreased sharply towards 164 DAFB.

There was a significant interaction for apple fruit peel total flavonoids (TF) levels with foliar B+Ca treatments and fruit maturity stages (DAFB) (Table 9). The control and treatments did not have significant differences at any maturity stage. At 122 DAFB, only treatments B`0.17+Ca`1.24 (lower) and B`0.08+Ca`1.24 (higher) differed significantly. At 136 DAFB only treatment B`0.02+Ca`1.24 (higher) and treatment B`0.17+Ca`1.24 (lower) significantly differed. At each of 150 and 164 DAFB fruit maturity stages, no significant differences occurred amongst the treatments and with the control. Across the maturity stages (122 to 164 DAFB), the control and treatment B`0.02+Ca`1.24 did not have significant differences. Whereas B`0.02+znCa`0.06 and B`0.08+Ca`1.24 had their lowest TF at 164 DAFB that was significantly different from that recorded at 122, 136 and 150 DAFB, save 122 DAFB with the Zn formulation. Only in B`0.17+Ca`1.24 was the TF characteristically lower at the earlier (122 and 136 DAFB) periods and significantly highest at 150 DAFB, but notably as well, these TF levels were not significantly different at 122, 136 and 164 DAFB.

The TP levels displayed decreasing trends from 122 to 164 DAFB in all treatments except for B`0.17+Ca`1.24 (Figure 2.1). Whereas B`0.17+Ca`1.24 showed a lower trend compared to other treatments until 150 DAFB, it surpassed all treatments except the control by 164 DAFB. Generally, TF level trends (Figure 2.2) rose and peaked at 150 DAFB, and decreased towards 164 DAFB. Treatment B`0.17+Ca`1.24, which initially trended lower than other treatments, remained almost constant towards 164 DAFB after 150 DAFB. The TF:TP trends (Figure 2.3), showed increasing trends and peaking at 150 DAFB, and then gradual decreases towards 164 DAFB.

b. 'Golden Delicious'

i. Phenolics and flavonoids

Significant interaction effects for the fruit peel total phenolics (TP) and total flavonoids (TF) (Tables 10 and 11) occurred between treatments and maturity stages (DAFB). Considering TP and TF levels together, at 80 DAFB, no treatment significantly differed with the control, only B^{0.02}+znCa^{0.06} (lower TP) differed significantly with B^{0.08}+Ca^{1.24} (higher TP), but none of these two treatments showed significant TP differences with other treatments and the control. At 94 DAFB, all TP and TF recorded with the treatments was not significantly different, the control (highest TP) was only significantly different from B^{0.08}+Ca^{1.24} (lower TP) and B^{0.02}+Ca^{1.24} (lowest TP). In addition, the control also had the highest TF at 94 DAFB that was significantly different from that recorded with all treatments. At 108 DAFB, the control and treatments B^{0.02}+Ca^{1.24}, B^{0.02}+znCa^{0.06} and B^{0.17}+Ca^{1.24} did not have significant differences for TP and TF levels. Treatments B^{0.08}+Ca^{1.24} and B^{0.17}+Ca^{1.24} respectively showed highest TP and TF levels that were not significantly different. The highest TP and TF levels of treatment B^{0.08}+Ca^{1.24} were significantly different from those of the control, as well as with values of treatments B^{0.02}+Ca^{1.24} and B^{0.02}+znCa^{0.06}. The TF of treatment B^{0.17}+Ca^{1.24} was only significantly different from that of B^{0.02}+znCa^{0.06}. At 122 DAFB, only treatment B^{0.08}+Ca^{1.24} had TP (lowest) significantly different from the control and as well to all other treatments, except B^{0.02}+Ca^{1.24}. But, the lowest TF of treatment B^{0.08}+Ca^{1.24} was only significantly different from that of treatments B^{0.02}+znCa^{0.06} and B^{0.17}+Ca^{1.24}, yet TF of this Zn and high B treatments was also not significantly different from each other and as well to the TF of the control and B^{0.02}+Ca^{1.24}.

As DAFB progressed, the control's highest TP and TF that were recorded at 94 DAFB, significantly differed from values at 80, 108 and 122 DAFB, showing an increase from 80 to 94 DAFB, and generally decreasing from 94 DAFB to both 108 and 122 DAFB periods with no significant changes. For treatment B^{0.02}+Ca^{1.24}, the TP and TF levels at both 80 and 94 DAFB (higher) were respectively not significantly different as was also values at both 108 and 122 DAFB (lower), but these two pairs significantly differed from each other, thus showing a significant decrease in TP and TF from 94 to 108 DAFB. The TP and TF levels recorded with B^{0.02}+znCa^{0.06} at 108 DAFB was only significantly different (lower) from that at both 80 and 94 DAFB, but not with that at 122 DAFB. Thus, with B^{0.02}+znCa^{0.06}, TP at 80, 94 and 122 DAFB was not significantly different, and so was TF at both 80 and 122 DAFB. The highest TF at 94 DAFB with B^{0.02}+znCa^{0.06} was significantly different from that at both 108 and 122 DAFB but not from the value at 80 DAFB, and the lower values recorded at both 108 and 122 DAFB were not significantly different. Treatment

B^{0.08}+Ca^{1.24} did not show significant TP and TF differences from 80 to 108 DAFB, but its lowest TP and TF values recorded at 122 DAFB were significantly different from all other DAFB periods. With treatment B^{0.17}+Ca^{1.24}, the TP was not significantly different across all DAFB periods, yet its TF levels showed non-significant differences from 80 to 108 DAFB, and its lowest TF at 122 DAFB was only significantly different from that at both 80 and 94 DAFB. The TP and TF trends (Figure 3.1 and 3.2) showed that all treatments gradually decreased towards fruit harvest, with B^{0.02}+znCa^{0.06} and B^{0.02}+Ca^{1.24} as the only ones lower than the control.

ii. Flavonoids to phenolics ratio

Treatments and maturity stage did not interact significantly for the total flavonoids to total phenolics ratio (TF:TP) (Table 12). Ratios did not differ significantly amongst the foliar B+Ca treatments, but significant differences occurred amongst the maturity stages (DAFB). The TF:TP recorded at 80, 94 and 122 DAFB was lowest and not significantly different, but did differ significantly with the highest TF:TP recorded at 108 DAFB. Thus, TF:TP increased with progress of fruit maturity or DAFB from 80 to 108 DAFB and then decreased again thereafter towards 122 DAFB. The TF:TP levels trended upwards from 94 to 108 DAFB where after it stayed relatively constant towards 122 DAFB with the exception of treatment B^{0.08}+Ca^{1.24} that showed a further increase after initially trending lower than other treatments. Treatment B^{0.02}+Ca^{1.24} trended higher than other treatments but trends of treatment B^{0.08}+Ca^{1.24} surpassed it at 122 DAFB (Figure 3.3).

c. 'Granny Smith'

i. Phenolics and flavonoids

Significant interactions for fruit peel total phenolic (TP) and total flavonoid (TF) levels occurred amongst treatments and maturity stages (DAFB) (Tables 13 and 14). At 94 DAFB, the control had the highest TP and TF levels that were only significantly different from only values of both treatments B^{0.02}+Ca^{1.24} and B^{0.17}+Ca^{1.24}. In addition, at 94 DAFB, treatments B^{0.02}+Ca^{1.24} and B^{0.17}+Ca^{1.24} did not significantly differ from each other for TF and TP, as well as treatments B^{0.02}+znCa^{0.06} and B^{0.08}+Ca^{1.24}. At 108 DAFB, the control again had the highest TP and TF levels that were only significantly different from only values of both treatments B^{0.02}+znCa^{0.06} and B^{0.17}+Ca^{1.24}. In addition, at 108 DAFB, treatments B^{0.02}+Ca^{1.24}, B^{0.02}+znCa^{0.06} and B^{0.17}+Ca^{1.24} did not significantly differ from each other for TF and TP. At 122 DAFB, the control still had the highest TP and TF levels that were only significantly different from only values of treatment B^{0.08}+Ca^{1.24}. In addition, at 122 DAFB, all treatments did not significantly differ from

each other for TF and TP. At 136 DAFB there were no significant TP and TF differences between the control and all treatments, but only treatments B`0.02+znCa`0.06 (lower) and B`0.17+Ca`1.24 (higher) differed significantly for both TP and TF levels.

Across the fruit maturity stages (94 to 136 DAFB), the control's highest TP and TF levels at 94 DAFB were only significantly different from that at 136 DAFB, thus the TP and TF for the control at 94, 108, and 122 DAFB was not significantly different, as well as across 108, 122 and 136 DAFB. The TP and TF of B`0.02+Ca`1.24 was not significantly different across all DAFB periods. The TP and TF recorded with B`0.02+znCa`0.06 at 94 DAFB was higher and significantly different (except TP at 122 DAFB) from all at other DAFB periods, and again across 108, 122 and 136 DAFB, TP and TF for this Zn formulation did not significantly differ. Treatment B`0.08+Ca`1.24 had non-significantly different and higher TP and TF at 94 and 108 DAFB, but these two values were significantly different from those at both 122 and 136 DAFB which also did not differ significantly. This indicates that a significant decrease in both TP and TF with treatment B`0.08+Ca`1.24 occurred at 108 to 122 DAFB, a characteristic that was only realised with this treatment. As was with B`0.02+Ca`1.24, treatment B`0.17+Ca`1.24 had TP and TF that was not significantly different across all DAFB periods.

The TP and TF trends (Figures 4.1 and 4.2) showed that values of the control were generally higher compared to all treatments across the fruit maturity stages, but decreased steadily as DAFB progressed, but remained highest towards 136 DAFB. The trends of treatments B`0.02+Ca`1.24 were rather constant from 108 to 136 DAFB. Treatment B`0.17+Ca`1.24, trends were also rather constant between 108 and 122 DAFB, and then rose towards 136 DAFB to exceed all treatments but not the control, yet the reverse was true for treatment B`0.02+znCa`0.06 where very sharp decreases from 108 to 122 DAFB were followed by rather constant trend towards 136 DAFB. Treatment B`0.08+Ca`1.24 had the most sharply decreasing TP and TF trends from the having the highest levels at 122 DAFB to the lowest at 136 DAFB.

ii. Flavonoids to phenolics ratio

Interaction between treatments and maturity stage was not significant for the 'Granny Smith' apple peel TF:TP, and significant differences were recorded only amongst the DAFB stages but not treatments (Table 15). At 94 DAFB, TF:TP was highest and was only non-significantly different to that at 108 DAFB. The fruit peel TF:TP at maturity stages 122 and 136 DAFB were the lowest and were not different from each other as well as to that at 108 DAFB. Generally, apple fruit peel total flavonoids to phenolics ratio (TF:TP) in 'Granny Smith' decreased with progression of DAFB contrary to the TF:TP trends in both 'Cripps Pink' and 'Golden Delicious' apples as also illustrated

in Figure 4.3. The TF:TP level of the control ($B^0.00+Ca^0.00$) and $B^0.17+Ca^1.24$ generally trended lower highest respectively.

d. Sunburn incidence

In 2014/15 ‘Cripps Pink’, experiments, the differences among the foliar B+Ca treatments for sunburn (total sunburn), no sunburn (class 0) and sunburn browning (class 1 and 2) incidence were not significant (Daiber, 2017; Table 7). In 2015/16 ‘Cripps Pink’ experiments, the differences among the foliar B+Ca treatments for sunburn data were only significant for class 1 type (Table 16). All the foliar B+Ca treatments showed class 1 sunburn browning that was significantly different from the control ($B^0.00+Ca^0.00$), but no treatment differed from the other significantly (Daiber, 2017). Total sunburn, no sunburn (class 0) and sunburn browning class 2 incidence were not significantly different among treatments.

In ‘Golden Delicious’, differences among the foliar B+Ca treatments for sunburn incidence data were only significant for class 0 (no sunburn) and class 1 type sunburn browning (Table 16). In class 0, treatments $B^0.08+Ca^1.24$ and $B^0.17+Ca^1.24$ had significantly lower than the control and treatment $B^0.02+znCa^0.06$. The incidence of SBB in class 1 in treatment $B^0.08+Ca^1.24$ was significantly lower compared to the control and treatment $B^0.02+znCa^0.06$. In ‘Granny Smith’, the differences among the foliar B+Ca treatments for sunburn incidence were not significant (Table 16).

e. Statistical characterisation of ‘Cripps Pink’ and Golden Delicious’ apple peel phenolic trends

i. ‘Cripps Pink’

The slope (b) for the total phenolics had a negative direction in all foliar treatments, including the control, suggesting that generally, this parameter decreased as fruit maturity advanced (Table 17). However, this decrease is only confirmed where the p -value is significant ($p < 0.05$), and that is only in treatments $B^0.02+Ca^1.24$, $B^0.02+znCa^0.06$ and $B^0.08+Ca^1.24$. The magnitude of the slope was also varying among treatments, suggesting variable decomposition of the phenolics, accordingly the rate of decomposition was highest in treatment $B^0.02+znCa^0.06$ and lowest in treatment $B^0.17+Ca^1.24$. Total flavonoids also decreased with fruit maturity variably among the foliar treatments, except in treatment $B^0.17+Ca^1.24$, which showed positive slope, although it was not significant ($p = 0.2019$). The negative slope was significant in treatments $B^0.02+znCa^0.06$ and $B^0.08+Ca^1.24$. The TF:TP ratio slope was positive in all treatments and the control, except for treatment $B^0.08+Ca^1.24$ that was negatively non-significant, only positive TF:TP slopes were significant in the control and $B^0.17+Ca^1.24$. All differences in the direction and magnitude of the

gradient of the peel phenolic biochemicals could not distinguish the various trends as the slope (*b*) contrasts were all not significant.

ii. ‘Golden Delicious’

The slope (*b*) for the total phenolics and flavonoids had a negative direction in all the foliar treatments including the control showing the decreasing trends with fruit maturity advance (Table 18). However, this decrease was significant only in the control, $B^{-0.02}+Ca^{1.24}$ and $B^{-0.08}+Ca^{1.24}$ for total phenolics, while the negative slope was significant in all treatments and control for total flavonoids. The TF:TP trends had a positive slope, except $B^{-0.08}+Ca^{1.24}$, and none was significant. As for ‘Cripps Pink’, trend slope differences with respect to direction and magnitude of the gradient of the peel phenolic biochemicals could not distinguish the various trends as the slope (*b*) contrasts were all not significant.

4. Discussion

a. Effect of the post-full-bloom foliar boron plus calcium treatments on apple peel phenolic levels

After the finding that boron plus calcium applied at post-full-bloom associated with sunburn incidence reduction in ‘Golden Delicious’ apples (Lötze & Hoffman, 2014), the most important questions that arose were how this phenomenon occurred (mode-of-action) and what would be the reaction of other cultivars to these post-full-bloom treatments. Two schools of thought emerged in respect to these questions, and in one, the traditional sunburn mitigation approaches mode-of-action of reducing the build-up of threshold fruit surfaces temperatures was borrowed as well as the reinforcement of cell structures especially walls and membranes by the supplementary boron and calcium. These factors were investigated by Daiber (2017). However, results did not support the findings as a possible mode-of-action (Daiber, 2017; Lötze et al., 2017; 2018). Quite a few of the products used in formulation of treatments did contain nitrogen (N), and higher peel N may induce higher chlorophyll levels and hence greener fruit peel with no sunburn browning symptoms, but apple peel mineral analyses in Daiber (2017) did not indicate such possibility. The other school of thought which became the investigation subject of this dissertation, related to studying the effect of the post-full-bloom foliar B+Ca treatments on the peel biochemistry. In particular, biochemical and/or metabolite aspects were the focus as it was established that their levels change within apple peels during fruit sunburn induction or development and these biochemical aspects are mainly phenolics,

oxidative stress and photosynthetic pigments (Solovchenko & Schmitz-Eiberger, 2003; Felicetti & Schrader, 2008; 2009; Yuri et al., 2010).

The 2014/15 experiments were largely exploratory on the questions or thoughts above and respective results informed the experimental practice for both the Daiber (2017) study as well the studies described in this dissertation for the next season 2015/16 experiments. The cultivar chosen in these experiments was the bi-colour 'Cripps Pink', quite distinct from 'Golden Delicious' but genetically related (Cripps et al., 1993). For the purpose of the peel biochemical investigation, two treatments were chosen to contrast to the control; treatment B^{0.02}+Ca^{0.10} had earlier been the best at reducing sunburn incidence in 'Golden Delicious' (Lötze & Hoffman, 2014). Treatment B^{0.02}+Ca^{1.29} carried extra calcium content in order to assess the importance of changing the levels of calcium concentration on both peel biochemistry and sunburn incidence reduction.

In 2014/15, the focus of the experiments with respect to boron plus calcium treatment induced changes in peel biochemistry targeted the early fruit maturity stages, as well as the position and orientation of the fruit within the tree canopy. No significant foliar B+Ca treatment or interaction effects on the peel phenolics were realised with early maturity stages, however the TF:TP showed significant interaction effects at 184 DAFB, with treatments showing variable TF:TP levels between West and East oriented fruit. This prompted the suggestion that foliar B+Ca treatment induced changes in peel biochemistry may occur later in the season, possibly towards the harvest maturity window, which became the focus in the 2015/16 experiments. In addition, 2015/16 experiments focused on the outer canopy positioned fruit and on both sides of the tree as results at harvest (214 DAFB) did not show any significant interaction effects amongst treatments, position or orientation of the fruit (Daiber, 2017).

With reference to 2015/16 results, the apple peel phenolic metabolites generally decreased as fruit matured, and these decreasing phenolic trends were associated with significant maturity stage (DAFB) effects on all respective parameters (TP, TF and TF:TP ratio) studied in the three ('Cripps Pink', 'Golden Delicious' and 'Granny Smith') apple cultivars. This is in agreement with several previous studies on phenolic levels of apple peels at varying fruit maturity (Lister et al., 1994; Mayr et al., 1995; Hamauzu et al., 1999; Awad et al., 2001; Kondo et al., 2002; Renard et al., 2007; Moyle, 2011; Van der Sluis et al., 2001; Mehrabani & Hassanpouraghdam, 2012). However, in this study, the decrease in phenolic levels as fruit maturity (DAFB) progressed was associated with statistically significant interaction effects between the foliar B+Ca treatments and fruit maturity stages (DAFB) for TP ('Golden Delicious' and 'Granny Smith') and TF for all the three cultivars studied (2015/16). This is evidence that foliar B+Ca treatments affect the natural apple peel phenolic synthesis and

decomposition as fruit maturity progresses in all the three apple cultivars. In addition, this occurred without a significant effect or a compromise on the internal apple fruit quality as suggested by results of Daiber (2017).

b. Does the foliar B plus Ca induced changes in apple peel phenolic levels explain sunburn browning incidence reduction outcomes in ‘Cripps Pink’ apple?

Most of the phenolic synthesis in apple fruit occurs between 30 and 60 DAFB (Renard et al., 2007). Thereafter, increasing fruit size leads to reduction (dilution) of this early maturity stage accumulated phenolic levels (Awad et al., 2001; Renard et al., 2007). Therefore, the decreasing trend of the fruit peel TP and TF levels observed starting at around 56 DAFB towards 70 DAFB is possibly a ‘dilution’ effect of earlier accumulated phenolic levels as result of increased fruit sizes. The apple fruit peel TF:TP ratio observed at different maturity stages (DAFB) ranged from 51-69 % (significantly different) and among foliar B+Ca treatments it was 54-57 % (not significantly different); TF concentrations were approximately two-thirds of those of TP in agreement with previous findings of Tsao et al. (2003), Wolfe et al. (2003) and Giomaro et al. (2014). Flavanols, that are a major group of flavonoids, account for 65-85 % of TP levels in the dessert apples (Guyot et al., 2002; 2003). The apple fruit peel TF:TP ratio trend was higher in treatment B`0.02+Ca`1.29 compared to both treatment B`0.02+Ca`0.10 and control (B`0.00+Ca`0.00) at harvest, but variability as par variance was not different from the control. Possibly if sampling continued towards fruit optimum harvest maturity, this statistics would differ, since in red-blushing cultivars like ‘Cripps Pink’ apple there is late season accumulation of peel anthocyanins that are part of flavonoids (Wolfe et al., 2003; Whale & Singh, 2007; Makedredza et al., 2015). The SBB incidence did not differ significantly amongst the foliar B+Ca treatments in 2014/15 purportedly due to a heavy sunburn-masking red blush effect (Daiber, 2017).

In 2015/16, TP levels at 122 DAFB were the highest across all the foliar B+Ca treatments and this decreased steadily during fruit maturity (DAFB) progressed. The decrease of the peel TP as the DAFB progressed possibly suggests a ‘dilution’ effect of the earlier (122 DAFB and below) accumulated phenolic levels as the apple fruit increased in size (Awad et al., 2001; Renard et al., 2007), as significant effects for the maturity stages (DAFB) were also recorded. However, the TF levels increased from maturity stage 122 DAFB and peaked between 136 to 150 DAFB in all treatments, and declined sharply towards 164 DAFB. This difference in trend of phenolic levels is unique to this cultivar among the three apple cultivars involved in this study, and probably is due to the fact that the second peak of anthocyanins (flavonoids) accumulation in the peel of this apple (‘Cripps Pink’ apple), occurs at maturity stage 150 to 160 DAFB (Whale & Singh, 2007).

Significant sunburn differences amongst the foliar B+Ca treatments were recorded but only for class 1 SBB incidence, in which all the foliar B+Ca treatments differed from the control (no B+Ca) but not from each other (Daiber, 2017). This suggests that all the foliar B+Ca treatments were equally efficient at reducing class 1 SBB incidence. A rational view is that higher TP and TF levels in apple fruit peel should associate with less class 1 SBB incidence, since these metabolites are powerful antioxidants and enable photoprotection of the photosynthetic apparatus in apple peel. However, phenolic aspects (TP, TF and TF:TP) do not correspond with this view as a possible mode-of-action as no significant treatment effects were recorded. In addition, the control (no B+Ca) had the highest TP and TF level trends at 164 DAFB, but a significantly higher incidence of class 1 SBB. In general, a mode-of-action was not realised, as the trend contrast of the foliar treatments was not significantly different from the control.

c. Does the foliar B plus Ca induced changes in apple peel phenolic levels explain sunburn browning incidence reduction outcomes in ‘Golden Delicious’ and ‘Granny Smith’ apples?

In ‘Golden Delicious’ apple, the peel TP and TF levels decreased as fruit matured and this as well is in agreement with the natural process of phenolic level changes in apple fruit growth and development (Awad et al., 2001; Renard et al., 2007). The reduction of these phenolic metabolites as fruit maturity progressed was variable, and had significant interaction effects between treatments and DAFB. This suggests an important effect of the foliar B+Ca, and as thus the importance of the TP, TF and derivative TF:TP trends across the DAFB amongst treatments. Treatments B`0.08+Ca`1.24 and B`0.17+Ca`1.24 increased the percentage of fruit without sunburn compared to the control. Whereas it was only B`0.17+Ca`1.24 showed a higher trend of peel TP and TF (Figures 3.1 and 3.2) at advanced maturity (122 DAFB) when apple fruit is most susceptible to SBB induction, and B`0.08+Ca`1.24 did not show the same higher trend compared to the control. Hence, the decrease in sunburn does not seem to relate to the trend in phenolic and flavonoid levels towards harvest. Again, a mode-of-action could not be determined, as the trend contrast of the foliar treatments were not different from the control.

Although flavonoids are crucial metabolites in protecting apple fruit peel against developing SBB (Solovchenko & Schmitz-Eiberger, 2003; Agati et al., 2013; Bi et al., 2014), peel flavonoid levels and trends over the fruit development of the foliar B+Ca treatments cannot explain the Class 1 SBB incidence reduction recorded for ‘Golden Delicious’ apples. Thus, Class 1 SBB incidence reduction as mediated by the foliar B+Ca treatments could be associated with another mechanism. Alternatively, the phenolic dynamics may not have ably explained the SBB incidence reduction explicitly due to the quantitative approach used in this study. Perhaps if a particular type of flavonoid

is the primary contributor in the mode-of-action by which the foliar B+Ca treatments reduce apple fruit SBB incidence, then qualitative assays may yield explicit trends and hence explicitly explain the SBB incidence reduction.

In ‘Granny Smith’, the peel TP and TF levels decreased with advancement of fruit maturity, and with significant interaction with treatments. The trends show that both TP and TF decreased in a very comparable manner across each foliar B+Ca treatment and control (no B+Ca), suggesting that flavonoids are the main phenolic components in the peel of this cultivar. Previous studies have also reported that flavonoids are the major phenolic metabolites in apples (Duenas et al., 2011; Yuri et al., 2014). As mentioned in the ‘Cripps Pink’ and ‘Golden Delicious’ apples experiment in this study, the decreasing phenolic levels with progress of fruit maturity (DAFB) in ‘Granny Smith’ apple suggests an increase in fruit size driven dilution effect of the earlier accumulated phenolic levels (Awad et al., 2001; Renard et al., 2007). Both ‘Golden Delicious’ and ‘Granny Smith’ apples experienced similar climatic conditions since they were at the same experiment site and side by side in orchard rows; however, unlike ‘Golden Delicious’, ‘Granny Smith’ apple fruit did not show significant SBB incidence reduction. This might have been due to the pre-optimum harvest of the experiment at 150 DAFB, as per the request of the producer, instead of the 2015/16 seasons’ anticipated harvest date of 178 DAFB for ‘Granny Smith’. And, despite the effect foliar B+Ca treatments had on ‘Granny Smith’ apple with respect to peel TP, TF and TF:TP levels, no significant difference between treatments was found in SBB incidence. Therefore, a change in phenolic levels during maturity as induced by foliar B+Ca treatments cannot serve as the mode-of-action whereby SBB incidence can be reduced in ‘Granny Smith’, or in other apples, involved in this study.

5. Conclusion

This study concludes that post-full-bloom foliar applications of a combination of B and Ca influence fruit peel phenolic metabolite levels in ‘Cripps Pink’, ‘Golden Delicious’ and ‘Granny Smith’ apples. Results from the 2014/15 ‘Cripps Pink’ experiment showed that foliar B+Ca treatments induce changes in peel phenolics, within different canopy positions of the tree, and a significant interaction for TF:TP ratio emerged at advanced maturity (184 DAFB). In 2015/16, with a focus on advanced fruit maturity, when susceptibility to SBB induction is most prominent, it was established that foliar B+Ca treatments and fruit maturity stages (DAFB) exerted significant interaction effects for total phenolics (‘Golden Delicious’ and ‘Granny Smith’), as well as total flavonoids for all cultivars. This implies a foliar B+Ca treatment effect on phenolic synthesis appears to be dependent on cultivar and individual treatment formulation effects. For instance, in ‘Cripps Pink’, no significant interactions were observed for total phenolics, in contrast to both ‘Golden Delicious’ and ‘Granny Smith’. Foliar B+Ca treatment effect on any of the three studied apple peel phenolic parameters did not pertinently explain the reduction in class 1 SBB incidence recorded in both ‘Cripps Pink’ and ‘Golden Delicious’.

Hence, in conclusion, phenolic changes in apple peel induced by particular foliar B+Ca treatments do not explain the mode-of-action of fruit SBB incidence suppression in apple orchards.

Acknowledgments

Support for this research was made possible through a capacity building competitive grant (RU/2015/DRRG/01/004) provided by Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) and Nulandis (Pty), South Africa. This study is part of a Ph.D. training program supported by RUFORUM members, Stellenbosch and Makerere Universities.

References

- Agati, G., Azzarello, E., Pollastri, S. & Tattini, M. 2012. Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science* 196:67-76.
- Agati, G., Brunetti, C., Ferdinando, M.D., Ferrini, F., Pollastri, S. & Tattini, M. 2013. Functional roles of flavonoids in photoprotection: New evidence, lessons from the past. *Plant Physiology and Biochemistry* 72:35-45.
- Awad, M.A., De Jager, A., Van Der Plas, L.H.W. & Van Der Krol, A.R. 2001. Flavonoid and chlorogenic acid changes in skin of 'Elstar' and 'Jonagold' apples during development and ripening. *Scientia Horticulturae* 90:69-83.
- Bi, X., Zhang, J., Chen, C., Zhang, D., Li, P. & Ma, F. 2014. Anthocyanin contributes more to hydrogen peroxide scavenging than other phenolics in apple peel. *Food Chemistry* 152:205-209.
- 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.
- Chen, L.S., Li, P. & Cheng, L. 2009. Comparison of thermotolerance of sun-exposed peel and shaded peel of 'Fuji' apple. *Environmental and Experimental Botany* 66:110-116.
- Cripps, J.E.L., Richards, L.A. & Mairata, A.M. 1993. 'Pink Lady' apple. *HortScience* 28:1057.
- Cook, N.C. & Jacobs, G. 2000. Progression of apple (*Malus x domestica* Borkh.) bud dormancy in two mild winter climates. *Journal of Horticultural Science and Biotechnology* 75:233-236.
- Daiber, S.H. 2017. Quantifying changes in tree physiology after amelioration to reduce sunburn on apples. MSc Thesis. Department of Horticultural Science, Stellenbosch University.
- Duenas, M., Surco-Laos, F., Gonzalez-Manzano, S., Gonzalez-Paramas, A.M. & Santos-Buelga, C. 2011. Antioxidant properties of major metabolites of quercetin. *European Food Research and Technology* 232:103-111.
- Falcone Ferreyra, M.L., Rius, S.P. & Casati, P. 2012. Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science* 3:222.
- Felicetti, D.A. & Schrader, L.E. 2008. Changes in pigment concentrations associated with the degree of sunburn browning of 'Fuji' apple. *Journal of the American Society of Horticultural Science* 133:27-34.

- Felicetti, D.A. & Schrader, L.E. 2009. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. II. Phenolics. *Plant Science* 176:84-89.
- Giomaro, G., Karioti, A., Bilia, A.R., Bucchini, A., Giamperi, L., Ricci, D. & Fraternali, D. 2014. Polyphenols profile and antioxidant activity of skin and pulp of a rare apple from Marche region (Italy). *Chemistry Central Journal* 8:45.
- Guyot, S., Le Bourvellec, C., Marnet, N. & Drilleau, J.F. 2002. Procyanidins are the most abundant polyphenols in dessert apples at maturity. *Food Science and Technology* 35:289-291.
- Guyot, S., Marnet, N., Sanoner, P. & Drilleau, J.F. 2003. Variability of the polyphenolic composition of cider apple (*Malus domestica*) fruits and juices. *Journal of Agricultural and Food Chemistry* 51:6240-6247.
- Hamauzu, Y., Iijima, E. & Banno, K. 1999. Changes in catechin and procyanidin contents during fruit development of two apple cultivars. *Journal of Japanese Society of Horticultural Science* 68:1184-1193.
- Khanizadeh, S., Tsao, R., Rekika, D., Yang, R., Charles, M.T. & Rupasinghe, H.P. V. 2008. Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. *Journal of Food Composition and Analysis* 21:396-401.
- Kondo, S., Tsuda, K., Muto, N. & Ueda, J.E. 2002. Antioxidative activity of apple skin or flesh extracts associated with fruit development on selected apple cultivars. *Scientia Horticulturae* 96:177-185.
- Lee, K.W., Kim, Y.J., Kim, D.O., Lee, H.J. & Lee, C.Y. 2003. Major phenolics in apple and their contribution to the total antioxidant capacity. *Journal of Agricultural and Food Chemistry* 51:6516-6520.
- Lister, C.E., Lancaster, J.E., Sutton, K.H. & Walker, J.R.L. 1994. Developmental changes in the concentration and composition of flavonoids in skin of a red and a green apple cultivar. *Journal of the Science of Food and Agriculture* 64:155-161.
- Lötze, E. & Hoffman, E.W. 2014. Foliar application of calcium plus boron reduces the incidence of sunburn in 'Golden Delicious' apple. *Journal of Horticultural Science and Biotechnology* 89:607-612.
- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2017. Boron in combination with calcium reduces sunburn in apple fruit. *Boron* 2:123-127.

- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2018. Evaluating the efficacy of a preharvest combination of calcium and boron as foliar application to reduce sunburn on 'Cripps Pink' apples. *Acta Horticulture* 1217:61-68.
- Makredza, B., Marais, H., Schmeisser, M., Lötze, E. & Steyn, W.J. 2015. Ripening associated red color development masks sunburn browning in apple peel. *HortScience* 50:814-818.
- Mayr, U., Treutter, D., Bauer, H. & Feucht, W. 1995. Developmental changes in the phenol concentrations of 'Golden Delicious' apple fruits and leaves. *Phytochemistry* 38:1151-1155.
- Mehrabani, L. V & Hassanpouraghdam, B.M. 2012. Developmental variation of phenolic compounds in fruit tissue of two apple cultivars. *Acta Scientiarum Polonorum* 11:259-264.
- Merzlyak, M.N., Solovchenko, A.E. & Chivkunova, O.B. 2002. Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. *Plant Physiology and Biochemistry* 40:679-684.
- Moyle, C.W.A. 2011. Polyphenols in apples and their interactions with vascular endothelial cells. PhD Dissertation, University of East Anglia.
- Pietta, P.G. 2000. Flavonoids as antioxidants. *Journal of Natural Products* 63:1035-1042.
- Renard, C.M.G.C., Dupont, N. & Guillermin, P. 2007. Concentrations and characteristics of procyanidins and other phenolics in apples during fruit growth. *Phytochemistry* 68:1128-1138.
- Slinkard, K. & Singleton, V.L. 1977. Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture* 28:49-55.
- Solovchenko, A. & Schmitz-Eiberger, M. 2003. Significance of skin flavonoids for UV-B-protection in apple fruits. *Journal of Experimental Botany* 54:1977-1984.
- Tsao, R., Yang, R., Young, J.C. & Zhu, H. 2003. Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). *Journal of Agricultural and Food Chemistry* 51:6347-6353.
- Tsao, R., Yang, R., Xie, S., Sockovie, E. & Khanizadeh, S. 2005. Which polyphenolic compounds contribute to the total antioxidant activities of apple? *Journal of Agricultural and Food Chemistry* 53:4989-4995.
- Van der Sluis, A.A., Dekker, M., De Jager, A. & Jongen, W.M.F. 2001. Activity and concentration of polyphenolic antioxidants in apple: Effect of cultivar, harvest year, and storage conditions. *Journal of Agricultural and Food Chemistry* 49:3606-3613.

- Whale, S.K. & Singh, Z. 2007. Endogenous ethylene and color development in the skin of 'Pink Lady' apple. *Journal of the American Society for Horticultural Science* 132:20-28.
- Wolfe, K., Wu, X. & Liu, R.H. 2003. Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry* 51:609-614.
- 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. *Journal of Food, Agriculture and Environment* 8:920-925.
- Yuri, J.A., Neira, A., Maldonado, F., Quilodran, A., Simeone, D., Razmilic, I. & Palomo, I. 2014. Total phenol and quercetin content and antioxidant activity in apples in response to thermal, light stress and to organic management. *Journal of Applied Botany and Food Quality* 87:131-138.

Tables

Table 1. Composition and sources of the products used to formulate the different B plus Ca foliar treatments.

	Product (Trade) name	¹ Active Ingredients (w/w)	Source
1	Manni-Plex [®] Ca	10 % Ca, 8 % N	Elim Kunsmis (Pty) Ltd., South Africa (2014/15)
2	Manni-Plex [®] Cal-Zn	6 % Ca, 6 % N, 3 % Zn	NexusAG (Pty) Ltd., South Africa (2015/16)
3	Manni-Plex [®] B	3.3 % B, 5 % N	
4	YaraLiva [™] Calcinit [™] (Ca(NO ₃) ₂)	19 % Ca, 26.3 % CaO, 15.5% N	Yara Business Unit Africa, South Africa
5	Calsol [®] (5Ca(NO ₃) ₂ NH ₄ NO ₃ .10H ₂ O)	19 % Ca, 15.5 % N	NexusAG (Pty) Ltd., South Africa
6	Spraybor [®] (Na ₂ B ₄ O ₇ .10H ₂ O)	16.5 % B	Nulandis (Pty) Ltd., South Africa

¹As per label on product packaging.

Table 2. Formulation protocol of the different B plus Ca foliar treatments.

Season	¹ Treatment formulation procedure	² Concentration (g.l ⁻¹)
	Control - no foliar B+Ca applications	0.00 g.l ⁻¹ B + 0.00 g.l ⁻¹ Ca (B`0.00+Ca`0.00 - Control)
2014/15	6 ml Manni-Plex [®] B plus 10 ml Manni-Plex [®] Ca	0.02 g.l ⁻¹ B + 0.10 g.l ⁻¹ Ca (B`0.02+Ca`0.10)
	6 ml Manni-Plex B [®] plus 68 ml Calcinit [™]	0.02 g.l ⁻¹ B + 1.29 g.l ⁻¹ Ca (B`0.02+Ca`1.29)
2015/16	Control - no foliar B+Ca applications	0.00 g.l ⁻¹ B + 0.00 g.l ⁻¹ Ca (B`0.00+Ca`0.00 - Control)
	6 ml Manni-Plex [®] B plus 10 ml Manni-Plex [®] Cal-Zn	0.02 g.l ⁻¹ B + 0.06 g.l ⁻¹ Ca (B`0.02+znCa`0.06)
	6 ml Manni-Plex [®] B plus 65 g Calsol [®]	0.02 g.l ⁻¹ B + 1.24 g.l ⁻¹ Ca (B`0.02+Ca`1.24)
	5 g Spraybor [®] plus 65 g Calsol [®]	0.08 g.l ⁻¹ B + 1.24 g.l ⁻¹ Ca (B`0.08+Ca`1.24)
	10 g Spraybor [®] plus 65 g Calsol [®]	0.17 g.l ⁻¹ B + 1.24 g.l ⁻¹ Ca (B`0.17+Ca`1.24)

¹The quantities were dissolved in 10 litres of water. ²Determined from weight per weight of B and Ca as indicated on the product label.

Table 3. Apple full bloom dates and B plus Ca foliar formulation treatment application dates at the Welgevallen and Applethwaite experimental sites.

Site and season	Full bloom dates	Treatment application times and dates					
		1 st	2 nd	3 rd	4 th	5 th	6 th
Welgevallen, 2014/15	13.10.2014	11.11.14	18.11.14	25.11.14	1.12.14	8.12.14	17.12.14
Welgevallen, 2015/16	05.10.2015	05.11.15	12.11.15	19.11.15	27.11.15	3.12.15	10.12.15
Applethwaite, 2015/16	16.10.2015	25.11.15	2.12.15	9.12.15	15.12.15	22.12.15	29.12.15

Table 4. 'Cripps Pink' apple peel phenolic parameters among three foliar B plus Ca treatments and at four early fruit maturity (DAFB) stages (2014/15).

Treatments (B+Ca)	TP	TF	TF:TP
B`0.00+Ca`0.00	465.65 ^{ns}	265.14 ^{ns}	56.53 ^{ns}
B`0.02+Ca`0.10	445.43	241.11	54.00
B`0.02+Ca`1.29	455.14	258.01	57.27
<i>p</i> -value	0.8439	0.2674	0.1618
Days after full bloom (DAFB)			
35	399.70 ^b	205.17 ^c	51.81 ^b
49	504.02 ^a	260.59 ^b	52.50 ^b
56	524.11 ^a	358.64 ^a	68.87 ^a
70	393.78 ^b	194.62 ^c	50.55 ^b
<i>p</i> -value	0.0018	0.0000	0.0000
B+Ca*DAFB (<i>p</i> -value)	0.4540	0.4190	0.1661

Mean values sharing a letter are not different, TP = total phenolics (mg GAE. 100 g⁻¹ peel FW), TF = total flavonoids (mg CE. 100 g⁻¹ peel FW) and TF:TP = total phenolics to total flavonoids ratio (%).

Table 5. 'Cripps Pink' apple peel phenolic parameters among three foliar B plus Ca treatments and at two fruit orientations of the outer canopy fruit position at 184 DAFB (2014/15).

Treatments (B+Ca)	TP	TF
B`0.00+Ca`0.00	547.73 ^{ns}	274.09 ^{ns}
B`0.02+Ca`0.10	496.93	253.41
B`0.02+Ca`1.29	516.19	256.03
<i>p</i> -value	0.5560	0.6000
Fruit orientation (O)		
West	481.53 ^{ns}	240.49 ^b
East	559.04	281.87 ^a
<i>p</i> -value	0.0620	0.0380
B+Ca*O (<i>p</i> -value)	0.2280	0.2050

Mean values sharing a letter are not different, TP = total phenolics (mg GAE. 100 g⁻¹ peel FW) and TF = total flavonoids (mg CE. 100 g⁻¹ peel FW).

Table 6. 'Cripps Pink' apple peel flavonoids to phenolics ratio (TF:TP) of outer canopy fruit position as a function of foliar B plus Ca and fruit orientation (West or East side of the tree) at 184 DAFB (2014/15).

Treatments (B+Ca)	West	East
B`0.00+Ca`0.00	48.67 ^c	51.00 ^b
B`0.02+Ca`0.10	54.38 ^a	48.76 ^{bc}
B`0.02+Ca`1.29	47.24 ^c	52.02 ^{ab}

Mean values sharing a letter are not different, B+Ca ($p = 0.2960$), Fruit orientation (O) ($p = 0.6470$) and B+Ca*O ($p = 0.0050$).

Table 7. 'Cripps Pink' apple peel phenolic parameters among three foliar B plus Ca treatments, two fruit positions and two fruit orientations at 214 DAFB (2014/15) plus sunburn incidence at harvest.

Treatments (B+Ca)	TP	TF	TF:TP
B`0.00+Ca`0.00	361.39 ^{ns}	198.53 ^{ns}	60.42 ^{ns}
B`0.02+Ca`0.10	328.69	182.35	63.44
B`0.02+Ca`1.29	294.64	177.83	70.39
<i>p</i> -value	0.6340	0.8110	0.3130
Fruit position (P)			
Inward	171.81 ^b	114.74 ^b	73.38 ^a
Outward	484.68 ^a	257.73 ^a	56.12 ^b
<i>p</i> -value	0.0000	0.0000	0.0040
Fruit orientation (O)			
West	283.83 ^{ns}	160.70 ^{ns}	62.78 ^{ns}
East	372.65	211.77	66.72
<i>p</i> -value	0.1290	0.0740	0.4690
B+Ca*P (<i>p</i> -value)	0.8980	0.8770	0.8330
B+Ca*O (<i>p</i> -value)	0.3280	0.4990	0.8900
P*O (<i>p</i> -value)	0.1450	0.1430	0.0580
B+Ca*P*O (<i>p</i> -value)	0.3920	0.4910	0.4780

Mean values sharing a letter are not different, TP = total phenolics (mg GAE. 100 g⁻¹ peel FW), TF = total flavonoids (mg CE. 100 g⁻¹ peel FW) and TF:TP = total phenolics to total flavonoids ratio (%).

Table 8. 'Cripps Pink' apple peel phenolic parameters among five foliar B plus Ca treatments and at four selected fruit maturity stages (DAFB).

Treatments (B+Ca)	TP	TF:TP
B`0.00+Ca`0.00	307.75 ^{ns}	45.20 ^{ns}
B`0.02+Ca`1.24	294.85	44.10
B`0.02+znCa`0.06	296.10	46.65
B`0.08+Ca`1.24	283.65	46.90
B`0.17+Ca`1.24	256.15	44.25
<i>p</i> -value	0.2876	0.6520
Days after full bloom (DAFB)		
122	357.32 ^a	34.68 ^d
136	302.04 ^b	48.52 ^b
150	296.12 ^b	56.96 ^a
164	195.32 ^c	41.52 ^c
<i>p</i> -value	0.0000	0.0000
B+Ca*DAFB (<i>p</i> -value)	0.1222	0.1822

Mean values sharing a letter are not different, TP = total phenolics (mg GAE. 100 g⁻¹ peel FW) and TF:TP = total phenolics to total flavonoids ratio (%).

Table 9. 'Cripps Pink' apple peel total flavonoids as a function of foliar B plus Ca and fruit maturity (DAFB) (2015/16).

Treatments B+Ca)	122 DAFB (26.01.2016)	136 DAFB (09.02.2016)	150 DAFB (23.02.2016)	164 DAFB (08.03.2016)
B`0.00+Ca`0.00	121 ^{abcde}	147 ^{abcde}	174 ^{abcde}	106 ^{abcde}
B`0.02+Ca`1.24	130 ^{abcde}	152 ^{ab}	155 ^{abcd}	76 ^{bcd}
B`0.02+znCa`0.06	153 ^{abcde}	147 ^{abc}	172 ^{ab}	69 ^e
B`0.08+Ca`1.24	130 ^{ab}	182 ^{abcd}	149 ^{abc}	66 ^e
B`0.17+Ca`1.24	77 ^{de}	95 ^{cde}	181 ^a	90 ^{abcde}

Mean values sharing a letter are not different, B+Ca ($p = 0.0830$), DAFB ($p = 0.0000$) and B+Ca*DAFB ($p = 0.0030$).

Table 10. 'Golden Delicious' apple peel total phenolics as a function of foliar B plus Ca and fruit maturity (DAFB).

Treatments (B+Ca)	80 DAFB (04.01.2016)	94 DAFB (18.01.2016)	108 DAFB (01.02.2016)	122 DAFB (15.02.2016)
B`0.00+Ca`0.00	249 ^{bcd}	333 ^a	166 ^{fgh}	191 ^{efg}
B`0.02+Ca`1.24	292 ^{abc}	243 ^{bcd}	161 ^{gh}	149 ^{gh}
B`0.02+znCa`0.06	234 ^{cdef}	265 ^{abcd}	148 ^{gh}	200 ^{defg}
B`0.08+Ca`1.24	305 ^{ab}	264 ^{bcd}	245 ^{bcd}	122 ^h
B`0.17+Ca`1.24	269 ^{abcd}	268 ^{abcd}	209 ^{defg}	204 ^{defg}

Mean values sharing a letter are not different, B+Ca ($p = 0.3449$), DAFB ($p = 0.0000$) and B+Ca*DAFB ($p = 0.0114$).

Table 11. 'Golden Delicious' apple peel total flavonoids as a function of foliar B plus Ca and fruit maturity (DAFB).

Treatments (B+Ca)	80 DAFB (04.01.2016)	94 DAFB (18.01.2016)	108 DAFB (01.02.2016)	122 DAFB (15.02.2016)
B`0.00+Ca`0.00	156 ^{bc}	206 ^a	130 ^{cdef}	115 ^{efgh}
B`0.02+Ca`1.24	174 ^b	162 ^b	119 ^{defg}	98 ^{gh}
B`0.02+znCa`0.06	146 ^{bcd}	165 ^b	112 ^{fgh}	116 ^{defg}
B`0.08+Ca`1.24	167 ^b	162 ^b	168 ^b	84 ^h
B`0.17+Ca`1.24	156 ^{bc}	172 ^b	147 ^{bcd}	123 ^{defg}

Mean values sharing a letter are not different, B+Ca ($p = 0.1825$), DAFB ($p = 0.0000$) and B+Ca*DAFB ($p = 0.0047$).

Table 12. 'Golden Delicious' apple peel flavonoids to phenolics ratio (TF:TP) among five foliar B plus Ca treatments and at four selected fruit maturity stages (DAFB).

Treatments (B+Ca)	TF:TP (%)
B`0.00+Ca`0.00	66.60 ^{ns}
B`0.02+Ca`1.24	69.15
B`0.02+znCa`0.06	65.80
B`0.08+Ca`1.24	66.95
B`0.17+Ca`1.24	64.90
<i>p</i> -value	0.8825
Days after full bloom (DAFB)	
80	60.48 ^b
94	64.28 ^b
108	76.44 ^a
122	65.52 ^b
<i>p</i> -value	0.0004
B+Ca*DAFB (<i>p</i> -value)	0.4126

Mean values sharing a letter are not different.

Table 13. 'Granny Smith' apple peel phenolics as a function of foliar B plus Ca and fruit maturity (DAFB).

Treatments (B+Ca)	94 DAFB (04.01.2016)	108 DAFB (18.01.2016)	122 DAFB (01.02.2016)	136 DAFB (15.02.2016)
B`0.00+Ca`0.00	523 ^a	431 ^{abcd}	441 ^{abcd}	122 ^{cdef}
B`0.02+Ca`1.24	320 ^{def}	337 ^{cdef}	327 ^{cdef}	309 ^{def}
B`0.02+znCa`0.06	458 ^{abc}	261 ^{ef}	331 ^{cdef}	229 ^f
B`0.08+Ca`1.24	503 ^{ab}	483 ^{ab}	278 ^{ef}	272 ^{ef}
B`0.17+Ca`1.24	346 ^{cdef}	284 ^{ef}	315 ^{def}	371 ^{bcd}

Mean values sharing a letter are not different, B+Ca ($p = 0.0025$), DAFB ($p = 0.0007$) and B+Ca*DAFB ($p = 0.0227$).

Table 14. 'Granny Smith' apple peel flavonoids as a function of foliar B plus Ca and fruit maturity (DAFB).

Treatments (B+Ca)	94 DAFB (04.01.2016)	108 DAFB (18.01.2016)	122 DAFB (01.02.2016)	136 DAFB (15.02.2016)
B`0.00+Ca`0.00	279 ^a	225 ^{abcd}	227 ^{abcd}	173 ^{def}
B`0.02+Ca`1.24	185 ^{cdef}	182 ^{def}	172 ^{def}	148 ^{ef}
B`0.02+znCa`0.06	257 ^{ab}	147 ^{ef}	170 ^{def}	123 ^f
B`0.08+Ca`1.24	272 ^a	244 ^{abc}	146 ^{ef}	146 ^{ef}
B`0.17+Ca`1.24	202 ^{bcde}	154 ^{ef}	170 ^{def}	188 ^{cde}

Mean values sharing a letter are not different, B+Ca ($p = 0.0025$), DAFB ($p = 0.0000$) and B+Ca*DAFB ($p = 0.0230$).

Table 15. 'Granny Smith' apple peel flavonoids to phenolics ratio (TF:TP) among five foliar B plus Ca treatments and at four selected fruit maturity stages (DAFB).

Treatments (B+Ca)	TF:TP (%)
B`0.00+Ca`0.00	52.16 ^{ns}
B`0.02+Ca`1.24	53.56
B`0.02+znCa`0.06	54.58
B`0.08+Ca`1.24	55.15
B`0.17+Ca`1.24	53.34
<i>p</i> -value	0.3700
Days after full bloom (DAFB)	
94	56.50 ^a
108	54.00 ^{ab}
122	53.02 ^b
136	51.51 ^b
<i>p</i> -value	0.0061
B+Ca*DAFB (<i>p</i> -value)	0.3583

Mean values sharing a letter are not different.

Table 16. Apple fruit sunburn incidence recorded at respective harvest dates from experiments with foliar B plus Ca.

Cultivar and Season	Treatments (B+Ca)	Sunburn incidence (%) [*]			
		Total	Class 0	Class 1	Class 2
'Cripps Pink' (2014/15)	B`0.00+Ca`0.00	20.46 ^{ns}	79.54 ^{ns}	7.00 ^{ns}	4.31 ^{ns}
	B`0.02+Ca`0.10	25.85	74.15	8.07	5.24
	B`0.02+Ca`1.29	18.19	81.81	6.75	4.21
	<i>p</i> -value	0.6097	0.6097	0.8841	0.5014
	'Cripps Pink' (2015/16)	B`0.00+Ca`0.00	56.10 ^{ns}	43.90 ^{ns}	15.90 ^a
B`0.02+Ca`1.24		46.70	53.30	8.92 ^b	14.40
B`0.02+znCa`0.06		48.60	51.40	9.26 ^b	16.20
B`0.08+Ca`1.24		46.70	53.30	8.02 ^b	15.20
B`0.17+Ca`1.24		52.14	47.86	11.01 ^b	20.74
<i>p</i> -value	0.4560	0.4564	0.0001	0.5842	
'Golden Delicious' (2015/16)	B`0.00+Ca`0.00	35.80 ^{ns}	64.20 ^b	16.43 ^a	10.56 ^{ns}
	B`0.02+Ca`1.24	23.93	76.07 ^{ab}	10.17 ^{ab}	5.74
	B`0.02+znCa`0.06	33.01	66.99 ^b	17.39 ^a	6.87
	B`0.08+Ca`1.24	12.48	87.52 ^a	5.65 ^b	3.33
	B`0.17+Ca`1.24	19.72	80.28 ^a	10.71 ^{ab}	4.47
<i>p</i> -value	0.1753	0.0087	0.0164	0.0642	
'Granny Smith' (2015/16)	B`0.00+Ca`0.00	31.85 ^{ns}	68.15 ^{ns}	15.73 ^{ns}	6.80 ^{ns}
	B`0.02+Ca`1.24	25.59	74.40	14.61	5.52
	B`0.02+znCa`0.06	28.64	71.36	12.47	8.20
	B`0.08+Ca`1.24	28.54	71.46	13.89	5.60
	B`0.17+Ca`1.24	22.98	77.02	12.57	4.54
<i>p</i> -value	0.5064	0.5064	0.2702	0.5853	

Mean values sharing a letter are not different. ^{*}Full data for sunburn classes 3- 5 available in Daiber (2017); Lötze et al. (2017; 2018).

Table 17: Trend contrasts of 'Cripps Pink' apple peel phenolic parameters at 122, 136, 150 and 164 days after full bloom among varying foliar B plus Ca treatments using the slope (*b*) statistics (*p*-value in parentheses).

Treatments (B+Ca)	Total phenolics (TP)	Total flavonoids (TF)	TF:TP ratio
Control (T ₀)	-2.8443 (0.0537)	-0.1143 (0.8809)	0.3143 (0.0383)
B`0.02+Ca`1.24 (T ₁)	-3.8614 (0.0006)	-1.1243 (0.0594)	0.0914 (0.6063)
B`0.02+znCa`0.06 (T ₂)	-5.4343 (0.0001)	-1.6286 (0.0137)	0.2300 (0.0796)
B`0.08+Ca`1.24 (T ₃)	-4.1900 (0.0012)	-1.5971 (0.0195)	-0.0114 (0.9421)
B`0.17+Ca`1.24 (T ₄)	-1.2386 (0.3204)	0.8829 (0.2019)	0.4100 (0.0312)
<i>b</i> contrasts (<i>p</i> -values)			
T ₀ versus T ₁	0.7891	0.8376	0.8812
T ₀ versus T ₂	0.8794	0.8718	0.9290
T ₀ versus T ₃	0.8692	0.8954	0.9444
T ₀ versus T ₄	0.9286	0.9327	0.8777
T ₁ versus T ₂	0.9028	0.9637	0.8146
T ₁ versus T ₃	0.9133	0.9394	0.9963
T ₁ versus T ₄	0.8544	0.9020	0.9357
T ₂ versus T ₃	0.9893	0.9755	0.8748
T ₂ versus T ₄	0.9496	0.9376	0.8113
T ₃ versus T ₄	0.9390	0.9620	0.9320

Table 18: Trend contrasts of 'Golden Delicious' apple peel phenolic parameters at 80, 94, 108 and 122 days after full bloom among varying foliar B plus Ca treatments using the slope (*b*) statistics (*p*-value in parentheses).

Treatments (B+Ca)	Total phenolics (TP)	Total flavonoids (TF)	TF:TP ratio
Control (T ₀)	-2.4405 (0.0253)	-1.4250 (0.0091)	0.0451 (0.7655)
B`0.02+Ca`1.24 (T ₁)	-3.6661 (0.0003)	-1.9352 (0.0001)	0.2367 (0.2382)
B`0.02+znCa`0.06 (T ₂)	-1.5586 (0.0627)	-1.0196 (0.0114)	-0.0142 (0.9294)
B`0.08+Ca`1.24 (T ₃)	-4.0556 (0.0003)	-1.7290 (0.0025)	0.5804 (0.0508)
B`0.17+Ca`1.24 (T ₄)	-1.8098 (0.0553)	-0.8831 (0.0487)	0.1310 (0.5068)
<i>b</i> contrasts (<i>p</i> -values)			
T ₀ versus T ₁	0.8814	0.8954	0.8543
T ₀ versus T ₂	0.8669	0.8375	0.9677
T ₀ versus T ₃	0.9387	0.9950	0.6854
T ₀ versus T ₄	0.9305	0.9140	0.8560
T ₁ versus T ₂	0.9848	0.9392	0.8851
T ₁ versus T ₃	0.9415	0.8905	0.8070
T ₁ versus T ₄	0.9498	0.9809	0.9982
T ₂ versus T ₃	0.9265	0.8328	0.7098
T ₂ versus T ₄	0.9348	0.9204	0.8868
T ₃ versus T ₄	0.9917	0.9090	0.8054

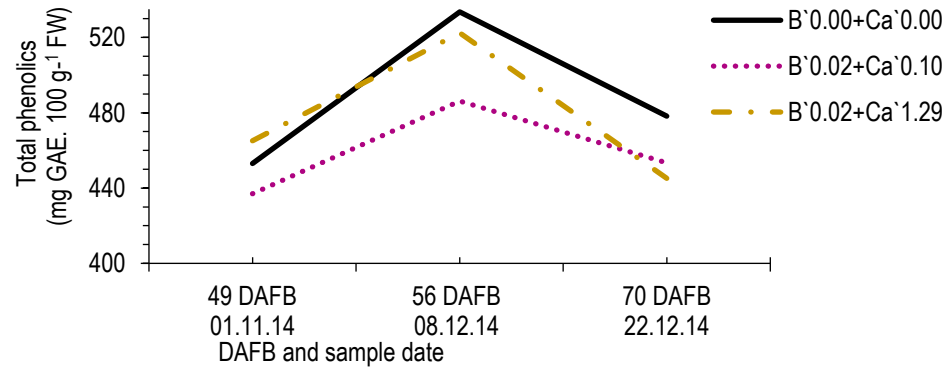


Figure 1.1: Moving mean of apple peel TP at early DAFB stages among the foliar B+Ca treatments in 'Cripps Pink'

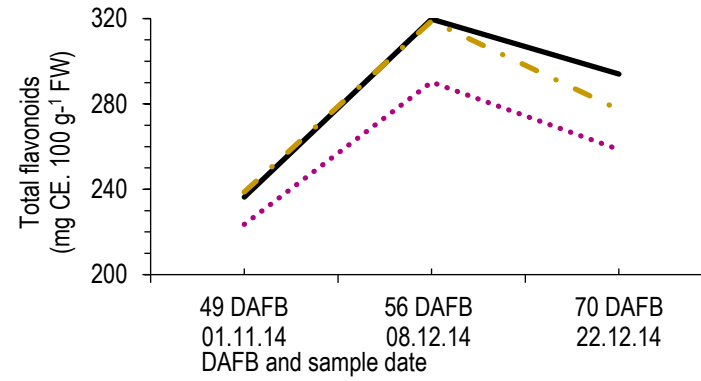


Figure 1.2: Moving mean of apple peel TF at early DAFB stages among the foliar B+Ca treatments in 'Cripps Pink'

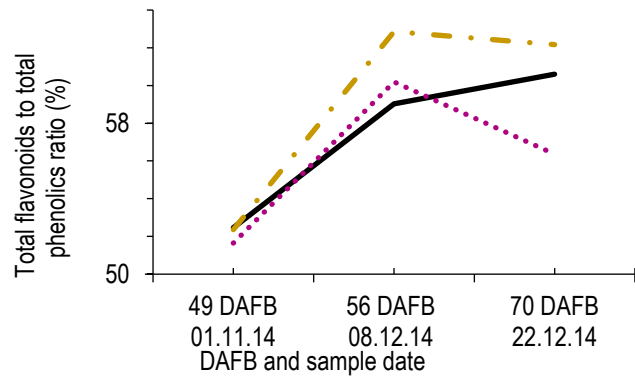


Figure 1.3: Moving mean of apple peel TF:TP towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

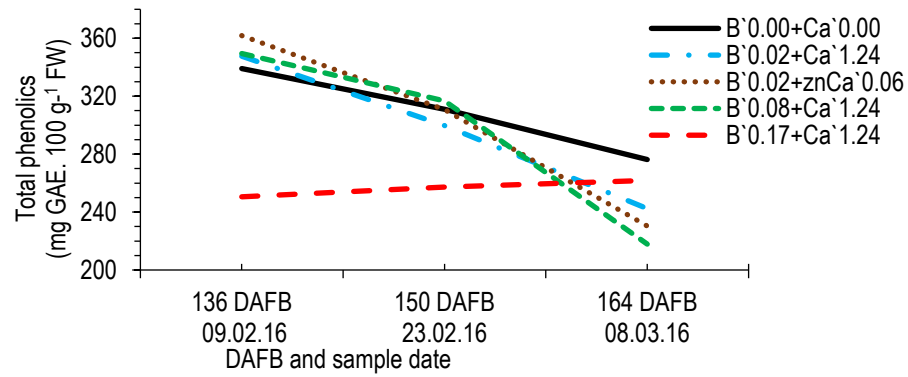


Figure 2.1: Moving mean of apple peel TP towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

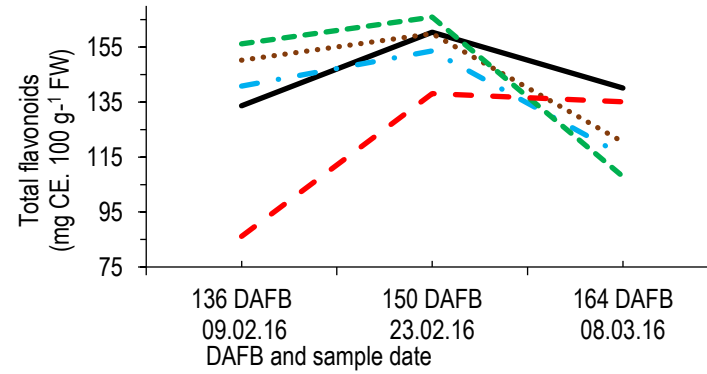


Figure 2.2: Moving mean of apple peel TF towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

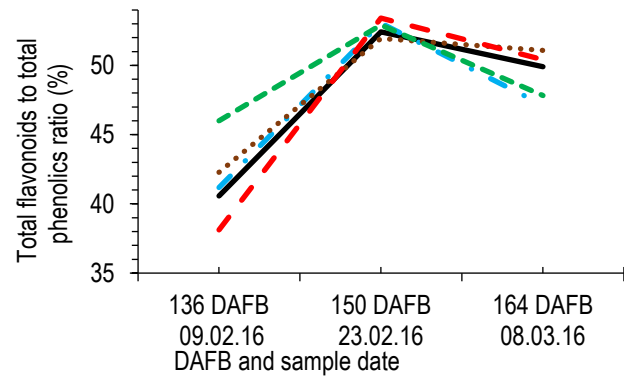


Figure 2.3: Moving mean of apple peel TF:TP towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

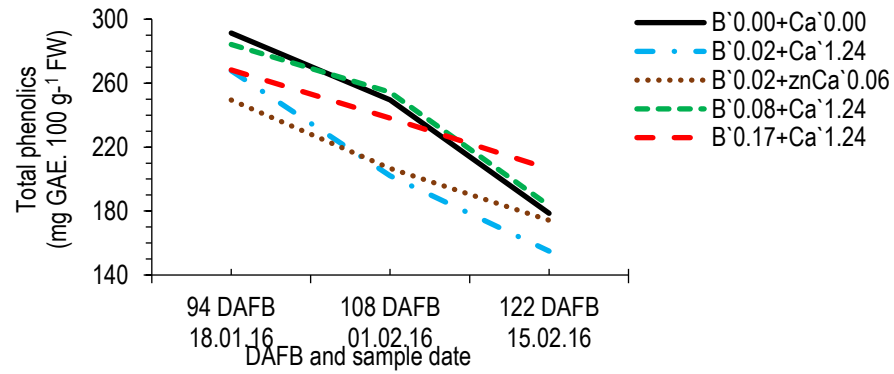


Figure 3.1: Moving mean of apple peel TP towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

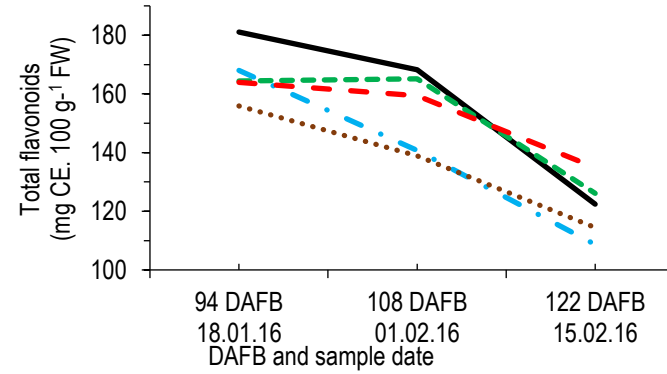


Figure 3.2: Moving mean of apple peel TF towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

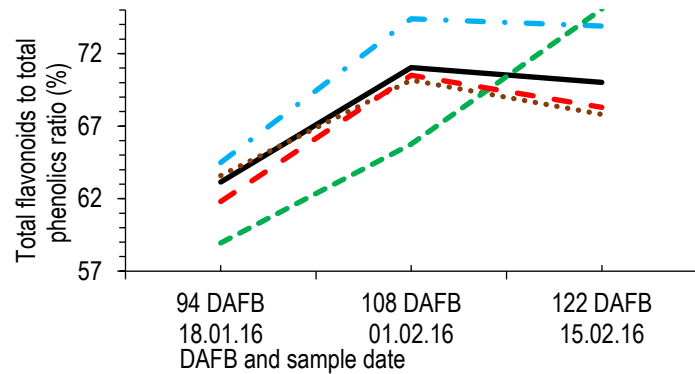


Figure 3.3: Moving mean of apple peel TF:TP towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

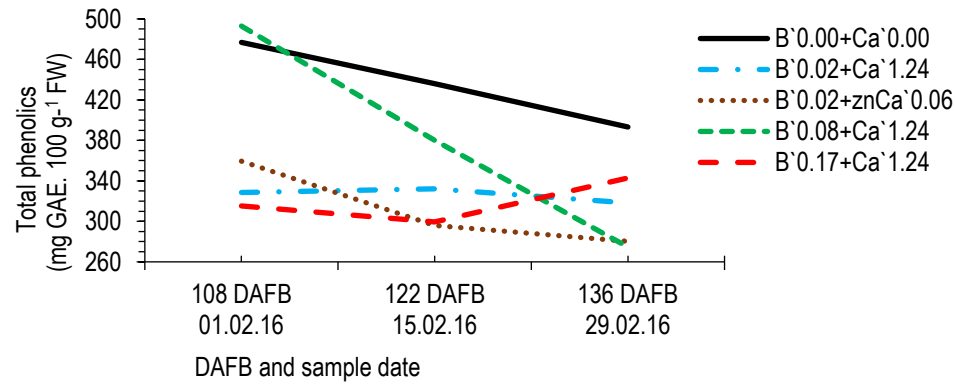


Figure 4.1: Moving mean of apple peel TP towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

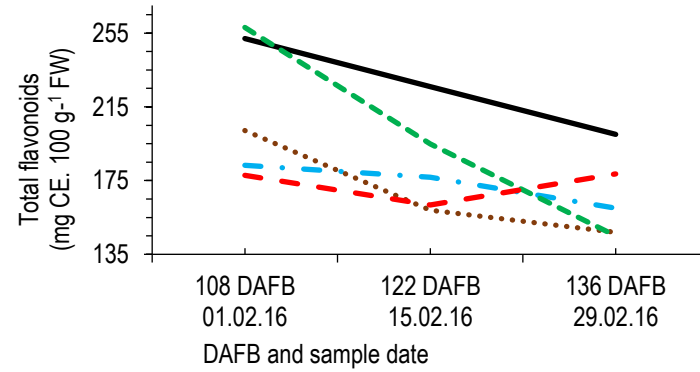


Figure 4.2: Moving mean of apple peel TF towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

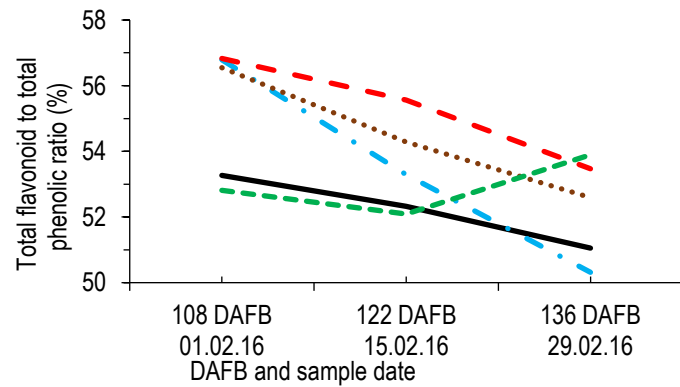


Figure 4.3: Moving mean of apple peel TF:TP towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

INVESTIGATING THE MODE-OF-ACTION BY WHICH POST-FULL-BLOOM FOLIAR APPLICATIONS OF A COMBINATION OF BORON AND CALCIUM IMPACT ON APPLE FRUIT SUNBURN BROWNING INCIDENCE

II. Boron plus calcium induced changes in apple peel photosynthetic pigment levels

Abstract

This study investigated the effect of post-full-bloom foliar boron plus calcium (B+Ca) treatments on apple fruit peel photosynthetic pigment levels, and related findings to sunburn browning (SBB) incidence. Overall, the study aimed at construing the mode-of-action by which such treatments reduce SBB incidence, which is a critical prerequisite to successful promotion this economic approach in the industry, for commercial farms. Four treatments were applied to ‘Cripps Pink’, ‘Golden Delicious’ and ‘Granny Smith’ bearing apple trees in experiments conducted in two orchards with a rich history of apple fruit sunburn disorders. Apple peel for pigment level analyses was sampled at four fruit maturity stages, represented as days after full bloom (DAFB), towards the harvest maturity window of each cultivar. Cases of significant ($p < 0.05$) interaction (only in ‘Granny Smith’), treatment and DAFB effects occurred, suggesting that fruit maturity (DAFB) and treatments jointly or singly, influenced apple peel photosynthetic pigment parameters studied. Evidence of foliar B+Ca treatment SBB incidence suppression was only significant in ‘Cripps Pink’ and ‘Golden Delicious’, however no dynamics in any peel photosynthetic attribute could explain this phenomenon to yield the sought mode-of-action. Therefore, the study concluded with the realisation that treatment formulation and cultivar differences do influence / impact apple peel photosynthetic pigments in ‘Cripps Pink’, ‘Golden Delicious’ and ‘Granny Smith’ apples. For instance, the inclusion of Zn was associated with significantly reduced magnitudes of the photosynthetic attributes, whereas ‘Granny Smith’ had higher levels compared to both ‘Cripps Pink’ and ‘Golden Delicious’, that are genetically related. Since the attributes studied generally showed strong and/or appreciable variability within treatments in the different cultivars, the differential behaviour suggests that an individual cultivar approach is justified in future research and development efforts of this foliar B+Ca approach to reduce SBB in these apple orchards.

Keywords: Carotenoids, Chlorophyll, ‘Cripps Pink’, ‘Golden Delicious’, ‘Granny Smith’

1. Introduction

Post-full-bloom foliar applications of combined B and Ca (B+Ca) is envisaged as a potential cost effective alternative to mitigate high apple fruit SBB incidence (Lötze & Hoffman, 2014; Daiber, 2017; Lötze et al., 2017). However, the mode-of-action of this approach is unknown, and this limits its deployment and refinement for the benefit of especially commercial growers who lose large revenues due to compromises on fruit quality by sunburn. Ca is a crucial co-factor in the reactions of the oxygen-evolving complex of photosystem (PS) II (Vrettos et al., 2001; Yachandra & Yano, 2011), and foliar Ca reportedly assisted in the *de novo* repair of PS structures and ultimately contributed to photoprotection in *Arachis hypogaea* (groundnut) plants that were under high heat and irradiance stress (Yang et al., 2015). The stresses that the groundnut plants were exposed are the same that would typically induce SBB in apple fruit. In addition, low quantities of B supplied to Mung beans (*Vigna radiata*) increased the chlorophyll and carotenoid levels coupled with a reduction in plant stress indicators (Seth & Aery, 2014).

Apple sunburn browning (SBB) is associated with a reduction of peel chlorophyll levels and this results in the yellow to bronze colours of carotenoids to be very conspicuous, which in turn corresponds with the severity of the SBB damage (Felicetti & Schrader, 2008; 2009; Racskó & Schrader, 2012). Carotenoids are essential components of plant photosynthetic systems with roles in antioxidant and photoprotection mechanisms (Dellapenna, 1999; Havaux et al., 1999; Mu et al., 2001; Bandurska et al., 2013; Racchi, 2013; Nisar et al., 2015). Apple peel carotenoids can potentially restrain fruit SBB since xanthophyll cycle contributes to photoprotection (Merzlyak & Solovchenko, 2002; Merzlyak et al., 2002; Felicetti & Schrader, 2008; Solovchenko et al., 2010).

Therefore, a possible increment or preservation of apple fruit peel chlorophyll and carotenoid pigments because of foliar B+Ca treatment might improve physiological mechanisms that may prevent SBB development. As thus, this study aimed at determining if foliar B+Ca treatments influence peel chlorophyll and carotenoid pigments towards fruit harvest maturity, a period when sunburn damage in apple fruit is most rife. The results on variability and trends of peel photosynthetic pigments were related to the SBB recorded at fruit harvest maturity to determine whether this might explain the effect of specific foliar B+Ca treatments on the sunburn incidence reduction.

2. Materials and methods

a. Experiment locations, treatments and sampling

This study was conducted on the same experimental locations and similar procedures were followed as described in Paper 1.

b. Determination of apple peel chlorophyll and carotenoid pigments

Chlorophyll and carotenoids pigments were extracted from freshly frozen apple peel samples following slightly modified method of Lichtenthaler (1987) and Lichtenthaler & Buschmann (2001). Extraction of the chlorophyll and carotenoid pigments occurred by stirring 500 mg of fresh frozen apple peel sample with 3 ml of cold, analytical grade acetone (99.9 %, Sigma Aldrich, South Africa) in a 50 ml capacity centrifuge tube at 4 °C for 24 hours in the dark. This was followed by centrifugation using the Eppendorf™ 5810R cooling centrifuge for 15 minutes at 3220 g. Then, the extract was decanted into a clean 10 ml capacity test tube and held at 4 °C in the dark. There after an additional 2 ml of cold acetone were added to the residues, followed by a vortexing and centrifuging step, using again the Eppendorf™ 5810R for 15 minutes at 3220 g, with a final decanting step to complete this phase. The two-decanted extracts were combined and centrifuged further still with the Eppendorf™ 5810R for 15 minutes at 3220 g, after which 2 ml of the extract were pipetted off.

Thereafter, the 2 ml extract was centrifuged further at a higher force using the Eppendorf™ 5417R cooling centrifuge at 20000 g at 4 °C for 15 minutes, after which 1 ml was carefully pipetted off and held at 4 °C in the dark until the determination of chlorophyll and carotenoid levels. Then, chlorophyll and carotenoid pigment levels were determined by measuring absorbance using spectrophotometer (Cary 50 Bio, Varian, Australia (Pty) Ltd) at 470, 645 and 670 nm, following Lichtenthaler (1987) and Lichtenthaler & Buschmann (2001). Acetone stable plastic cuvettes (Lasec® (Pty) Ltd, Cape Town, South Africa) were used, and with the extraction solvent (acetone) as the blank. The levels of chlorophyll *a* (CHLa), chlorophyll *b* (CHLb), total chlorophyll (TCHL) and total carotenoids (TCAR) were expressed as $\mu\text{g g}^{-1}$ fresh weight (FW) of apple peel.

c. Data analyses

The variability in the parameters of the apple peel photosynthetic pigments was analysed using Statistica software. Differences were deemed significant for $p < 0.05$, where appropriate means across treatments and fruit maturity (DAFB) were separated with the Fisher's LSD posthoc test. The

respective means plus standard errors of each pigment parameter among the foliar B+Ca treatments with progression of fruit maturity or DAFB were illustrated as trends with XLSTAT software (Appendixes 6, 7 and 8). These curves were further smoothed, with the two-point moving average technique, to enable easier seasonal comparisons among treatments. In the experiments where SBB incidence suppression occurred, the respective phenolic parameter trends were evaluated and contrasted amongst varying foliar B+Ca treatments, using statistical characteristics of respective trend gradient and/or slope (*b*) obtained with XLSTAT software. The statistical analyses results and behaviour of the pigments in their respective trend curves, were then related to the SBB incidence recorded at harvest maturity (Paper 1, Table 16) to identify the foliar B+Ca treatments that influenced apple peel phenolic parameters whilst associating with significant SBB incidence reduction.

3. Results

a. ‘Cripps Pink’

i. Chlorophyll *a*, chlorophyll *b* and total chlorophyll

‘Cripps Pink’ apple peel chlorophyll *a* (CHLa), chlorophyll *b* (CHLb) and total chlorophyll (TCHL) showed similar variability patterns amongst the foliar B+Ca treatments and DAFB levels (Table 1). All these chlorophyll pigment parameters showed significant differences amongst the foliar B+Ca treatments and DAFB; however, no significant interaction effects occurred. CHLa, CHLb and TCHL showed similar patterns in variability among the foliar B+Ca treatments, including the control. Treatments B`0.02+Ca`1.24, B`0.08+Ca`1.24 and B`0.17+Ca`1.24 showed the highest and non-significantly different CHLa, CHLb and TCHL. Treatment B`0.02+znCa`0.06 had lowest levels of CHLa, CHLb and TCHL that were not significantly different from only the control. In addition, B`0.08+Ca`1.24 was not significantly different from the control for CHLa and TCHL, while B`0.02+Ca`1.24 showed the same behaviour as CHLb. There was a sharp contrast between treatment B`0.02+znCa`0.06 and the other treatments. CHLa, CHLb and TCHL further showed steady decline with progress of DAFB (Figures 1.1, 1.2 and 1.3). Table 1 shows that the values recorded at 122 DAFB were significantly higher in comparison to values at all DAFB periods. At 136 DAFB, CHLa, CHLb and TCHL were significantly higher (not significant) from values recorded at both 150 and 164 DAFB, except that TCHL at 136 and 164 DAFB was not significantly different from one another.

ii. Chlorophyll *a* to chlorophyll *b* ratio

‘Cripps Pink’ apple peel chlorophyll *a* to chlorophyll *b* ratio (CHLa:CHLb) was only associated with significant differences among the fruit maturity stages (DAFB), and no significant interaction or treatment effect was recorded (Table 1). Although treatment differences were non-significant ($p = 0.1305$), CHLa:CHLb levels of the control and treatment B`0.02+znCa`0.06 were highest, and treatment B`0.17+Ca`1.24, lowest with (Table 1). CHLa:CHLb appeared to increase with progression of DAFB, where the levels at 150 and 164 DAFB were highest and not significantly different from each other, but were significantly different from the almost similar levels at 122 and 136 DAFB. The respective seasonal trends of these CHLa:CHLb levels further showed a general increase with DAFB, and that the control had a higher moving mean trend, followed by B`0.02+znCa`0.06 (Figure 1.4).

iii. Total carotenoids

‘Cripps Pink’ apple peel total carotenoids (TCAR) levels did not record significant interactions or treatment differences among treatments, but did for maturity stages (DAFB) (Table 1). TCAR at 122 DAFB was the highest and was significantly different from all DAFB, except 150 DAFB. Figure 1.5 shows that, towards harvest maturity, TCAR seemed to be increasing in treatments B`0.17+Ca`1.24 and B`0.08+Ca`1.24.

iv. Chlorophyll to carotenoids ratio

‘Cripps Pink’ apple peel total chlorophyll to total carotenoids ratio (TCHL:TCAR) differed significantly between treatments and DAFB, and no significant interaction effect was recorded (Table 1). TCHL:TCAR levels of treatments B`0.08+Ca`1.24 and B`0.17+Ca`1.24 were significantly higher than all other treatments, except B`0.02+Ca`1.24. The lowest TCHL:TCAR levels were recorded with treatment B`0.02+znCa`0.06, and this was significantly different from all treatments, with exception of the control. TCHL:TCAR at 122 and 136 DAFB was significantly higher compared to TCHL:TCAR levels at 150 and 164 DAFB, which were not significantly different from each other. This indicates that TCHL:TCAR decreased with progress of DAFB, which is also illustrated in the seasonal trend curves of Figure 1.6. Treatment B`0.02+znCa`0.06 and the control (B`0.00+Ca`0.00) decreased slowly from low initial ratios, while the other three treatments decreased more rapidly from higher initial ratios to reach comparable ratios at 164 DAFB.

b. ‘Golden Delicious’

i. Chlorophyll *a*, chlorophyll *b* and total chlorophyll

‘Golden Delicious’ apple peel chlorophyll *a* (CHLa), chlorophyll *b* (CHLb) and total chlorophyll (TCHL) differences were only significant for CHLa and TCHL (Table 2), but not for CHLb, and no significant interaction effects between treatments and DAFB were recorded for these chlorophyll pigments parameters. Treatment B`0.02+znCa`0.06 had significantly lower CHLa and TCHL levels compared to all other treatments, except treatment B`0.02+Ca`1.24. CHLa, CHLb and TCHL levels were the highest at 80 DAFB, differed significantly from all other DAFB (Table 2), and decreased with progression of DAFB. Differences between treatments and the gradual decrease of CHL during fruit development were also evident from the seasonal trend lines (Figures 2.1, 2.2 and 2.3). Unlike other treatments, CHLa, CHLb and TCHL levels trended upwards in B`0.17+Ca`1.24 from 108 to 122 DAFB (Figures 2.1, 2.2 and 2.3).

ii. Chlorophyll *a* to chlorophyll *b* ratio

‘Golden Delicious’ apple peel chlorophyll *a* to chlorophyll *b* ratio (CHLa:CHLb) did not have significant differences amongst treatments, but did differ significantly within fruit maturity stages (DAFB) (Table 2), where the lowest levels recorded at 108 DAFB were significantly different from that recorded at all other maturity stages (DAFB). The control had the highest CHLa:CHLb levels at all maturity stages albeit that treatment differences and interaction with maturity stage were not significant (Figures 2G, H). Treatment B`0.02+znCa`0.06 showed a considerably lower running moving mean of CHLa:CHLb until 108 DAFB where after it showed a steady increase towards 122 DAFB (Figure 2H).

iii. Total carotenoids

‘Golden Delicious’ apple peel total carotenoids (TCAR) levels showed significant differences within foliar B+Ca treatments and fruit maturity stages (DAFB), and no significant interactions occurred (Table 2). Treatment B`0.02+znCa`0.06 had the lowest TCAR level which was significantly different from that recorded within all other treatments, which were not significantly different from each other (Table 2). TCAR levels recorded 80 DAFB was the highest, and was significantly different from that recorded at all other fruit maturity stages. The TCAR levels at 94 DAFB was also significantly different from that at both 108 and 122 DAFB, which were not different from each other, with a

general indication of TCAR decreasing with progress of DAFB. Figure 7.2 illustrate this decreasing trend (Figure 2.5). Treatment B`0.02+znCa`0.06 showed the lowest moving mean of TCAR at all fruit maturity stages (Figure 2.5) in accordance with the statistical analysis.

iv. Chlorophyll to carotenoids ratio

‘Golden Delicious’ apple peel total chlorophyll to total carotenoids ratio (TCHL:TCAR) was not significantly different among treatments, but was among fruit maturity stages (DAFB) (Table 2), where the levels recorded at 108 DAFB was the highest and significantly different from that at all other DAFB. No significant interaction effects between treatments and DAFB occurred. B`0.02+Ca`1.24 seemed to trend higher for TCHL:TCAR throughout fruit development. B`0.02+znCa`0.06 initially trended even higher, but decreased considerably towards 122 DAFB (Figure 2.6).

c. ‘Granny Smith’

i. Chlorophyll *a*, chlorophyll *b* and total chlorophyll

‘Granny Smith’ apple peel chlorophyll *a* (CHLa), chlorophyll *b* (CHLb) and total chlorophyll (TCHL) showed significant interaction between treatments and maturity stage (Tables 3, 4, 5). At 94 DAFB there was no treatment that differed significantly from the control for CHLa, CHLb and TCHL, and the only significant treatment differences were between B`0.02+Ca`1.24 (lower) and B`0.08+Ca`1.24 (higher). At 108 DAFB, all treatments did not significantly differ with the control, except treatment B`0.02+znCa`0.06 (lowest values), in addition, lowest levels recorded with treatment B`0.02+znCa`0.06 at 108 DAFB were significantly lower compared to all treatments. Again, at 122 DAFB, the control did not significantly differ with any treatment, and at this maturity stage, significant treatment differences for CHLa, CHLb and TCHL were only recorded with treatment B`0.02+Ca`1.24 (higher) against both treatments B`0.02+znCa`0.06 and B`0.08+Ca`1.24. At 136 DAFB, all treatments did not significantly differ with the control, except treatment B`0.02+znCa`0.06 (lowest values), in addition, the lowest levels recorded with treatment B`0.02+znCa`0.06 at 136 DAFB were significantly lower compared to all treatments, with exception of treatment B`0.08+Ca`1.24. Notably, values of treatment B`0.02+znCa`0.06 generally stood out as lower values and common points of significant differences with all other B+Ca treatments.

Across the maturity stages (94 to 136 DAFB), CHLa, CHLb and TCHL levels did not significantly differ within the control and treatment B`0.17+Ca`1.24 (Tables 3, 4 and 5). With treatment B`0.02+Ca`1.24, CHLa, CHLb and TCHL levels at 94 and 136 DAFB were lower and not significantly different, and the reverse was true for this treatment values recorded at both 108 and 122 DAFB. This indicates that the pigments increased from 94 DAFB and peaked at 122 DAFB, but decreased towards 136 DAFB. With treatment B`0.02+znCa`0.06, CHLa, CHLb and TCHL levels at 94 and 122 DAFB were higher and not significantly different, and the reverse was true for values recorded at both 108 and 136 DAFB. The treatment B`0.08+Ca`1.24 had non-significantly differing CHLa, CHLb and TCHL values at 94 to 108 DAFB. However, CHLa was also not significantly different between 108 and 122 DAFB, and as well 122 and 136 DAFB. Treatment B`0.08+Ca`1.24 CHLb and TCHL levels were lower and not significantly different at 122 and 136 DAFB, indicating that CHLb and TCHL decreased significantly as fruit maturity progressed.

The low levels of these photosynthetic pigment aspects with treatment B`0.02+znCa`0.06, are also prominently illustrated in the trends (Figures 3.1, 3.2 and 3.3). In addition, unlike other treatments, B`0.17+Ca`1.24 showed an increasing trend towards fruit harvest while B`0.08+Ca`1.24 showed a strong decrease.

ii. Total carotenoids

‘Granny Smith’ apple peel total carotenoids (TCAR) levels showed significant interaction effects between treatments and fruit maturity stages (Table 6). At 94 DAFB, no treatment significantly differed with the control, and the only treatment significant differences were with treatment B`0.08+Ca`1.24 (higher) versus treatment B`0.02+Ca`1.24 (lower). At 108 DAFB, no treatment significantly differed with the control, and the only treatment significant differences were recorded with treatment B`0.08+Ca`1.24 (higher) versus treatment B`0.02+Ca`1.24 (lower). Again, at 122 DAFB, no treatment significantly differed with the control, and treatment significant differences were realised with treatment B`0.02+Ca`1.24 (higher) versus treatment B`0.08+Ca`1.24 (lower). Lastly, at 136 DAFB, again no treatment significantly differed with the control except the Zn treatment, and further treatment significant differences were recorded with treatment B`0.17+Ca`1.24 (higher) versus both treatments B`0.02+znCa`0.06 and B`0.08+Ca`1.24 that had lower levels but non-significantly differing.

Across the maturity stages (94 to 136 DAFB), TCAR levels did not significantly differ within the control and treatment B`0.17+Ca`1.24 (Table 6). With treatment B`0.02+Ca`1.24, TCAR at 94 and

136 DAFB was lowest and not significantly different, and the reverse was true for values recorded at both 108 and 122 DAFB. Although B`0.02+Ca`1.24 levels at 94 and 108 DAFB were not also significantly different, there was indication that TCAR increased from 94 DAFB to 122 DAFB, but sharply decreased towards 136 DAFB. With treatment B`0.02+znCa`0.06, TCAR levels at 94 and 122 DAFB were higher and not significantly different, and the reverse was true for values recorded at both 108 and 136 DAFB. Treatment B`0.08+Ca`1.24 had higher non-significantly differing TCAR values at 94 and 108 DAFB, and the reverse was true for values recorded at both 122 and 136 DAFB. The lowest levels recorded for TCAR were with treatment B`0.02+znCa`0.06 at 108 DAFB (significantly different from other treatments except B`0.17+Ca`1.24) and 136 DAFB (significantly different from other treatments except B`0.08+Ca`1.24) and these, as for CHLa, CHLb and TCHL, were major points of significant differences for TCAR levels. The low levels of TCAR with treatment B`0.02+znCa`0.06 are further illustrated with Figure 3.4. As for TCHL (Figure 3.3), B`0.08+Ca`1.24 also showed a sharply decreasing seasonal trend for TCAR from 108 to 136 DAFB.

iii. Chlorophyll *a* to chlorophyll *b* ratio

‘Granny Smith’ apple peel chlorophyll *a* to chlorophyll *b* ratio (CHLa:CHLb) showed non-significant differences amongst treatments, but differences were significant within fruit maturity stages, and no significant interaction occurred (Table 7). Levels at 94 and 108 DAFB were the highest and lowest respectively, and were significantly different from one another, as well as to the levels at both 122 and 136 DAFB, that were not significantly different. Treatment B`0.17+Ca`1.24 showed a flatter seasonal trend in ratio during fruit development, but generally treatments all responded similarly (Figure 3.5).

iv. Chlorophyll to carotenoids ratio

‘Granny Smith’ apple peel total chlorophyll to total carotenoids ratio (TCHL:TCAR) was significantly different among the foliar B+Ca treatments, but not with the fruit maturity / DAFB stages (Table 7). The TCHL:TCAR ratio were significantly lower in B`0.02+znCa`0.06 compared to all other treatments (Table 7) as also illustrated in Figures 3.6 in which treatment B`0.17+Ca`1.24 trended higher than other treatments at 108 and 122 DAFB.

d. Statistical characterisation of ‘Cripps Pink’ and Golden Delicious’ apple peel photosynthetic pigments trends

i. ‘Cripps Pink’

The slope (*b*) for CHLa, CHLb and TCHL had a negative direction for all foliar treatments, including the control, suggesting that these aspects decreased with progression of fruit maturity with significant values (Table 8). CHLa:CHLb trends were all positive-sloped, but significant slopes from zero were recorded with only treatments B`0.02+Ca`1.24, B`0.08+Ca`1.24 and B`0.17+Ca`1.24. The TCAR and TCHL:TCAR slopes were all negative, except the TCAR slope of treatment B`0.08+Ca`1.24. Significant slopes in TCAR were recorded for the control and B`0.02+znCa`0.06, while in TCHL:TCAR slopes, only the control and B`0.02+znCa`0.06 did not have significant values from zero. All differences with respect to the direction and magnitude of the gradient of the peel photosynthetic pigments could not distinguish the various seasonal trends from one another, as the slope (*b*) contrasts were all not significantly different.

ii. ‘Golden Delicious’

The slope (*b*) for the CHLa, CHLb, TCHL and TCAR was negative for all foliar treatments, including the control, showing the decreasing trends with fruit maturity advance (Table 9), which were significant except CHLb, TCHL and TCAR slope values of treatment B`0.17+Ca`1.24. The CHLa:CHLb slope was negative except for B`0.02+znCa`0.06. The only CHLa:CHLb significant slope was recorded with treatment B`0.08+Ca`1.24. All the TCHL:TCAR slopes were non-significant and negative, except for treatments B`0.08+Ca`1.24 and B`0.17+Ca`1.24. Again, the trend slope differences with respect to direction and magnitude of the gradient of the peel phenolic biochemicals could not distinguish the various seasonal trends as none of the slope (*b*) contrasts were significant.

4. Discussion

a. Does the foliar B plus Ca induced changes in apple peel photosynthetic pigment attributes explain sunburn browning incidence reduction in ‘Cripps Pink’?

i. Chlorophyll and sunburn browning incidence ‘Cripps Pink’

Peel chlorophyll *a* (CHLa), chlorophyll *b* (CHLb) and total chlorophyll (TCHL) levels decrease in ‘Cripps Pink’ as fruit mature and with fruit expansion. As fruit maturity progresses, chlorophyll

transition to carotenoids in the chromoplasts occurs (Bian et al., 2011; Egea et al., 2011). Such decrease of chlorophyll and concomitant increase of carotenoids progress throughout fruit maturity, especially after harvest (Merzlyak, 2006; Delgado-Pelayo et al., 2014). However, with respect to the chlorophyll pigments, if the foliar B+Ca treatments can assist in preservation or increase of chlorophyll pigments in the apple peel, three benefits could arise to favour the reduction in class 1 SBB incidence as recorded in experiments of this study.

First, higher chlorophyll levels, especially CHLb, are linked to physiological plant defence systems and/or biochemical processes in the apple peel that can potentially reduce sunburn (Chen et al., 2008; Iams et al., 2009). Secondly, an increased chlorophyll to carotenoid ratio may result in decreased apple fruit sunburn (Chen et al., 2008). Thirdly, higher chlorophyll content would probably sustain fruit photosynthetic activity, whereby facilitating increased flavonoids through boosting the second peak of anthocyanin accumulation of ‘Cripps Pink’ that occurs towards the harvest maturity window (Whale & Singh, 2007). Increased anthocyanins (which partly constitute flavonoids) in apple fruit peel could be of benefit to the occurrence of reduced class 1 SBB, as observed in this study in ‘Cripps Pink’ and ‘Golden Delicious’, through two ways: (i) their known photoabatement roles (Smillie & Hetherington, 1999) and/or (ii), anthocyanins masking SBB disorders in ‘Cripps Pink’ (Makredza et al., 2015). However, this perceived benefit would result in an unacceptable blushed visual appearance of ‘Granny Smith’ at harvest.

The ‘Cripps Pink’ experiment in this study showed a significant and lower class 1 SBB incidence in B+Ca treatments compared to the control. However, among treatments, differences in class 1 SBB were not significant, suggesting that all treatments were equally efficient at reducing the class 1 SBB incidence. Two treatments (B^{0.02}+Ca^{1.24} and B^{0.17}+Ca^{1.24}) significantly increased TCHL levels in comparison to the control, while two treatments (B^{0.02}+znCa^{0.06} and B^{0.08}+Ca^{1.24}) did not differ from the control. Therefore, it is unlikely that a decrease in Class 1 SBB can be attributed to the effect of foliar B+Ca on peel chlorophyll levels, as even the trend contrast of the foliar treatments were not different from the control.

ii. Chlorophyll *a* to chlorophyll *b* ratio in ‘Cripps Pink’

Chen et al. (2008) indicated that chlorophyll *b* (CHLb) is rapidly lost during apple fruit sunburn development, however this may be part of the process of the preferential destruction of chlorophyll pigments compared to carotenoids as further noted by the same author. Chlorophyll destruction process is established to be preceded by conversion of CHLb to chlorophyll *a* (CHLa) (Kim et al.,

2009; Hortensteiner & Krautler, 2011; Tanaka & Tanaka, 2011), whereas a non-detectable and/or lower peel CHLb, reflected as high peel CHLa:CHLb associated with increasing SBB severity in apple fruit (Felicetti & Schrader, 2008; 2009). Delineating CHLa:CHLb variability amongst foliar B+Ca treatments towards the harvest maturity window in ‘Cripps Pink’ may aid towards understanding the role played by this chlorophyll aspect in the class 1 SBB incidence reduction phenomenon. This then, will be relevant in the effort to understand the mode-of-action as aimed in this study.

Even though not significant ($p = 0.1305$), the control had a higher CHLa:CHLb ratio than the foliar B+Ca treatments, which might correspond with the recent findings that foliar Ca promoted photosynthetic integrity in peanut leaves under heat and high irradiance (Yang et al., 2015). Similarly, Felicetti and Schrader (2008) found that increased SBB severity was associated with increasing CHLa:CHLb ratios, although, it is noted that fruit without visible sunburn was used for the assessment of pigments in the current study. The higher levels of chlorophyll compared to the control in some of the foliar B+Ca treatments, as well the higher CHLa:CHLb levels in the control ($p = 0.1305$), does not suffice as a sole explanation as to how foliar B+Ca decreased Class 1 SBB in ‘Cripps Pink’. This result suggests that CHLa:CHLb levels recorded in this study confirm the importance of higher peel chlorophyll levels for apple fruit SBB incidence suppression, but unfortunately CHLa:CHLb does not explain how foliar B+Ca decreased Class 1 SBB, given that also the trend contrast of the foliar treatments were not significantly different from the control.

iii. Carotenoids and sunburn browning incidence in ‘Cripps Pink’

Carotenoids are very important photosynthetic pigments with vital roles in plant antioxidant and photoprotection mechanisms as well as particularly in apple fruit peels (Merzlyak & Solovchenko, 2002; Merzlyak et al., 2002; Felicetti & Schrader, 2008; Solovchenko et al., 2010; Bandurska et al., 2013; Racchi, 2013; Nisar et al., 2015). The carotenoid based xanthophyll cycle is involved in limiting apple fruit SBB development (Ma & Cheng, 2004; Chen et al., 2008). Apple peel carotenoid levels towards harvest appear to play a contributory role, instead of major roles, in protection against SBB causative agents. This is because apple fruit is more susceptible to SBB damage towards harvest (Glenn et al., 2002; Schrader et al., 2003; Glenn, 2009; Fan et al., 2011; Zupan et al., 2014). Yet, there is an accompanying transition of chloroplasts to chromoplasts that should, ultimately, increase carotenoids and reduces chlorophyll (Bian et al., 2011). This argument of a seemingly only contributory role to SBB resistance is supported by observations

that increase in apple fruit SBB severity is associated with declining peel carotenoid levels particularly, beta-carotene and xanthophylls, but not lutein (Felicetti & Schrader, 2008; Zhang et al., 2015). In this regard, relative increments or preservations of carotenoids may be of particular interest to understand the underlying mode-of-action as aimed in this study.

Unfortunately, incremental differences in TCAR levels amongst treatments were not significant ($p = 0.0923$), although the control and the B 0.02+znCa`0.06 had decreasing TCAR levels towards fruit harvest, compared to the foliar B+Ca treatments. Increasing of carotenoids in peel tissues could actually be a manifestation of the SBB symptoms as golden-yellow blemishes on the apple fruit skin (Racskó & Schrader, 2012), but phenolic accumulation or reduction in chlorophyll compared to carotenoid levels can also contribute towards yellowing of the fruit. However, in this study, the relative increases of the TCAR levels observed with the foliar B+Ca treatments, except where Zn was included, seems to be beneficial, as all treatments registered a low and significantly different SBB incidence compared to the control, with the lowest TCAR levels. Evidently, Class 1 SBB incidence reduction could not be explained by changes in TCAR levels. Again, the trend contrast of the foliar treatments did not differ from the control.

iv. Chlorophyll to carotenoids ratio and sunburn browning incidence in ‘Cripps Pink’

The total chlorophyll to carotenoids ratio (TCHL:TCAR) estimates the integrity of photosynthetic systems (Hannoufa & Hossain, 2012). Lower levels of this ratio associate with severity of sunburn in apple fruit as chlorophyll is more easily destroyed compared to carotenoids, leading to increased conspicuousness of the carotenoid-colours on the apple fruit skin, hence a manifest of SBB damage and disorders (Chen et al., 2008; Lolicato, 2011; Racsko & Schrader, 2012). With respect to the aim of this study as seeking a respective mode-of-action by which foliar B+Ca treatments mediate SBB reduction in apple fruit, the focus is on the magnitude of this ratio in comparison to the control. A high magnitude of this ratio indicates high TCHL and the reverse is true. Yet, naturally, chlorophyll will decrease as carotenoids increase with fruit maturity, especially after harvest (Bian et al., 2011). Foliar B+Ca treatments with capacity to promote apple fruit SBB resistance should preserve and/or increase chlorophyll in peel tissues, resulting in increased TCHL:TCAR levels in comparison to the control.

As expected from the above discussion on apple fruit peel TCHL:TCAR, the magnitude of this ratio decreased with fruit maturity, and significant differences amongst DAFB were recorded. However, the TCHL:TCAR levels realised in this experiment also cannot be related directly with the class 1

SBB incidence reduction in a way that elucidates explicitly the sought underlying mode-of-action. Two foliar B+Ca treatments, B^{0.08}+Ca^{1.24} and B^{0.17}+Ca^{1.24}, stood out with highest and significantly different TCHL:TCAR levels compared to the control. Class 1 SBB incidence reduction in these treatments as recorded in the experiment could have possibly resulted from the physiological relevance of this high ratio in apple fruit sunburn dynamics as alluded to earlier above. However, treatment B^{0.02}+Ca^{1.24} showed TCAR levels that did not differ significantly from the control and B^{0.08}+Ca^{1.24} and B^{0.17}+Ca^{1.24}. In addition, treatment B^{0.02}+znCa^{0.06} had TCAR levels that were significantly different from B^{0.08}+Ca^{1.24} and B^{0.17}+Ca^{1.24}. Yet, with these differences amongst treatments, class 1 SBB incidence was not significantly different amongst them. Particularly, the lowest TCHL:TCAR levels of treatment B^{0.02}+znCa^{0.06} and the decreasing trends in all treatments towards fruit harvest. This strongly suggest that TCHL:TCAR does not explain the SBB incidence reduction, with the trend contrast of the foliar treatments not differing from the control.

b. Does the foliar B plus Ca induced changes in apple peel photosynthetic pigment attributes explain sunburn browning incidence reduction in ‘Golden Delicious’ and ‘Granny Smith’ apples?

i. Chlorophyll and sunburn browning incidence in ‘Golden Delicious’

Variability of ‘Golden Delicious’ apple peel chlorophyll *a* (CHLa), chlorophyll *b* (CHLb) and total chlorophyll (TCHL) appeared similar in both treatments and fruit maturity stages, except that CHLb was the only aspect not associated with significant differences amongst the treatments (Table 5). As expected, these aspects of chlorophyll pigments decreased with fruit maturity (Figures 2.1, 2.2 and 2.3). In contrast with ‘Cripps Pink’, in ‘Golden Delicious’ significant differences for SBB incidence occurred for class 0 (no sunburn) in addition to class 1. Overall, ‘Golden Delicious’ recorded better sunburn incidence reduction with these foliar B+Ca treatments in comparison to ‘Cripps Pink’. However, still in ‘Golden Delicious’, the CHLa, CHLb and TCHL levels contested the sole likelihood that higher chlorophyll levels alone explain the SBB incidence reduction in ‘Golden Delicious’. This is because the control (no B+Ca), with the highest SBB incidence, showed CHLa, CHLb and TCHL levels that were not significantly different from treatment B^{0.08}+Ca^{1.24}, with the lowest sunburn incidence. In addition, the moving mean trends show that actually B^{0.08}+Ca^{1.24} had lower CHLa and TCHL levels towards fruit harvest compared to the control. This potentially shows that the benefits of higher chlorophyll levels with respect to SBB development, as explained earlier under ‘Cripps Pink’, are not aligned with the sought mode-of-action in ‘Golden Delicious’. Further still,

the trend contrast of the foliar treatments were not different from the control or even among the treatments that showed variable SBB suppression outcomes.

ii. Carotenoids and sunburn browning incidence in ‘Golden Delicious’

Apple peel total carotenoids (TCAR) levels do not appear to offer the sought mode-of-action in the ‘Golden Delicious’ experiment, as there was no significant differences between the control and treatments that were observed to promote the highest SBB incidence reduction. In particular, with regard to the control, decreasing chlorophyll with increasing carotenoids as apple fruit maturity advances might contribute to the manifest of SBB damage or disorders (Chen et al., 2008; Lolicato, 2011; Racskó & Schrader, 2012). The sharply decreasing TCHL:TCAR trend from 108 DAFB towards fruit harvest point to the inference that the control and treatment B`0.02+znCa`0.06 were poised for more carotenoid per unit of chlorophyll than any other treatment. However, differences were not significant for the TCHL:TCAR amongst treatments, and could not fully confirm this relation to SBB.

The idea of carotenoid-level manifestation of SBB in this experiment is also not supported by the behaviour of TCHL:TCAR seasonal trends of treatments B`0.02+Ca`1.24 and B`01.7+Ca`1.24, which had rising TCHL:TCAR by 164 DAFB. A true carotenoid-level manifesting SBB in this experiment would infer that treatments B`0.02+Ca`1.24 and B`01.7+Ca`1.24 would promote SBB incidence reduction compared to B`0.08+Ca`1.24, yet this was not the case. Charoenchongsuk et al. (2015) observed that, in some pome fruit where peel background colour changes from green to yellow with advance of fruit maturity, there is loss of both chlorophyll and carotenoid pigments. Similarly, this process in ‘Golden Delicious’ where such change of fruit peel colour is known, may confound the observed changes in carotenoid levels presented as TCAR and TCHL:TCAR and therefore complicates the mapping on SBB incidence reduction. As before, the trend contrast of the foliar treatments were similar to the control, as well as among the treatments that showed variable SBB suppression outcomes.

In ‘Cripps Pink’, treatment B`0.02+znCa`0.06 was associated with severely reduced integrity of the photosynthetic systems compared to other B+Ca treatments when considering the significant differences of TCHL:TCAR magnitudes. In ‘Golden Delicious’, TCAR levels in treatment B`0.02+znCa`0.06 were the lowest and significantly different from all other treatments, including the control. The same applied to chlorophyll (TCHL) levels. At first instance, it appears as though this infers that addition of Zn to the foliar B+Ca treatments as well compromises the photosynthetic

integrity of ‘Golden Delicious’ peel tissues. The addition of Zn, and not the low Ca content in the treatments, seems to be the key factor in the results, as the control had no Ca but had higher TCAR and TCHL levels in comparison to treatment B`0.02+znCa`0.06. Since this contrasting finding occurs within the genetically related ‘Golden Delicious’ and ‘Cripps Pink’ (Cripps et al., 1993), as well as in ‘Granny Smith’ (significantly lower TCHL:TCAR with the Zn treatment), this strongly provides evidence that cultivar and/or genotype differences influence the response to post-full-bloom foliar B+Ca treatments with regard to changes in peel biochemistry. This could also be the basis as to why cultivars respond variably concerning SBB incidence reduction outcomes observed in this study.

Photosynthetic integrity as indicated by TCHL:TCAR levels (Hannoufa & Hossain, 2012) and its dynamics associate with apple fruit SBB induction and/or severity development via reduction of chlorophyll and increase in carotenoids (Chen et al., 2008) should result in a lower TCHL:TCAR in apple sunburn development. In this study, where the control serves as reference point, the TCHL:TCAR indicated significantly lower integrity, which also related to a significantly higher class 1 sunburn incidence in the control in ‘Cripps Pink’ compared to treatments B`0.02+Ca`1.24 and B`0.08+Ca1.24. However, this aspect is not clear in ‘Golden Delicious’, where TCHL:TCAR did not differ significantly among treatments, even though the control had the lowest values.

However, all treatments, except B`0.02+znCa`0.06, had significantly higher photosynthetic integrity levels (based on TCHL and TCHL:TCAR magnitudes and control as reference point) and showed similar capacities to promote SBB incidence reduction in ‘Cripps Pink’, as well as the Zn containing treatment. In ‘Golden Delicious’, there were no significances in photosynthetic integrity as displayed in TCHL:TCAR, yet significant differences in SBB occurred within treatments. Thus, using the TCHL:TCAR ratios as indication of photosynthetic integrity do not acceptably explain the SBB reduction between cultivars. These inconsistencies suggest that the possible mode-of-action for SBB reduction cannot be explained by the TCHL:TCAR values only, or realised from the seasonal trend contrasts of the foliar treatments that were not different from the control or between treatments, with variable SBB suppression outcomes.

In ‘Granny Smith’, a significantly higher photosynthetic integrity in all treatments compared B`0.02+znCa`0.06 was recorded as reflected in the TCHL:TCAR ratio. However, no differences in SBB occurred between treatments, resonation with previous findings in ‘Golden Delicious’, again indicating the need for an alternative approach to explain the role of TCHL:TCAR ratio in SBB mitigation. Generally, due to cultivar differences, ‘Granny Smith’ showed higher photosynthetic pigments and respective ratios compared to both ‘Cripps Pink’ and ‘Golden Delicious’. In the study

of Charoenchongsuk et al. (2015), stay-green pome fruit kept higher levels of chlorophyll and carotenoids towards advanced maturity. Ampomah-Dwamena et al. (2012) also noticed that in ‘Granny Smith’ high levels of chlorophyll and carotenoids prevail together.

5. Conclusion

This study concludes with the realisation that treatment formulation differences influence and/or impact apple peel photosynthetic pigments in ‘Cripps Pink’, ‘Golden Delicious’ and ‘Granny Smith’ apples. The inclusion of Zn in the foliar B+Ca treatments was associated with significantly reduced magnitudes of the photosynthetic pigments and ratios respectively. The photosynthetic pigments attributes studied generally showed strong and/or appreciable variability within the foliar B+Ca treatments in each study cultivar, and significant interaction between treatment and maturity stage occurred for CHLa, CHLb, TCHL and TCAR levels in ‘Granny Smith’. There was no photosynthetic pigment dynamics that could explain the significant SBB incidence reduction that occurred in both ‘Cripps Pink’ and ‘Golden Delicious’ apples, therefore the mode-of-action was not realised. However, as these differential behaviour trends of the foliar B+Ca treatments on fruit peel chlorophyll and carotenoid pigments varied between cultivars, an individual cultivar approach is warranted in future research and development efforts of this foliar B+Ca approach in suppressing SBB in apple orchards.

Acknowledgments

Support for this research was made possible through a capacity building competitive grant (RU/2015/DRRG/01/004) provided by Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) and Nulandis (Pty), South Africa. This study is part of a Ph.D. training program supported by RUFORUM members, Stellenbosch and Makerere Universities.

References

- Ampomah-Dwamena, C., Dejnopratt, S., Lewis, D., Sutherland, P., Volz, R.K. & Allan, A.C. 2012. Metabolic and gene expression apple (*Malus X domestica*) carotenogenesis. *Journal of Experimental Botany* 63:4497-4511.
- Bandurska, H., Niedziela, J. & Chadzinikolau, T. 2013. Separate and combined responses to water deficit and UV-B radiation. *Plant Science* 213:98-105.
- Bian, W., Barsan, C., Egea, I., Purgatto, E., Chervin, C., Zouine, M., Latche, A., Bouzayen, M. & Pech, J C. 2011. Metabolic and molecular events occurring during chromoplast biogenesis. *Journal of Botany* 2011:1-13.
- Charoenchongsuk, N., Ikeda, K., Itai, A., Oikawa, A. & Murayama, H. 2015. Comparison of the expression of chlorophyll-degradation-related genes during ripening between stay-green and yellow-pear cultivars. *Scientia Horticulturae* 181:89-94.
- 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.
- Daiber, S.H. 2017. Quantifying changes in tree physiology after amelioration to reduce sunburn on apples. MSc Thesis. Department of Horticultural Science, Stellenbosch University.
- Delgado-Pelayo, R., Gallardo-Guerrero, L. & Hornero-Mendez, D. 2014. Chlorophyll and carotenoid pigments in the peel and flesh of commercial apple fruit varieties. *Food Research International* 65:272-281.
- Dellapenna, D. 1999. Carotenoid synthesis and function in plants: Insights from mutant studies in *Arabidopsis*. *Pure and Applied Chemistry* 71:2205-2212.
- Egea, I., Bian, W., Barsan, C., Jauneau, A., Pech, J.C., Latche, A., Li, Z. & Chervin, C. 2011. Chloroplast to chromoplast transition in tomato fruit: Spectral confocal microscopy analyses of carotenoids and chlorophylls in isolated plastids and time-lapse recording on intact live tissue. *Annals of Botany* 108:291-297.
- Fan, L., Song, J., Forney, C.F. & Jordan, M.A. 2011. Fruit maturity affects the response of apples to heat stress. *Postharvest Biology and Technology* 62:35-42.

- Felicetti, D.A. & Schrader, L.E. 2008. Changes in pigment levels associated with the degree of sunburn browning of 'Fuji' apple. *Journal of the American Society for Horticultural Science* 133:27-34.
- Felicetti, D.A. & Schrader, L.E. 2009. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. I. Chlorophylls and Carotenoids. *Plant Science* 176:78-83.
- Glenn, D.M. 2009. Particle film mechanisms of action that reduce the effect of environmental stress in 'Empire' apple. *Scientia Horticulturae* 134:314-321.
- 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. *Journal of the American Society for Horticultural Science* 127:188-193.
- Hannoufa, A. & Hossain, Z. 2012. Regulation of carotenoid accumulation in plants. *Biocatalysis and Agricultural Biotechnology* 1:198-202.
- Havaux, M. & Niyogi, K.K. 1999. The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proceedings of the National Academy of Sciences of the United States of America* 96:8762-8767.
- Hipps, N.A. & Davies, M.J. 2001. Effects of foliar zinc applications at different times in the growing season on tissue zinc concentrations, fruit set, yield and grade out of culinary apple trees. *Acta Horticulturae* 564:145-151.
- Hortensteiner, S. & Krautler, B. 2011. Chlorophyll breakdown in higher plants. *Biochimica et Biophysica Acta* 1807:977-988.
- 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. *Journal of Food, Agriculture and Environment* 7:446-452.
- Kim, E.H., Li, X.P., Razeghifard, R., Anderson, J.M., Niyogi, K.K., Pogson, B.J. & Chow, W.S. 2009. The multiple roles of light-harvesting chlorophyll a/b -protein complexes define structure and optimize function of *Arabidopsis* chloroplasts: A study using two chlorophyll b-less mutants. *Biochimica et Biophysica Acta* 1787:973-984.
- Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology* 148:350-382.

- Lichtenthaler, H.K. & Buschmann, C. 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Current Protocols in Food Analytical Chemistry* F4.3: F4.3.1-F4.3.8.
- Lolicato, S. 2011. Sun Protection for Fruit - A practical manual for protecting sunburn on fruit. Department of Primary Industries, Farm Services Victoria Division, Australia.
- Lötze, E. & Hoffman, E.W. 2014. Foliar application of calcium plus boron reduces the incidence of sunburn in 'Golden Delicious' apple. *Journal of Horticultural Science and Biotechnology* 89:607-612.
- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2017. Boron in combination with calcium reduces sunburn in apple fruit. *Boron* 2:123-127.
- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2018. Evaluating the efficacy of a preharvest combination of calcium and boron as foliar application to reduce sunburn on 'Cripps Pink' apples. *Acta Horticulture* 1217:61-68.
- Ma, F. & Cheng, L. 2004. Exposure of the shaded side of apple fruit to full sun leads to up-regulation of both the xanthophyll cycle and the ascorbate-glutathione cycle. *Plant Science* 166:1479-1486.
- Makredza, B., Marais, H., Schmeisser, M., Lötze, E. & Steyn, W.J. 2015. Ripening associated red color development masks sunburn browning in apple peel. *HortScience* 50:814-818.
- Merzlyak, M.N. 2006. Modeling pigment contributions to spectral reflection of apple fruit. *Photochemical & Photobiological Sciences* 5:748-754.
- Merzlyak, M.N. & Solovchenko, A.E. 2002. Photostability of pigments in ripening apple fruit: A possible photoprotective role of carotenoids during plant senescence. *Plant Science* 163:881-888.
- Merzlyak, M.N., Solovchenko, A.E. & Chivkunova, O.B. 2002. Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. *Plant Physiology and Biochemistry* 40:679-684.
- Mu, P., Li, X.P. & Niyogi, K.K. 2001. Update on photosynthesis non-photochemical quenching. A response to excess light energy. *Plant Physiology* 125:1558-1566.
- Murata, N., Allakhverdiev, S.I. & Nishiyama, Y. 2012. The mechanism of photoinhibition in vivo: Re-evaluation of the roles of catalase, α -tocopherol, non-photochemical quenching, and electron transport. *Biochimica et Biophysica Acta* 1817:1127-1133.

- Neilsen, G.H. & Neilsen, D. 2002. Effect of foliar Zn, form and timing of Ca sprays on fruit Ca concentration in new apple cultivars. *Acta Horticulturae* 594:435-443.
- Nisar, N., Li, L., Lu, S., Khin, N.C. & Pogson, B.J. 2015. Carotenoid metabolism in plants. *Molecular Plant* 8:68-82.
- Racchi, M.L. 2013. Antioxidant defences in plants with attention to *Prunus* and *Citrus* spp. *Antioxidants* 2:340-369.
- Rahayu, Y.S., Romheld, V. & Bangerth, F. 2001. Does zinc nutrition affect calcium disorder of fruits? *Acta Horticulturae* 564:135-143.
- Schrader, L.E. 2011. Scientific basis of a unique formulation for reducing sunburn of fruits. *HortScience* 46:6-11.
- Schrader, L.E., Sun, J., Felicetti, D.A., Seo, J.H., Jedlow, L. & Zhang, J. 2003. Stress-induced disorders: Effects on apple fruit quality. Washington Tree Fruit Postharvest Conference. 1:1-7.
- Seth, K. & Aery, N.C. 2014. Effect of boron on the contents of chlorophyll, carotenoid, phenol and soluble leaf protein in mung Bean, *Vigna radiata* (L.) Wilczek. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences* 84:713-719.
- Smillie, R.M. & Hetherington, S.E. 1999. Photoabatement by anthocyanin shields photosynthetic systems from light stress. *Photosynthetica* 36:451-463.
- Solovchenko, A.E., Chivkunova, O.B., Gitelson, A.A. & Merzlyak, M.N. 2010. Non-destructive estimation pigment content, ripening, quality and damage in apple fruit with spectral reflectance in the visible range. *Fresh Produce* 4:91-102.
- Sotiropoulos, T., Petridis, A., Koukourikou-Petridou, M. & Koundouras, S. 2016. Evaluation of 'Sun Protect' in protecting apples (*Malus × domestica* Borkh.) against sunburn. *Horticultural Science (Prague)* 43:175-180.
- Tanaka, R. & Tanaka, A. 2011. Chlorophyll cycle regulates the construction and destruction of the light-harvesting complexes. *Biochimica et Biophysica Acta* 1807:968-976.
- Vrettos, J.S., Limburg, J. & Brudvig, G.W. 2001. Mechanism of photosynthetic water oxidation: combining biophysical studies of photosystem II with inorganic model chemistry. *Biochimica et Biophysica Acta* 1503:229-245.

- Whale, S.K. & Singh, Z. 2007. Endogenous ethylene and color development in the skin of 'Pink Lady' apple. *Journal of the American Society for Horticultural Science* 132:20-28.
- Yachandra, V.K. & Yano, J. 2011. Calcium in the oxygen-evolving complex: Structural and mechanistic role determined by X-ray spectroscopy. *Journal of Photochemistry and Photobiology B - Biology* 104:51-59.
- Yang, S., Wang, F., Guo, F., Meng, J.J., Li, X.G. & Wan, S.B. 2015. Calcium contributes to photoprotection and repair of photosystem II in peanut leaves during heat and high irradiance. *Journal of Integrative Plant Biology* 57:486-495.
- Zhang, J., Niu, J., Duan, Y., Zhang, M., Liu, J., Li, P. & Ma, F. 2015. Photoprotection mechanism in the 'Fuji' apple peel at different levels of photooxidative sunburn. *Physiologia Plantarum* 154:54-65.
- Zupan, A., Mikulic-Petkovsek, M., Slatnar, A., Stampar, F. & Veberic, R. 2014. Individual phenolic response and peroxidase activity in peel of differently sun-exposed apples in the period favorable for sunburn occurrence. *Journal of Plant Physiology* 171:1706-1712.

Tables

Table 1. 'Cripps Pink' apple peel photosynthetic pigments and their respective ratios among five foliar B plus Ca treatments and at four selected fruit maturity stages (DAFB).

Treatments (B+Ca)	CHLa	CHLb	TCHL	CHLa:CHLb	TCAR	TCHL:TCAR
B`0.00+Ca`0.00	27.19 ^{bc}	18.12 ^{bc}	45.31 ^{bc}	2.01 ^{ns}	12.21 ^{ns}	3.77 ^{bc}
B`0.02+Ca`1.24	29.39 ^a	20.95 ^{ab}	50.33 ^a	1.53	12.63	4.08 ^{ab}
B`0.02+znCa`0.06	26.09 ^c	16.65 ^c	42.74 ^c	1.86	12.86	3.38 ^c
B`0.08+Ca`1.24	28.25 ^{ab}	21.66 ^a	49.90 ^{ab}	1.53	11.29	4.60 ^a
B`0.17+Ca`1.24	30.04 ^a	22.78 ^a	52.82 ^a	1.41	12.43	4.43 ^a
<i>p</i> -value	0.0019	0.0028	0.0007	0.1305	0.0923	0.0024
Days after full bloom (DAFB)						
122	35.40 ^a	27.55 ^a	62.95 ^a	1.36 ^b	13.67 ^a	4.77 ^a
136	29.45 ^b	24.26 ^b	53.72 ^b	1.38 ^b	11.33 ^b	4.98 ^a
150	23.89 ^c	13.77 ^c	37.66 ^c	2.04 ^a	12.52 ^{ab}	3.07 ^b
164	24.01 ^c	14.54 ^c	38.55 ^{bc}	1.90 ^a	11.62 ^b	3.37 ^b
<i>p</i> -value	0.0000	0.0000	0.0001	0.0056	0.0001	0.0000
B+Ca*DAFB (<i>p</i> -value)	0.6232	0.4092	0.5722	0.4819	0.2332	0.2942

Mean values sharing a letter are not different, CHLa = chlorophyll *a* ($\mu\text{g g}^{-1}$ peel FW), CHLb = chlorophyll *b* ($\mu\text{g g}^{-1}$ peel FW), TCHL = total chlorophyll ($\mu\text{g g}^{-1}$ peel FW), CHLa:CHLb = chlorophyll *a* to chlorophyll *b* ratio and TCAR = total carotenoids ($\mu\text{g g}^{-1}$ peel FW) and TCHL:TCAR = total chlorophyll to total carotenoids ratio.

Table 2. 'Golden Delicious' apple peel photosynthetic pigments and their respective ratios among five foliar B plus Ca treatments and at four selected fruit maturity stages (DAFB).

Treatments (B+Ca)	CHLa	CHLb	TCHL	CHLa:CHLb	TCAR	TCHL:TCAR
B`0.00+Ca`0.00	55.25 ^a	39.14 ^{ns}	94.39 ^a	1.41 ^{ns}	28.95 ^a	3.27 ^{ns}
B`0.02+Ca`1.24	54.85 ^a	39.43	94.27 ^a	1.40	27.37 ^a	3.45
B`0.02+znCa`0.06	48.18 ^b	35.64	83.82 ^b	1.36	24.36 ^b	3.47
B`0.08+Ca`1.24	52.12 ^{ab}	38.21	90.33 ^{ab}	1.36	27.91 ^a	3.26
B`0.17+Ca`1.24	55.10 ^a	40.06	95.16 ^a	1.38	28.45 ^a	3.37
<i>p</i> -value	0.0056	0.1079	0.0218	0.0529	0.0011	0.1899
Days after full bloom (DAFB)						
80	65.18 ^a	46.39 ^a	111.57 ^a	1.41 ^a	33.22 ^a	3.37 ^b
94	52.41 ^b	37.90 ^b	90.30 ^b	1.39 ^a	27.35 ^b	3.33 ^b
108	49.90 ^b	37.16 ^b	87.06 ^b	1.35 ^b	24.48 ^c	3.57 ^a
122	44.92 ^c	32.53 ^c	77.45 ^c	1.39 ^a	24.59 ^c	3.18 ^b
<i>p</i> -value	0.0000	0.0000	0.0000	0.0158	0.0000	0.0029
B+Ca*DAFB (<i>p</i> -value)	0.1024	0.2245	0.1481	0.1251	0.0866	0.5475

Mean values sharing a letter are not different, CHLa = chlorophyll *a* ($\mu\text{g g}^{-1}$ peel FW), CHLb = chlorophyll *b* ($\mu\text{g g}^{-1}$ peel FW), TCHL = total chlorophyll ($\mu\text{g g}^{-1}$ peel FW), CHLa:CHLb = chlorophyll *a* to chlorophyll *b* ratio, TCAR = total carotenoids ($\mu\text{g g}^{-1}$ peel FW) and TCHL:TCAR = total chlorophyll to total carotenoids ratio.

Table 3. 'Granny Smith' apple peel chlorophyll *a* as a function of foliar B plus Ca and fruit maturity (DAFB).

Treatments (B+Ca)	94 DAFB (04.01.2016)	108 DAFB (18.01.2016)	122 DAFB (01.02.2016)	136 DAFB (15.02.2016)
B`0.00+Ca`0.00	161.06 ^{abc}	154.71 ^{abcd}	153.33 ^{abcd}	145.58 ^{cde}
B`0.02+Ca`1.24	144.21 ^{cde}	159.32 ^{abcd}	172.36 ^a	138.48 ^{def}
B`0.02+znCa`0.06	155.26 ^{abcd}	119.74 ^{fg}	145.59 ^{cde}	113.37 ^g
B`0.08+Ca`1.24	168.45 ^{ab}	157.80 ^{abcd}	139.21 ^{def}	128.63 ^{efg}
B`0.17+Ca`1.24	151.87 ^{abcd}	146.86 ^{cde}	158.26 ^{abcd}	150.68 ^{bcd}

Mean values sharing a letter are not different, B+Ca ($p = 0.0012$), DAFB ($p = 0.0002$) and B+Ca*DAFB ($p = 0.0067$).

Table 4. 'Granny Smith' apple peel chlorophyll *b* as a function of foliar B plus Ca and fruit maturity (DAFB).

Treatments (B+Ca)	94 DAFB (04.01.2016)	108 DAFB (18.01.2016)	122 DAFB (01.02.2016)	136 DAFB (15.02.2016)
B`0.00+Ca`0.00	121.96 ^{abcd}	124.53 ^{abc}	119.17 ^{abcd}	112.86 ^{bcde}
B`0.02+Ca`1.24	108.71 ^{cdef}	126.68 ^{ab}	133.71 ^a	107.47 ^{cdef}
B`0.02+znCa`0.06	116.99 ^{abcd}	94.74 ^{fg}	112.53 ^{bcde}	87.14 ^g
B`0.08+Ca`1.24	128.19 ^{ab}	127.05 ^{ab}	106.17 ^{def}	99.28 ^{efg}
B`0.17+Ca`1.24	115.92 ^{bcde}	117.45 ^{abcd}	122.55 ^{abcd}	118.86 ^{abcd}

Means sharing a letter are not different, B+Ca ($p = 0.0008$), DAFB ($p = 0.0000$) and B+Ca*DAFB ($p = 0.0050$).

Table 5. 'Granny Smith' apple peel total chlorophyll as a function of foliar B plus Ca and fruit maturity (DAFB).

Treatments (B+Ca)	94 DAFB (04.01.2016)	108 DAFB (18.01.2016)	122 DAFB (01.02.2016)	136 DAFB (15.02.2016)
B`0.00+Ca`0.00	283 ^{abcd}	279 ^{abcd}	272 ^{abcd}	258 ^{bcde}
B`0.02+Ca`1.24	253 ^{cdef}	286 ^{abc}	306 ^a	246 ^{def}
B`0.02+znCa`0.06	272 ^{abcd}	214 ^{fg}	258 ^{bcde}	201 ^g
B`0.08+Ca`1.24	297 ^{ab}	285 ^{abc}	245 ^{def}	228 ^{efg}
B`0.17+Ca`1.24	268 ^{abcd}	264 ^{bcde}	281 ^{abcd}	270 ^{abcd}

Means sharing a letter are not different, B+Ca ($p = 0.0010$), DAFB ($p = 0.0005$) and B+Ca*DAFB ($p = 0.0057$).

Table 6. 'Granny Smith' apple peel total carotenoids as a function of foliar B plus Ca and fruit maturity (DAFB).

Treatments (B+Ca)	94 DAFB (04.01.2016)	108 DAFB (18.01.2016)	122 DAFB (01.02.2016)	136 DAFB (15.02.2016)
B`0.00+Ca`0.00	63 ^{abc}	62 ^{abcd}	63 ^{abcd}	58 ^{cdef}
B`0.02+Ca`1.24	57 ^{cdef}	63 ^{abc}	67 ^a	55 ^{def}
B`0.02+znCa`0.06	61 ^{abcd}	51 ^{fg}	61 ^{abcd}	47 ^g
B`0.08+Ca`1.24	67 ^{ab}	64 ^{abc}	55 ^{def}	53 ^{efg}
B`0.17+Ca`1.24	60 ^{bcde}	56 ^{cdef}	62 ^{abcd}	62 ^{abcd}

Means sharing a letter are not different, B+Ca ($p = 0.0115$), DAFB ($p = 0.0006$) and B+Ca*DAFB ($p = 0.0009$).

Table 7. 'Granny Smith' apple peel photosynthetic pigment ratios among five foliar B plus Ca treatments and at four selected fruit maturity stages (DAFB).

Treatments (B+Ca)	CHLa:CHLb	TCHL:TCAR
B`0.00+Ca`0.00	1.29 ^{ns}	4.45 ^a
B`0.02+Ca`1.24	1.29	4.49 ^a
B`0.02+znCa`0.06	1.30	4.29 ^b
B`0.08+Ca`1.24	1.29	4.42 ^a
B`0.17+Ca`1.24	1.28	4.50 ^a
<i>p</i> -value	0.1230	0.0081
Days after full bloom (DAFB)		
94	1.32 ^a	4.45 ^{ns}
108	1.25 ^c	4.48
122	1.30 ^b	4.42
136	1.29 ^b	4.36
<i>p</i> -value	0.0000	0.1330
B+Ca*DAFB (<i>p</i> -value)	0.0009	0.4705

Mean values sharing a letter are not different, CHLa:CHLb = chlorophyll *a* to chlorophyll *b* ratio and TCHL:TCAR = total chlorophyll to total carotenoids ratio.

Table 8 Trend contrasts of 'Cripps Pink' apple peel photosynthetic pigment parameters at 122, 136, 150 and 164 days after full bloom among varying foliar B plus Ca treatments using the slope (*b*) statistic (*p*-value in parentheses).

Treatments (B+Ca)	CHLa	CHLb	CHLa:CHLb	TCHL	TCAR	TCHL:TCAR
Control (T ₀)	-0.2628 (0.0001)	-0.2612 (0.0207)	0.020 (0.5847)	-0.5240 (0.0026)	-0.0708 (0.0075)	-0.0236 (0.1666)
B`0.02+Ca`1.24 (T ₁)	-0.2563 (0.0001)	-0.3428 (0.0004)	0.0114 (0.0480)	-0.5992 (0.0001)	-0.0316 (0.2426)	-0.0395 (0.0221)
B`0.02+znCa`0.06 (T ₂)	-0.3350 (0.0000)	-0.2959 (0.0014)	0.0213 (0.1114)	-0.6310 (0.0001)	-0.0782 (0.0148)	-0.0276 (0.0528)
B`0.08+Ca`1.24 (T ₃)	-0.3255 (0.0000)	-0.5152 (0.0000)	0.0257 (0.0038)	-0.8408 (0.0000)	0.0161 (0.5792)	-0.0799 (0.0004)
B`0.17+Ca`1.24 (T ₄)	-0.2399 (0.0000)	-0.3531 (0.0001)	0.0116 (0.0204)	-0.5930 (0.0000)	-0.0125 (0.7182)	-0.0476 (0.0111)
<i>b</i> contrasts (<i>p</i> -values)						
T ₀ versus T ₁	0.9378	0.8567	0.4716	0.8698	0.9411	0.9791
T ₀ versus T ₂	0.9436	0.8492	0.7257	0.8984	0.8841	0.8841
T ₀ versus T ₃	0.8362	0.8746	0.5500	0.8788	0.8947	0.9394
T ₀ versus T ₄	0.9707	0.8018	0.4456	0.7604	0.7984	0.9859
T ₁ versus T ₂	0.8825	0.9921	0.5992	0.9703	0.9419	0.9044
T ₁ versus T ₃	0.9089	0.9811	0.8048	0.9906	0.9528	0.9650
T ₁ versus T ₄	0.8955	0.9405	0.9075	0.8803	0.8525	0.9188
T ₂ versus T ₃	0.9728	0.9731	0.7402	0.9797	0.9891	0.8269
T ₂ versus T ₄	0.7853	0.9483	0.5502	0.8521	0.9084	0.8706
T ₃ versus T ₄	0.8094	0.9218	0.7260	0.8713	0.8977	0.9534

KEY: CHLa = chlorophyll a, CHLb = chlorophyll b, TCHL = Total chlorophyll, TCAR = Total carotenoids

Table 9: Trend contrasts of 'Golden Delicious' apple peel photosynthetic pigment parameters at 80, 94, 108 and 122 days after full bloom among varying foliar B plus Ca treatments using the slope (*b*) statistic (*p*-value in parentheses)

Treatments (B+Ca)	CHLa	CHLb	CHLa:CHLb	TCHL	TCAR	TCHL:TCAR
Control (T ₀)	-0.5601 (0.0000)	-0.3793 (0.0001)	-0.0005 (0.4390)	-0.9395 (0.0000)	-0.2580 (0.0000)	-0.0032 (0.5632)
B`0.02+Ca`1.24 (T ₁)	-0.4840 (0.0003)	-0.3224 (0.0026)	-0.0007 (0.4348)	-0.8064 (0.0007)	-0.2056 (0.0031)	-0.0040 (0.3970)
B`0.02+znCa`0.06 (T ₂)	-0.4645 (0.0002)	-0.3592 (0.0001)	0.0009 (0.5103)	-0.8237 (0.0001)	-0.1766 (0.0131)	-0.0086 (0.1473)
B`0.08+Ca`1.24 (T ₃)	-0.4533 (0.0000)	-0.2672 (0.0015)	-0.0024 (0.0357)	-0.7204 (0.0002)	-0.2643 (0.0000)	0.0044 (0.3192)
B`0.17+Ca`1.24 (T ₄)	-0.2982 (0.0449)	-0.1840 (0.0875)	-0.0008 (0.5100)	-0.4822 (0.0568)	-0.1233 (0.0908)	0.0000 (0.9969)
<i>b</i> contrasts (<i>p</i> -values)						
T ₀ versus T ₁	0.9501	0.8963	0.7831	0.9283	0.8475	0.9034
T ₀ versus T ₂	0.9797	0.9462	0.6081	0.9745	0.8141	0.9806
T ₀ versus T ₃	0.9127	0.9599	0.6849	0.9229	0.9642	0.8635
T ₀ versus T ₄	0.8103	0.8440	0.6653	0.8293	0.7781	0.8670
T ₁ versus T ₂	0.9704	0.8449	0.7735	0.9033	0.9637	0.8846
T ₁ versus T ₃	0.8645	0.8578	0.8794	0.8535	0.8145	0.9586
T ₁ versus T ₄	0.8564	0.9453	0.8533	0.8975	0.9235	0.7783
T ₂ versus T ₃	0.8929	0.9863	0.8854	0.9482	0.7823	0.8453
T ₂ versus T ₄	0.8288	0.7951	0.9120	0.8061	0.9594	0.8856
T ₃ versus T ₄	0.7351	0.8073	0.9726	0.7609	0.7478	0.7434

KEY: CHLa = chlorophyll *a*, CHLb = chlorophyll *b*, TCHL = Total chlorophyll, TCAR = Total carotenoids

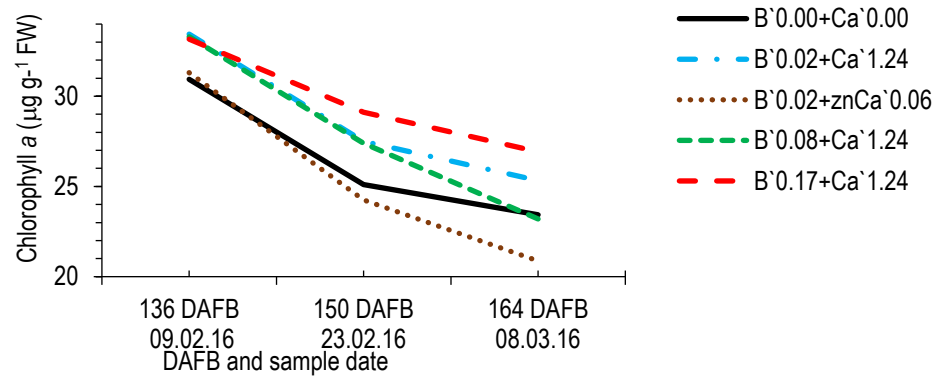


Figure 1.1. Moving mean of apple peel CHLa towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

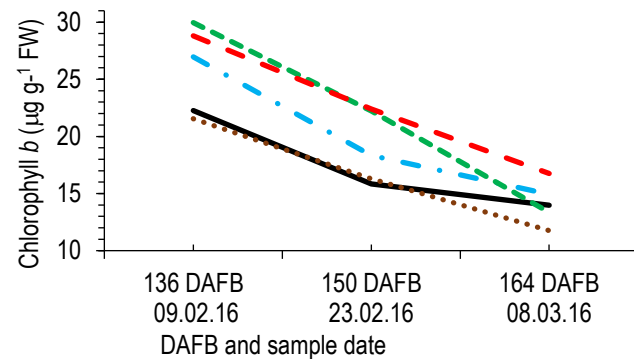


Figure 1.2: Moving mean of apple peel CHLb towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

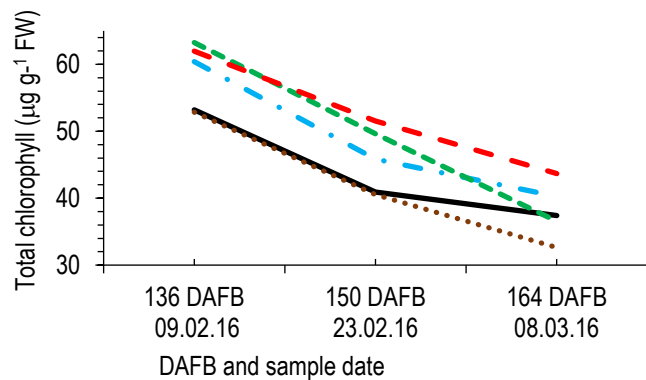


Figure 1.3: Moving mean of apple peel TCHL towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

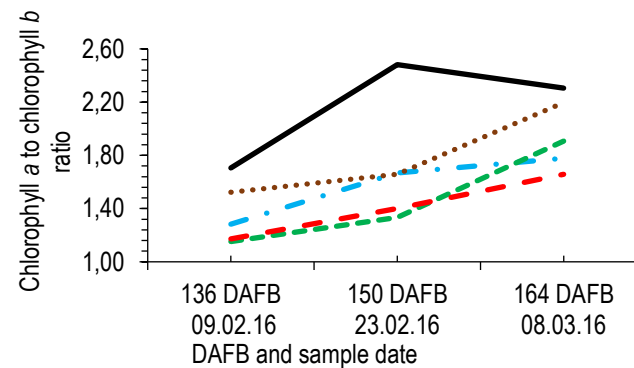


Figure 1.4: Moving mean of apple peel CHLa:CHLb towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

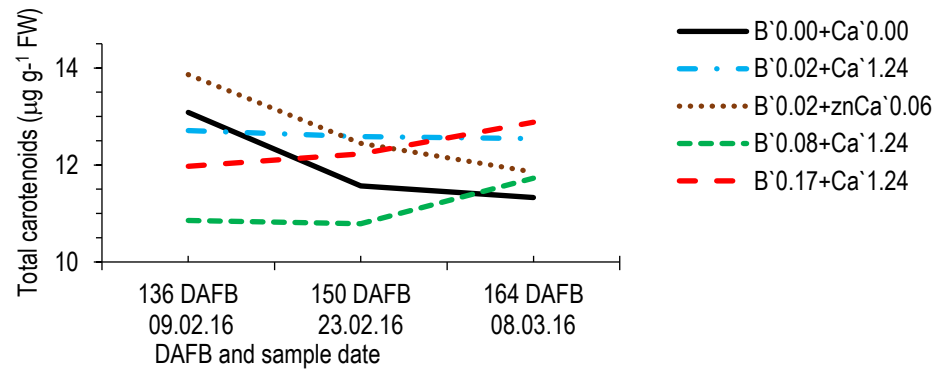


Figure 1.5: Moving mean of apple peel TCAR towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

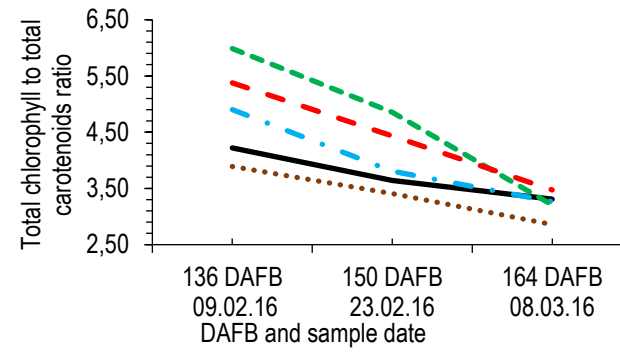


Figure 1.6: Moving mean of apple peel TCHL:TCAR towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'.

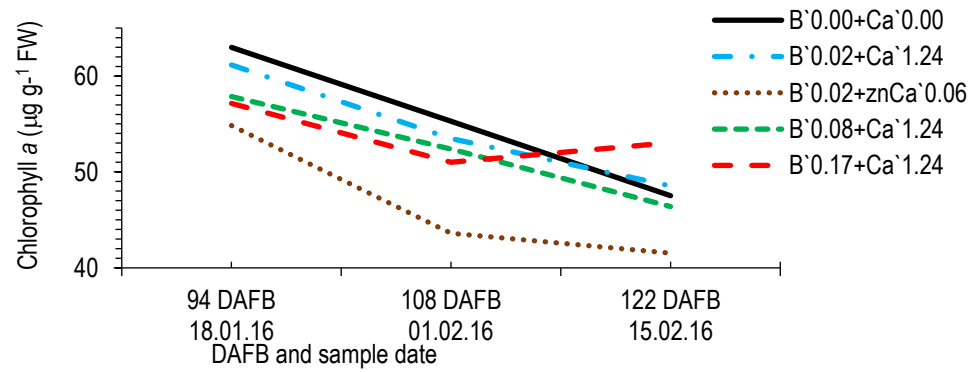


Figure 2.1. Moving mean of apple peel CHLa towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

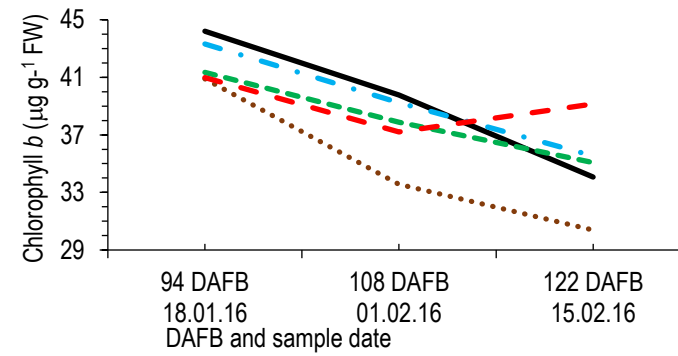


Figure 2.2: Moving mean of apple peel CHLb towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'.

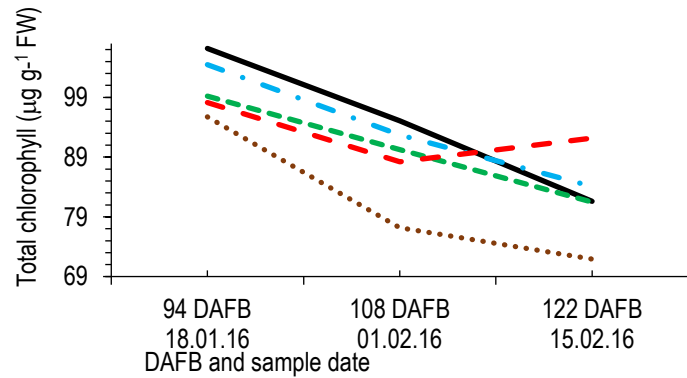


Figure 2.3: Moving mean of apple peel TCHL towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

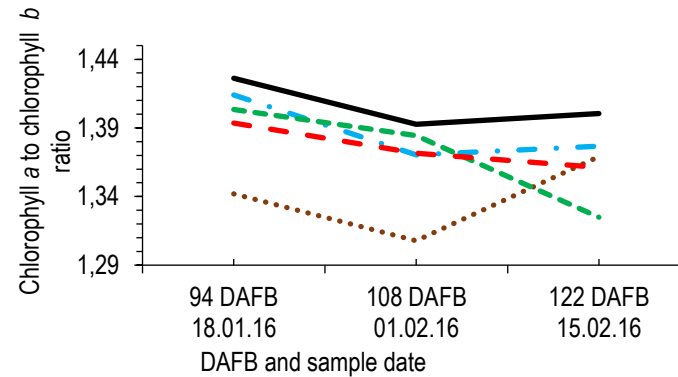


Figure 2.4: Moving mean of apple peel CHLa:CHLb towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'.

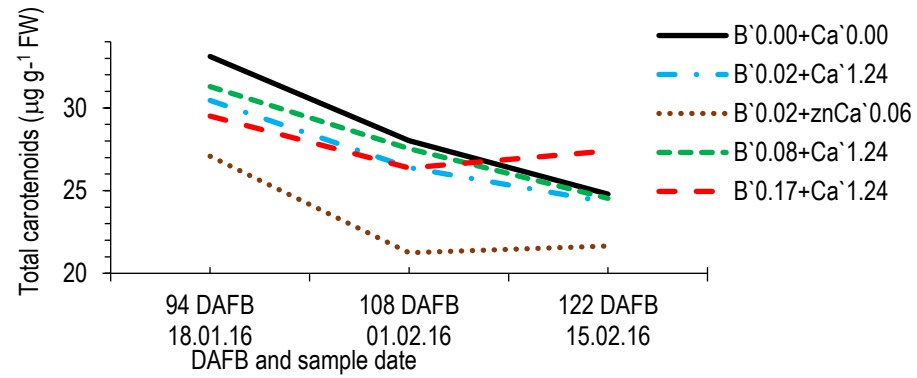


Figure 2.5: Moving mean of apple peel TCAR towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

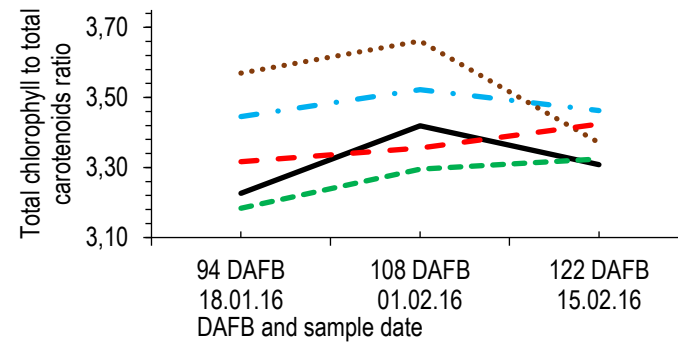


Figure 2.6: Moving mean of apple peel TCHL:TCAR towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

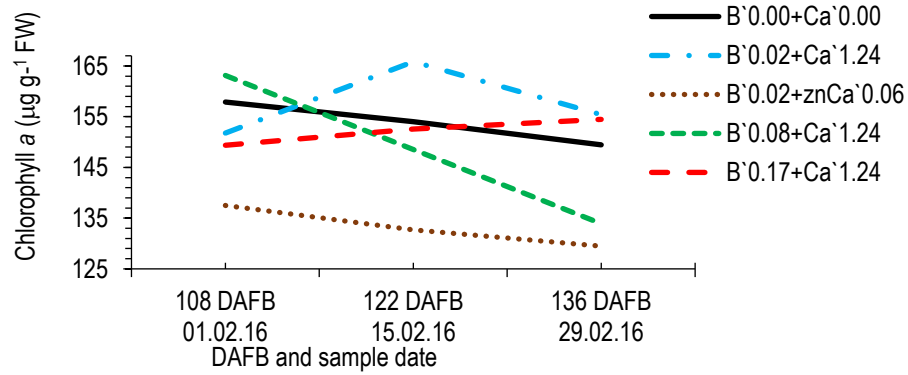


Figure 3.1: Moving mean of apple peel CHLa towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

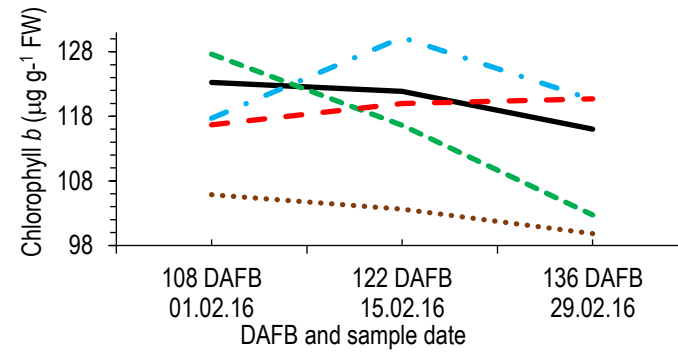


Figure 3.2: Moving mean of apple peel CHLb towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

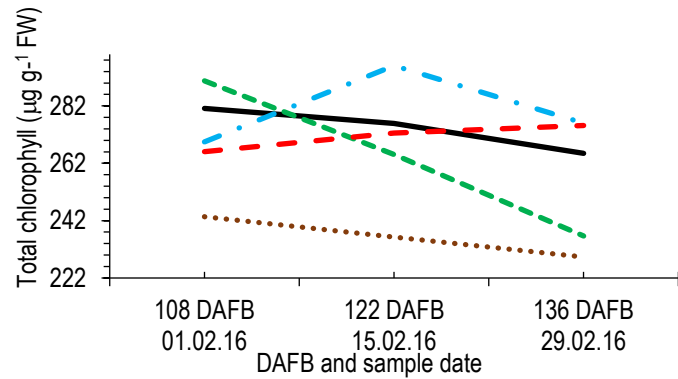


Figure 3.3: Moving mean of apple peel TCHL towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

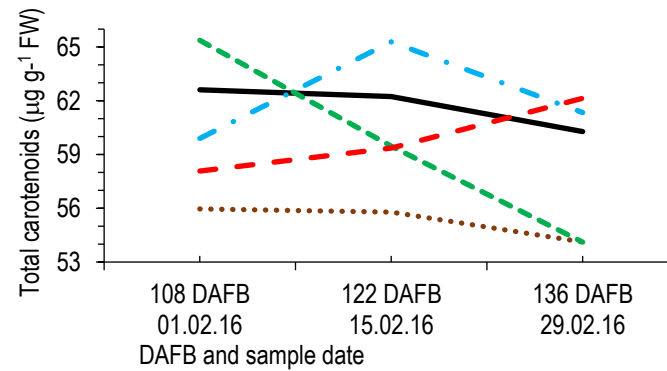


Figure 3.4: Moving mean of apple peel TCAR towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

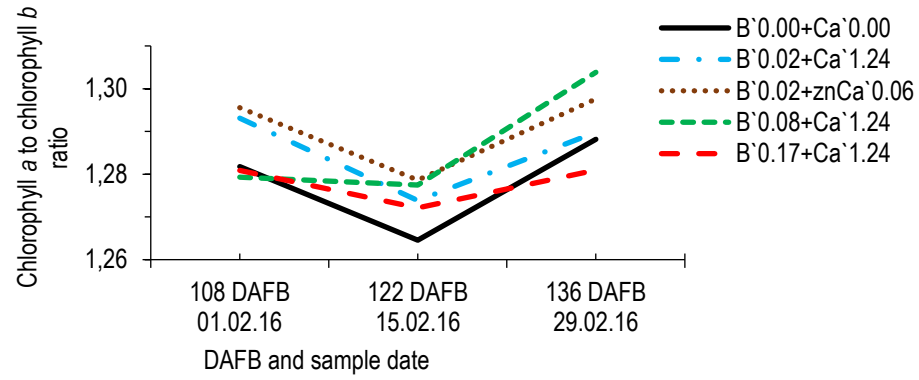


Figure 3.5: Moving mean of apple peel CHLa:CHLb towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

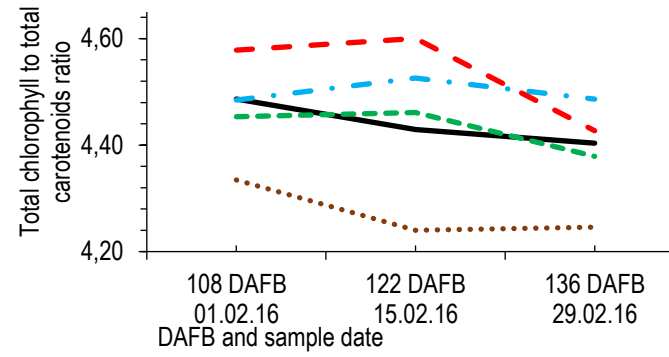


Figure 3.6: Moving mean of apple peel TCHL:TCAR towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

INVESTIGATING THE MODE-OF-ACTION BY WHICH POST-FULL-BLOOM FOLIAR APPLICATIONS OF A COMBINATION OF BORON AND CALCIUM IMPACT ON APPLE FRUIT SUNBURN BROWNING INCIDENCE

III. Boron plus calcium induced changes in apple peel oxidative stress levels

Abstract

This study investigated apple peel total peroxides levels as a proxy for oxidative stress status, and related findings to sunburn browning (SBB) incidence of fruit at harvest, following post-full-bloom foliar applications of boron plus calcium (B+Ca) treatments. The overall aim was to determine possible treatment-induced reduction in oxidative stress levels, and whether that offers a possible mode-of-action for the SBB incidence suppression associated with such treatments. Increased apple peel tissue oxidative stress is strongly associated with the induction fruit sunburn browning (SBB) disorders. Four treatments were applied to one bi-colour ('Cripps Pink') and two green ('Golden Delicious' and 'Granny Smith') apples in randomised complete block design experiments. The treatments were applied for six times on weekly intervals starting at 28 in the bi-colour and 39 days after full bloom (DAFB) in the green apples. Apple fruit peel for analyses of total peroxides (TPERO) were sampled at two-week intervals from 122 to 164 ('Cripps Pink'), 80 to 122 ('Golden Delicious') and 94 to 136 DAFB ('Granny Smith'). Generally, TPERO decreased with progression of apple fruit maturity (DAFB). Significant effects on peel TPERO levels occurred for treatments and maturity stages (DAFB) in 'Cripps Pink' and 'Granny Smith', while a significant interaction was recorded in 'Golden Delicious'. The control (no B+Ca) had the highest apple peel TPERO levels and fruit SBB incidence in all cultivars. This could have inferred that treatments reduce peel oxidative stress, possibly explaining SBB incidence reduction phenomenon. However, results across the treatments did not support this suggestion. This was mainly due to the inability to reflect the rather 'mild' oxidative stress trends and variability of SBB class 1 and 2 types. Therefore, alternative and much more robust methods should be investigate and/or developed to evaluate peel tissue oxidative stress

in investigations with varying foliar B+Ca treatments and apple fruit sunburn browning suppression dynamics.

Keywords: ‘Cripps Pink’, ‘Golden Delicious’, ‘Granny Smith’, lipid peroxidation, total peroxides

1. Introduction

Excessive solar irradiance and temperature leads to elevated levels of oxidative stress in plant tissues, which impairs cellular physiology mainly through compromising photosynthetic electron transport systems (Barber & Andersson, 1992; Mishra & Singhal, 1992; Smillie, 1992; Nawkar et al., 2013). This oxidative stress is characterized by increased levels of reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂) (Scandalios, 1993; Asada, 1999; Gill & Tuteja, 2010; Weisany et al., 2012). The excessive accumulation of these ROS will further cause cellular damage and/or death by the destruction (peroxidation) of the cell’s phospholipid membranes (lipid peroxidation), proteins, nucleic acids and chlorophyll pigments (Aro et al., 1993; Murata et al., 2007; Wahid et al., 2007; Hideg et al., 2013). Such oxidative stress induced by high light and temperature in apple peel tissue causes fruit sunburn browning (SBB) disorders (Wünsche et al., 2001; 2004; Schrader et al., 2008).

In apple peel tissue, innate defence mechanisms are often not sufficient to prevent the oxidative stress induced by the high light and temperature stresses, hence SBB development that greatly compromises quality and marketability of apple fruit (Wünsche et al., 2001; Schrader et al., 2003). The increase in oxidative stress in apple peel tissue degrades chlorophyll, while increasing phenolic and carotenoid metabolites (Felicetti & Schrader, 2009a, b; Yuri et al., 2010). The destruction of apple peel chlorophyll pigments renders the yellow to bronze carotenoid colours to be highly conspicuous on the fruit skin, and in varying severity levels that leads to distinct apple fruit SBB classes (Felicetti & Schrader, 2008; Schrader et al., 2008; Racskó & Schrader, 2012). In addition, SBB disorders are also most prominent on green fruit cultivars like ‘Granny Smith’, making them appear most susceptible (Lolicato, 2011; Sotiropoulos et al., 2016), in comparison to red blushing apples where there is a masking of symptoms (Makedredza et al., 2015). Overall, the apple fruit SBB phenomenon leads to large revenue losses, as markets reject such fruit with these undesirable blemishes, and depending on the severity, potential fruit for export and/or fresh market is culled, downgraded to juice making and/or sold at low prices (Schrader, 2011; Hamadziripi, 2012; Racskó & Schrader, 2012).

Lötze & Hoffman (2014) reported on a post-full-bloom foliar B plus Ca (B + Ca) approach to reduce apple fruit SBB incidence in orchards. This new approach is of interest for its envisaged benefits as being economical to implement, as well as having a low water footprint across the value chain compared to existing approaches like kaolin applications and overhead cooling. In addition, this alternative foliar B+Ca approach does not induce negative effects on apple fruit and tree physiology (Daiber, 2017). However, the mode-of-action by which these foliar B+Ca treatments reduce apple fruit SBB incidence is not yet explained (Daiber, 2017; Lötze et al., 2017), and this seriously compromises protocol refinement which is essential for successful promotion of this new approach particularly amongst commercial growers.

Typical plant stress factors like high solar irradiance and heat cause apple fruit SBB by elevating peel oxidative stress and/or reactive oxygen species (ROS) levels (Gill & Tuteja, 2010). In particular, the ROS specie H_2O_2 is also elevated (Majer et al., 2014; Zhang et al., 2014; 2015), and by means of two pathways: (i) chloroplast pathway (Asada, 1999; Schmitt et al., 2014) and (ii), plasma membrane based NADPH oxidase pathway (Uzilday et al., 2014; Zhang et al., 2014). Hydrogen peroxide (H_2O_2) as a ROS is key in oxidative-stress-induced damages, first, by its ability to form secondary and highly harmful species like the hydroxyl radical (OH^\bullet) (Scandalios, 1993; Blokhina et al., 2003; Gill & Tuteja, 2010; Schmitt et al., 2014). And, secondly because of its unavoidable production during cellular metabolic activities (Karuppanapandian et al., 2011; Czegeny et al., 2014; Yokawa & Baluska, 2015; You & Chan, 2015). As thus, antioxidant mechanisms in plants as well as in apple fruit tissues mainly target detoxification of H_2O_2 (Bi et al., 2014; Majer et al., 2014), confirming its key importance in plant oxidative stress dynamics (Czegeny et al., 2014), and as well as for this study.

The mineral elements B and Ca are associated with maintenance of cell membranes (Blevins & Lukaszewski, 1998; Kobayashi et al., 1999; Bolanos et al., 2004; Camacho-Cristobal et al., 2008). Cell membranes are main targets of oxidative-stress-driven lipid peroxidation (Scandalios, 1993; Blokhina, 2000; Blokhina et al., 2003), suggesting that foliar B+Ca treatments may reduce dynamics of oxidative stress in apple peel tissue. In addition, either foliar B or Ca applications in plants and/or fruit trees reportedly promotes increased tissue antioxidant capacity (Jiang & Huang, 2001; Policarpo et al., 2002; Agarwal et al., 2005; Garc et al., 2007; Thurzo et al., 2010; Singh et al., 2012). This study aimed to establish whether the mode-of-action of the foliar B+Ca treatments in reducing apple

fruit SBB incidence possibly operates by protecting membrane integrity through decreasing the peel tissue oxidative stress (H_2O_2), at four selected periods (days after full bloom (DAFB)) as maturity stages towards harvest maturity of the respective cultivars.

2. Materials and methods

a. Experiment locations, treatments and sampling

This study was carried out according to the procedures covered in full in Paper 1.

b. Determination of oxidative stress levels

Total peroxides (TPERO) levels, which in principle is analogous to hydrogen peroxide (H_2O_2) levels (Brennan & Frenkel, 1977; Du & Bramlage, 1995; Rao et al., 1998), was the proxy for the evaluation of dynamics of peel tissue oxidative stress levels in three apple cultivars towards fruit harvest maturity. Apple fruit sampling at four selected maturity stages (DAFB) occurred between 06:00 and 07:00, and samples were taken to the laboratory within two hours in a well-insulated cool box. Thereafter, the whole fruit had the peel extracted off carefully using small hand-held kitchen knives. ‘Golden Delicious’ and ‘Cripps Pink’ apple fruit samples were peeled immediately on arrival at the laboratory, however due to manpower constraints ‘Granny Smith’ apple samples were kept at -0.5 °C and processed the next day. For each foliar B+Ca treatment replicate, the peeled samples consisted of four to six fruit. The peel samples were milled with a standard laboratory pestle and mortar under liquid nitrogen, where after it was stored at -80 °C until extraction and determination of TPERO levels using analytical grade chemicals that were sourced from Sigma Aldrich, South Africa.

Apple fruit peel tissue TPERO was extracted and levels determined with slightly modified protocols of Brennan & Frenkel (1977) and Du & Bramlage (1995). Briefly, TPERO were extracted from 1 g of fresh frozen apple peel sample with 5 ml of cold acetone in a 50 ml centrifuge tube, with the contents homogenized using the Ultra Turrax, IKA T18 basic (14000 min^{-1}) for 30 seconds. Fresh acetone was used to rinse the Ultra Turrax after each sample. Thereafter, the homogenized mixture was centrifuged using Eppendorf™ 5810R cooling centrifuge for 15 minutes at 4 °C and $3220 g$. In a next step, 2 ml of supernatant were pipetted into an Eppendorf tube and were further centrifuged at 4 °C and $20000 g$ in an Eppendorf™ 5417R cooling centrifuge for 15 minutes. Then, to 1 ml from

the supernatant above, was added to 12 ml of double distilled water in a centrifuge tube, followed by vortexing. Thereafter, 1 ml of Titanium reagent, consisting of 20 % Titanic Tetrachloride in concentrated Hydrochloric acid (37 %) volume per volume was added, and the tube contents held for 5 minutes at room temperature, after vortexing. Thereafter, 1 ml was pipetted into a plastic cuvette and absorbance was measured using spectrophotometer (Cary 50 Bio, Varian, Australia (Pty) Ltd, Melbourne, Australia) at 415 nm against a blank that contained distilled water in place of the extract. TPERO levels were determined with standard curve made using freshly prepared commercial H₂O₂, which ranged from 0.2 to 1.8 µM and diluted from a stock of 2 µM and kept at 4 °C. TPERO levels were expressed as µmoles (µmol) of H₂O₂ per gram of fresh weight (FW) apple peel.

c. Data analyses

The variability of apple peel total peroxides (TPERO) was analysed using Statistica software. Differences were significant for $p < 0.05$ and the means across B+Ca treatments and fruit maturity (DAFB) levels were separated with the Fisher's LSD posthoc test. The respective means plus standard errors of TPERO among the foliar B+Ca treatments with progression of DAFB were illustrated in trends with using XLSTAT software (Appendix 9) as described before. The statistical analyses results and behaviour of the total peroxides in their respective trend curves were related to the SBB incidence recorded at harvest maturity (Paper 1, Table 16) to identify foliar B+Ca treatments that influenced peel oxidative stress whilst associating with significant SBB incidence reduction.

3. Results

a. 'Cripps Pink'

'Cripps Pink' apple peel TPERO values did not show significant interaction effects for treatments and DAFB periods (Table 1), but had significant effects for both treatments and DAFB periods. Treatment B^{0.17}+Ca^{1.24} had the lowest TPERO that was statistically different to all treatments, with exception of treatment B^{0.08}+Ca^{1.24}. The TPERO of control, B^{0.02}+Ca^{1.24}, B^{0.02}+znCa^{0.06} and B^{0.08}+Ca^{1.24} did not differ significantly. Among fruit maturity stages (DAFB), the highest and lowest TPERO levels were respectively associated with 150 and 164 DAFB, and were significantly different from each other. The TPERO levels recorded at both 122 and 136 was not

significantly different, and the same applied to levels recorded at both 136 and 150 DAFB. Generally, treatment B`0.17+Ca`1.24 trended much lower than other treatments until 150 DAFB, where after its TPERO levels, unlike other treatments, further increased until 164 DAFB (Figure 1). The TPERO in treatments B`0.17+Ca`1.24 and B`0.02+znCa`0.06 seemed to decrease at a faster rate from 150 to 164 DAFB compared to other treatments.

b. ‘Golden Delicious’

‘Golden Delicious’ apple peel total peroxides levels (TPERO) showed a significant interaction effect between treatments and fruit maturity stages (DAFB) (Table 2). Generally, apple peel TPERO levels decreased with fruit maturity (Figure 2). At 80 DAFB, no significant differences occurred between treatments. At 94 DAFB, the control had the highest TPERO that did not differ significantly from the B`0.08+Ca`1.24 and B`0.17+Ca`1.24 treatments. Treatments B`0.02+Ca`1.24 and B`0.02+znCa`0.06 had lowest and non-significantly differing TPERO values, but both were significantly different from the higher values recorded with the B`0.08+Ca`1.24 and B`0.17+Ca`1.24 treatments. At 108 DAFB, no significant differences were recorded between treatments. Treatment B`0.08+Ca`1.24, with the highest TPERO value, was only significantly different from B`0.02+znCa`0.06, with lowest value. At 122 DAFB, TPERO for treatment B`0.08+Ca`1.24 differed significantly from B`0.08+Ca`1.24. Treatments B`0.02+Ca`1.24, B`0.08+Ca`1.24 and B`0.17+Ca`1.24 showed TPERO levels that were not significantly different. The treatment B`0.02+znCa`0.06 had the highest TPERO value, and this was significantly different from both values of B`0.02+Ca`1.24 and B`0.08+Ca`1.24 treatments, but not with that of treatment B`0.17+Ca`1.24.

All treatments showed a declining trend towards harvest, except B`0.02+znCa`0.06, that showed an increasing trend towards harvest (Figure 2). In addition Table 2 shows that values recorded at 80 DAFB were significantly higher than those at 122 DAFB, with exception of the control and treatment B`0.02+znCa`0.06. TPERO across all fruit maturity stages was only non-significant for treatment B`0.02+znCa`0.06. Treatments B`0.08+Ca`1.24 and B`0.17+Ca`1.24 showed non-significant differences for TPERO from 80 to 108 DAFB, whereas in this period, the control showed the overall highest TPERO at 94 DAFB, which was significantly different from the value at 108 DAFB, but not at 80 DAFB, that was also not different from the 108 DAFB value. Treatment B`0.02+Ca`1.24 showed a significant decreasing TPERO from 80 to 94 DAFB, but levels at 94 and 108 DAFB periods

were not significantly different. The lowest TPERO with this treatment recorded at 122 DAFB was also not significantly different from that at 94 DAFB. Thus, in all treatments and the control, TPERO at 80 and 108 DAFB periods did not differ significantly. At 108 and 122 DAFB, only treatments B`0.08+Ca`1.24 and B`0.02+Ca`1.24 showed significant TPERO differences.

c. ‘Granny Smith’

‘Granny Smith’ apple peel TPERO values did not show significant interaction effects for treatments and fruit maturity stages (Table 3), but had significant effects for both treatments and DAFB periods. The highest TPERO level was recorded with the control, and was significantly different from all except B`0.08+Ca`1.24. The lowest TPERO level was recorded with treatment B`0.17+Ca`1.24, but this did not differ from any treatment, except the control. TPERO levels at 94 DAFB were the lowest and significantly different from all other maturity stages. Differences in TPERO levels at 108, 122 and 136 DAFB were not significant. ‘Granny Smith’ TPERO at 108, 122 and 136 DAFB periods was not significantly different. Trends showed that generally the control had the highest TPERO levels at all times (Figure 3), whereas treatment B`0.02+znCa`0.06 showed the lowest levels. TPERO levels in treatment B`0.17+Ca`1.24 increased with fruit maturity, whereas B`0.08+Ca`1.24 appeared to decrease with fruit maturity (Figure 3).

d. Statistical characterisation of ‘Cripps Pink’ and Golden Delicious’ apple peel total peroxides content trends

In ‘Cripps Pink’, the slope (*b*) for the total peroxides content had a negative direction in all the foliar treatments including the control, except treatment B`0.17+Ca`1.24 (Table 4). In addition, only treatments B`0.08+Ca`1.24 and B`0.17+Ca`1.24 had significant slopes values from zero. In ‘Golden Delicious’, the slope was negative and was only not significant with treatment B`0.02+znCa`0.06. However, in none of the treatments of these two cultivars, the peroxides slope differences with respect to direction and magnitude of the gradient of the peel phenolic biochemicals could distinguish between the various seasonal trends, as the slope (*b*) contrasts were all not significant.

4. Discussion

a. Importance of apple fruit peel total peroxides in apple fruit sunburn browning

Apple peel total peroxides (TPERO) as quantified following Du & Bramlage (1995) and Brennan & Frenkel (1977) constitutes of 90-95 % H_2O_2 , the remaining 5-10 % are hydroperoxides and/or peroxy radicals (Rao et al., 1998; Blokhina et al., 2003). This total peroxides content is a potential and often used indicator of oxidative stress in plant tissues (Du & Bramlage, 1995; Rao et al., 1998). Hydroperoxides are very harmful intermediate products of an oxidative stress induced process of lipid peroxidation and/or membrane oxidation (Rao et al., 1998; Blokhina et al., 2003). This literally infers that the protocol used in this study determined H_2O_2 levels in the apple fruit peel tissues, which allows for the interchangeable use of ' H_2O_2 levels' and 'TPERO levels' in this study as analogous terms.

Following the 'duality' principle of ROS as well as of the H_2O_2 in plant response to abiotic stresses (Dat et al., 2000), it is construed that during apple fruit SBB development, H_2O_2 levels in peel tissue may become important in two ways. Firstly, like other ROS and related species, H_2O_2 may act and/or participate in signal transduction processes to induce stress-resistance mechanisms. For instance, elevated H_2O_2 levels in apple fruit peel were associated with an induction of anthocyanin synthesis and defence mechanisms (Torres et al., 2003; Bi et al., 2014; Zhang et al., 2014; 2015); however, this is a scenario of delicate balance between signalling and cellular destruction (Suzuki & Mittler, 2006). Secondly, the heat and/or temperature stress that heralds apple fruit sunburn development can also increase H_2O_2 levels in the peel tissue (Asada, 1999; Ma et al., 2008; Schmitt et al., 2014; Zhang et al., 2015). This can potentially exacerbate oxidative stress levels in the apple peel, likely through photo-conversion of H_2O_2 generating highly lipid peroxidizing OH^\bullet radicals (Blokhina, 2000; Blokhina et al., 2003; Czegeny et al., 2014).

b. Expected versus observed trends of peel total peroxides towards apple fruit harvest

Elevated oxidative stress in the peel as induced by effects of high light and heat stresses is established as the major physiological event in apple fruit SBB development (Wünsche et al., 2004; Chen et al., 2008; Zhang et al., 2015). Therefore, at the inception of this study it was envisaged that occurrence of significant apple fruit SBB incidence differences amongst treatments would associate with clearly segregating respective peel oxidative stress levels, particularly towards fruit harvest, a period commonly associated with a high SBB susceptibility (Racsó & Schrader, 2012; Fan et al., 2011). As thus, this would ably provide a basis to construe a possible mode-of-action by which respective foliar B+Ca treatments promote fruit SBB incidence reduction in apple orchards. Although significant

SBB incidence reduction among the foliar B+Ca treatments occurred in ‘Cripps Pink’ and ‘Golden Delicious’, peel oxidative stress variability did not match to relative abilities of different treatments in reducing sunburn incidence. This does not fully invalidate the methodology of oxidative stress determination used in this study, as results obtained generally showed higher oxidative stress trends with the control (no B+Ca), most especially towards fruit harvest (Figures 1, 2 and 3) and hence, logically relating to higher SBB incidence of the control. In addition, individual cultivar differences for the apple peel TPERO levels occurred.

Furthermore, at inception of the study, it was envisaged that the apple peel oxidative stress trends would show rising trends with advanced DAFB, as susceptibility to heat and associated oxidative stresses and/or even SBB increases with maturity (Dussi et al., 2005; Fan et al., 2011). However, results from this study showed otherwise, whereby TPERO levels decreased with fruit maturity entirely in ‘Golden Delicious’ and some cases were also observed in both ‘Cripps Pink’ and ‘Granny Smith’ (Figures 1.2, 2.2 & 3.2). This could be a result of declining photosynthesis capacity with fruit maturity, as it was found that the TPERO were generally lower in ‘Cripps Pink’ (red-blushing with maturity), moderate in ‘Golden Delicious’ (carotene-yellowing with maturity) and highest in ‘Granny Smith’ a more green apple at maturity. In Paper 2 of this dissertation, quantities of total chlorophyll recorded followed the same trend in these cultivars, strongly suggesting that the oxidative stress parameter evaluated in the current study largely constituted of apple fruit peel H₂O₂ levels generated via the chloroplast pathway.

c. Does the foliar B plus Ca induced changes in apple peel total peroxides levels explain sunburn browning incidence reduction outcomes in ‘Cripps Pink’ and ‘Golden Delicious’ apples?

In ‘Cripps Pink’ apple, the treatments were associated with comparable capacity to reduce class 1 SBB incidence, because differences were significantly different from the control but not amongst treatments. Oxidative stress was lowest in the treatment with the highest B, but this does not explain the class 1 SBB incidence reduction. In addition, low oxidative stress could not explain the SBB reduction in ‘Cripps Pink’, as TPERO in treatment B`0.02+Ca`1.24 also did not differ significantly from the control (Table 1) and showed matching trends (Figures 1 and 2). Yet, SBB incidence in the control and B`0.02+Ca`1.24 significantly differed. Overall, observation (s) above strongly indicate that the class 1 SBB incidence reduction phenomenon in ‘Cripps Pink’ is not explainable by trends

of TPERO (H_2O_2) levels as a measure of the apple peel oxidative stress towards fruit harvest. Further still, the trend contrast of the foliar treatments were not different from the control. In ‘Golden Delicious’, apple peel tissue TPERO levels trends as a measure of oxidative stress also do not explain observed SBB incidence reduction phenomenon, because trends do not agree based on almost similar concessions raised in ‘Cripps Pink’. The trend contrast of the foliar treatments did not differ from the control or among treatments with variable SBB suppression outcomes. These observations of TPERO trends and sunburn browning showed that TPERO dynamics towards fruit harvest, as a measure of oxidative stress, could not be related directly with sunburn browning incidence reduction amongst the treatments in ‘Cripps Pink’ or ‘Golden Delicious’. Yet it is an indisputable fact that oxidative stress in apple peels associates with SBB development. This raises the question on the suitability of TPERO or lipid peroxidation to access oxidative stress associated with SBB in apple.

d. Suitability of lipid peroxidation product assays to predict sunburn browning (SBB)

The protocol applied in this study to assess membrane stability has mostly been reported in fruit in storage, harvested at a specified maturity stage (Brennan & Frenkel, 1977; Du & Bramlage, 1995; Rao et al., 1998). However, in this study, apple peel TPERO or H_2O_2 levels assays were conducted on young fruit as it progressed to maturity. A particular challenge was that apple fruit contains a significant amount of phenolic compounds and ascorbic acid, which vary with cultivar and fruit maturity. For instance, blushed cultivars have a relative high phenolic content due to anthocyanin accumulation in the apple peel with progression in fruit maturity (Wolfe et al., 2003; Tsao et al., 2005; Moyle, 2011; Kalinowska et al., 2014). In the determination of TPERO or H_2O_2 levels following the Du & Bramlage (1995) and Brennan & Frenkel (1977) protocols, the high phenolic and ascorbic content of the apple peel tissues resulted in a quenching effect on the spectrophotometer readings. Therefore, modifications of the Du & Bramlage (1995) and Brennan & Frenkel (1977) protocols were required, and this was achieved by extended sample extract dilutions indicated in the materials and methods section. The manipulation of sample extract dilution was previously reported in a comparable study as a very good method to reduce the quenching effect of the high apple peel ascorbic and phenolic content (Perez & Rubio, 2006).

This study also primarily focused on class 1 and 2 SBB types, because of their economic importance. However, a previous study did not find significant differences in cell membrane integrity between

apple fruit with and without SBB disorders (Schrader et al., 2001). Schrader et al. (2001) furthermore showed that the lipid peroxidation process and its products are limited in class 1 and 2 SBB types. However, if the oxidative stress associated with apple fruit sunburn successfully induces lipid peroxidation at appreciable levels in class 1 and 2 SBB types, Schrader et al. (2001) would have detected significant differences in cell membrane conductivity between apple fruit with and without SBB disorders (Bhattacharjee, 1998; Verma & Dubey, 2003; Rucinska & Gwozdz, 2005). Furthermore, the lipid peroxidation intermediate products (hydroperoxides and/or peroxy radicals) that are very harmful (Rao et al., 1998) and would have exacerbated oxidative stress in the apple peel tissue (Asada, 1999; Xu et al., 2006; Schmitt et al., 2014). Possibly, this would have resulted in a clear segregation of the foliar B+Ca treatments associated TPERO or H₂O₂ levels with respect to the SBB incidence reduction at harvest in all three cultivars. These observations suggest that the assay used in this study to determine lipid peroxidation products (TPERO or H₂O₂) was not able to produce a clear distinction in dynamics of oxidative stress in order to identify fruit expected to develop the less severe class 1 and 2 SBB and which fruit would be unlikely to develop it.

Du and Bramlage (1995) quantified oxidative stress in 'Golden Delicious' apple peel based on two lipid peroxidation products: the thiobarbituric acid assay reactive substances (TBARS) and TPERO levels estimated in terms of malondialdehyde (MDA) and H₂O₂ levels respectively. These parameters were used to study oxidative stress levels in plant, fruit as well as apple peel tissues (Mishra & Singhal, 1992; Du & Bramlage, 1995; Rao et al., 1998; Vilaplana et al., 2006; Duan et al., 2011; Song et al., 2016). However, in this study, the MDA assays performed following both Du and Bramlage (1995) and Du and Bramlage (1992) protocols did not yield trustworthy results as per the spectrophotometer readings obtained. This could have been due to the highly fluctuating sugars and phenolic metabolites that occurs in the apple peel tissue with progression of fruit maturity and/or due to the exposure to SBB causative elements and/or agents (Bakhshi & Arakawa, 2006; Jakopic et al., 2009; Yuri et al., 2010; Moyle, 2011). These varying and abundant metabolites of the apple peel (Wolfe et al., 2003; Tsao et al., 2005; Kalinowska et al., 2014) are known to compromise the MDA assay output (Du & Bramlage, 1992; Hodges et al., 2015). Apple fruit peel tissue oxidative stress analysis via the MDA assay seems best suited for studies concerned only with a population of fruit at a single maturity stage (Du & Bramlage, 1995; Rao et al., 1998).

5. Conclusion

Post-full-bloom foliar B+Ca treatments induced changes in dynamics of apple peel tissue oxidative stress as evidenced by statistically significant effects in ‘Cripps Pink’ and ‘Granny Smith’ apples, as well as significant interaction effects between treatments and fruit maturity in ‘Golden Delicious’. However, apple peel tissue total peroxides levels dynamics, as a measure of oxidative stress, did not account for SBB incidence reduction observed amongst treatments in ‘Cripps Pink’ and ‘Golden Delicious’. This probably resulted from the inadequacy of lipid peroxidation assays to yield oxidative stress information of young, developing fruitlets that could be related to the incidence of class 1 and 2 SBB types, as per objectives of this study. However, this does not negate the role of oxidative stress in the development of apple fruit class 1 and 2 SBB types established in literature. Rather, it highlights the need to find and preferably use non-destructive methods in investigations of oxidative stress dynamics when developing or refining specific post-full-bloom foliar B+Ca treatments intended to mitigate high fruit SBB incidence in apple orchards.

Acknowledgments

Support for this research was made possible through a capacity building competitive grant (RU/2015/DRRG/01/004) provided by Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) and Nulandis (Pty), South Africa. This study is part of a Ph.D. training program supported by RUFORUM members, Stellenbosch and Makerere Universities.

References

- Agarwal, S., Sairam, R.K., Srivastava, G.C., Tyagi, A. & Meena, R.C. 2005. Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. *Plant Science* 169:559-570.
- Aro, E.M., Virgin, I. & Andersson, B. 1993. Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. *Biochimica et Biophysica Acta* 1143:113-134.
- Asada, K. 1999. The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology* 50:601-639.
- Bakhshi, D. & Arakawa, O. 2006. Induction of phenolic compounds biosynthesis with light irradiation in the flesh of red and yellow apples. *Journal of Applied Horticulture* 8:101-104.
- Barber, J. & Andersson, B. 1992. Too much of a good thing: light can be bad for photosynthesis. *Trends in Biochemical Sciences* 17:61-66.
- Bhattacharjee, S. 1998. Membrane lipid peroxidation, free radical scavengers and ethylene evolution in *Amaranthus* as affected by lead and cadmium. *Biologia Plantarum* 40:131-135.
- Bi, X., Zhang, J., Chen, C., Zhang, D., Li, P. & Ma, F. 2014. Anthocyanin contributes more to hydrogen peroxide scavenging than other phenolics in apple peel. *Food Chemistry* 152:205-209.
- Blevins, D.G. & Lukaszewski, K.M. 1998. Boron in plant structure and function. *Annual Review of Plant Physiology and Plant Molecular Biology* 49:481-500.
- Blokhina, O. 2000. Anoxia and oxidative stress: Lipid peroxidation, antioxidant status and mitochondrial functions in plants. PhD Dissertation. Department of Biosciences, Division of Plant Physiology, University of Helsinki.
- Blokhina, O., Virolainen, E. & Fagerstedt, K. V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals of Botany* 91:179-194.
- Bolanos, L., Lukaszewski, K., Bonilla, I. & Blevins, D. 2004. Why boron? *Plant Physiology and Biochemistry* 42:907-912.

- Brennan, T. & Frenkel, C. 1977. Involvement of hydrogen peroxide in the regulation of senescence in pear. *Plant Physiology* 59:411-416.
- Camacho-Cristobal, J.J., Herrera-Rodriguez, M.B., Beato, V.M., Rexach, J., Navarro-Gochicoa, M.T., Maldonado, J.M. & Gonzalez-Fontes, A. 2008. The expression of several cell wall-related genes in *Arabidopsis* roots is down-regulated under boron deficiency. *Environmental and Experimental Botany* 63:351-358.
- 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.
- Czegeny, G., Wu, M., Der, A., Eriksson, L.A., Strid, A. & Hideg, E. 2014. Hydrogen peroxide contributes to the ultraviolet-B (280-315 nm) induced oxidative stress of plant leaves through multiple pathways. *FEBS Letters* 588:2255-2261.
- Daiber, S.H. 2017. Quantifying changes in tree physiology after amelioration to reduce sunburn on apples. MSc Thesis. Department of Horticultural Science, Stellenbosch University.
- Dat, J., Vandenabeele, S., Vranova, E., Van Montagu, M., Inze, D. & Van Breusegem, F. 2000. Dual action of the active oxygen species during plant stress responses. *Cellular and Molecular Life Sciences* 57:779-795.
- Du, Z. & Bramlage, W.J. 1992. Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. *Journal of Agricultural and Food Chemistry* 40:1566-1570.
- Du, Z. & Bramlage, W.J. 1995. Peroxidative activity of apple peel in relation to development of poststorage disorders. *HortScience* 30:205-209.
- Duan, X., Liu, T., Zhang, D., Su, X., Lin, H. & Jiang, Y. 2011. Effect of pure oxygen atmosphere on antioxidant enzyme and antioxidant activity of harvested litchi fruit during storage. *Food Research International* 44:1905-1911.
- Dussi, M.C., Giardina, G. & Reeb, P. 2005. Shade nets effect on canopy light distribution and quality of fruit and spur leaf on apple cv. Fuji. *Spanish Journal of Agricultural Research* 3:253-260.

- Fan, L., Song, J., Forney, C.F. & Jordan, M.A. 2011. Fruit maturity affects the response of apples to heat stress. *Postharvest Biology and Technology* 62:35-42.
- Felicetti, D.A. & Schrader, L.E. 2008. Changes in pigment concentrations associated with the degree of sunburn browning of 'Fuji' apple. *Journal of the American Society of Horticultural Science* 133:27-34.
- Felicetti, D.A. & Schrader, L.E. 2009a. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. II. Phenolics. *Plant Science* 176:84-89.
- Felicetti, D.A. & Schrader, L.E. 2009b. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. I. Chlorophylls and Carotenoids. *Plant Science* 176:78-83.
- Garc, M.F., Hern, J.A., Lopez-Gomez, E., San Juan, M.A., Diaz-Vivancos, P., Mataix Beneyto, J., Garcia-Legaz, M.F. & Hernandez, J.A. 2007. Effect of rootstocks grafting and boron on the antioxidant systems and salinity tolerance of loquat plants (*Eriobotrya japonica* Lindl.). *Environmental and Experimental Botany* 60:151-158.
- Gill, S.S. & Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* 48:909-930.
- Hamadziripi, E.T. 2012. The effect of canopy position on the fruit quality and consumer preference of apples. MSc Thesis. Department of Horticultural Science, Stellenbosch University.
- Hamim, H., Violita, V., Triadiati, T. & Miftahudin, M. 2017. Oxidative stress and photosynthesis reduction of cultivated (*Glycine max* L.) and wild soybean (*G. tomentella* L.) exposed to drought and Paraquat. *Asian Journal of Plant Sciences* 16:65-77.
- Hideg, E., Jansen, M.A.K. & Strid, A. 2013. UV-B exposure, ROS, and stress: Inseparable companions or loosely linked associates? *Trends in Plant Science* 18:107-115.
- Hodges, D.M., DeLong, J.M., Forney, C.F. & Prange, R.K. 2015. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* 207:604-611.
- Jakopic, J., Stampar, F. & Veberic, R. 2009. The influence of exposure to light on the phenolic content of 'Fuji' apple. *Scientia Horticulturae* 123:234-239.

- Jiang, Y. & Huang, B. 2001. Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. *Journal of Experimental Botany* 52:341-349.
- Kalinowska, M., Bielawska, A., Lewandowska-Siwkiewicz, H., Priebe, W. & Lewandowski, W. 2014. Apples: Content of phenolic compounds vs. variety, part of apple and cultivation model, extraction of phenolic compounds, biological properties. *Plant Physiology and Biochemistry* 84:169-188.
- Karuppanapandian, T., Moon, J.C., Kim, C., Manoharan, K. & Kim, W. 2011. Reactive oxygen species in plants: Their generation, signal transduction, and scavenging mechanisms. *Australian Journal of Crop Science* 5:709-725.
- Kobayashi, M., Nakagawa, H., Asaka, T. & Matoh, T. 1999. Borate-rhamnogalacturonan II bonding reinforced by Ca^{2+} retains pectic polysaccharides in higher-plant cell walls. *Plant Physiology* 119:199-204.
- Lolicato, S. 2011. Sun Protection for Fruit - A practical manual for protecting sunburn on fruit. Department of Primary Industries, Farm Services Victoria Division, Victoria, Australia.
- Lötze, E. & Hoffman, E.W. 2014. Foliar application of calcium plus boron reduces the incidence of sunburn in 'Golden Delicious' apple. *Journal of Horticultural Science and Biotechnology* 89:607-612.
- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2017. Boron in combination with calcium reduces sunburn in apple fruit. *Boron* 2:123-127.
- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2018. Evaluating the efficacy of a preharvest combination of calcium and boron as foliar application to reduce sunburn on 'Cripps Pink' apples. *Acta Horticulture* 1217:61-68.
- Ma, Y.H., Ma, F.W., Zhang, J.K., Li, M.J., Wang, Y.H. & Liang, D. 2008. Effects of high temperature on activities and gene expression of enzymes involved in ascorbate-glutathione cycle in apple leaves. *Plant Science* 175:761-766.

- Majer, P., Czegeny, G., Sandor, G., Dix, P.J. & Hideg, E. 2014. Antioxidant defence in UV-irradiated tobacco leaves is centred on hydrogen-peroxide neutralization. *Plant Physiology and Biochemistry* 82:239-243.
- Makeredza, B., Marais, H., Schmeisser, M., Lötze, E. & Steyn, W.J. 2015. Ripening associated red color development masks sunburn browning in apple peel. *HortScience* 50:814-818.
- Mishra, R.K. & Singhal, G.S. 1992. Function of photosynthetic apparatus of intact wheat leaves under high light and heat stress and its relationship with peroxidation of thylakoid lipids. *Plant Physiology* 98:1-6.
- Moyle, C.W.A. 2011. Polyphenols in apples and their interactions with vascular endothelial cells. PhD Dissertation, University of East Anglia.
- Murata, N., Takahashi, S., Nishiyama, Y. & Allakhverdiev, S.I. 2007. Photoinhibition of photosystem II under environmental stress. *Biochimica et Biophysica Acta* 1767:414-421.
- Nawkar, G.M., Maibam, P., Park, J.H., Sahi, V.P., Lee, S.Y. & Kang, C.H. 2013. UV-induced cell death in plants. *International Journal of Molecular Sciences* 14:1608-1628.
- Perez, F.J. & Rubio, S. 2006. An improved chemiluminescence method for hydrogen peroxide determination in plant tissues. *Plant Growth Regulation* 48:89-95.
- Policarpo, M., Di Marco, L., Farina, V. & Tagliavini, S. 2002. Effect of foliar nutrition on Peach (*Prunus persica* L. Batsch) yield and fruit quality as related to different crop loads. *Acta Horticulturae* 594:659-666.
- Racsó, J. & Schrader, L.E. 2012. Sunburn of apple fruit: Historical background, recent advances and future perspectives. *Critical Reviews in Plant Sciences* 31:455-504.
- Rao, M. V, Watkins, C.B., Brown, S.K. & Weeden, N.F. 1998. Active oxygen species metabolism in 'White Angel' x 'Rome Beauty' apple selections resistant and susceptible to superficial scald. *Journal of the American Society for Horticultural Science* 123:299-304.
- Rucinska, R. & Gwozdz, E.A. 2005. Influence of lead on membrane permeability and lipoxygenase activity in lupine roots. *Biologia Plantarum* 49:617-619.
- Scandalios, J.G. 1993. Oxygen stress and superoxide dismutases. *Plant Physiology* 101:7-12.

- Schmitt, F.J., Renger, G., Friedrich, T., Kreslavski, V.D., Zharmukhamedov, S.K., Los, D.A., Kuznetsov, V. V & Allakhverdiev, S.I. 2014. Reactive oxygen species: Re-evaluation of generation, monitoring and role in stress-signaling in phototrophic organisms. *Biochimica et Biophysica Acta* 1837:835-848.
- Schrader, L.E. 2011. Scientific basis of a unique formulation for reducing sunburn of fruits. *HortScience* 46:6-11.
- Schrader, L., Zhang, J. & Sun, J. 2003. Environmental stresses that cause sunburn of apple. *Acta Horticulturae* 618:397-405.
- Schrader, L., Sun, J., Zhang, J., Felicetti, D. & Tian, J. 2008. Heat and light-induced apple skin disorders: Causes and prevention. *Acta Horticulturae* 772:51-58.
- Schrader, L. E., Zhang, J., and Duplaga, W. K. 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Online. *Plant Health Progress* doi:10.1094/PHP-2001-1004-01-RS.
- Singh, D.P., Beloy, J., Mcinerney, J.K. & Day, L. 2012. Impact of boron, calcium and genetic factors on vitamin C, carotenoids, phenolic acids, anthocyanins and antioxidant capacity of carrots (*Daucus carota*). *Food Chemistry* 132:1161-1170.
- Smillie, R.M. 1992. Calvin cycle activity in fruit and the effect of heat stress. *Scientia Horticulturae* 51:83-95.
- Song, X., Wang, Y. & Lv, X. 2016. Responses of plant biomass, photosynthesis and lipid peroxidation to warming and precipitation change in two dominant species (*Stipa grandis* and *Leymus chinensis*) from North China Grasslands. *Ecology and Evolution* 6:1871-1882.
- Sotiropoulos, T., Petridis, A., Koukourikou-Petridou, M. & Koundouras, S. 2016. Evaluation of 'Sun Protect' in protecting apples (*Malus × domestica* Borkh.) against sunburn. *Horticultural Science (Prague)* 43:175-180.
- Suzuki, N. & Mittler, R. 2006. Reactive oxygen species and temperature stresses: A delicate balance between signalling and destruction. *Physiologia Plantarum* 126:41-51.

- Thurzo, S., Szabo, Z., Nyeki, J., Nagy, P.T., Silva, A.P. & Goncalves, B. 2010. Effect of boron and calcium sprays on photosynthetic pigments, total phenols and flavonoid content of sweet cherry (*Prunus avium* L.). *Acta Horticulturae* 868:457-462.
- Torres, R., Valentines, M.C., Usall, J., Vinas, I. & Larrigaudiere, C. 2003. Possible involvement of hydrogen peroxide in the development of resistance mechanisms in ‘Golden Delicious’ apple fruit. *Postharvest Biology and Technology* 27:235-242.
- Tsao, R., Yang, R., Xie, S., Sockovie, E. & Khanizadeh, S. 2005. Which polyphenolic compounds contribute to the total antioxidant activities of apple? *Journal of Agricultural and Food Chemistry* 53:4989-4995.
- Uzilday, B., Turkan, I., Ozgur, R. & Sekmen, A.H. 2014. Strategies of ROS regulation and antioxidant defense during transition from C3 to C4 photosynthesis in the genus *Flaveria* under PEG-induced osmotic stress. *Journal of Plant Physiology* 171:65-75.
- Van Breusegem, F., Bailey-Serres, J. & Mittler, R. 2008. Unraveling the tapestry of networks involving reactive oxygen species in plants. *Plant Physiology* 147:978-984.
- Verma, S. & Dubey, R.S. 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Science* 164:645-655.
- Vilaplana, R., Valentines, M.C., Toivonen, P. & Larrigaudiere, C. 2006. Antioxidant potential and peroxidative state of ‘Golden Smoothie’ apples treated with 1-Methylcyclopropene. *Journal of the American Society for Horticultural Science* 131:104-109.
- Wahid, A., Gelani, S., Ashraf, M. & Foolad, M.R. 2007. Heat tolerance in plants: An overview. *Environmental and Experimental Botany* 61:199-223.
- Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A. & Ghassemi-Golezani, K. 2012. Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omics Journal* 5:60-67.
- Wolfe, K., Wu, X. & Liu, R.H. 2003. Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry* 51:609-614.

- Wünsche, J.N., Greer, D.H., Palmer, J.W., Lang, A. & McGhie, T. 2001. Sunburn - The cost of a high light environment. *Acta Horticulturae* 557:349-356.
- Wünsche, J.N., Bowen, J., Ferguson, I., Woolf, A. & Mcghie, T. 2004. Sunburn on apples - Causes and control mechanisms. *Acta Horticulturae* 636:631-636.
- Xu, S., Li, J., Zhang, X., Wei, H. & Cui, L. 2006. Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. *Environmental and Experimental Botany* 56:274-285.
- Yokawa, K. & Baluska, F. 2015. Pectins, ROS homeostasis and UV-B responses in plant roots. *Phytochemistry* 112:80-83.
- You, J. & Chan, Z. 2015. ROS regulation during abiotic stress responses in crop plants. *Frontiers in Plant Science* 6:1092.
- 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. *Journal of Food, Agriculture and Environment* 8:920-925.
- Zhang, J., Chen, C., Zhang, D., Li, H., Li, P. & Ma, F. 2014. Reactive oxygen species produced via plasma membrane NADPH oxidase regulate anthocyanin synthesis in apple peel. *Planta* 240:1023-1035.
- Zhang, J., Niu, J., Duan, Y., Zhang, M., Liu, J., Li, P. & Ma, F. 2015. Photoprotection mechanism in the 'Fuji' apple peel at different levels of photooxidative sunburn. *Physiologia Plantarum* 154:54-65.

Tables

Table 1. 'Cripps Pink' apple peel total peroxides among five foliar B plus Ca treatments and at four selected fruit maturity stages (DAFB).

Treatments (B+Ca)	Total peroxides (nmol.g ⁻¹ peel FW)
B`0.00+Ca`0.00	5.89 ^a
B`0.02+Ca`1.24	6.06 ^a
B`0.02+znCa`0.06	5.87 ^a
B`0.08+Ca`1.24	5.32 ^{ab}
B`0.17+Ca`1.24	4.58 ^b
<i>p</i> -value	0.0033
Days after full bloom (DAFB)	
122	5.60 ^b
136	5.71 ^{ab}
150	6.37 ^a
164	4.49 ^c
<i>p</i> -value	0.0000
B+Ca*DAFB (<i>p</i> -value)	0.1780

Mean values sharing a letter are not different.

Table 2. 'Golden Delicious' apple peel total peroxides as a function of foliar B plus Ca and fruit maturity (DAFB).

Treatments (B+Ca)	80 DAFB (04.01.2016)	94 DAFB (18.01.2016)	108 DAFB (01.02.2016)	122 DAFB (15.02.2016)
B`0.00+Ca`0.00	9.4 ^{abcd}	10.7 ^a	8.3 ^{bcd}	7.7 ^{cde}
B`0.02+Ca`1.24	10.4 ^{ab}	7.6 ^{cde}	8.9 ^{abcd}	5.6 ^{ef}
B`0.02+znCa`0.06	9.4 ^{abcd}	7.6 ^{cde}	7.4 ^{cdef}	8.3 ^{bcd}
B`0.08+Ca`1.24	9.6 ^{abc}	9.4 ^{abcd}	10.1 ^{ab}	5.1 ^f
B`0.17+Ca`1.24	10.5 ^{ab}	9.1 ^{abcd}	9.5 ^{abcd}	7.3 ^{def}

Mean values sharing a letter are not different, B+Ca ($p = 0.2788$), DAFB ($p = 0.0000$) and B+Ca*DAFB ($p = 0.0276$).

Table 3. 'Granny Smith' apple peel total peroxides among five foliar B plus Ca treatments and at four selected fruit maturity stages (DAFB).

Treatments (B+Ca)	Total peroxides (nmol.g ⁻¹ peel FW)
B`0.00+Ca`0.00	16.60 ^a
B`0.02+Ca`1.24	12.44 ^b
B`0.02+znCa`0.06	11.87 ^b
B`0.08+Ca`1.24	14.66 ^{ab}
B`0.17+Ca`1.24	13.21 ^b
<i>p</i> -value	0.0278
Days after full bloom (DAFB)	
94	10.23 ^b
108	14.77 ^a
122	15.77 ^a
136	14.25 ^a
<i>p</i> -value	0.0011
B+Ca*DAFB (<i>p</i> -value)	0.2979

Mean values sharing a letter are not different.

Table 4: Trend contrasts of 'Cripps Pink' (122, 136, 150 and 164 DAFB) and 'Golden Delicious' (80, 94, 108 and 122 DAFB) apple peel total peroxides content among varying foliar B plus Ca treatments using the slope (*b*) statistic (*p*-value in parentheses)

Treatments (B+Ca)	'Cripps Pink'	'Golden Delicious'
Control (T ₀)	-0.0046 (0.8445)	-0.0545 (0.0160)
B`0.02+Ca`1.24 (T ₁)	-0.0148 (0.4834)	-0.0914 (0.0059)
B`0.02+znCa`0.06 (T ₂)	-0.0505 (0.0532)	-0.0247 (0.3221)
B`0.08+Ca`1.24 (T ₃)	-0.0572 (0.0040)	-0.0917 (0.0158)
B`0.17+Ca`1.24 (T ₄)	0.0318 (0.0178)	-0.0666 (0.0471)
<i>b</i> contrasts (<i>p</i> -values)		
T ₀ versus T ₁	0.9407	0.8082
T ₀ versus T ₂	0.9668	0.9072
T ₀ versus T ₃	0.8467	0.7307
T ₀ versus T ₄	0.6807	0.7761
T ₁ versus T ₂	0.9080	0.8958
T ₁ versus T ₃	0.9035	0.9097
T ₁ versus T ₄	0.7259	0.9636
T ₂ versus T ₃	0.8161	0.8105
T ₂ versus T ₄	0.6573	0.8609
T ₃ versus T ₄	0.8085	0.9456

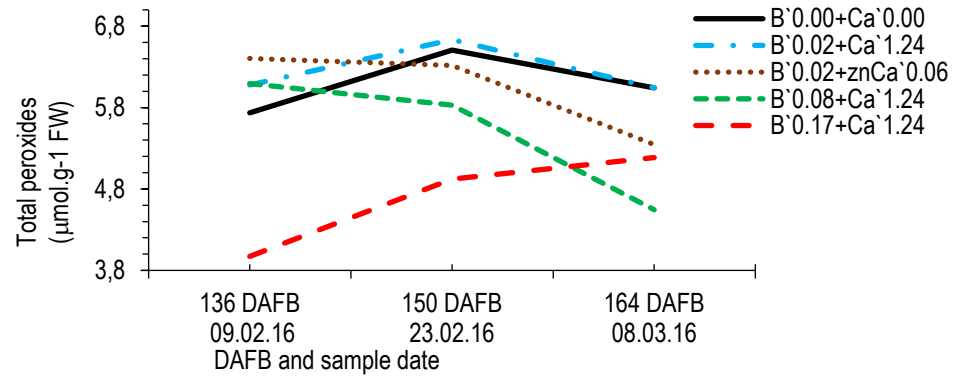


Figure 1. Moving mean of apple peel TPERO towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

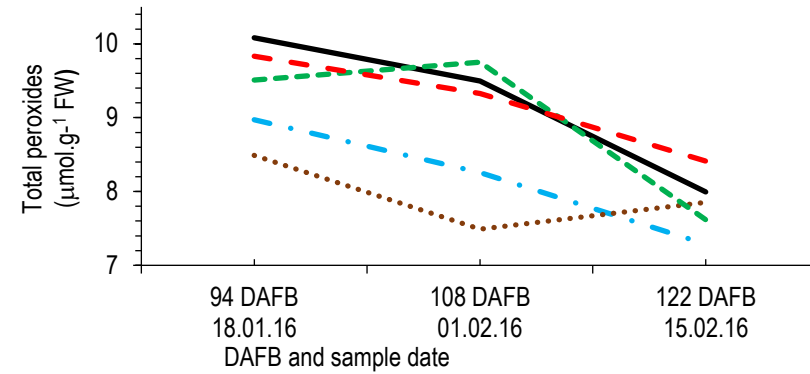


Figure 2. Moving mean of apple peel TPERO towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

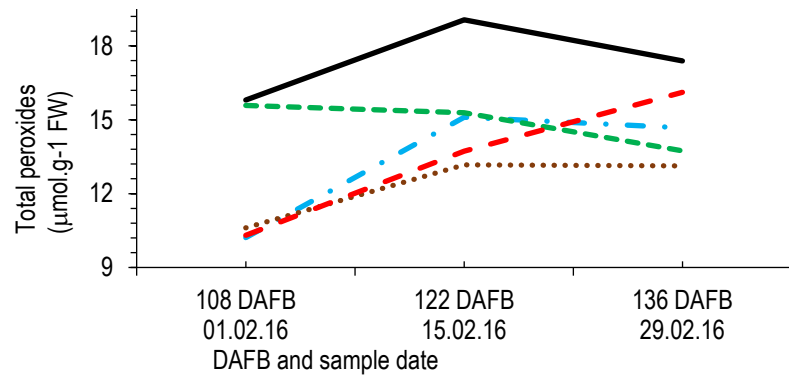


Figure 3. Moving mean of apple peel TPERO towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

RELATING FOLIAR BORON PLUS CALCIUM INDUCED PEEL BIOCHEMICAL CHANGES TO SUPPRESSION OF SUNBURN BROWNING INCIDENCE IN 'GOLDEN DELICIOUS' APPLES

Abstract

Apple peel biochemical variances as induced by different foliar boron plus calcium (B+Ca) treatment formulations did not explain (mode-of-action) the sunburn browning (SBB) suppression phenomenon in 'Golden Delicious' apples. In this study, a multivariate approach, the canonical correlation analysis (CCorA), was undertaken to further study the nature of the relationship (s) between the respective peel biochemical aspects and SBB incidence suppression among foliar B+Ca treatments, to construe the unknown mode-of-action alluded to above. The study utilised apple peel biochemical and SBB incidence data collected in the 2015/16 season experiment on suppression of SBB incidence at Applethwaite (Pty) farm, Elgin, Western Cape, South Africa. The CCorA was conducted as: (i) data sets associated with the full study, (ii) data sets from two foliar B+Ca treatments with the least SBB incidence reduction and (iii), data sets from two foliar B+Ca treatments with the best SBB incidence reduction. Ordinarily, it was expected that CCorA in (i) should result in a significant correlation in agreement with the physiology of apple fruit SBB and peel biochemical and/or metabolite changes. However, CCorA in (ii) and (iii) were intended to facilitate insights into the foliar B+Ca treatments influences in respect to the relationship between apple peel biochemistry and SBB incidence recorded, where it was envisaged that a strong relationship between the data sets would be realised in (ii), and a weak relationship in (iii). The envisaged result in (iii) would infer that foliar B+Ca treatments prime apple peel biochemistry with relevant metabolites resulting in SBB resistance, hence a weak relationship between the data sets. The results affirmed these envisaged relationships, where in (i), CCorA model explained 56 % of the variance shared between data sets, with significant function 1 ($\lambda = 0.441$, $F(36, 421.5) = 2.861$, $p < 0.0001$) and function 2 ($\lambda = 0.706$, $F(24, 328.3) = 1.747$, $p = 0.018$). In (ii), CCorA model fully explained 81 % of the variance shared between data sets, with statistically significant function 1 ($\lambda = 0.189$, $F(36, 140.4) = 2.184$, $p < 0.001$) and function 2 ($\lambda = 0.382$, $F(24, 110.8) = 1.818$, $p = 0.020$). In scenario (iii), the overall CCorA

model was not significant at the 5 % confidence level. The contribution of different domains of metabolites in significant canonical functions of scenario (ii) suggests that several metabolites are additively involved in mediation of SBB incidence, because of appropriately formulated foliar B+Ca treatments. Therefore, findings from this study enable the postulation that an additive-metabolite-action underscores the observed SBB incidence suppression phenomenon as a mode-of-action in ‘Golden Delicious’ apples.

Keywords: Additive-metabolite-action, Canonical correlation analysis, ‘Golden Delicious’

1. Introduction

The need to investigate the mode-of-action by which selective post-full-bloom foliar applications of boron plus calcium (B+Ca) suppress fruit sunburn browning (SBB) incidence in apple fruit, has recently received strong attention (Daiber, 2017; Lötze et al., 2017; Mwije, 2017). This relative new foliar B+Ca treatments approach to reduce apple fruit SBB incidence sustainably holds potential, particularly in South Africa, where the increased frequency of severe summer droughts and temperatures are threatening the profitability of the pome fruit sector (Midgley & Lötze, 2011; Botai et al., 2017). Currently, no negative effects either on fruit or tree physiology is associated with any of these foliar B+Ca treatments (Daiber, 2017). In addition, the foliar B+Ca treatment approach is economical to implement, compared to existing approaches like shade netting, overhead cooling and kaolin applications (Wünsche et al., 2004; Dussi et al., 2005; Gindaba & Wand, 2005; 2007a, b). However, to ably implement this foliar B+Ca treatments approach and exploit its envisaged benefits, particularly on commercial scale, the elucidation of its mode-of-action is very important.

A scientific elucidation of the mode-of-action associated with the foliar B+Ca treatment mediated apple fruit SBB incidence reduction will enhance further refinement of the protocol, as well as promote its commercialisation as evidenced from a comparable study of Schrader (2011). In addition, defining its mode-of-action will enable sound recommendations to manage cultivar specific requirements in response

to the foliar B+Ca treatments, as was suggested by the results of Daiber (2017), as well as identifying prerequisites for adopting the approach in other horticultural crops affected by sunburn damage.

Results from Daiber (2017) showed that similar foliar B+Ca treatments yielded different SBB incidence suppression outcomes, and cultivars varied in their responses to these treatments. Studies preceding this paper also showed that apple peel biochemistry was variably influenced by treatment formulation and cultivar differences. However, no single peel metabolite change, as associated with the varying foliar B+Ca treatments, could explain the reduction of class 1 and 2 SBB in ‘Golden Delicious’ and ‘Cripps Pink’ apples. Trend contrasts were not significantly different from each other, although the magnitude and direction of the slopes differed. Thus, an alternative possibility to yield a mode-of-action utilising a multivariate approach (to relate the peel biochemical aspects to SBB suppression outcomes) among the varying foliar B+Ca treatments was investigated. After all, apple fruit SBB is indeed a manifestation of several (multivariate) biochemical changes, starting with increased peel tissue oxidative stress and its resultant chlorophyll degradation (Chen et al., 2008; Felicetti & Schrader, 2008; 2009a, b), followed by upregulation of peel antioxidant biochemicals to ostensibly counteract the developing damages in peel tissues (Yuri et al., 2010; Racskó & Schrader, 2012).

In the context of this study, canonical correlation analysis CCorA attains superiority over other available multivariate techniques, as it is specifically and suitable for studies involving multiple variables and it critically reduces the risk of type 1 error (Knapp, 1978; Akbas & Takma, 2005; Rinn et al., 2009; Nimon et al., 2010). Essentially, the core purpose of conducting a CCorA is to generate concepts to inform theory (Hair et al., 1998; Akbas & Takma, 2005; Sherry & Henson, 2005; Zientek & Thompson, 2006; Rinn et al., 2009; Nimon et al., 2010; Lee & Lee, 2012; Bringula, 2016; Chung et al., 2017; Fox & Hammond, 2017). This fundamental purpose of CCorA resonates very well with the aim and context of this particular study. As thus, CCorA is considered a most appropriate multivariate tool, given that apple fruit SBB phenomenon is associated with multiple peel metabolite changes, as well as several severity levels (SBB types) (Felicetti & Schrader, 2008; 2009a, b; Yuri et al., 2010). This makes it possible to have two different data sets for the CCorA, with one containing the multiple independent (metabolites) and another, consisting of the multiple dependent (SBB incidence by classes) variables.

In addition, CCorA specifies that a worthy (significant) relationship between the two data sets warrant further interpretation of the variables and/or observations (Hair et al., 1998; Henson, 2002; Onwuegbuzie & Daniel, 2003; Sherry & Henson, 2005). Thus, in relation to the background and aim of this particular study, CCorA can signify whether or not the apple peel metabolites levels during fruit development are related to the SBB incidence reduction recorded at harvest. This paper therefore explored subjecting the data sets of (i) seasonal (at different maturity stages) peel metabolites of ‘Golden Delicious’ apple under different B+Ca treatments, and (ii), the SBB incidence (class 0, 1, 2 and total SBB (TSBB)) as recorded at fruit harvest maturity to CCorA. The overall aim was to study and characterise the nature of the relationship in these data sets in effort to explain a mode-of-action for the post-full-bloom foliar B+Ca mediated fruit SBB incidence suppression in apple orchards.

2. Materials and methods

Details of the experiments as conducted in 2015/16 season are fully described in paper 1. However, biochemical data on an earlier sampling date (66 DAFB) was included to enable five samplings for this study to increase the sample size for CCorA. Therefore, the fruit samples were collected at the following dates and fruit maturity stages (DAFB): (i) 21.12.2015 (66 DAFB), (ii) 04.01.2015 (80 DAFB), (iii) 18.01.2016 (94 DAFB), (iv) 01.02.2016 (108 DAFB) and finally (v), 15.02.2016 (122 DAFB). The biochemical data used in this study included total phenolic (TP), total flavonoid (TF) and the TF:TP ratio, chlorophyll *a* (CHLa), chlorophyll *b* (CHLb), total chlorophyll (TCHL), total carotenoids (TCAR) and ratio values for CHLa:CHLb and TCHL:TCAR determined as described in the preceding papers 1 and 2 of this dissertation.

Only ‘Golden Delicious’ data could be used for the CCorA. Even though these same treatments had SBB incidence in ‘Cripps Pink’, the ‘Cripps Pink’ data could not form adequate sample sizes to subject to an acceptable CCorA. Two variable data sets: (i) nine variables of ‘Golden Delicious’ apple peel biochemical aspects and (ii), four variables of respective SBB incidence (classes), were subjected to the multivariate CCorA technique to evaluate the presumed relationship amongst them. CCorA was done as: (i) all treatment plots in the experiment as observations ($\beta\delta\rho = 125$), where, β (5) are the levels of the foliar B+Ca treatments including the control (no B+Ca), δ (5) are the levels of maturity stages or days

after full bloom (DAFB) and ρ (5) is the number of replications, (ii) CCorA was done for the least two performing foliar B+Ca treatments in SBB incidence reduction and (iii), for the best two performing B+Ca treatments in reducing SBB incidence. In each CCorA case of (ii) and (iii), 50 observations ($\beta\delta\rho = 50$) were considered, where, β (2) are the levels of foliar B+Ca treatments including the control (no B+Ca), δ (5) are the levels of maturity stages or days after full bloom (DAFB) and ρ (5) is the number of replications. In each CCorA scenario, each observation had a data set of nine parameters of apple peel biochemistry and data set of four sunburn browning incidence (at harvest). The CCorA was conducted using XLSTAT Version 2010.4.01.

3. Results

a. Statistical significance of the CCorA model and respective canonical functions

Four canonical functions (Cf) were realised in each CCorA scenario, because of the four-variable SBB data set as the number of Cf must be equal the variables in the smaller data set (Gunderson & Muirhead, 1997; Hair et al., 1998; Sherry & Henson, 2005). The canonical functions were also tested in a hierarchal approach (dimension reduction analysis), starting with the full model (1- n), where n is number of resultant canonical functions from the CCorA (Sherry & Henson, 2005). The significance of the full model (1 to 4) in all the three scenarios of the CCorA conducted in this study was evaluated using the Wilks' lambda (λ), which is most recommended approach and with general applicability (Hair et al., 1998; Sherry & Henson, 2005; Rinn et al., 2009; Bringula, 2016).

i. CCorA with all foliar B+Ca treatments in the full experiment

The CCorA dimension reduction analysis of the full experiment (all observations =125) is given in Table 1a. The full model (Cf 1) and Cf 2 were significant: Cf 1 ($\lambda = 0.441$, $F(36, 421.5) = 2.861$, $p < 0.0001$) and Cf 2 ($\lambda = 0.706$, $F(24, 328.3) = 1.747$, $p = 0.018$). This infers that the null hypothesis of no relationship (Canonical correlation (R_C) = 0) among the data sets, is rejected (Hair et al., 1998; Sherry & Henson, 2005). The Cf 3 and Cf 4 were not significant. The overall effect size of the model ($1 - \lambda$) was 56%, which infers that the model was able to explain an adequate/sufficient variance among the CCorA

input variables or data sets (Sherry & Henson, 2005; Rinn et al., 2009). In addition to significance, Cf 1 and Cf 2 displayed appreciable R_c values of 61 % and 45 % (Table 1a), suggesting that they only explained (R_c^2) 38 % and 20 % of the variance between the data sets. Thus, only Cf 1 and Cf 2 justified further interpretation (Hair et al., 1998; Sherry & Henson, 2005). The orthogonal nature of the canonical functions in CCorA as elaborated by Sherry & Henson (2005), explains the discrepancy of the effect sizes between parts of the model with a value of 58 % (summation of R_c^2 values associated with Cf 1 and Cf 2). Yet, the overall effect size of the model ($1-\lambda$) was 56 %. This discrepancy and orthogonality are typical of CCorA (Hair et al., 1998; Sherry & Henson, 2005) and was observed with CCorA, in the least SBB incidence reducing foliar B+Ca treatments, where summation of R_c^2 values of Cf 1 and Cf 2 showed 90 %, yet the standard $1-\lambda$ approach yielded 81 %.

ii. CCorA with two foliar B+Ca treatments associated with the least SBB incidence reduction

The control (no B+Ca) and the treatment B`0.02+znCa`0.06 were associated with the least SBB incidence reduction (Daiber, 2017; Lötze et al., 2017). The CCorA dimension reduction analysis of the associated observations (50) is given in Table 1b. The full model (Cf 1) and Cf 2 were significant: Cf 1 ($\lambda = 0.189$, $F(36, 140.4) = 2.184$, $p < 0.001$) and Cf 2 ($\lambda = 0.382$, $F(24, 110.8) = 1.818$, $p = 0.020$). Again in this scenario, the null hypothesis, of no relationship ($R_c = 0$) among the data sets is rejected (Hair et al., 1998; Sherry & Henson, 2005). The Cf 3 and Cf 4 were not significant. The overall effect size of the model ($1 - \lambda$) was 81 %, which infers that the model was able to explain a large portion of variance among the data sets (Sherry & Henson, 2005; Rinn et al., 2009). In addition, Cf 1 and Cf 2 also showed high R_c values of 71 % and 63 % (Table 1b), suggesting that they explained (R_c^2) 51 % and 40 % of the variance between the data sets. Thus, only Cf 1 and Cf 2 warranted further interpretation (Hair et al., 1998; Sherry & Henson, 2005).

iii. CCorA with two foliar B+Ca treatments associated with best SBB incidence reduction

The treatments B`0.08+Ca`1.24 and B`0.17+Ca`1.24 were associated with the best SBB incidence reduction in this experiment (Daiber, 2017; Lötze et al., 2017). In this particular scenario of the CCorA (50 observations), the overall model was not significant (Table 1c). Thus, the null hypothesis, of no

relationship ($R_C = 0$) among the data sets is true (Hair et al., 1998; Sherry & Henson, 2005). The model particulars were Cf 1 ($\lambda = 0.422$, $F(36, 140.4) = 1.010$, $p < 0.464$) and Cf 2 ($\lambda = 0.647$, $F(24, 110.8) = 0.748$, $p = 0.791$) (Table 1c). This CCorA will thus not be interpreted further, as there is no sound relationship among the data sets (Hair et al., 1998; Sherry & Henson, 2005; Rinn et al., 2009).

b. Standardized canonical function (weights), structure and communality coefficients

Occurrence of a significant CCorA model permits further interpretation, especially in the quest to inform theory (Sherry & Henson, 2005), as in this case the present study attempts to achieve in relation to the AMA postulate as a mode-of-action of the foliar B+Ca mediated apple fruit SBB incidence reduction. The guiding principle for considering the Cf for interpretation is availability of evidence of significance, appreciable effect sizes (R_C^2 at least 10%) as well as considerable (at least 10%) cumulative contribution (Hair et al., 1998; Sherry & Henson, 2005; Rinn et al., 2009). In this regard, the CCorA in this study showed that Cf 1 and Cf 2 of the full experiment and the least SBB incidence reducing foliar B+Ca treatments could be considered (Tables 4a, 4b and 4c). Interpretation of CCorA involves close analysis of the degree or magnitude and direction of either the standardised canonical function (weights), structure and communality coefficients associated with the data set (input) variables (Hair et al., 1998; Courville & Thompson, 2001; Sherry & Henson, 2005; Zientek & Thompson, 2006; Nimon et al., 2010). Therefore, only coefficients of Cf 1 and 2 are presented as results in this section (Tables 5a, 5b and 5c), highlighting their magnitude and direction where applicable.

i. Full experiment

The standardized canonical function (S_C) or the weights and the structure coefficients for this CCorA scenario are given in Table 2a, following the recommended format as detailed in Hair et al. (1998) and Sherry & Henson (2005). Considering the SBB data (Table 2a), for Cf 1, TSBB (-2.379), class 0 (-2.003) and class 2 (1.059) variables had the highest absolute S_C values. For Cf 2, variables class 0 (-1.402) and 2 (-1.299) had the highest S_C values. In addition, for Cf 1, and in the SBB data set, only class 1 and 2 variables had positive S_C values, whereas, in Cf 2, only class 1 had positive S_C value (Table 2a). In the apple peel metabolites data set (Table 2a), variables TCAR (-3.859), CHLb (1.960), TCHL:TCAR (-

1.581), TCHL (1.468) and CHLa (1.104) had the highest S_C values for Cf 1. For Cf 2, the highest S_C values were with TCAR (5.670), CHLa (-2.726), TCHL:TCAR (2.362) and CHLb (-1.703) variables. In addition, for Cf 1, and in the apple peel metabolite data set, all S_C values were positive, except for that of TF:TP ratio, TCHL:TCAR, TF and TCAR variables, whereas, in Cf 2, all S_C values were negative, except for that of TCHL:TCAR, TP, TF and TCAR variables (Table 2a).

Varying cut-off values are reported in literature for the magnitudes of structure coefficients (R_S) and communality (H^2), with some authors suggesting 0.3 (Lee & Lee, 2012; Bringula, 2016; Chung et al., 2017). Other authors used 0.4 (Fox & Hammond, 2017) as well as 0.45 (Sherry & Henson, 2005). This particular study adopted the R_S cut-off value of 0.45 and H^2 values above 45 % following recommendations by Sherry & Henson (2005), since these cut-off values are also mostly used in other factor analyses. In Table 2a, the R_S and H^2 values that reached these magnitudes are underlined. Considering the SBB data set (Table 2a), for Cf 1, all the four input variables were associated with very high absolute R_S values of class 2 (0.94), class 0 (-0.91), TSBB (0.888) and class 1 (0.615), while for Cf 2, the variable class 1 (0.723) had the highest and most important R_S value. In addition, for Cf 1, and in the SBB data set, all variables were important (> 0.45) and had positive R_S values, except class 0, whereas, in Cf 2, the only important R_S value of variable class 1 was positive (Table 2a). In the apple peel metabolites data set (Table 2a), variables CHLa (0.87), TCHL (0.87), CHLb (0.86), TP (0.85), TCAR (0.82), TF (0.78) and TF:TP ratio (-0.55) had the highest R_S values for Cf 1. F, while for Cf 2, the highest R_S values were with TF (0.53) and TP (0.52). Furthermore, for Cf 1, and in the apple peel metabolite data set, all R_S values that were important (> 0.45) in magnitude, as mentioned above, were positive, except the TF:TP ratio, whereas, in Cf 2, all important R_S values (TP and TF) were positive (Table 2a).

Considering the SBB data set (Table 2a), across Cf 1 and 2 (H^2 is a summation of Cf R^2_S values), all the four input variables were associated with very high H^2 values of class 2 (95.14), class 0 (92.89), class 1 (90.16) and TSBB (88.78). In the apple peel metabolites data set (Table 2a), across Cf 1 and 2, important H^2 values were observed with TP (97.91), TF (88.167), CHLa (78.41), TCHL (77.83), CHLb (75.35) and TCAR (67.78).

ii. Two least SBB incidence reducing foliar B+Ca treatments

The weights (S_C), structure coefficients for this CCorA scenario are given in Table 2b (Hair et al., 1998; Sherry & Henson, 2005), as well as the squared structure coefficients (R_S^2) and communality (H^2) values. The important structure coefficients (above 0.45) and communality values (> 45 %) following Sherry & Henson (2005) are underlined in Table 2b. In the SBB data set (Table 2b), for Cf 1, variables class 0 (-1.31) and TSBB (-1.16) had the highest absolute S_C values, whereas, for Cf 2, the S_C value of the variable class 1 (1.27) was the highest. In addition, for Cf 1 and in the SBB data set, only variables class 1 and 2 had positive S_C values, where as in Cf 2, only class 0 and 1 had positive S_C values (Table 2b). In the apple peel metabolites data set (Table 2b), variables CHLb (2.19), TCAR (-1.52), CHLa (-0.862) and TP (0.81) had the highest S_C values for Cf 1. With Cf 1, all apple peel metabolite S_C values were negative, except CHLa:CHLb, TP, CHLb and TCHL, whereas, for Cf 2, all S_C values were positive, except TF:TP ratio, CHLa:CHLb, CHLb and TCHL (Table 2b).

The important (> 0.45) structure coefficients (R_S) are underlined (Table 2b). In the SBB data set, for Cf 1, all the four input variables were associated with very high absolute R_S values of class 0 (-0.95), TSBB (0.94), class 1 (0.94) and class 2 (0.69), while, for Cf 2, the variable class 2 (-0.69) had the highest and most important R_S value. In addition, for Cf 1, and in the SBB data set, all variables were important (> 0.45) and had positive R_S values, except class 0, whereas, in Cf 2, the only important R_S value of variable class 2 was negative (Table 2b). In the apple peel metabolites data set (Table 2b), variables TP (0.91), TF (0.80), CHLb (0.71), TF:TP ratio (-0.70), TCHL (0.69), CHLa (0.67) and TCAR (0.64) had the important R_S values for Cf 1, while for Cf 2, the important R_S values were with CHLa (-0.56), TCHL (-0.55), CHLb (-0.54) and TCAR (-0.53). For Cf 1, and in the apple peel metabolite data set, all R_S values that were important (> 0.45) in magnitude as mentioned above were positive, except the TF:TP ratio, whereas, in Cf 2, all important R_S values (CHLa, TCHL, CHLb and TCAR) were negative (Table 2b).

All communality (H^2) values of the variables in the SBB data set (Table 2b), were important (> 45 %) across Cf 1 and 2, with class 1 (98.34), class 0 (97.94), TSSB (96.02) and class 2 (94.43). In the apple peel metabolites data set (Table 2b), across Cf 1 and 2, important H^2 values were observed with TP

(84.40), CHLb (80.41), TCHL (78.30), CHLa (75.72), TCAR (68.90), TF (64.66) and TF:TP ratio (51.19).

iii. Two best SBB incidence reducing foliar B+Ca treatments

The weights (S_C), structure coefficient (R_S), squared structure coefficients (R_S^2) and communality (H^2) values for this CCorA scenario are given in Table 2c, however these values are only for comparison purposes to those of Table 2b (CCorA in least SBB incidence reducing foliar B+Ca treatments). No magnitude or direction of these values is important as the recommended and true purpose of CCorA as the associated dimension reduction analysis in Table 1c revealed that the model was not significant.

4. Discussion

a. The focus of the canonical correlation analyses in this study

The different post-full-bloom foliar B+Ca treatments displayed varying efficiencies in reducing fruit SBB incidence at harvest maturity in ‘Golden Delicious’ apple, where the control (no B+Ca) was associated with the highest SBB incidence (Daiber, 2017; Lötze et al., 2017). In addition, foliar B+Ca treatment formulation differences were associated with varying changes in apple peel tissue metabolites (Papers 1 & 2) as well as varying SBB suppression outcomes (Daiber, 2017). It was thus hypothesised respective data sets of peel metabolites and SBB incidence variables would yield significant relationships when subjected to CCorA. This was investigated, first with all observations, for the full experiment (involving varying foliar B+Ca treatments). Results yielded a significant relationships evidenced by significant Cf 1 and Cf 2. This was expected, because SBB occurred and provided a potential suggestion of a ‘multivariate’ based phenomenon in SBB suppression with different foliar B+Ca treatments.

The most important focus of the CCorA technique in this study was on comparing CCorA outputs from selected foliar B+Ca treatments associated with the least (LEAST) and best (BEST) SBB incidence suppression of ‘Golden Delicious’ fruit at harvest. The control (no B+Ca) and B`0.02+znCa`0.06 treatment constituted the LEAST combination, while the BEST combination was treatments

$B^{-0.08}+Ca^{1.24}$ and $B^{-0.17}+Ca^{1.24}$. In each of these LEAST and BEST combinations of the foliar B+Ca treatments for their respective CCorA analyses, SBB incidence suppression outcomes at harvest maturity were generally comparable (Daiber, 2017; Lötze et al., 2017). Thus, examining the correlations and interactions of the input variables in the LEAST CCorA (Table 2b), whilst comparing it to that of BEST (Table 2c), would explain/quantify the strength of these relationships and involvement of several biochemical aspects in the sought mode-of-action, by which the post-full-bloom foliar B+Ca treatments mediate apple fruit SBB at harvest.

b. Apple peel metabolite variables as hypothetical predictors of the SBB incidence variables

A theoretical support is necessary for the respective grouping of variables into data sets prior to conducting CCorA (Nimon et al., 2010). One data set is designated as predictor (independent), while another data set becomes the criterion (dependent) variables (Hair et al., 1998; Akbas & Takma, 2005; Sherry & Henson, 2005; Bringula, 2016). However, the execution procedures of the CCorA do not require or consider such predictor or criterion data set classification (Hair et al., 1998; Sherry & Henson, 2005). In this study, apple peel metabolites data set would suit best as the predictor, since it is established that some of these components of the peel biochemistry like the flavonoids (anthocyanins) can mask SBB disorders in maturing bi-coloured apple cultivars like ‘Cripps Pink’ (Makaredza et al., 2015). Ratios of chlorophyll *a* to chlorophyll *b* and total chlorophyll to total carotenoids, are also parameters known to variably change with levels of SBB (Chen et al., 2008; Felicetti & Schrader, 2008; 2009a, b). It is also acceptable in CCorA to identify the two variable data sets discretely (Zientek & Thompson, 2006), an approach this particular study adopted, as the data sets were merely referred to by their general description as apple peel biochemical variables data set and the SBB incidence variables data set.

However, variables that constitute each of the CCorA data sets usually have a relationship or correlation amongst them (Henson, 2002), which results in the multi-collinearity phenomenon. For instance, in this study, there is a relationship amongst the phenolic parameters, as well as amongst the chlorophyll parameters. Multi-collinearity creates challenges in the interpretation of CCorA output (Henson, 2002; Nimon et al., 2010), especially, if the interpretation is based on standardised canonical coefficients (weights) (S_C) and structure coefficients (R_s), as these fall short when knowledge about the full canonical

effects is of interest. The multi-collinearity problem can be averted by considering the commonality (H^2) values (Sherry & Henson, 2005; Nimon et al., 2010). In this study, respective H^2 values were determined and consulted following Sherry & Henson (2005), but the emphasis was mostly on the canonical variate associated with apple peel metabolite (predictor) variables.

c. Interpretation of full experiment CCorA with respect to unravelling the sought mode-of-action

The central statistic in CCorA is the canonical correlation (R_C) value, together with its square, the R_C^2 , which are both analogous to Pearson r or multiple regression R and R^2 respectively (Hair et al., 1998; Sherry & Henson, 2005). The $1-\lambda$ statistic is also analogous to the multiple regression R^2 (Hair et al., 1998; Henson, 2002; Sherry & Henson, 2005). The strength of the relationship between apple peel metabolites and SBB incidence is very strong as indicated by the R_C value of 0.613 for the full model or Cf 1. A summation of the two R_C^2 values for Cf 1 and 2, or going by the full model statistic of $1-\lambda$, reveals that the apple peel metabolites (predictor variables) accounts for close to 56 to 58 % of the variance in the SBB incidence data set (criterion variables). This is considered as a large size effect, since it is above 50 % (Sherry & Henson, 2005). From this, and in relation to the aim of this study, it is important to focus on determining the contribution of particular predictor (apple peel metabolites) variables to their own canonical variate, with emphasis of finding several of them contributing in almost equal measure to satisfy a ‘multivariate’ hypothesised mode-of-action.

To assess the contribution of the predictor/metabolite variables to their canonical variate, the standardised canonical coefficients/weights (S_C) in Table 2a, were consulted first. The S_C in essence are analogous to beta (β) weights in regression, and showed which predictor was stronger in the specific canonical variate (Sherry & Henson, 2005). However, the S_C are known to be unstable, and it is recommended to examine them alongside their associated structure coefficient (R_S) values (Hair et al., 1998; Courville & Thompson, 2001; Akbas & Takma, 2005; Sherry & Henson, 2005). The structure coefficient (R_S) value is a correlation between an input variable and its canonical variate (Hair et al., 1998; Sherry & Henson, 2005; Rinn et al., 2009).

Only those variables with threshold value should be considered as important (Sherry & Henson, 2005), therefore these were underlined in Table 2a for Cf 1 and 2. The R_S values aid interpretation of the CCorA

output by identifying the input variables that contribute most to the canonical variate, while the squared value (R_S^2) indicates the proportion of variance an input variable shares with its canonical variate and can in essence be interpreted like any other R^2 type effect size (Sherry & Henson, 2005; Rinn et al., 2009). The communality coefficients (H^2) show the proportion of variance in each input variable that is explained by the complete CCorA across the interpreted canonical functions (Sherry & Henson, 2005; Rinn et al., 2009). It is the sum of the R_S^2 values and in broad sense provides how useful an observed variable was for the entire CCorA model (Sherry & Henson, 2005; Rinn et al., 2009).

Particularly, of interest as related to the aim of this study, are the predictor (apple peel metabolites) variables with large S_C values coupled with large R_S and squared structure coefficient (R_S^2) values, as these features infer good predictors (Zientek & Thompson, 2006). The highest R_S and R_S^2 values were respectively observed with CHLa, TCHL, CHLb, TP, TCAR and TF variables. These six parameters contributed mainly to Cf 1, with again, two of them (TP and TF) showing importance in Cf 2, suggesting that they are the primary predictor variables in this canonical solution. In addition, these six parameters are from different domains of pigments (chlorophylls, carotenoids) and phenolics. This indicated that SBB suppression involves several biochemical components, preferably acting additively to each other, in a postulate to the sought mode-of-action. In this aspect, this CCorA provides evidence that different metabolites combine together to account for the 56 % of the variance in the SBB incidence data set. The variable TF was associated with a lower S_C value, but with one of the large R_S values for Cf 1 and a strong secondary contribution from Cf 2, suggesting that TF is a good predictor, although affected by multi-collinearity (Zientek & Thompson, 2006). In addition, the six variables showed high H^2 values ranging from 68 % (TCAR) to 98 % (TP), suggesting that they were main factors in the canonical approach, which further validates and underscores an additive ‘multivariate’ phenomenon in the SBB suppression of respective foliar B+Ca treatments.

d. Interpretation of CCorA outputs of the least and best SBB incidence reducing foliar B+Ca treatments with respect to unravelling the sought mode-of-action

From the above discussion, evidence validating a possible multivariate and additive phenomenon as a postulate to the sought a mode-of-action is realised from the fact that different domains of pigments

contributed very strongly to their respective canonical variate. However, this does not adequately root the presumed postulate on appropriate or specific foliar B+Ca treatments, as it would ordinarily be expected that, with apple fruit SBB the respective contributions by the six pigment parameters is expected (Felicetti & Schrader, 2008; 2009a, b; Solovchenko et al., 2010; Yuri et al., 2010; 2014; Racskó & Schrader, 2012; Zhang et al., 2015). Therefore, to implicate and/or affirm particular foliar B+Ca treatment formulation effect and as such the presumed additive-mode-of-action postulate, two outputs of CCorA from the least and best SBB incidence reducing foliar B+Ca treatments were compared side by side. This was performed specifically on the significance status of the full model in the two CCorA outputs, as well as the components of the canonical variates, with regard to the peel metabolites in each respective canonical solution.

To the commendation of a specific post-full-bloom foliar B+Ca treatment as an alternative approach for apple fruit SBB mitigation, the least SBB incidence reducing foliar B+Ca treatments were the control (no B+Ca) and the treatment B`0.02+znCa`0.06 (Daiber, 2017; Lötze et al., 2017). The Zn treatment had Ca levels at 0.06 g.l⁻¹ compared to the 1.24 g.l⁻¹ and significantly suppressed levels of peel photosynthetic pigments (Paper 2). In this study, the canonical correlation (R_C) value of the least SBB incidence reducing foliar B+Ca treatments (LEAST), was higher than that in the best SBB incidence reducing foliar B+Ca treatments (BEST), being 71 % versus 59 %. This shows that the relationship between the apple peel metabolites and SBB data sets in the LEAST was higher than that in the BEST scenario. In addition, the relationship in the LEAST was significant, while in the BEST case, it was not. Adding the two squared canonical correlation (R_C^2) values for Cf 1 and Cf 2, or comparing the (R_C^2) and the $1-\lambda$ statistic values, revealed that the model in the LEAST had a very large effect size of 81 % compared to 58 %, in the BEST scenario. These differences in R_C , R_C^2 and $1-\lambda$ values in the two CCorA scenarios suggest that apple peel metabolites (predictor variables) in the LEAST case account for 81 % of the variance in the respective SBB data set (criterion variables), compared to the 58 % in the BEST scenario.

In the BEST CCorA scenario, the lack of a sound relationship among the data sets was interpreted as an effect of the respective foliar B+Ca treatments (B`0.08+Ca`1.24 plus B`0.17+Ca`1.24). In the BEST data set, that was associated with a high B and Ca concentration, a higher incidence of class 0 and lower class 1, 2 SBB incidence and TSBB was observed. By comparison, treatments with the LEAST scenario,

with lower B and Ca or with Zn (control and $B^{-0.02} + ZnCa^{0.06}$), were associated with a lower class 0 and higher class 1, 2 SBB incidence and TSBB (Daiber, 2017; Lötze et al., 2017). If additive peel metabolite effects and/or action in SBB suppression are present in the BEST than in the LEAST scenario, then this can be investigated further. This can be done by using respective standardised canonical coefficients or weights (S_C), structure coefficient (R_S), squared structure coefficient (R_S^2) and communality (H^2) values among the LEAST and BEST CCorA scenarios, particularly, the composition dynamics of the canonical variate of predictor (apple peel metabolites) variables in each CCorA case.

As in the full experiment CCorA scenario, in the LEAST CCorA case, the highest R_S and R_S^2 values were respectively observed with the same six metabolite parameters. However thus was observed in a different ranking: TP, TF, CHLb, TCHL, CHLa and TCAR, in the respective canonical variate (as per R_S values) and to the whole canonical solution (as per the H^2 values). TP and TF made the primary contribution and the chlorophyll parameters gave a vital secondary contribution (in both Cf 1 and Cf 2). This suggests that several apple peel metabolites (predictors) jointly account for the 81 % ($1-\lambda$) variance in the respective SBB incidence data set. This observation is in agreement with a multivariate additive postulate in SBB suppression outcomes associated with specific foliar B+Ca treatments. This additive metabolite postulate is an effect of particular formulated foliar B+Ca treatments, because the R_S and R_S^2 of the six mentioned variables were of no value in the Cf 2 of the BEST CCorA case. This is presumably because the associated sunburn data set did not show a sufficiently high SBB incidence or severity in class 1, 2 SBB incidence and TSBB variables than experienced in this experiment.

5. Conclusion

Previous papers clearly indicated that metabolic changes in apple peel after application of B+Ca foliar treatments could not fully explain the mode-of-action of SBB reduction for classes 1 and 2 in ‘Golden Delicious’. This study demonstrated that some of these metabolites, in combination, contribute to the significant CCorA solution between data of variables from apple peel metabolites and SBB incidence in ‘Golden Delicious’ apple fruit. Findings agree with established facts where peel biochemical profile of

apple fruit changes with SBB induction and severity development. As thus, a strong relationship with the SBB and peel biochemical data sets was realised where the foliar B+Ca treatments did not suppress SBB incidence adequately, and a weak relationship was realised where particular B+Ca formulations did suppress SBB incidence significantly. The involvement of several metabolites in the SBB dynamics of apple fruit peel treated with particular foliar B+Ca treatments that did not suppress the SBB, resulting into a significant canonical correlation analysis outcomes in contrast to where SBB incidence was suppressed, yields evidence of an underlying multivariate-based mode-of-action. This further shows that dynamics in several peel biochemicals as induced by the foliar B+Ca treatments do additively contribute to the yet un known physiological mechanism by which the SBB incidence is suppressed. These apple peel tissue metabolites may be predictors of potential SBB suppression outcomes associated with specific foliar B+Ca treatments in ‘Golden Delicious’ apple fruit. However, as this experiment was only conducted during one season due to logistics, it is recommended that the postulate of additive-metabolite-action is further evaluated for at least one more season and if possible, in an orchard with a higher incidence of class 1 and 2 SBB.

Acknowledgments

Support for this research was made possible through a capacity building competitive grant (RU/2015/DRRG/01/004) provided by Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) and Nulandis (Pty), South Africa. This study is part of a Ph.D. training program supported by RUFORUM members, Stellenbosch and Makerere Universities.

References

- Agati, G., Azzarello, E., Pollastri, S. & Tattini, M. 2012. Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science* 196:67-76.
- Agati, G., Brunetti, C., Ferdinando, M.D., Ferrini, F., Pollastri, S. & Tattini, M. 2013. Functional roles of flavonoids in photoprotection: New evidence, lessons from the past. *Plant Physiology and Biochemistry* 72:35-45.
- Akbas, Y. & Takma, C. 2005. Canonical correlation analysis for studying the relationship between egg production traits and body weight, egg weight and age at sexual maturity in layers. *Czech Journal of Animal Science* 50:163-168.
- Botai, C., Botai, J., De Wit, J.P., Ncongwane, K.P. & Adeola, A.M. 2017. Drought Characteristics over the Western Cape Province, South Africa. *Water* 9:876.
- Bringula, R.P. 2016. Factors affecting web portal information services usability: A canonical correlation analysis. *International Journal of Human-Computer Interaction* 32:814-826.
- 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.
- Chung, P.K., Zhao, Y., Liu, J.D. & Quach, B. 2017. A canonical correlation analysis on the relationship between functional fitness and health-related quality of life in older adults. *Archives of Gerontology and Geriatrics* 68:44-48.
- Courville, T. & Thompson, B. 2001. Use of structure coefficients in published multiple regression articles: β is not enough. *Educational and Psychological Measurement* 61:229-248.
- Daiber, S.H. 2017. Quantifying changes in tree physiology after amelioration to reduce sunburn on apples. MSc Thesis. Department of Horticultural Science, Stellenbosch University.
- Dussi, M.C., Giardina, G. & Reeb, P. 2005. Shade nets effect on canopy light distribution and quality of fruit and spur leaf on apple cv. Fuji. *Spanish Journal of Agricultural Research* 3:253-260.
- Felicetti, D.A. & Schrader, L.E. 2008. Changes in pigment concentrations associated with the degree of sunburn browning of 'Fuji' apple. *Journal of the American Society of Horticultural Science* 133:27-34.
- Felicetti, D.A. & Schrader, L.E. 2009a. Changes in pigment concentrations associated with sunburn

- browning of five apple cultivars. II. Phenolics. *Plant Science* 176:84-89.
- Felicetti, D.A. & Schrader, L.E. 2009b. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. I. Chlorophylls and Carotenoids. *Plant Science* 176:78-83.
- Fox, S. & Hammond, S. 2017. Investigating the multivariate relationship between impulsivity and psychopathy using canonical correlation analysis. *Personality and Individual Differences* 111:187-192.
- 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. *HortScience* 40:592-596.
- Gindaba, J. & Wand, S.J.E. 2007a. Do fruit sunburn control measures affect leaf photosynthetic rate and stomatal conductance in 'Royal Gala' apple? *Environmental and Experimental Botany* 59:160-165.
- Gindaba, J. & Wand, S.J.E. 2007b. Climate-ameliorating measures influence photosynthetic gas exchange of apple leaves. *Annals of Applied Biology* 150:75-80.
- Gunderson, B.K. & Muirhead, R.J. 1997. On estimating the dimensionality in canonical correlation analysis. *Journal of Multivariate Analysis*. 62:121-136.
- Hair, J.F., Anderson, R.E., Tatham, R.L. & Black, W.C. 1998. *Multivariate Data Analysis*. 5th Edition. Prentice Hall, Inc.
- Hamadziripi, E.T. 2012. The effect of canopy position on the fruit quality and consumer preference of apples. MSc Thesis. Department of Horticultural Science, Stellenbosch University.
- Henson, R.K. 2002. The logic and interpretation of structure coefficients in multivariate general linear model analyses. *American Educational Research Association* TM034307:1-35.
- Knapp, T.R. 1978. Canonical correlation analysis: A general parametric significance-testing system. *Psychological Bulletin* 85:410-416.
- Lee, J. & Lee, H. 2012. Canonical correlation analysis of online video advertising viewing motivations and access characteristics. *New Media and Society* 14:1358-1374.
- Lolicato, S. 2011. *Sun Protection for Fruit - A practical manual for protecting sunburn on fruit*. Department of Primary Industries, Farm Services Victoria Division, Victoria, Australia.
- Lötze, E. & Hoffman, E.W. 2014. Foliar application of calcium plus boron reduces the incidence of sunburn in 'Golden Delicious' apple. *Journal of Horticultural Science and Biotechnology* 89:607-612.

- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2017. Boron in combination with calcium reduces sunburn in apple fruit. *Boron* 2:123-127.
- Makaredza, B., Marais, H., Schmeisser, M., Lötze, E. & Steyn, W.J. 2015. Ripening associated red color development masks sunburn browning in apple peel. *HortScience* 50:814-818.
- Midgley, S.J.E. & Lötze, E. 2011. Climate change in the Western Cape of South Africa: Trends, projections and implications for chill unit accumulation. *Acta Horticulturae* 903:1127-1134.
- Mwije, A. 2017. Pioneering a new paradigm of sunburn mitigation in apples. In *Bringing Science to Communities: Voices from the Field (Issue 1)* (Ed. Withers, J). A RUFORUM Publication. Kampala, Uganda. 20-23 pp.
- Nimon, K., Henson, R.K. & Gates, M.S. 2010. Revisiting interpretation of canonical correlation analysis: A tutorial and demonstration of canonical commonality analysis. *Multivariate Behavioral Research* 45:702-724.
- Onwuegbuzie, A.J. & Daniel, L.G. 2003. Typology of analytical and interpretational errors in quantitative and qualitative educational research. *Current Issues in Education* 6:1-53.
- Racskó, J. & Schrader, L.E. 2012. Sunburn of apple fruit: Historical background, recent advances and future perspectives. *Critical Reviews in Plant Sciences* 31:455-504.
- Rinn, A.N., Jamieson, K.M., Gross, C.M. & McQueen, K.S. 2009. A canonical correlation analysis of the influence of social comparison, gender, and grade level on the multidimensional self-concepts of gifted adolescents. *Social Psychology of Education* 12:251-269.
- Schrader, L.E. 2011. Scientific basis of a unique formulation for reducing sunburn of fruits. *HortScience* 46:6-11.
- Schrader, L. E., Zhang, J., and Duplaga, W. K. 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. *Plant Health Progress* DOI:10.1094/PHP-2001-1004-01-RS
- Sherry, A. & Henson, R.K. 2005. Conducting and interpreting canonical correlation analysis in personality research: A user-friendly primer. *Journal of Personality Assessment* 84:37-48.
- Solovchenko, A.E., Chivkunova, O.B., Gitelson, A.A. & Merzlyak, M.N. 2010. Non-destructive estimation pigment content, ripening, quality and damage in apple fruit with spectral reflectance in the visible range. *Fresh Produce* 4:91-102.
- Wünsche, J.N., Lombardini, L. & Greer, D.H. 2004. 'Surround' particle film applications - Effects on

whole canopy physiology of apple. *Acta Horticulturae* 636:565-571.

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. *Journal of Food, Agriculture and Environment* 8:920-925.

Yuri, J.A., Neira, A., Maldonado, F., Quilodran, A., Simeone, D., Razmilic, I. & Palomo, I. 2014. Total phenol and quercetin content and antioxidant activity in apples in response to thermal, light stress and to organic management. *Journal of Applied Botany and Food Quality* 87:131-138.

Zhang, J., Niu, J., Duan, Y., Zhang, M., Liu, J., Li, P. & Ma, F. 2015. Photoprotection mechanism in the ‘Fuji’ apple peel at different levels of photooxidative sunburn. *Physiologia Plantarum* 154:54-65.

Zientek, L.R. & Thompson, B. 2006. Commonality analysis: Partitioning variance to facilitate better understanding of data. *Journal of Early Intervention* 28:299-307.

Tables

Table 1a. CCorA dimension reduction analysis of full experiment observations.

Cf	Ev	Cv (%)	R _C	R _C ²	λ	F	HDf	EDf	<i>p</i>
1 to 4	0.601	54.088	0.613	0.375	0.441	2.861	36	421.453	< 0.0001
2 to 4	0.253	83.152	0.449	0.202	0.706	1.747	24	328.336	0.018
3 to 4	0.120	98.625	0.328	0.107	0.884	1.035	14	228	0.419
4 to 4	0.010	100.000	0.098	0.010	0.990	0.185	6	115	0.981

Table 1b. CCorA dimension reduction analysis of B`0.00+Ca`0.00 plus B`0.02+znCa`0.06 observations.

Cf	Ev	Cv (%)	R _C	R _C ²	λ	F	HDf	EDf	<i>p</i>
1 to 4	1.021	38.809	0.711	0.505	0.189	2.184	36	140.3934	0.001
2 to 4	0.653	69.154	0.629	0.395	0.382	1.818	24	110.8128	0.020
3 to 4	0.405	91.291	0.537	0.288	0.631	1.442	14	78	0.154
4 to 4	0.128	100.000	0.337	0.113	0.887	0.852	6	40	0.538

Table 1c. CCorA dimension reduction analysis of B`0.08+Ca`1.24 plus B`0.17+Ca`1.24 observations.

Cf	Ev	Cv (%)	R _C	R _C ²	λ	F	HDf	EDf	<i>p</i>
1 to 4	0.533	47.070	0.590	0.348	0.422	1.010	36	140.3934	0.464
2 to 4	0.334	80.975	0.501	0.251	0.647	0.748	24	110.8128	0.791
3 to 4	0.115	94.964	0.322	0.103	0.863	0.425	14	78	0.962
4 to 4	0.039	100.000	0.193	0.037	0.963	0.258	6	40	0.953

Cf = canonical function, Ev = Eigen value and is equal to L (1-L), where L is Ev derived with XLSTAT, Cv = cumulative variability, R_C = canonical correlation, R_C² = squared R_C, λ = Wilks lambda, F = the F value (statistic), HDf = hypothesis (R_C = 0) degrees of freedom, EDf = error degrees of freedom and *p* = significance of F value (Pr>F).

Table 2a. Standardised canonical function (weights) (S_c), structure (R_s), squared structure (R_s^2) and communality (H^2) coefficients associated with canonical functions 1 and 2 of the CCorA with full experiment observations.

CCorA input variable	Function 1			Function 2			H^2 (%)
	S_c	R_s	R_s^2 (%)	S_c	R_s	R_s^2 (%)	
<u>Sunburn browning data set</u>							
Class 0	-2.003	<u>-0.905</u>	81.924	-1.402	-0.331	10.966	<u>92.890</u>
Class 1	0.489	<u>0.615</u>	37.839	0.449	<u>0.723</u>	52.321	<u>90.160</u>
Class 2	1.059	<u>0.943</u>	88.877	-1.299	-0.250	6.259	<u>95.137</u>
TSBB	-2.379	<u>0.888</u>	78.787	-0.361	0.316	9.988	<u>88.775</u>
<u>Apple peel metabolites data set</u>							
TF:TP	-0.052	<u>-0.547</u>	29.902	-0.124	-0.337	11.386	41.288
CHLa:CHLb	0.076	0.119	1.418	-0.155	-0.098	0.961	2.379
TCHL:TCAR	-1.581	0.128	1.632	2.362	-0.068	0.463	2.095
TP	0.582	<u>0.845</u>	71.387	0.531	<u>0.515</u>	26.523	<u>97.910</u>
TF	-0.110	<u>0.778</u>	60.576	0.514	<u>0.525</u>	27.591	<u>88.167</u>
CHLa	1.104	<u>0.873</u>	76.195	-2.726	-0.149	2.211	<u>78.406</u>
CHLb	1.960	<u>0.858</u>	73.638	-1.703	-0.131	1.710	<u>75.347</u>
TCHL	1.468	<u>0.871</u>	75.812	-2.309	-0.142	2.012	<u>77.825</u>
TCAR	-3.859	<u>0.820</u>	67.295	5.670	-0.070	0.487	<u>67.781</u>

Structure coefficients (R_s) and communality coefficients (H^2) greater than 0.45 and 45 % respectively are underlined.

Table 2b. Standardised canonical function (weights) (S_c), structure (R_s), squared structure (R_s^2) and communality (H^2) coefficients for canonical functions 1 and 2 of the CCorA with B`0.00+Ca`0.00 plus B`0.02+znCa`0.06 observations.

CCorA input variable	Function 1			Function 2			H^2 (%)
	S_c	R_s	R_s^2 (%)	S_c	R_s	R_s^2 (%)	
Sunburn browning data set							
Class 0	-1.313	<u>-0.948</u>	89.895	0.400	0.284	<u>8.046</u>	<u>97.942</u>
Class 1	0.714	<u>0.935</u>	87.445	1.271	0.330	<u>10.894</u>	<u>98.339</u>
Class 2	0.264	<u>0.686</u>	47.122	-0.474	<u>-0.688</u>	<u>47.311</u>	<u>94.433</u>
TSBB	-1.163	<u>0.941</u>	88.516	-0.514	-0.274	<u>7.498</u>	<u>96.015</u>
Apple peel metabolites data set							
TF:TP	-0.189	<u>-0.700</u>	49.022	-0.551	-0.147	<u>2.171</u>	<u>51.193</u>
CHLa:CHLb	0.033	-0.150	2.253	-1.782	-0.216	<u>4.675</u>	6.928
TCHL:TCAR	-0.536	0.167	2.802	4.146	-0.010	<u>0.010</u>	2.813
TP	0.805	<u>0.909</u>	82.703	0.028	0.130	<u>1.696</u>	<u>84.398</u>
TF	-0.085	<u>0.801</u>	64.102	0.181	0.075	<u>0.559</u>	<u>64.662</u>
CHLa	-0.862	<u>0.669</u>	44.799	4.862	<u>-0.556</u>	<u>30.924</u>	<u>75.723</u>
CHLb	2.194	<u>0.714</u>	51.032	-12.457	<u>-0.542</u>	<u>29.376</u>	<u>80.408</u>
TCHL	0.402	<u>0.691</u>	47.749	-2.300	<u>-0.553</u>	<u>30.552</u>	<u>78.302</u>
TCAR	-1.520	<u>0.638</u>	40.683	8.962	<u>-0.531</u>	<u>28.217</u>	<u>68.900</u>

Structure coefficients (R_s) and communality coefficients (H^2) greater than 0.45 and 45 % respectively are underlined

Table 2c. Standardised canonical function (weights) (S_c), structure (R_s), squared structure (R_s^2) and communality (H^2) coefficients for canonical functions 1 and 2 of the CCorA with B`0.08+Ca`1.24 plus B`0.17+Ca`1.24 observations.

CCorA input variable	Function 1			Function 2			H^2 (%)
	S_c	R_s	R_s^2 (%)	S_c	R_s	R_s^2 (%)	
Sunburn browning data set							
Class 0	0.483	-0.653	53.529	0.498	0.732	2.367	55.896
Class 1	1.170	0.454	28.237	1.517	-0.531	4.577	32.814
Class 2	1.991	0.884	15.334	1.886	-0.392	0.273	15.607
TSBB	-1.454	0.671	51.925	-3.025	-0.721	0.244	52.169
Apple peel metabolites data set							
TF:TP	-0.503	-0.396	15.660	-0.533	0.598	35.711	51.371
CHLa:CHLb	0.235	0.066	0.429	-0.245	-0.504	25.422	25.851
TCHL:TCAR	-3.406	0.038	0.145	-1.672	0.179	3.211	3.356
TP	-0.458	0.594	35.265	-3.050	-0.679	46.151	81.416
TF	0.325	0.526	27.700	1.836	-0.531	28.164	55.863
CHLa	-0.391	0.739	54.570	-0.292	-0.162	2.639	57.209
CHLb	6.200	0.731	53.443	3.238	-0.068	0.467	53.909
TCHL	2.377	0.739	54.676	1.190	-0.124	1.531	56.207
TCAR	-7.030	0.709	50.278	-3.269	-0.220	4.829	55.107

GENERAL DISCUSSION AND CONCLUSION

1. General discussion

a. The increasing risk of fruit sunburn disorders in apple farming systems

The apple fruit market is increasingly becoming competitive, and with strict enforcements of high quality criteria because consumers are demanding superior quality produce (Jaeger et al., 1998; Harker et al., 2003; 2008). Thus, worldwide there is a growing emphasis and efforts to produce superior and high quality apple fruit, albeit, the changing environments and resource constraints that increase the risk of quality compromising challenges like fruit sunburn browning (SBB) disorders. The increasing worldwide trend of adopting the intensive orchard system will exacerbate apple fruit SBB incidence, if appropriate interventions are not implemented, as dwarfing rootstocks and high tree densities causes a reduction in tree canopy necessary to protect fruit from sunburn under natural conditions (Wünsche et al., 2004).

Apart from intensive orchard systems, apple fruit production is also increasingly spreading to more warm and dry areas (Turyomurugyendo et al., 2004; Griesbach, 2007; Ashebir et al., 2010). In these new apple-producing regions, growers are often resource constrained to implement adequately the currently available SBB mitigation strategies, yet these environments are rife with SBB causing conditions especially high solar temperatures and irradiance and frequent droughts (Wünsche et al., 2004; Baroniya et al., 2014). Further still, in traditional apple growing areas like the Western Cape, South Africa, harsher climatic conditions are exacerbating fruit SBB incidence due to climate change (Rouault & Richard, 2003; Midgley & Lötze, 2011; Botai et al., 2017). Thus, irrespective of where apple production systems are being implemented anywhere worldwide, growers are facing serious challenges to prevent high incidence of fruit sunburn disorders (Wünsche et al., 2001; Schrader et al., 2008; Racskó & Schrader, 2012). Probably, the inevitability and ever-increasing risk of apple fruit sunburn requires that future approaches towards SBB incidence suppression in the various orchard systems should embrace a multi-faceted, preferably a mix of cheaper, easier to implement, and overall much more sustainable approaches.

b. Post-full-bloom foliar boron plus calcium approach can complement existing practices

The main methods to reduce fruit SBB incidence in apple orchards are climate-ameliorating strategies that include the very expensive shade netting (Gindaba & Wand, 2007a; Racskó & Schrader, 2012).

Kaolin applications, with limited efficiency under conditions of extreme temperature conditions of above 40 °C, also require additional water to clean kaolin residues from fruit. Furthermore, overhead cooling, apart from expensive initial and maintenance costs, in areas with limited water supply, may have dire challenges in implementing these approaches (Lolicato, 2011). In addition, despite reported negative effects of these approaches on fruit and tree physiology and/or canopy (Wünsche et al., 2004; Dussi et al., 2005; Gindaba & Wand, 2005; 2007b; Feng et al., 2014), shade netting is considered a future mandatory investment especially in intensive apple orchard systems (Tanny et al., 2009; Tanny, 2013). Shade netting results in both reduction of solar radiation and wind speeds in apple orchards, hence creating a modified crop microclimate that leads to significant reduction of irrigation water usage and expenses (Tanny et al., 2009; Tanny, 2013). However, shade netting may increase the risk of low calcium uptake by the apple trees (De Freitas et al., 2013), thus predisposing fruit to bitter pit and other calcium-related disorders (Miqueloto et al., 2014). These disorders compromise the quality of the produce, and require mandatory attention through foliar applications in apple fruit orchards (Saure, 1996; Lötze & Theron, 2007; Joubert et al., 2008). Applications of sunburn mitigating post-full-bloom foliar B+Ca treatments under shadenetting may be of benefit in this regard.

Further still, where two different cultivars are planted in the same orchard, for instance ‘Golden Delicious’ and ‘Cripps Pink’ apples, it poses the question of the preferred net colour, as the shade net colour requirements differ among cultivars. It is likely that the implementation of SBB incidence reducing post-full-bloom foliar B+Ca treatments may reduce the costs of having different colour nets for different cultivars established in same orchards, as is common practice for pollination purposes in older plantings. Perhaps, combining shade netting with foliar B+Ca treatments on susceptible apples like ‘Granny Smith’ may yield even better and sustainable SBB incidence reduction; however, this needs further experimentations to ascertain its true practicability. Nutrient deficiencies may exacerbate photodamage in plants (Agati et al., 2013), which closely associates with apple fruit SBB induction (Chen et al., 2008). Foliar Ca reportedly maintains the integrity of photosynthetic systems of plant tissues experiencing high light and temperature stresses (Yang et al., 2015). In addition, the micronutrient boron, included in the foliar B+Ca treatments, is a well-known micronutrient for vascular plants, and optimum nutrition of this element is important for apple fruit trees (Peryea & Drake, 1991; Blevins & Lukaszewski, 1998; Herrera-Rodriguez et al., 2010; Wang et al., 2015). As such, the post full foliar B+Ca treatments application gains a further recommendation as a routine farming practice for apple fruit orchards. Overall, post-full-bloom foliar B+Ca approach has potential to complement the existing practices particularly shade netting in the effort of reducing incidences of apple fruit SBB in intensive orchards. Foliar application of nutrients is a standard practice in apple

fruit orchards to improve yield, fruit quality and as well boost physiology of the tree or crop plant against biotic or abiotic stress factors (Peryea, 2002; Murtic et al., 2012). Treutter (2010) envisages the exploitation of foliar nutrition practices for phytochemical farming objectives to increase secondary metabolites involved in plant abiotic stress resistance, and in apple orchards this can possibly be advanced by development of such foliar B+Ca approaches.

c. Refinement of the post-full-bloom foliar boron plus calcium approach

i. Treatment formulation

The treatment B`0.02+znCa`0.06 did not reduce apple fruit SBB, except in ‘Cripps Pink’ (2015/16), where class 1 percentage was reduced significantly. Although Zn is also relevant in promoting abiotic stress tolerance and quite relevant for apples (Murtic et al., 2012), the presence of Zn in foliar applications containing Ca may compromise the Ca (Hippis & Davies, 2001; Neilsen & Neilsen, 2002). This was followed up with further experiments, but no appreciable conclusions were reached (Rahayu et al., 2001). Therefore, Zn and Ca antagonism with respect to such foliar B+Ca treatments remains to be resolved. Current papers (Daiber, 2017; Lötze et al., 2017; 2018), as well as this dissertation, recommends the exclusion of zinc (Zn) in such post-full-bloom foliar treatments, because across all cultivars, there was a notable characteristic of low chlorophyll pigments associated with this B`0.02+znCa`0.06 treatment. Two issues to take note of are i) the inclusion of Zn and ii), the level of Ca at 0.06 g.l⁻¹ compared to 1.24 g.l⁻¹ of other foliar B+Ca treatments. However, in this dissertation, the control often had a higher chlorophyll content than B`0.02+znCa`0.06 treatment, and this therefore infers that the compromise of the Zn treatment was not due its low level of Ca in the formulation. In addition, the same treatment promoted class 1 SBB incidence reduction and showed evidence of a stable photosynthetic system in ‘Cripps Pink’, but not in ‘Golden Delicious and ‘Granny Smith’. Thus, probably ‘Cripps Pink’ may be less sensitive or more tolerant to the Zn inclusion than ‘Golden Delicious’ and ‘Granny Smith’. Therefore, different cultivars may require different formulations of the foliar B+Ca treatments for effective sunburn incidence suppression.

ii. Assessment of apple fruit peel oxidative stress

Certainly, low apple peel oxidative stress level is important to realise low SBB incidence in fruit at harvest (Chen et al., 2008; 2009; Zhang et al., 2015). However, the method used in the respective study of this dissertation failed to capture relevant oxidative stress dynamics, most likely due to lower detection sensitivity of the methodology used as adopted from Du and Bramlage (1995) and Rao et al. (1998), who utilised fruit at same maturity stage in contrast with the study conducted in this

research. Schrader et al. (2001) noted that cell membrane integrity indicators were not significantly different in apple fruit with lower classes of SBB and those without. Certainly, with this study it is now very evident that lipid peroxidation assays do not have the ability to distinguish foliar B+Ca treatments with respect to reducing oxidative stress in apple peels. It is recommended that future endeavours in relation to this aspect should consider the use of confocal microscopic analyses of oxidative stress species (Macarisin et al., 2007; Sabban-Amin et al., 2011; Mditshwa et al., 2016) or non-destructive hyperspectral imaging (Kong et al., 2016).

Regarding the above recommendation, in this research it was also realised that non-enzymatic sources of oxidative stress may be the most important in the apple fruit SBB phenomenon. Oxidative stress in plant tissues can arise from non-enzymatic and enzymatic pathways where the enzymatic source is via catalytic activities of lipoxygenases (EC 1.13.11.12), while the non-enzymatic sources are due to the ROS formed within different cell compartments (Axelrod et al., 1981; Dix & Aikens, 1993; Skorzynska-Polit, 2007). Lipoxygenase-derived oxidative stress is more associated with plant organ senescence (Lynch & Thompson, 1984; He et al., 2002), although non-enzymatic oxidative stress was reported to be involved as well (Berger et al., 2001). An important aspect in relation to this study, is that the oxidative stress species or intermediates generated in the lipoxygenase-enzymatic pathway are deactivated in respective complex enzymatic processes, while those generated in non-enzymatic pathway, are not subjected to enzymatic deactivation (Spiteller, 2003). This exacerbates their effects on cellular life and physiology (Gill & Tuteja, 2010). This observation suggests that non-enzymatic oxidative stress predominates the apple fruit SBB phenomenon as the respective plant organ (the apple fruit) is certainly not in senescence, even up to commercial harvest, which is in agreement with previous research findings (Bi et al., 2014; Zhang et al., 2014; 2015).

The envisaged role of non-enzymatic oxidative stress in apple fruit SBB as alluded to above adds more credit to the recommendation made of analysing oxidative stress species in apple peels in future research efforts to refine this foliar B+Ca approach. It is important to note that ascorbic acid and related cycle including glutathione are also part of the non-enzymatic antioxidant and important in the scavenging of ROS (Łata et al., 2005). Initially it seemed beneficial to evaluate the ascorbate pool and reduction levels in apple peels under varying foliar B+Ca, but preliminary data with such treatments was not significantly different for ascorbate parameters and glutathione as well (E. Lotze and E.W.Hoffman, Stellenbosch University, Unpublished data, 2014). With respect to this and following studies, the apple peel phenolic aspects seems more versatile in curtailing the oxidative stress inducers as well as the ROS (Solovchenko & Schmitz-Eiberger, 2003; Bi et al., 2014; Zhang et al., 2014). Future research may require to include the dynamics of Flavanone 3-hydroxylase (F3H, EC 1.4.11.9) within particular post-full-bloom foliar B+Ca treatments associated with significant

sunburn incidence reduction. This is because flavanone 3-hydroxylase is a major enzyme involved in the biosynthesis of flavonoids and has an important role in resistance to abiotic stress (Liu et al., 2013; Song et al., 2016). These aspects could be part of further research to refine particular post-full-bloom foliar B+Ca treatments by employing biochemical studies, but also by adopting a non-destructive approach for flavones following recommendation by Merzlyak et al. (2005).

iii. Adopting non-destructive assessment of chlorophyll

In ‘Cripps Pink’ and in ‘Golden Delicious’, the trends of CHLa:CHLb of the control were higher, albeit the treatment effects were not statistically significant, and the Zn containing treatment effect was mostly on reduction of photosynthetic pigment parameters across all cultivars. This suggests that differences between foliar B+Ca treatments existed but the resolution of the methods used was not robust enough to elucidate them in this study. In fact, higher chlorophyll *a* to chlorophyll *b* ratios was associated with higher incidence of SBB (Felicetti & Schrader, 2008; 2009). Such higher CHLa:CHLb ratio trend or value would indicate the destruction of chlorophyll or be indicative of higher H₂O₂ or oxidative stress levels (Somashekaraiah et al., 1992; Zhang et al., 2015). Therefore, in future efforts to refine the foliar B+Ca treatment protocols for commercialisation, it is envisaged that robust methods possibly involving non-destructive methods for chlorophyll should be adopted perhaps following examples or modifications in studies of Merzlyak et al. (2003), Solovchenko et al. (2010a) and Torres et al. (2016).

iv. Consideration of carotenoids and emphasis on non-photochemical quenching function

Treatment B`0.02+znCa`0.06 may have achieved class 1 SBB incidence reduction in ‘Cripps Pink’ because of the higher carotenoids (TCAR) content, indicated by the significantly lower TCHL:TCAR. But, this Zn treatment had TCHL:TCAR that was indifferent from the control and the only further distinguishing aspect between the treatments and control would be its higher but non-significant TCAR level. This raises possibility of the role of carotenoids in the sought mode-of-action, but most probably for only ‘Cripps Pink’. Early apple fruit development is associated with high chlorophyll and carotenoid levels, and are preserved in stay-green fruit or severely reduced in non-stay-green fruit during maturation (Ampomah-Dwamena et al., 2012; Charoenchongsuk et al., 2015). However, as apple fruit mature the accompanying high irradiances leads to the degradation of chlorophyll along with the increase or revealing of carotenoids as a symptom of SBB (Felicetti & Schrader, 2008). This is more true of the experimental conditions involved in this study as fruit was harvested from the tree instead of fruit under storage which usually accumulates carotenoids. Generally, as much as the increased conspicuousness of carotenoid colours is an indication of SBB,

the presence of carotenoids, including the xanthophylls can protect the fruit peel against both heat and light stresses (Jahns & Holzwarth, 2012). Ma & Cheng (2003) reported outer canopy apple fruit peel to have contained relatively lower chlorophyll and higher carotenoids, purportedly as a defence mechanism to SBB causative agents. Yet, high irradiance reportedly increased carotenoid levels in ‘Fuji’ and ‘Delicious’ but not in ‘Granny Smith’ apples (Felicetti & Schrader, 2009). There is no doubt about the antioxidant potential of carotenoids, which is manifested in terminating chain reactions producing harmful and highly reactive intermediate oxidative stress species, while scavenging for the same. In addition, they prevent the formation of these species from the excited chlorophyll (quenching), while dissipating excess energy through the xanthophyll cycle (Havaux, 1998; Havaux & Niyogi, 1999; Collins, 2001; Mortensen et al., 2001; Karuppanapandian et al., 2011; Azqueta & Collins, 2012).

In relation to this B+Ca treatment study, carotenoids present a conundrum in that the accumulation, although beneficial as antioxidants, is itself a manifestation of SBB. This suggests that carotenoids should be assessed non-destructively, to detect them at an initial level, before they become indicators and/or symptoms of SBB disorders. At such levels, the ratio of chlorophylls to carotenoids will still be optimum for maintaining the integrity of the photosynthesis system or there will be a metabolic equilibrium between biosynthesis and catabolism of carotenoids, thus maintaining the carotenoids at adequate physiological levels (Hannoufa & Hossain, 2012). In the refinement of the foliar B+Ca treatments approach, dynamics of carotenoids may be amply studied non-destructively perhaps following examples of Merzlyak et al. (2003) and Solovchenko et al. (2005). Alternatively, non-photochemical quenching ability may be determined non-destructively and used to discern which treatment sustains relevant carotenoid levels (Li & Cheng, 2008; Solovchenko et al., 2010b; Solovchenko & Chivkunova, 2011).

2. General conclusion

Foliar nutrient formulations has a biological or physiological impact on several metabolites in the apple fruit peel. Since our B+Ca treatments are applied very early in the season and significant differences in peel biochemical aspects are observed towards harvest maturity, this infers that the physiological treatment effect continues through the season, more especially towards harvest maturity when SBB susceptibility is high.

A clear conclusion from this study is that a significant suppression of class 1 SBB is dependent on cultivar and foliar formulation. All treatments (high or low B and Ca) considerably reduced class 1 SBB in ‘Cripps Pink’, but only the treatment with high B and similar Ca level reduced class 1 SBB

in 'Golden Delicious'. In contrast, 'Granny Smith' no significant results was recorded for any of the treatment combinations on pre-mature fruit. Cultivar and foliar treatment formulation differences influence the peel biochemical dynamics, for instance, (i) total flavonoids and total phenolics (TF:TP ratio) trends were in similar in related cultivars ('Cripps Pink' and 'Golden Delicious' apples), but not in 'Granny Smith' apple. In addition, (ii) the occurrence of significant interaction effects for apple peel biochemical aspects also differed between cultivars and (iii), treatment B`0.02+znCa`0.06 caused reduced photosynthetic pigments levels in all cultivars. Overall, this suggests that different cultivars require unique post-full-bloom foliar B+Ca treatment formulations to reduce class 1 SBB significantly and as thus further research to optimise and/or refine this approach should proceed with appropriate specificity of cultivars.

Finally, the aim of the research described in this dissertation was to contribute to the understanding of the mode-of-action by which post-full-bloom foliar applications of combined B and Ca reduce class 1 and 2 apple fruit sunburn browning. This knowledge would enable the adoption and extension of this approach to other susceptible cultivars and crops. The literature review of this dissertation yielded strong and feasible justifications for a biochemical mode-of-action. The overall aim of this research to yield a mode-of-action is general, however, the specific investigation activities undertaken as described in the three research papers generated important insights into the sought biochemical mode-of-action and the importance of considering cultivar and treatment formulation differences.

There is an increasing trend in horticultural science research of adopting non-destructive techniques, for instance Near Infra-Red spectroscopy tools. Our findings provided the primary apple peel biochemical indicators to be investigated further with non-destructive techniques that can be more efficient and economical to uses, but also very reliable when investigating complex and multi-dimensional phenomenon during plant function with or without stress and in controlled or field environments. This can greatly assist in future research on these aspects.

References

- Agati, G., Brunetti, C., Ferdinando, M.D., Ferrini, F., Pollastri, S. & Tattini, M. 2013. Functional roles of flavonoids in photoprotection: New evidence, lessons from the past. *Plant Physiology and Biochemistry* 72:35-45.
- Ampomah-Dwamena, C., Dejnopratt, S., Lewis, D., Sutherland, P., Volz, R.K. & Allan, A.C. 2012. Metabolic and gene expression apple (*Malus X domestica*) carotenogenesis. *Journal of Experimental Botany* 63:4497-4511.
- Ashebir, D., Deckers, T., Nyssen, J., Bihon, W., Tsegay, A., Tekie, H., Poesen, J., Haile, M., Wondumagegnehu, F, Raes, D, Behailu, M and Deckers, J. 2010. Growing apple (*Malus domestica*) under tropical mountain climate conditions in Northern Ethiopia. *Experimental Agriculture* 46:53-65.
- Axelrod, B., Cheesbrough, T.M. & Laakso, S. 1981. Lipoxygenase from Soybeans: EC 1.13.11.12 Linoleate: Oxygen oxidoreductase. *Methods in Enzymology* 71:441-451.
- Azqueta, A. & Collins, A.R. 2012. Carotenoids and DNA damage. *Mutation Research* 733:4-13.
- Baroniya, S.S., Kataria, S., Pandey, G.P. & Guruprasad, K.N. 2014. Growth, photosynthesis and nitrogen metabolism in soybean varieties after exclusion of the UV-B and UV-A/B components of solar radiation. *Crop Journal* 2:388-397.
- Berger, S., Weichert, H., Porzel, A., Wasternack, C., Kuhn, H. & Feussner, I. 2001. Enzymatic and non-enzymatic lipid peroxidation in leaf development. *Biochimica et Biophysica Acta* 1533:266-276.
- Bi, X., Zhang, J., Chen, C., Zhang, D., Li, P. & Ma, F. 2014. Anthocyanin contributes more to hydrogen peroxide scavenging than other phenolics in apple peel. *Food Chemistry* 152:205-209.
- Blevins, D.G. & Lukaszewski, K.M. 1998. Boron in plant structure and function. *Annual Review of Plant Physiology and Plant Molecular Biology* 49:481-500.
- Botai, C., Botai, J., De Wit, J.P., Ncongwane, K.P. & Adeola, A.M. 2017. Drought Characteristics over the Western Cape Province, South Africa. *Water* 9:876.
- 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.
- Chen, L.S., Li, P. & Cheng, L. 2009. Comparison of thermotolerance of sun-exposed peel and shaded

- peel of 'Fuji' apple. *Environmental and Experimental Botany* 66:110-116.
- Collins, A.R. 2001. Carotenoids and genomic stability. *Mutation Research* 475:21-28.
- De Freitas, S.T., Do Amarante, C.V.T., Dandekar, A.M. & Mitcham, E.J. 2013. Shading affects flesh calcium uptake and concentration, bitter pit incidence and other fruit traits in 'Greensleeves' apple. *Scientia Horticulturae* 161:266-272.
- Dix, T.A. & Aikens, J. 1993. Mechanisms and biological relevance of lipid peroxidation initiation. *Chemical Research in Toxicology* 6:2-18.
- Du, Z. & Bramlage, W.J. 1995. Peroxidative activity of apple peel in relation to development of poststorage disorders. *HortScience* 30:205-209.
- Dussi, M.C., Giardina, G. & Reeb, P. 2005. Shade nets effect on canopy light distribution and quality of fruit and spur leaf on apple cv. Fuji. *Spanish Journal of Agricultural Research* 3:253-260.
- Felicetti, D.A. & Schrader, L.E. 2008. Changes in pigment concentrations associated with the degree of sunburn browning of 'Fuji' apple. *Journal of the American Society of Horticultural Science* 133:27-34.
- Felicetti, D.A. & Schrader, L.E. 2009. Changes in pigment concentrations associated with sunburn browning of five apple cultivars. I. Chlorophylls and Carotenoids. *Plant Science* 176:78-83.
- Feng, F., Li, M., Ma, F. & Cheng, L. 2014. The effects of bagging and debagging on external fruit quality, metabolites, and the expression of anthocyanin biosynthetic genes in 'Jonagold' apple (*Malus domestica* Borkh.). *Scientia Horticulturae* 165:123-131.
- Gill, S.S. & Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* 48:909-930.
- 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. *HortScience* 40:592-596.
- Gindaba, J. & Wand, S.J.E. 2007a. Climate-ameliorating measures influence photosynthetic gas exchange of apple leaves. *Annals of Applied Biology* 150:75-80.
- Gindaba, J. & Wand, S.J.E. 2007b. Do fruit sunburn control measures affect leaf photosynthetic rate and stomatal conductance in 'Royal Gala' apple? *Environmental and Experimental Botany* 59:160-165.
- Griesbach, J. 2007. *Growing Temperate Fruit Trees in Kenya*. World Agroforestry Centre (ICRAF). Nairobi, Kenya.

- Hannoufa, A. & Hossain, Z. 2012. Regulation of carotenoid accumulation in plants. *Biocatalysis and Agricultural Biotechnology* 1:198-202.
- Harker, F.R., Gunson, F.A. & Jaeger, S.R. 2003. The case for fruit quality: An interpretive review of consumer attitudes, and preferences for apples. *Postharvest Biology and Technology* 28:333-347.
- Harker, F.R., Kupferman, E.M., Marin, A.B., Gunson, F.A. & Triggs, C.M. 2008. Eating quality standards for apples based on consumer preferences. *Postharvest Biology and Technology* 50:70-78.
- Havaux, M. 1998. Carotenoids as membrane stabilizers in chloroplasts. *Trends in Plant Science* 3:147-151.
- Havaux, M. & Niyogi, K.K. 1999. The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. *Proceedings of the National Academy of Sciences of the United States of America* 96:8762-8767.
- Havaux, M., Eymery, F., Porfirova, S., Rey, P. & Dormann, P. 2005. Vitamin E protects against photoinhibition and photooxidative stress in *Arabidopsis thaliana*. *The Plant Cell* 17:3451-3469.
- He, Y., Fukushige, H., Hildebrand, D.F. & Gan, S. 2002. Evidence supporting a role of Jasmonic acid in *Arabidopsis* leaf senescence. *Plant Physiology* 128:435-441.
- Herrera-Rodriguez, M.B., Gonzalez-Fontes, A., Rexach, J., Camacho-Cristobal, J.J., Maldonado, J.J. & Navarro-Gochicoa, M.T. 2010. Role of boron in vascular plants and response mechanisms to boron stresses. *Plant Stress* 4:115-122.
- Hipps, N.A. & Davies, M.J. 2001. Effects of foliar zinc applications at different times in the growing season on tissue zinc concentrations, fruit set, yield and grade out of culinary apple trees. *Acta Horticulturae* 564:145-151.
- Jaeger, S.R., Andani, Z., Wakeling, I.N. & Macfie, H.J.H. 1998. Consumer preferences for fresh and aged apples: a cross-cultural comparison. *Food Quality and Preference* 9:355-366.
- Jahns, P. & Holzwarth, A.R. 2012. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. *Biochimica et Biophysica Acta* 1817:182-193.
- Joubert, J., Theron, K.I. & Lötze, E. 2008. Evaluating pre-harvest foliar calcium applications to increase fruit calcium and reduce bitter pit in 'Golden Delicious' apples. *Scientia Horticulturae* 116:299-304.
- Karuppanapandian, T., Moon, J.C., Kim, C., Manoharan, K. & Kim, W. 2011. Reactive oxygen species in plants: Their generation, signal transduction, and scavenging mechanisms. *Australian*

Journal of Crop Science 5:709-725.

- Kong, W., Liu, F., Zhang, C., Zhang, J. & Feng, H. 2016. Non-destructive determination of Malondialdehyde (MDA) distribution in oilseed rape leaves by laboratory scale NIR hyperspectral imaging. *Scientific Reports* 6:35393.
- Łata, B., Trąpczyńska, A., Oleś, M. 2005. Antioxidant content in the fruit peel, flesh and seeds of selected apple cultivars during cold storage. *Folia Horticulturae* 17(1): 47-60.
- Li, P. & Cheng, L. 2008. The shaded side of apple fruit becomes more sensitive to photoinhibition with fruit development. *Physiologia Plantarum* 134:282-292.
- Liu, M., Li, X., Liu, Y. & Cao, B. 2013. Regulation of flavanone 3-hydroxylase gene involved in the flavonoid biosynthesis pathway in response to UV-B radiation and drought stress in the desert plant, *Reaumuria soongorica*. *Plant Physiology and Biochemistry* 73:161-167.
- Lolicato, S. 2011. Sun Protection for Fruit - A practical manual for protecting sunburn on fruit. Department of Primary Industries, Farm Services Victoria Division, Victoria, Australia.
- Lötze, E. & Theron, K.I. 2007. Evaluating the effectiveness of pre-harvest calcium applications for bitter pit control in 'Golden Delicious' apples under South African conditions. *Journal of Plant Nutrition* 30:471-485.
- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2017. Boron in combination with calcium reduces sunburn in apple fruit. *Boron* 2:123-127.
- Lötze, E., Daiber, S.H. & Midgley, S.J.E. 2018. Evaluating the efficacy of a preharvest combination of calcium and boron as foliar application to reduce sunburn on 'Cripps Pink' apples. *Acta Horticulture* 1217:61-68.
- Lynch, D. V & Thompson, J.E. 1984. Lipoxygenase-mediated production of superoxide anion in senescing plant tissue. *FEBS Letters* 173:251-254.
- Ma, F. & Cheng, L. 2003. The sun-exposed peel of apple fruit has higher xanthophyll cycle-dependent thermal dissipation and antioxidants of the ascorbate-glutathione pathway than the shaded peel. *Plant Science* 165:819-827.
- Macarasin, D., Cohen, L., Eick, A., Rafael, G., Belausov, E., Wisniewski, M. & Droby, S. 2007. *Penicillium digitatum* suppresses production of hydrogen peroxide in host tissue during infection of citrus fruit. *Postharvest Pathology and Mycotoxins* 97:1491-1500.
- Makeredza, B., Marais, H., Schmeisser, M., Lötze, E. & Steyn, W.J. 2015. Ripening associated red color development masks sunburn browning in apple peel. *HortScience* 50:814-818.

- Mditshwa, A., Fawole, O.A., Vries, F., van Der Merwe, K., Crouch, E. & Opara, U.L. 2016. Classification of 'Granny Smith' apples with different levels of superficial scald severity based on targeted metabolites and discriminant analysis. *Journal of Applied Botany and Food Quality* 89:49-55.
- Merzlyak, M.N., Solovchenko, A.E. & Gitelson, A.A. 2003. Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. *Postharvest Biology and Technology* 27:197-211.
- Merzlyak, M.N., Solovchenko, A.E., Smagin, A.I. & Gitelson, A.A. 2005. Apple flavonols during fruit adaptation to solar radiation: Spectral features and technique for non-destructive assessment. *Journal of Plant Physiology* 162:151-160.
- Midgley, S.J.E. & Lötze, E. 2011. Climate change in the Western Cape of South Africa: Trends, projections and implications for chill unit accumulation. *Acta Horticulturae* 903:1127-1134.
- Miqueloto, A., Do Amarante, C.V.T., Steffens, C.A., Dos Santos, A. & Mitcham, E. 2014. Relationship between xylem functionality, calcium content and the incidence of bitter pit in apple fruit. *Scientia Horticulturae* 165:319-323.
- Mortensen, A., Skibsted, L.H. & Truscott, T.G. 2001. The interaction of dietary carotenoids with radical species. *Archives of Biochemistry and Biophysics* 385:13-19.
- Murtic, S., Civic, H., Duric, M., Sekularac, G., Kojovic, R., Kulina, M. & Krsmanovic, M. 2012. Foliar nutrition in apple production. *African Journal of Biotechnology* 11:10462-10468.
- Neilsen, G.H. & Neilsen, D. 2002. Effect of foliar Zn, form and timing of Ca sprays on fruit Ca concentration in new apple cultivars. *Acta Horticulturae* 594:435-443.
- Parveen, M., Asaeda, T. & Rashid, M.H. 2017. Hydrogen sulfide induced growth, photosynthesis and biochemical responses in three submerged macrophytes. *Flora* 230:1-11.
- Peryea, F.J. 2002. Properties and performance of boron spray products for apple. *Acta Horticulturae* 594:211-215.
- Peryea, F.J. & Drake, S.R. 1991. Influence of mid-summer boron sprays on boron content and quality indices of 'Delicious' apple. *Journal of Plant Nutrition* 14:825-840.
- Racskó, J. & Schrader, L.E. 2012. Sunburn of apple fruit: Historical background, recent advances and future perspectives. *Critical Reviews in Plant Sciences* 31:455-504.
- Rahayu, Y.S., Romheld, V. & Bangerth, F. 2001. Does zinc nutrition affect calcium disorder of fruits? *Acta Horticulturae* 564:135-143.

- Rao, M. V., Watkins, C.B., Brown, S.K. & Weeden, N.F. 1998. Active oxygen species metabolism in 'White Angel' x 'Rome Beauty' apple selections resistant and susceptible to superficial scald. *Journal of the American Society for Horticultural Science* 123:299-304.
- Rouault, M. & Richard, Y. 2003. Intensity and spatial extension of drought in South Africa at different time scales. *Water SA* 29:489-500.
- Rouphael, Y., Colla, G., Giordano, M., El-Nakhel, C., Kyriacou, M.C. & De Pascale, S. 2017. Foliar applications of a legume-derived protein hydrolysate elicit dose-dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars. *Scientia Horticulturae* 226:353-360.
- Sabban-Amin, R., Feygenberg, O., Belausov, E. & Pesis, E. 2011. Low oxygen and 1-MCP pretreatments delay superficial scald development by reducing reactive oxygen species (ROS) accumulation in stored 'Granny Smith' apples. *Postharvest Biology and Technology* 62:295-304.
- Saure, M.C. 1996. Reassessment of the role of calcium in development of bitter pit in apple. *Australian Journal of Plant Physiology* 23:237-243.
- Schrader, L., Sun, J., Zhang, J., Felicetti, D. & Tian, J. 2008. Heat and light-induced apple skin disorders: Causes and prevention. *Acta Horticulturae* 772:51-58.
- Schrader, L. E., Zhang, J., and Duplaga, W. K. 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Online. *Plant Health Progress* doi:10.1094/PHP-2001-1004-01-RS.
- Skorzynska-Polit, E. 2007. Lipid peroxidation in plant cells, its physiological role and changes under heavy metal stress. *Acta Societatis Botanicorum Poloniae* 76:49-54.
- Solovchenko, A. & Schmitz-Eiberger, M. 2003. Significance of skin flavonoids for UV-B-protection in apple fruits. *Journal of Experimental Botany* 54:1977-1984.
- Solovchenko, A.E. & Chivkunova, O.B. 2011. Physiological role of anthocyanin accumulation in common Hazel juvenile leaves. *Russian Journal of Plant Physiology* 58:674-680.
- Solovchenko, A.E., Chivkunova, O.B., Merzlyak, M.N. & Gudkovsky, V.A. 2005. Relationships between chlorophyll and carotenoid pigments during on- and off-tree ripening of apple fruit as revealed non-destructively with reflectance spectroscopy. *Postharvest Biology and Technology* 38:9-17.
- Solovchenko, A.E., Chivkunova, O.B., Gitelson, A.A. & Merzlyak, M.N. 2010a. Non-destructive estimation pigment content, ripening, quality and damage in apple fruit with spectral reflectance

in the visible range. *Fresh Produce* 4:91-102.

- Solovchenko, A.E., Merzlyak, M.N. & Pogosyan, S.I. 2010b. Light-induced decrease of reflectance provides an insight in the photoprotective mechanisms of ripening apple fruit. *Plant Science* 178:281-288.
- Somashekaraiah, B. V., Padmaja, K. & Prasad, A.R.K. 1992. Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. *Physiologia Plantarum* 85:85-89.
- Song, X., Diao, J., Ji, J., Wang, G., Guan, C., Jin, C. & Wang, Y. 2016. Molecular cloning and identification of a flavanone 3-hydroxylase gene from *Lycium chinense*, and its overexpression enhances drought stress in tobacco. *Plant Physiology and Biochemistry* 98:89-100.
- Spiteller, G. 2003. The relationship between changes in the cell wall, lipid peroxidation, proliferation, senescence and cell death. *Physiologia Plantarum* 119:5-18.
- Tanny, J. 2013. Microclimate and evapotranspiration of crops covered by agricultural screens: A review. *Biosystems Engineering* 114:26-43.
- Tanny, J., Cohen, S., Grava, A., Naor, A. & Lukyanov, V. 2009. The effect of shading screens on microclimate of apple orchards. *Acta Horticulturae* 807:103-108.
- Torres, C.A., Leon, L. & Sanchez-Contreras, J. 2016. Spectral fingerprints during sun injury development on the tree in Granny Smith apples: A potential non-destructive prediction tool during the growing season. *Scientia Horticulturae* 209:165-172.
- Treutter, D. 2010. Managing phenol contents in crop plants by phytochemical farming and breeding-visions and constraints. *International Journal of Molecular Sciences* 11:807-857.
- Turyomurugyendo, L., Boffa, J.M. & Hakiza, J.J. 2004. Introduction of deciduous fruit tree growing in the tropical highlands of Kabale, Uganda. *Uganda Journal of Agricultural Sciences* 9:470-479.
- Wang, C., Zhang, S.H., Wang, P.F., Li, W. & Lu, J. 2010. Effects of ammonium on the antioxidative response in *Hydrilla verticillata* (L.) Royle plants. *Ecotoxicology and Environmental Safety* 73:189-195.
- Wünsche, J.N., Greer, D.H., Palmer, J.W., Lang, A. & McGhie, T. 2001. Sunburn - The cost of a high light environment. *Acta Horticulturae* 557:349-356.
- Wünsche, J.N., Lombardini, L. & Greer, D.H. 2004. 'Surround' particle film applications - Effects on whole canopy physiology of apple. *Acta Horticulturae* 636:565-571.

- Yang, S., Wang, F., Guo, F., Meng, J.J., Li, X.G. & Wan, S.B. 2015. Calcium contributes to photoprotection and repair of photosystem II in peanut leaves during heat and high irradiance. *Journal of Integrative Plant Biology* 57:486-495.
- Zhang, J., Chen, C., Zhang, D., Li, H., Li, P. & Ma, F. 2014. Reactive oxygen species produced via plasma membrane NADPH oxidase regulate anthocyanin synthesis in apple peel. *Planta* 240:1023-1035.
- Zhang, J., Niu, J., Duan, Y., Zhang, M., Liu, J., Li, P. & Ma, F. 2015. Photoprotection mechanism in the 'Fuji' apple peel at different levels of photooxidative sunburn. *Physiologia Plantarum* 154:54-65.

APPENDIXES

Appendix 1. Sunburn incidence assessment charts

Sunburn incidence assessment in 'Cripps Pink' at harvest of the experiments



0

1

2

3

4

5

6

Source: Daiber (2017). Class 0 (no sunburn). Class 1 to class 4 (progression of sunburn browning). Class 5 (sunburn necrosis). Class 6 (photo oxidative sunburn).

Sunburn incidence assessment in 'Golden Delicious' at harvest



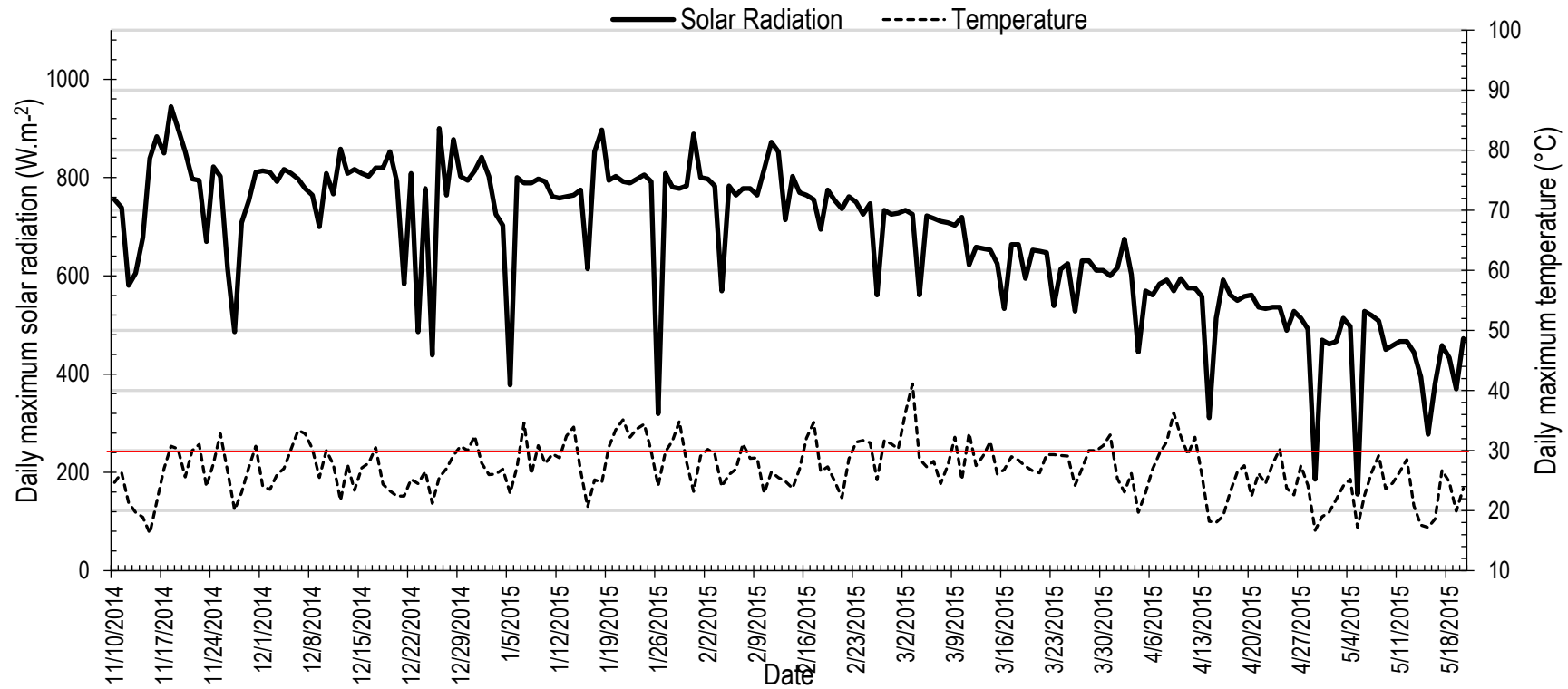
Sunburn incidence scoring chart in 'Granny Smith' at harvest



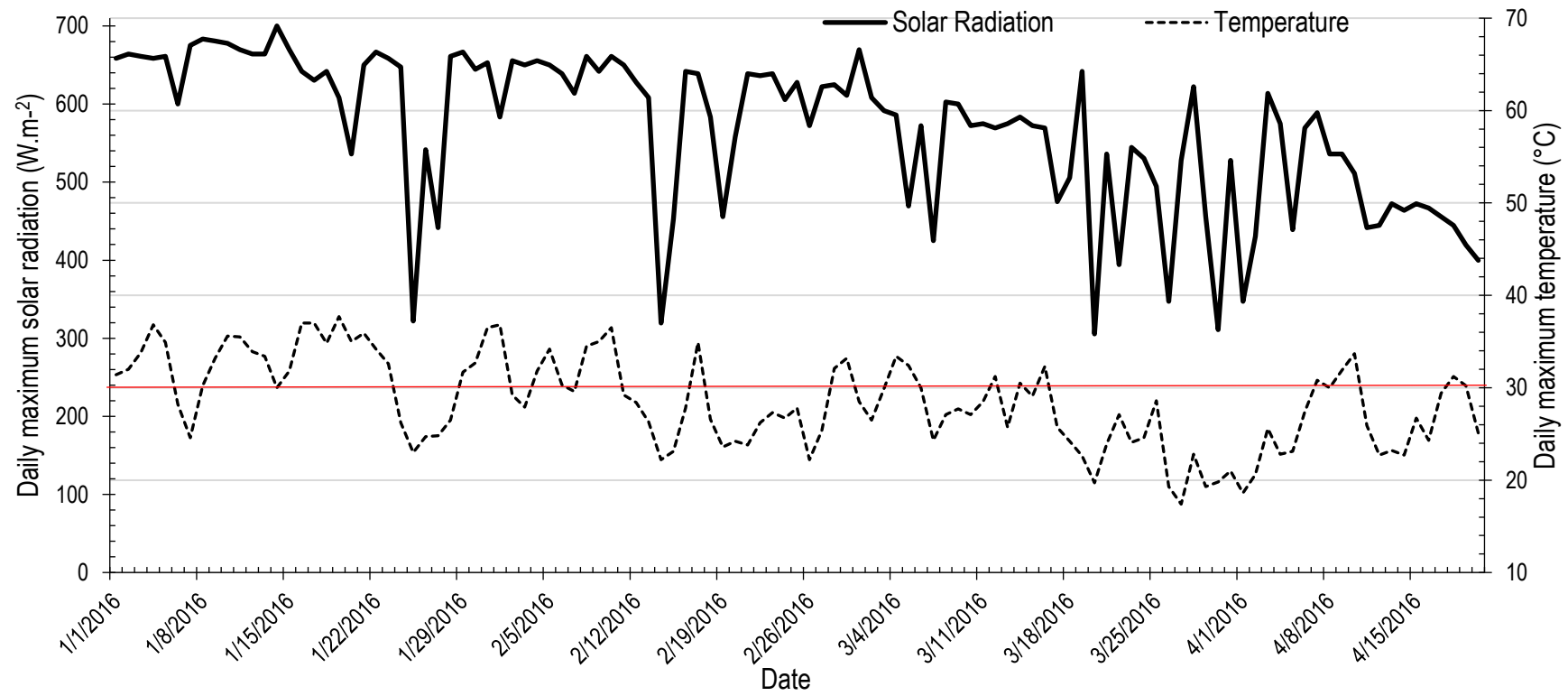
Source: Daiber (2017). Class 0 (no sunburn). Class 1 to class 4 (progression of sunburn browning). Class 5 (sunburn necrosis).

Appendix 2. Daily maximum solar radiation and temperature at the experimental locations

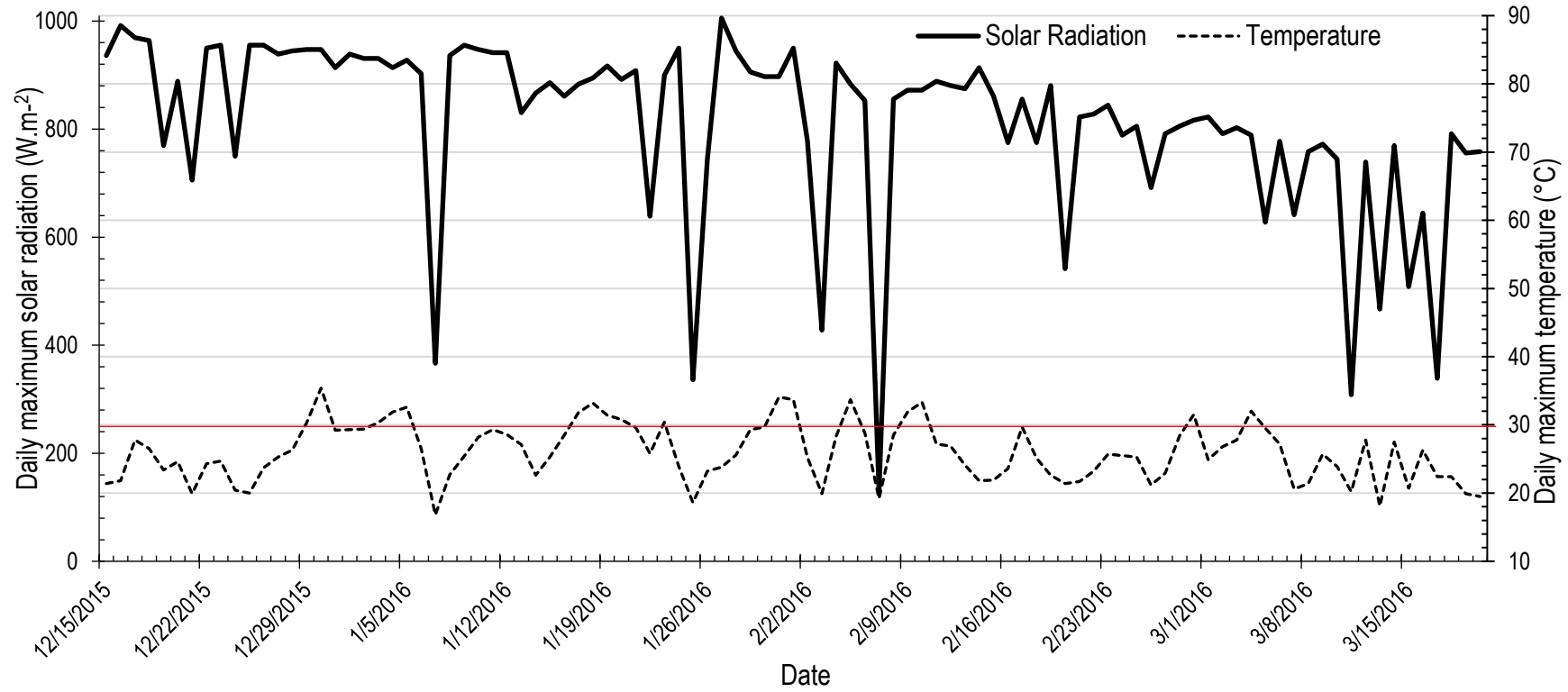
Daily maximum solar radiation ($\text{W}\cdot\text{m}^{-2}$) and temperature ($^{\circ}\text{C}$) at Welgevallen farm during 2014/15.



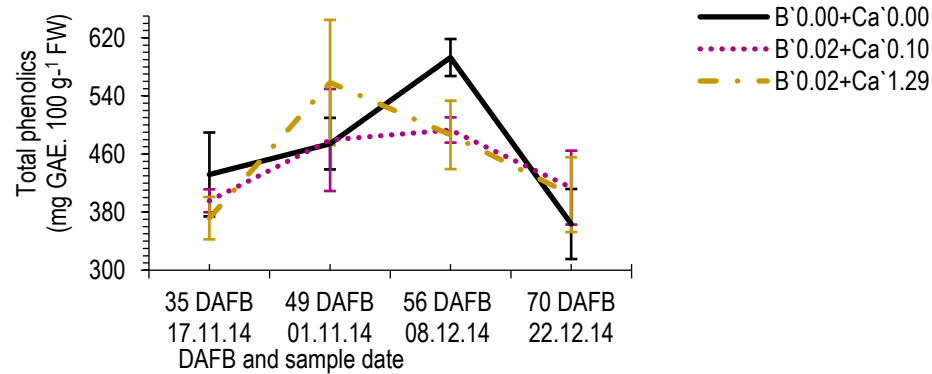
Daily maximum solar radiation (W.m^{-2}) and temperature ($^{\circ}\text{C}$) at Welgevallen farm during 2015/16.



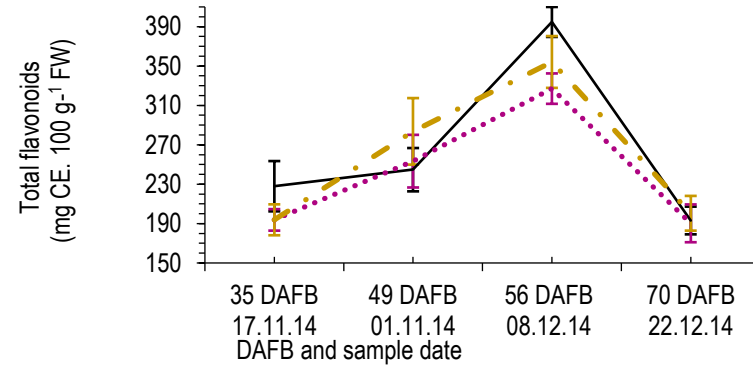
Daily maximum for solar radiation (W.m^{-2}) and temperature ($^{\circ}\text{C}$) at Applethwaite farm during 2015/16.



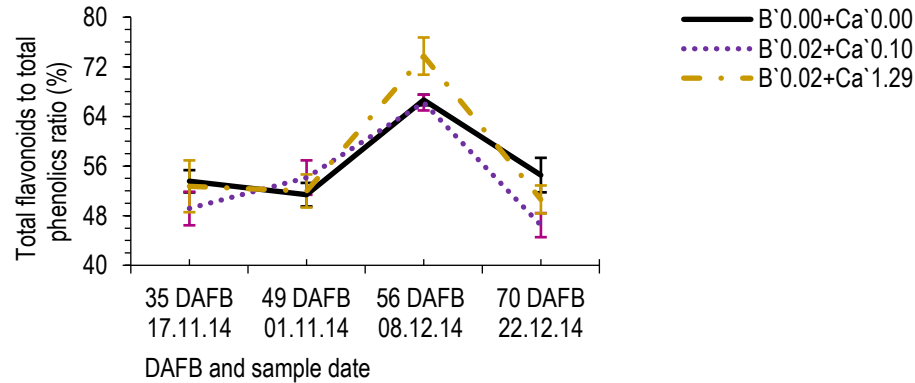
Appendix 3. 'Cripps pink' apple peel phenolics



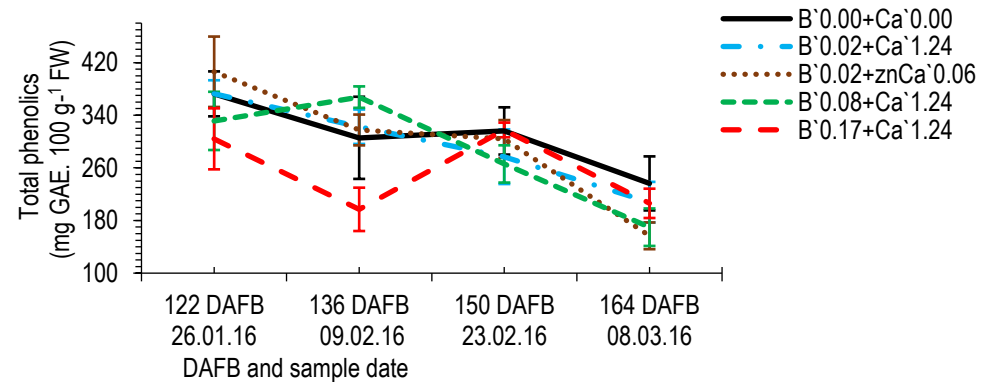
Trends of apple peel phenolics (TP) at early DAFB stages among the foliar B+Ca treatments in 'Cripps Pink'



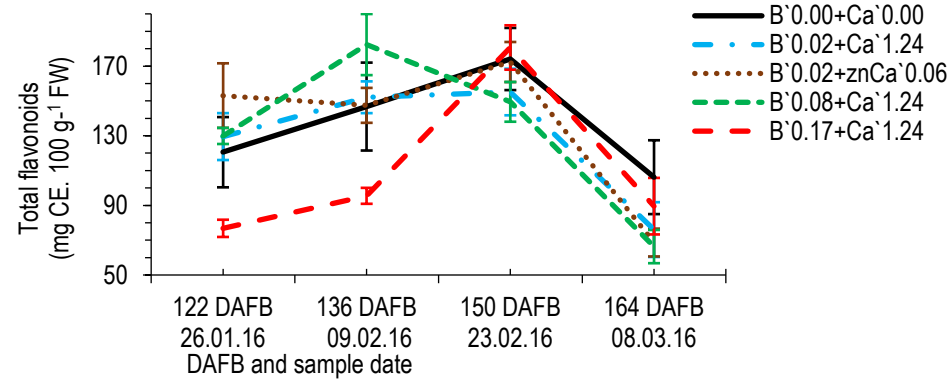
Trends of apple peel flavonoids (TF) at early DAFB stages among the foliar B+Ca treatments in 'Cripps Pink'



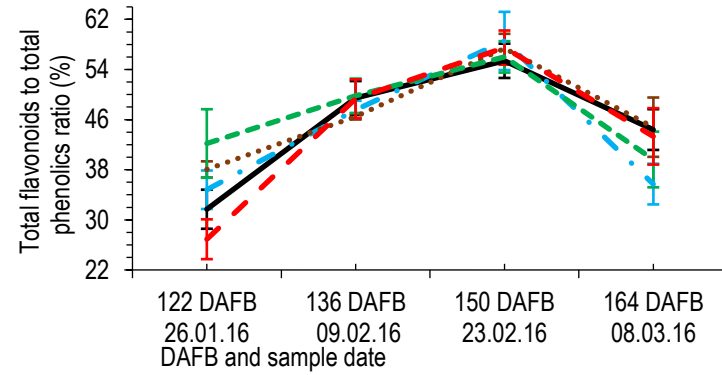
Trends of apple peel flavonoids to phenolics ratio (TF:TP) at early DAFB stages among the foliar B+Ca treatments in 'Cripps Pink'



Trends of apple peel phenolics (TP) towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

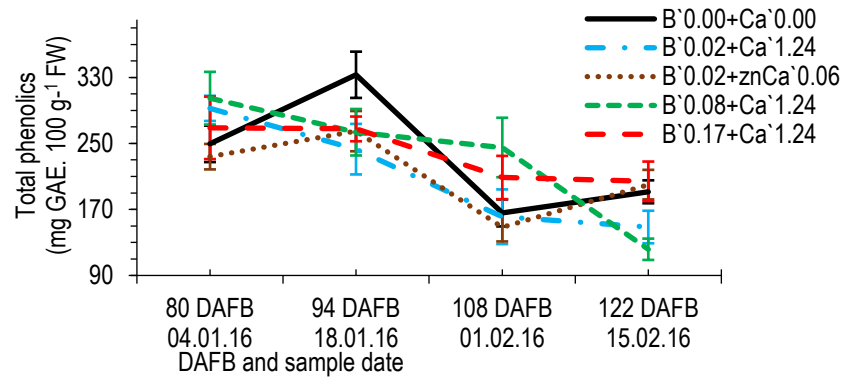


Trends of apple peel flavonoids (TF) towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

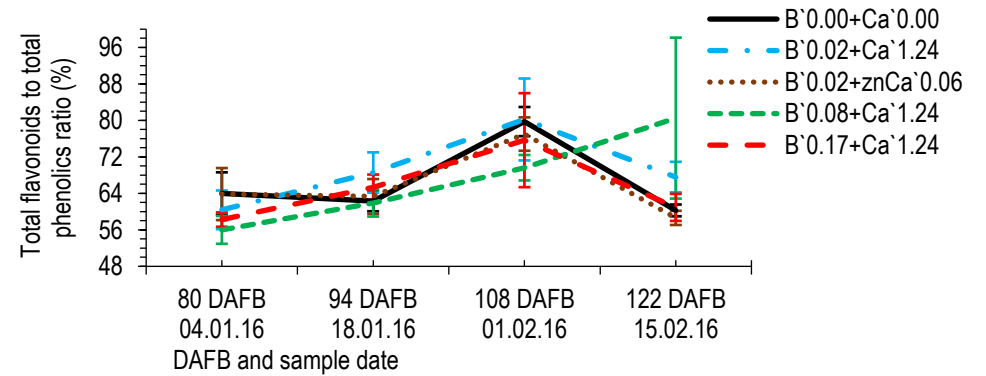


Trends of apple peel flavonoids to total phenolics ratio (TF:TP) towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

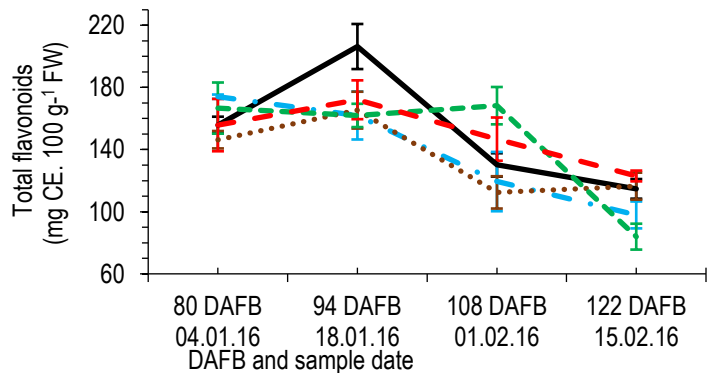
Appendix 4. 'Golden Delicious' apple peel phenolics



Trends of apple peel phenolics (TP) towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

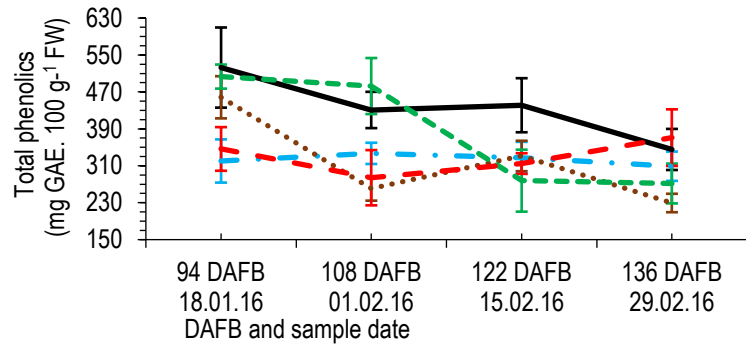


Trends of apple peel flavonoids to phenolics ratio (TF:TP) towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

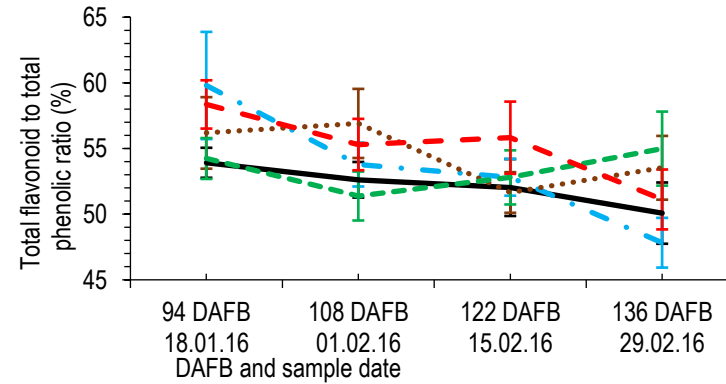


Trends of apple peel flavonoids (TF) towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

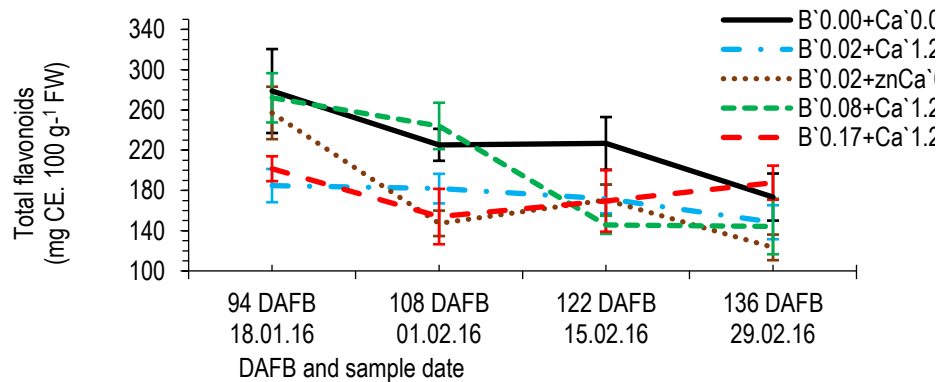
Appendix 5. 'Granny Smith' apple peel phenolics



Trends of apple peel phenolics (TP) towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

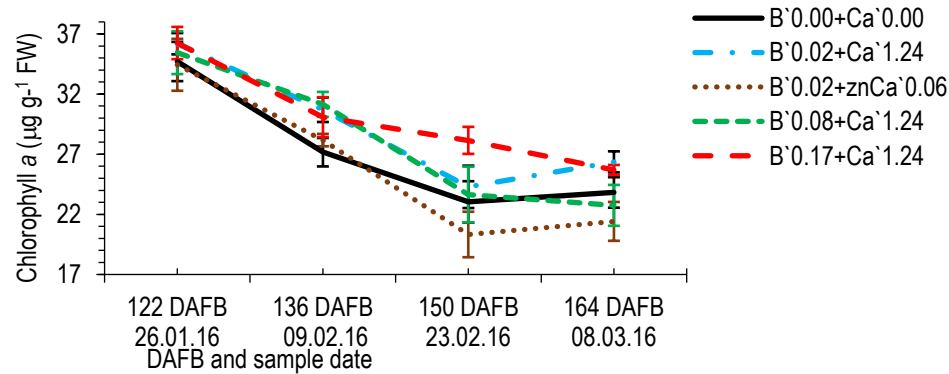


Trends of apple peel flavonoids to phenolics (TF:TP) towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

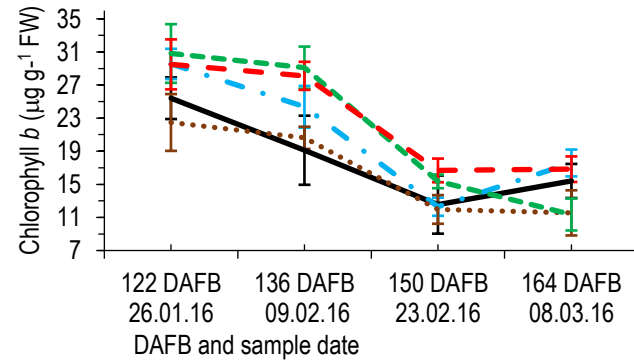


Trends of apple peel flavonoids (TF) trend towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

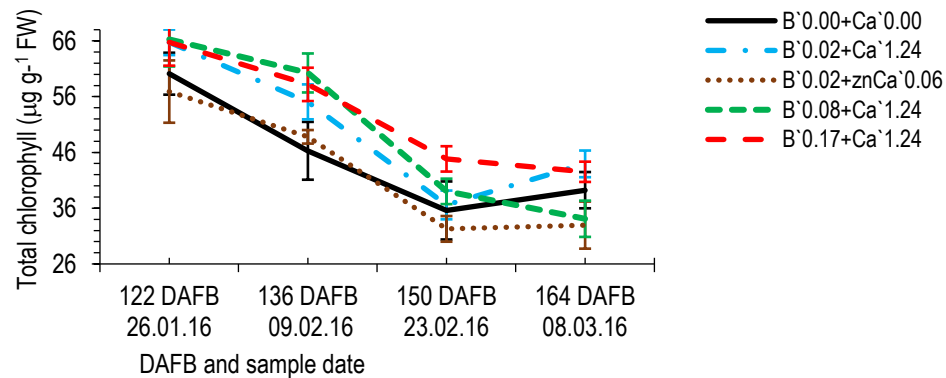
Appendix 6. 'Cripps pink' apple peel photosynthetic pigments



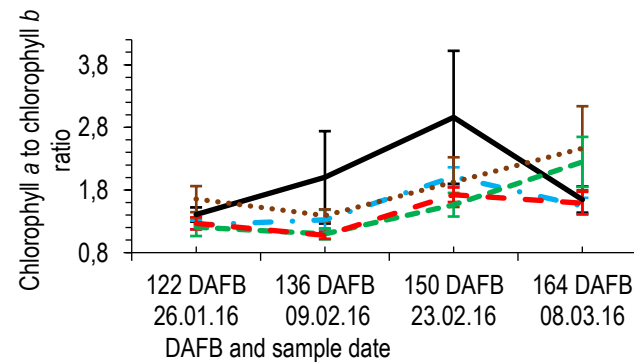
Trends of apple peel chlorophyll a (CHLa) towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'



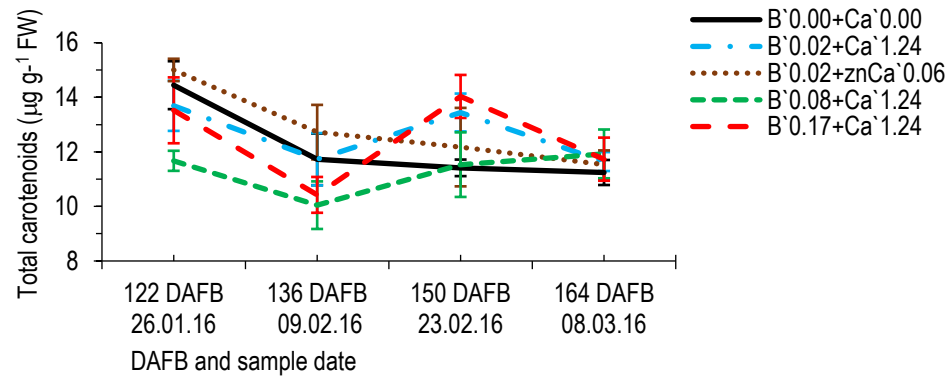
Trends of apple peel chlorophyll b (CHLb) towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'



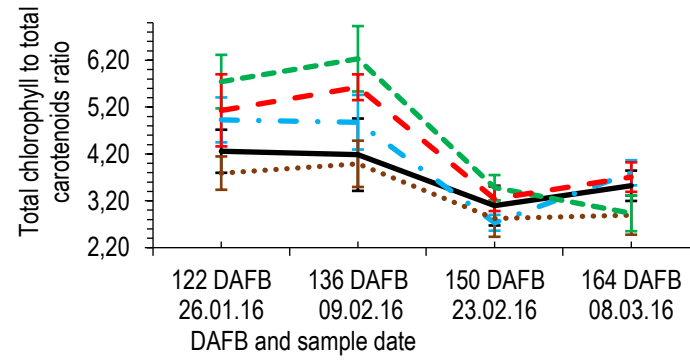
Trends of apple peel total chlorophyll (TCHL) towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'



Trends of apple peel chlorophyll a to chlorophyll b ratio (CHLa:CHLb) towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

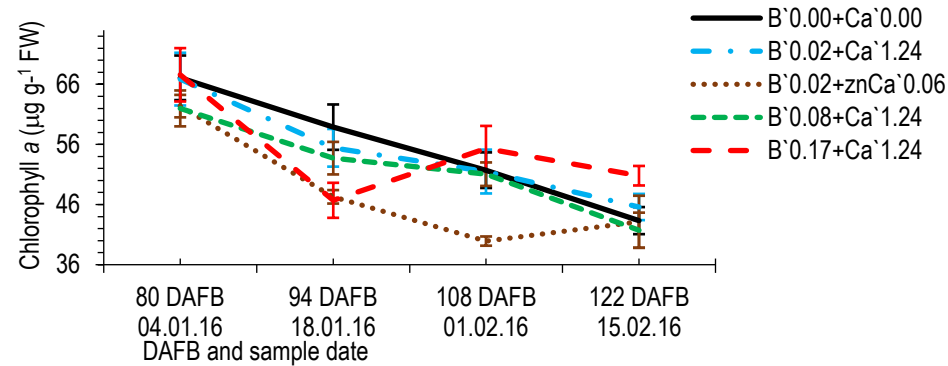


Trends of apple peel carotenoids (TCAR) towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

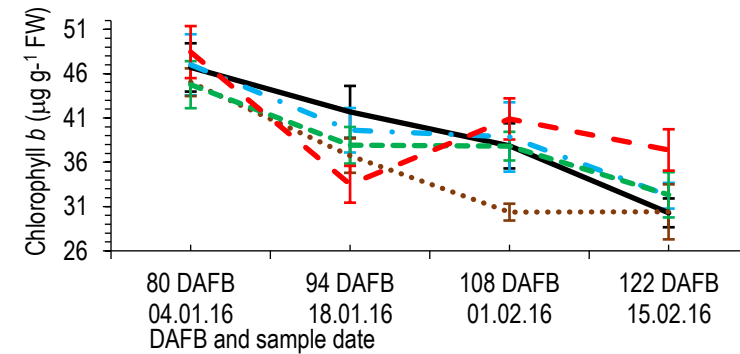


Trends of apple peel chlorophyll to carotenoids ratio (TCHL:TCAR) towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'

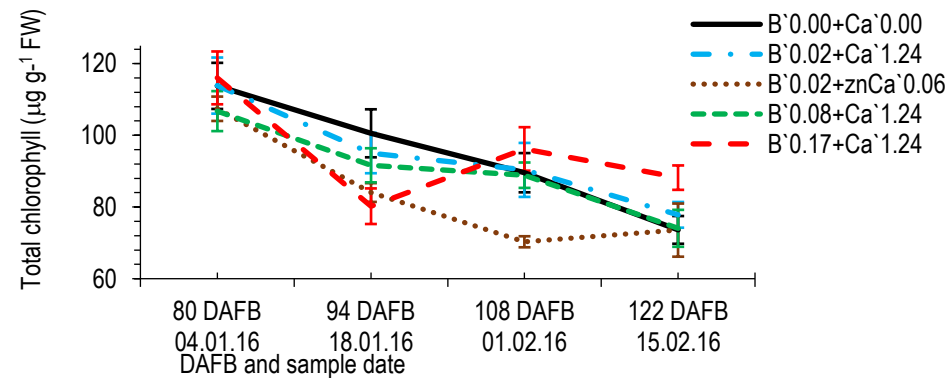
Appendix 7. 'Golden Delicious' apple peel photosynthetic pigments



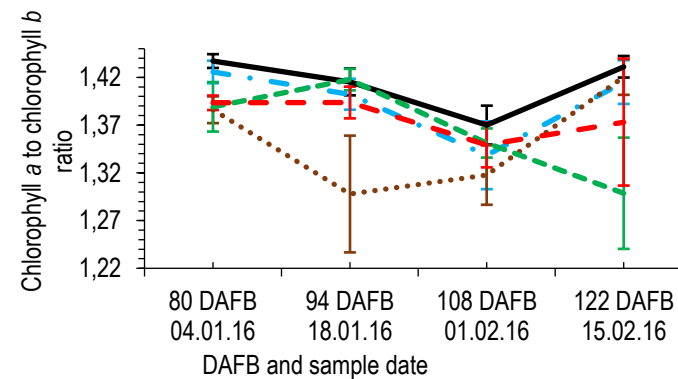
Trends of apple peel chlorophyll a (CHLa) towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'



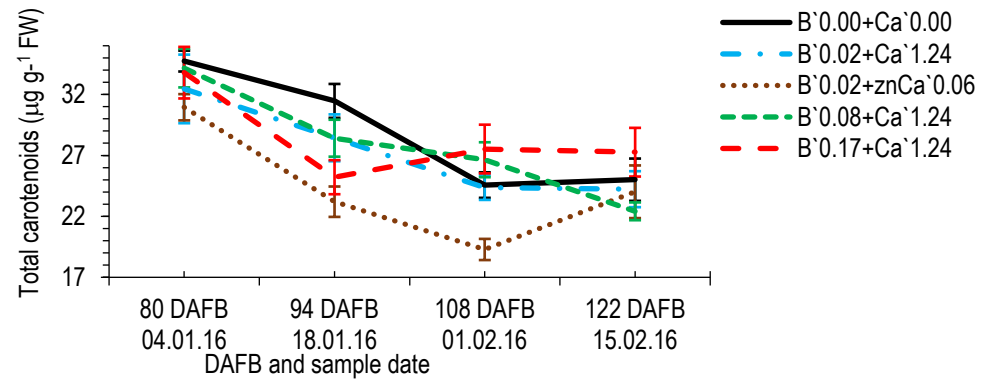
Trends of apple peel chlorophyll b (CHLb) towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'



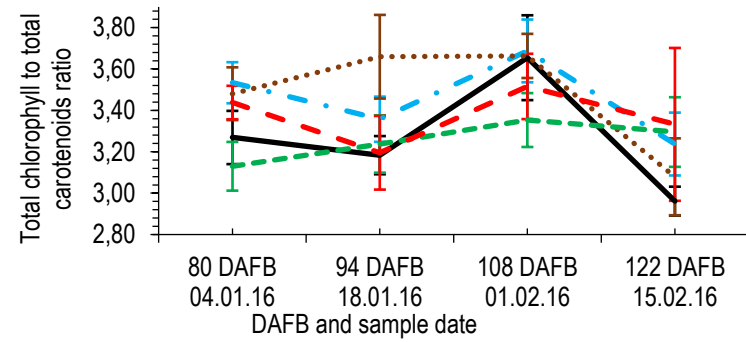
Trends of apple peel total chlorophyll (TCHL) towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'



Trends of apple peel chlorophyll a to chlorophyll b ratio (CHLa:CHLb) towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

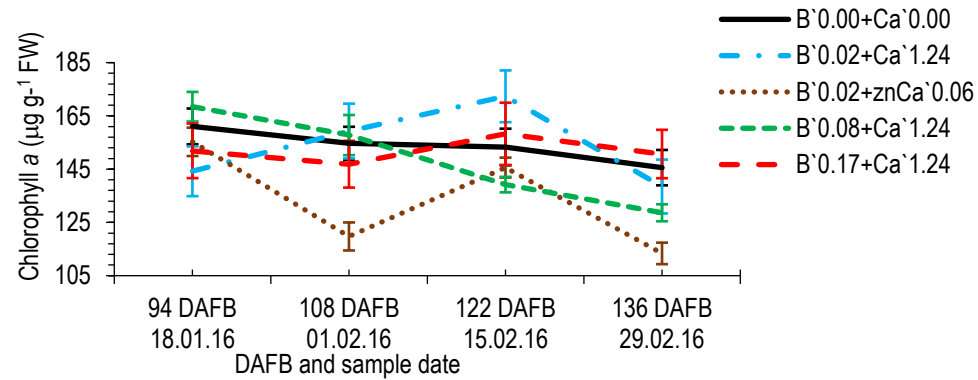


Trends of apple peel carotenoids (TCAR) towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

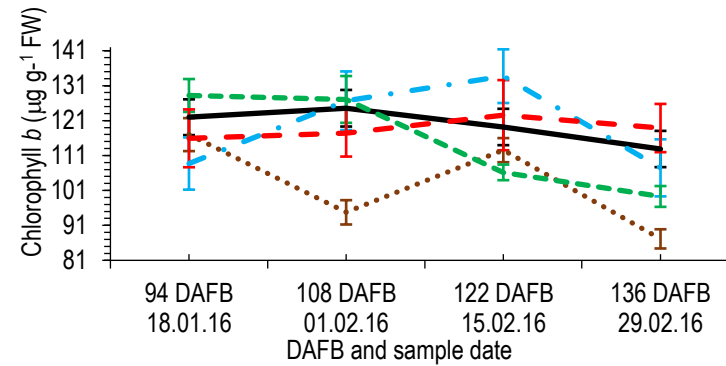


Trends of apple peel chlorophyll to carotenoids ratio (TCHL:TCAR) towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'

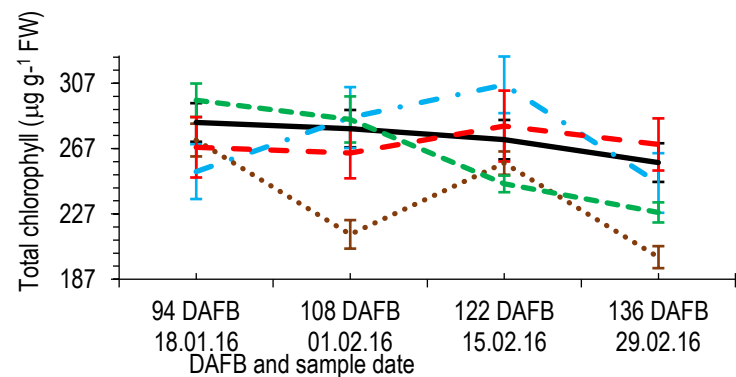
Appendix 8. 'Granny Smith' apple peel photosynthetic pigments



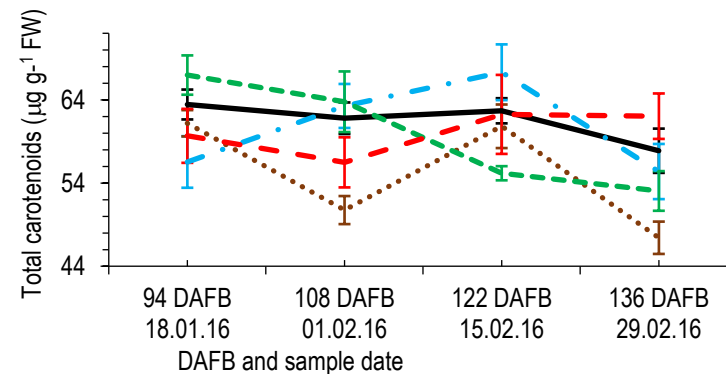
Trends of apple peel chlorophyll a (CHLa) towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'



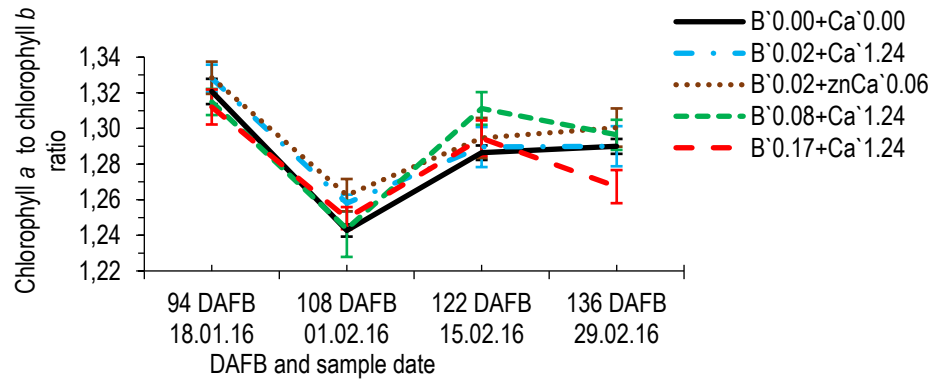
Trends of apple peel chlorophyll b (CHLb) towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'



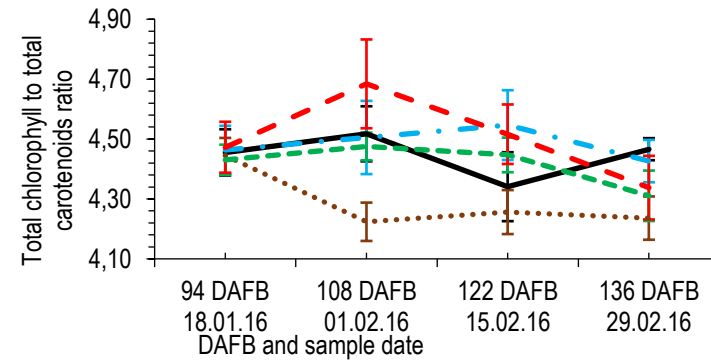
Trends of apple peel chlorophyll (TCHL) towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'



Trends of apple peel carotenoids (TCAR) towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

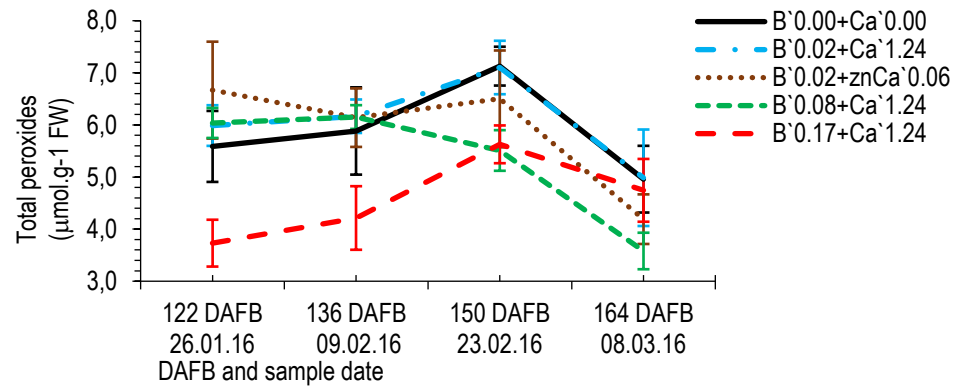


Trends of apple peel chlorophyll a to chlorophyll b ratio (CHLa:CHLb) towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

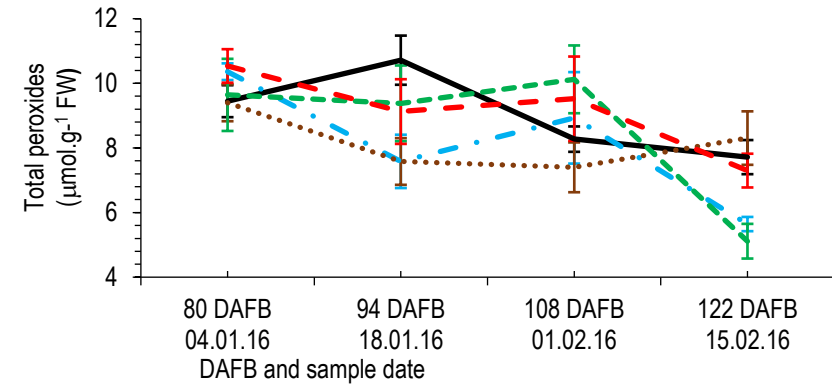


Trends of apple peel chlorophyll to carotenoids ratio (TCHL:TCAR) towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'

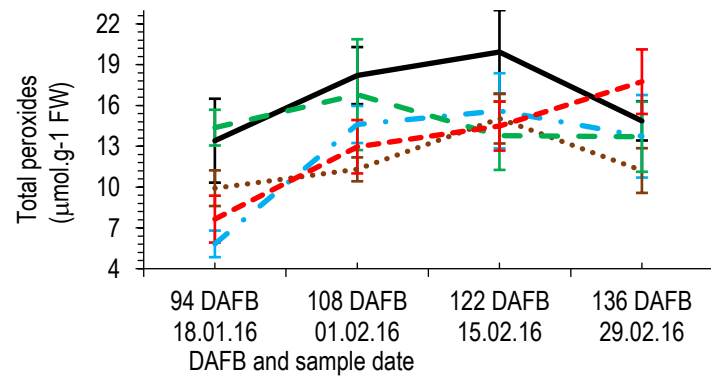
Appendix 9. 'Cripps pink', 'Golden Delicious' and 'Granny Smith' apple peel total peroxides content



Trends of apple peel peroxides (TPERO) towards fruit harvest maturity among the foliar B+Ca treatments in 'Cripps Pink'



Trends of apple peel peroxides (TPERO) towards fruit harvest maturity among the foliar B+Ca treatments in 'Golden Delicious'



Trends of apple peel peroxides (TPERO) trend towards fruit harvest maturity among the foliar B+Ca treatments in 'Granny Smith'