

Impact of shade netting on internal and external quality of 'Nadorcott' mandarin fruit

by

Johané Botes

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



Supervisor: Dr. P.J.R. Cronje

Citrus Research International

Dept. of Horticultural Science

Stellenbosch University



Co-supervisor: Dr. E.W. Hoffman

Dept. of Horticultural Science

Stellenbosch University

South Africa

Co-supervisor: Prof. L. Zacarías

Extraordinary professor: Dept. of Horticultural Science

Stellenbosch University

South Africa

December 2018

DECLARATION

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

December 2018

Copyright © 2018 Stellenbosch University

All rights reserved

ACKNOWLEDGEMENTS

I would like to give my sincere gratitude to the following people (in no particular order) who have contributed to my thesis:

My supervisor, Dr. Paul Cronje and Co-supervisor Dr. Lynn Hoffman, thank you for all the guidance, support and encouragement with this project. I truly appreciate every minute you have put into reading my work and all the valuable inputs into each chapter. Thank you for keeping me motivated till the end.

Professor Martin Kidd, from the Centre for Statistical Consultation at Stellenbosch University for the statistical analyses of my data and for the patience with all the questions.

Gustav Lötze and all the lab personnel. Special thanks to the lab ladies, for all the help with my trials and for making lab days way more fun with all the jokes and stories, I will miss you all a lot.

Jade North for all the admin behind the project and making me familiar with the trial site. Thanks for always offering to help where you can, it is much appreciated.

To the lecturers and staff from the department of Horticulture, Stellenbosch University. Special thanks to Mrs. Carin Pienaar, Petra Mouton and Jakkie Stander.

Professor Karen Theron, thank you for always being willing to help with the statistical questions I had and all the knowledge you shared with me.

Dr. Olaniyi Fawole for assistance with the texture analyzer and making me familiar with the machine and **Ukar Maharaj** for always being available to answers questions regarding the analyzer.

Dr. Rémy Rosalie for the development of the protocol for pigment analysis and **Dr. Elizabeth Rohwer** for all your insightful knowledge you shared with me in the lab, I truly appreciate it.

The Citrus Academy for my bursary throughout my studies at Stellenbosch University.

Citrus Research International, HORTSCI and the **Department of Science and Technology** for the financial support.

Mouton Citrus, for allowing me to use the trial site at Houtkaprug Farm.

#Team Citrus: Du Toit, Helen, Leila and Robert. You guys made this project so much fun, I will miss all our jokes. I wish you all the best for the future. #ek_kort_n_vakansie.

My fellow postgrad students in the department, thanks for lightening the mood during the final phases of this project and fun conversations in the office. Good luck with the rest of your studies.

To my parents, Louw and Joan and brother Carlo, thank you for the prayers, lots of laughter and support in your own unique manner throughout my project. I love you all very much.

Gerhard, for all your love and support throughout the two years and especially during those frustrating days. Thanks for always listening, I bet your citrus jargon has improved a lot.

My home away from home, The Niemann's and Van Der Merwes, thank you for all your love and support.

To all my friends and Fisichem housemates, thank you for the moral support, laughter and all the activities which contributed to a balanced life.

Last but not the least, to my Heavenly Father for giving me my all-day strength to pursue this project and my passion for citrus. *Fill 4:13. I can do all this through Him who gives me strength.*

SUMMARY

In the export-focused citrus industry of southern Africa, the production of high quality fruit i.e. good size, well-developed rind color, blemish-free fruit and good taste is of utmost importance to remain sustainable. Shade nets are a preharvest technique implemented to protect crops against excessive sunlight, wind and hail damage. Shade nets are effective in reducing sunburn, but inconsistent results from previous studies arise about fruit size, rind color and internal quality. Furthermore, the impact on postharvest fruit quality with regard to developing rind physiological disorders and to maintain the physical integrity of the rind as a protection layer against moisture loss and decay development, is not known. Three experiments were conducted to determine how 20% white permanent shade netting would influence, firstly the fruit quality development, secondly the postharvest behavior and lastly the rind physical properties of 'Nadorcott' mandarin fruit produced in Citrusdal, over two seasons (2016 and 2017). In the first experiment, results indicated that the fruit size, rind color and internal quality development patterns were similar for both shade-net exposed and control fruit. Shade netting, however, resulted in an increased fruit size over the 2017 development period, but with no influence on rind color or maturity. Sunburn incidence was effectively reduced by shade nets. During cold storage at 4 and -0.6 °C over a period of 34 days, storage duration did not influence the postharvest quality of shade net fruit differently compared to control fruit in terms of rind color, internal quality parameters and fruit weight loss. In addition, no negative effect of shade netting was evident for the above-mentioned parameters or the incidence of staining. In the third experiment inconsistency occurred with regards to the effect of shade netting on fruit rind strength at harvest. A higher firmness was recorded for shaded fruit in the first season. However, during cold storage, there was some indication that the shade net fruit was more susceptible to deformation and required a lower force over the whole storage duration to puncture the rind, compared to the control fruit. However, a lower force required may also be beneficial as it could be indicative that the fruit may be easier to peel. The firmness of shade net-produced fruit was differently influenced by cold storage in 2016, within the first 14 days of storage, compared to the control. In 2017, control fruit were recorded to have a higher firmness over the storage duration. Both results indicated that the control fruit possibly stored better than shade-net fruit. To conclude, shade net was effective in reducing sunburn without negatively affecting any external and internal quality parameters. The postharvest storage potential of fruit from shade netting did not differ from the control at both storage regimes. Results regarding the impact of shade netting on the physical properties of the rind provides some first guideline threshold force values required before damage is inflicted on the fruit. Knowledge of typical forces applied during the commercial harvest- and pack house processes is, however, required before these values can be compared to commercial practices to determine its importance. The use of shade-netting shows potential as a preventative technology ensuring high quality, unblemished fruit, but requires future studies taking into account the effect of various cultivars, tree age, bearing positions and the microclimatic effect on fruit production and postharvest storage behavior.

OPSOMMING

Die produksie van hoë gehalte vrugte met die verlangde vruggrootte, goed ontwikkelde skilkleur, vlekkelose voorkoms en goeie smaak is van uiterste belang vir die uitvoer-gefokusde sitrusbedryf van suiderlike Afrika om volhoubaar te bly. Skadunette is 'n voor-oes tegniek wat toegepas word om gewasse te beskerm teen oormatige sonlig-, wind- en haelskade. Skadunette is doeltreffend om sonbrand te verminder, maar teenstrydige resultate van vorige studies bestaan egter ten opsigte van vruggrootte, skilkleur, en interne kwaliteit. Optimale na-oes vrugkwaliteit moet ook volgehou word sonder dat fisiologiese skilafwykings ontwikkel, asook die behoud van die fisiese integriteit van die skil om sodoende vogverlies en verrotting te beperk. Drie eksperimente was uitgevoer om vas te stel hoe 20% wit permanente skadunet eerstens die vrugkwaliteit ontwikkeling beïnvloed, tweedens die na-oes gedrag en laastens die fisiese eienskappe van die skil van 'Nadorcott' mandaryn vrugte soos geproduseer in Citrusdal oor twee seisoene (2016 en 2017) affekteer. In die eerste eksperiment het vrugte van beide behandelinge dieselfde ontwikkelingspatroon gevolg in terme van vruggrootte, skilkleur en interne kwaliteit. Skadunet het gelei tot 'n hoër gemiddelde vruggrootte oor die ontwikkelingsperiode van 2017, maar met geen invloed op skilkleur of rypheid nie. Sonbrand was egter drasties verminder onder die net. Tydens koue opberging van vrugte oor 'n tydperk van 34 dae by 4 en -0.6 °C onderskeidelik, was bevind dat die stoortydperk nie die skadunet geproduseerde vrugte anders beïnvloed het teenoor die kontrole vrugte in terme van skilkleur, interne kwaliteit parameters en die vogverlies van vrugte nie. Daarbenewens was geen negatiewe effek van skadunette sigbaar op bogenoemde vrugeienskappe sowel as met die teenwoordigheid van bevlekking ('staining') van vrugte nie. In die derde eksperiment was daar teenstrydige resultate met betrekking tot die skadunet-effek op die skilsterkte by oes. Vrugte blootgestel aan skadunet was fermier by oes in 2017. Tydens koue stoor, het resultate getoon dat die skadunet vrugte moontlik meer geredelik vervorm kan word en 'n laer toegepaste krag benodig het om die skil te prik in vergelyking met die kontrole vrugte. 'n Laer krag kan moontlik 'n aanduiding wees dat dié vrugte makliker sal skil wat voordelig kan wees. Koue opberging het 'n groter effek op die fermheid van skadunet geproduseerde vrugte gehad binne die eerste 14 dae van opberging in vergelyking met kontrole vrugte. In 2017 het kontrole vrugte weer 'n hoër fermheid vertoon oor die opbergingsperiode, wat moontlik aandui dat kontrole vrugte opberging beter hanteer as skadunet geproduseerde vrugte. Die gevolgtrekking van hierdie studie was dat die skadunet effektief was om sonbrand te verlaag, sonder om egter die interne en eksterne kwaliteitseienskappe negatief te beïnvloed. Verder het skadunet geen effek gehad op die opbergingspotensiaal van vrugte by 4 en -0.6 °C nie. Resultate rondom die impak van skadunette op die fisiese eienskappe van die vrugskil was 'n eerste rapportering en dien dus as 'n riglyn van die drempelkrag wat toegepas kan word voordat vrugbeskadiging intree. Kennis van die kommersiële krag wat tydens die oes- en pakhuisproses van toegepassing is, word egter benodig om hierdie waardes te vergelyk met kommersiële waardes om die impak daarvan vas te stel. Die gebruik van skadunette toon potensiaal as voorkomende tegnologie wat hoë kwaliteit, vleklose vrugte verseker, maar verdere studies word vereis waar die effek van verskillende kultivars, boom ouderdom, draerposisies en mikroklimaat op vrug produksie en na-oes opbergingspotensiaal in ag geneem moet word.

NOTE

This thesis is a compilation of chapters, starting with a literature review, followed by three research papers. Each paper is prepared as a scientific paper for submission to the *Journal of the American Society for Horticultural Science*. Repetition or duplication between papers might therefore be necessary. The required spelling is English (United States).

TABLE OF CONTENTS

Declaration	i
Acknowledgements	ii
Summary	iv
Opsomming	v
Note	vi
Table of contents	vii
Chapter 1: General Introduction	1
Chapter 2: Literature Review- Light as an important determinant in <i>Citrus</i> fruit phenology, development and quality	9
1. Introduction	9
2. Characteristics of light	11
3. Light, the driver of photosynthesis	12
4. Citrus flower- and fruit development	13
4.1. Citrus flowering and floral development	13
4.1.1. The effect of light on flower initiation and development	15
4.1.2. Other factors influencing citrus flowering	17
4.2. Fruit formation.....	18
4.2.1. Factors influencing fruit set	19
4.2.2. Fruit structure	20
4.2.3. Fruit development phases	21
4.2.4. Fruit development in <i>Citrus</i> as affected by light levels	22
5. Impact of light on citrus fruit quality	24
5.1. External fruit quality parameters	25
5.1.1. Effect of light on the morphology and anatomy of plants	25

5.1.2. Rind color.....	26
5.2. Internal fruit quality parameters	34
5.2.1. Soluble Solid Content	35
5.2.2. Acidity.....	37
5.2.3. Ascorbic Acid	37
5.3. Biochemical changes in the rind due to light	38
5.3.1. Carbohydrates	38
5.3.2. Secondary metabolites	39
5.4. Environmental damage to the rind.....	41
6. Conclusion.....	42
7. Literature Cited	43
Chapter 3: The influence of 20% white shade netting on ‘Nadorcott’ mandarin fruit development and quality parameters	56
Chapter 4: Cold storage behavior of ‘Nadorcott’ mandarin fruit as affected by preharvest shading	91
Chapter 5: Rind physical properties of shade net produced ‘Nadorcott’ mandarin at harvest and following long-term cold storage.....	121
Chapter 6: General Discussion and Conclusion	148
Addendum A:.....	154

CHAPTER 1

General Introduction

South Africa is currently the 10th largest citrus producing country and the second largest exporter, with a total of 76% (990 749 ton) being exported annually, while 18% is used in processing and the remaining 6% consumed locally. Soft citrus accounts for 16% of the South African citrus production, with ‘Nadorcott’ mandarin being the most widely cultivated (CGA, 2017). ‘Nadorcott’, an easy peeler, has a characteristic orange/red rind color with a good flavor, high sugars and moderate acids, all attributes that makes this cultivar very popular amongst growers and consumers. Regrettably, ‘Nadorcott’ is also very prone to sunburn damage.

The production of high quality citrus fruit is important to assure continued purchasing in the discerning export market. In citrus fruit, rind color and fruit size are the main determinants of external quality, while sugars, organic acids, vitamin C and flavor compounds contributes to the internal quality (Iglesias et al., 2007). In addition, the sugar to acid ratio [soluble solid content (SSC) to acid ratio] is not only used as a maturity index, but also determines the taste (sweet, tart, or insipid) of the juice (Goodrich, 2000). The consumers’ first perception, however, is primarily based on the rind coloration of the mature fruit (Rodrigo et al., 2013) as well as on the absence of any blemishes on the rind (Kays, 1999) such as physiological disorders, or lesions caused by sunburn, wind or insect damage.

Harsh environmental factors are difficult to control, with temperature and sunlight being the main role players influencing fruit quality (Lado et al., 2018). Climate plays an important role in how fruit develop its quality standards, with temperature in particular having a strong influence on the rate of maturation (Reuther, 1988). Kimball (1984) found a strong, positive linear relationship between the °Brix/Acid ratio and accumulated heat for ‘Washington navel’ orange [*C. sinensis* (L.) Osbeck], whilst Richardson et al. (2000) similarly reported that a rise in temperature improved the SSC/acid ratio for ‘MihoWase’ Satsuma mandarin. Furthermore, the influence of light on fruit quality (both external and internal) is evident from studies where canopy positions were compared (Ehara et al., 1981; Khalid et al., 2012; Moon et al., 2011; Sites and Reitz, 1949), since the external canopy is exposed to higher light levels compared to the inside canopy (Cronje et al., 2013; Grant, 1997). Syvertsen and Albrigo (1980) reported grapefruit from sunlit canopy positions to mature earlier and with better juice quality than shaded fruit. Verreyne et al. (2004) concluded in their study where the top, inside and outside

bottom tree sectors of various mandarin types were compared, that the best juice quality [SSC:Total Acidity (TA)] were found on the top and outside bottom sectors.

With emphasis on achieving optimum external quality, low light and temperature levels close to harvest are required for the required chloro-chromoplast conversion associated with rind color development (Goldschmidt, 1988; Thomson et al., 1967). Low temperatures lead to rapid loss of chlorophyll in 'Red Blush' grapefruit and thereby enhance rind coloration (Meredith and Young, 1969), with similar effects reported for 'Nules Clementine' mandarin (Barry and Van Wyk, 2006). High light during fruit development in phase I and II on the other hand increases carotenoid levels, the pigment responsible for the yellow-orange coloration (Gross, 1987; Lewis and Coggins, 1964), thereby improving the rind coloration (Cronje et al., 2013).

In contrast to the cool temperature and light required for color development, excessive sunlight/Ultraviolet -B (UV-B) and temperature exposure are responsible for sunburn in fruit (Ketchie and Ballard, 1968; Racsko and Schrader, 2012; Schrader et al., 2003), a condition which affects the fruit appearance (Schrader et al., 2001) and consequently reduces the packout percentage.

Shade netting is a relatively new orchard technology that is implemented especially to protect fruit from extreme climatic events such as excessive sunlight, hail and wind, with additional protection against insects (Iglesias and Alegre, 2006; Rajapakse and Shahak, 2007). However, shade nets alter the microclimate by reducing light levels (Jifon and Syvertsen, 2001, 2003; Lee et al., 2015), increasing relative humidity (Wachsmann et al., 2014), but with inconsistent results reported with respect to how air temperature is affected (Iglesias and Alegre, 2006; Lee et al., 2015; Wachsmann et al., 2014).

Shade nets are generally considered effective in reducing sunburn on fruit crops (Gindaba and Wand, 2005; Lee et al., 2015). However, with regards to fruit quality parameters, some contrasting results were reported on fruit size (Shahak et al., 2008; Syvertsen et al., 2003), rind coloration (Gindaba and Wand, 2005; Shahak et al., 2004; Syvertsen et al., 2003) and SSC to acid ratio in citrus fruit (Jifon and Syvertsen, 2001; Syvertsen et al., 2003; Wachsmann et al., 2014).

The storage life and quality of the fruit are affected by preharvest conditions in addition to the handling and environmental factors they are exposed to during the postharvest chain (El-Otmani et al., 2011). In the citrus industry in particular, fruit are subjected to physical handling from harvest right through the postharvest chain, which can be deleterious to the fruits appearance (Davies and Albrigo, 1994). The rind, however, serves as a protective layer of the

fruit (El-Otmani et al., 2011). With South Africa exporting the majority of its produce, it is important that cold storage and shipping conditions are such to ensure that the fruit retains its quality and no rind physiological disorders and defects develop during the cold chain, prior to delivery to the consumer of export markets (El-Otmani et al., 2011).

Currently soft citrus from South Africa is shipped between $-0.6\text{ }^{\circ}\text{C}$ and $4\text{ }^{\circ}\text{C}$ (PPECB, 2017). Research conducted on the impact of postharvest storage have recorded changes in rind coloration, SSC, titratable acids and fruit weight loss over time (Çandir et al., 2013; Tietel et al., 2012; Van Wyk et al., 2009). In addition, the fruit firmness is also inversely affected in relation to the storage period (Singh and Reddy, 2006). Furthermore, during postharvest storage, the fruit are susceptible to the development of rind physiological disorders i.e. chilling injury due to low temperature exposure (below $10\text{ }^{\circ}\text{C}$) (Bassal and El-Hamahmy, 2011; Petracek et al., 2006) and non-chilling temperature disorders, such as rind staining/rind breakdown (RB) (Cronje et al., 2011; Lafuente and Zacarías, 2006), all which affect the external appearance, thereby reducing the marketability of the fruit (El-Otmani et al., 2011). Cronje et al. (2011) proposed that the sensitivity of fruit to RB are largely determined by preharvest conditions, with postharvest signals as the possible trigger to initiate and promote symptom development.

Furthermore, light seems to have an impact on rind and/or cuticle thickness of fruit surfaces and leaves (Fallahi and Moon, 1988; Juniper and Jeffree, 1983). It can be postulated that a thicker and stronger rind will be able to withstand physical handling better and therefore prevent rind injuries.

Research reports are limited as to how shade netting affects citrus fruit growth and rind development in terms of the commercial important quality attributes as pertaining to rind color, SSC and TA. This study aims to determine and quantify the impact of 20% white permanent shade netting on ‘Nadorcott’ mandarin fruit quality during development in addition to establishing the rind characteristics (strength and fruit firmness) of control (no netting) compared to shaded fruit, along with how the fruit subjected to netting respond to long-term postharvest cold storage at 4 and $-0.6\text{ }^{\circ}\text{C}$.

This research forms part of a more extended research program on the effect of shade nets on ‘Nadorcott’ mandarin in Citrusdal, South Africa, with other studies addressing aspects of how shade nets influence the orchard microclimate, together with the physiology of the crop (Prins, 2018) as well as the tree phenology and productivity (Brown, 2018).

The first objective was to provide a literature review on the importance of light as a determinant in citrus fruit phenology, development and quality. Emphasis was placed on

aspects of flowering, fruit set, and fruit development (internal and external quality) in addition to sugar accumulation in the rind and rind anatomy as influenced by light.

The second objective was to determine if shade netting has a negative impact on the external and internal quality variables of ‘Nadorcott’ fruit during fruit development.

The third objective was to determine whether shade netting affects the postharvest storage potential of the fruit.

The fourth objective was to determine the effect shade netting has on rind strength and fruit firmness at harvest as well as after cold storage.

This study thus aims to provide better insights into the use of permanent shade netting on ‘Nadorcott’ fruit and its effect on fruit development, fruit quality, postharvest storability and the rind properties under South African conditions.

Literature Cited

- Barry, G.H. and A.A. van Wyk. 2006. Low-temperature cold shock may induce rind colour development of ‘Nules Clementine’ mandarin (*Citrus reticulata* Blanco) fruit. *Postharvest Biol. Technol.* 40:82–88.
- Bassal, M. and M. El-Hamahmy. 2011. Hot water dip and preconditioning treatments to reduce chilling injury and maintain postharvest quality of Navel and Valencia oranges during cold quarantine. *Postharvest Biol. Technol.* 60:186–191.
- Brown, R. 2018. Effect of permanent shade netting on ‘Nadorcott’ mandarin tree phenology and productivity. Stellenbosch Univ., South Africa. MSc. Thesis. (*submitted*).
- Çandir, E., M. Kamiloğlu, D. Üstün, and G.T. Kendir. 2013. Comparison postharvest quality of conventionally and organically grown ‘Washington Navel’ oranges. *J. App. Bot. Food Quality* 86:59–65.
- Citrus Growers Association of Southern Africa (CGA). 2017. Key industry statistics for citrus growers 2017. 7 November 2017. <<http://www.citrusresourcewarehouse.org.za/home/document-home/information/cga-key-industry-statistics/4589-cga-key-industry-statistics-2017>>
- Cronje, P.J.R., G.H. Barry, and M. Huysamer. 2011. Postharvest rind breakdown of ‘Nules Clementine’ mandarin is influenced by ethylene application, storage temperature and storage duration. *Postharvest Biol. Technol.* 60:192–201.
- Cronje, P.J.R., G.H. Barry, and M. Huysamer. 2013. Canopy position affects pigment expression and accumulation of flavonoid carbohydrates of ‘Nules Clementine’ mandarin fruit, thereby affecting rind condition. *J. Amer. Soc. Hort. Sci.* 138:217–224.

- Davies, F.S. and L.G. Albrigo. 1994. *Citrus*. CAB International, Wallingford.
- Ehara, T., T. Nogata, and T. Nakamuta. 1981. Studies on fruit-bearing branches of 'Satsuma' mandarins. *Proc. Int. Soc. Citricult.* 1:209–214.
- El-Otmani, M., A. Ait-Oubahou, and L. Zacarías. 2011. *Citrus spp.*: Orange, mandarin, tangerine, clementine, grapefruit, pomelo, lemon and lime, p. 437–514. In: E.M. Yahia (ed.). *Postharvest biology and technology of tropical and subtropical fruits*. Vol 2. Woodhead Publishing, Cambridge, UK.
- Fallahi, E. and J.W. Moon, Jr. 1988. Effect of canopy position on quality, photosynthesis and mineral nutrition of four citrus varieties. *Univ. of Arizona Citrus Rpt.* P-76:5–12. 28 September 2017. < <http://hdl.handle.net/10150/215697/>>.
- Gindaba, J. and S.J.E. Wand. 2005. Comparative effects of evaporative cooling, kaolin particle film, and shade net on sunburn and fruit quality in apples. *HortScience* 40:592–596.
- Goldschmidt, E.E. 1988. Regulatory aspects of chloro-chromoplast interconversions in senescing *Citrus* fruit peel. *Isr. J. Bot.* 37:123–130.
- Goodrich, R. 2000. Consumer product quality attributes and contribution of citric acid in juices. *Proc. Intl. Soc. Citricult. IX Congr.* 1:639.
- Grant, R.H. 1997. Partitioning of biologically active radiation in plant canopies. *Int. J. Biometeorol.* 40:26–40.
- Gross, J. 1987. *Pigments in fruits*. Academic Press, London.
- Iglesias, D.J., M. Cercós, J.M. Colmenero-Flores, M.A. Naranjo, G. Ríos, E. Carrera, O. Ruiz-Rivero, I. Lliso, R. Morillon, F.R. Tadeo, and M. Talon. 2007. Physiology of citrus fruiting. *Braz. J. Plant Physiol.* 19:333–362.
- Iglesias, I. and S. Alegre. 2006. The effect of anti-hail nets on fruit protection, radiation, temperature, quality and profitability of 'Mondial Gala' apples. *J. Appl. Hort.* 8:91–100.
- Jifon, J.L. and J.P. Syvertsen. 2001. Effects of moderate shade on citrus leaf gas exchange, fruit yield and quality. *Proc. Fla. State Hort. Soc.* 114:177–181.
- Jifon, J.L. and J.P. Syvertsen. 2003. Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves. *Tree physiol.* 23:119–27.
- Juniper, B.E. and C.E. Jeffree. 1983. *Plant surfaces*. Edward Arnold, London.
- Kays, S.J. 1999. Preharvest factors affecting appearance. *Postharvest Biol. Technol.* 15:233–247.
- Ketchie, D.O. and A.L. Ballard. 1968. Environments which cause heat injury to 'Valencia' oranges. *Proc. Amer. Soc. Hort. Sci.* 93:166–172.

- Khalid, S., A.U. Malik, B.A. Saleem, A.S. Khan, M.S. Khalid, and M. Amin. 2012. Tree age and canopy position affect rind quality, fruit quality and rind nutrient content of ‘Kinnow’ mandarin (*Citrus nobilis* Lour \times *Citrus deliciosa* Tenora). *Scientia Hort.* 135:137–144.
- Kimball, D.A. 1984. Factors affecting the rate of maturation of citrus fruits. *Proc. Fla. State Hort. Soc.* 97:40–44.
- Lado, J., G. Gambetta, and L. Zacarias. 2018. Key determinants of citrus fruit quality: Metabolites and main changes during maturation. *Scientia Hort.* 233:238–248.
- Lafuente, M.T. and L. Zacarías. 2006. Postharvest physiological disorders in citrus fruit. *Stewart Postharvest Rev.* 1:1–9.
- Lee, T.C., P.J. Zhong, and P.T. Chang. 2015. The effects of preharvest shading and postharvest storage temperatures on the quality of ‘Ponkan’ (*Citrus reticulata* Blanco) mandarin fruits. *Scientia Hort.* 188:57–65.
- Lewis, L.N. and C.W. Coggins. 1964. The inhibition of carotenoid accumulation in Navel oranges by gibberellin A₃, as measured by thin layer chromatography. *Plant and Cell Physiol.* 5:457–463.
- Meredith, F.I. and R.H. Young. 1969. Effect of temperature on pigment development in ‘Red Blush’ grapefruit and ‘Ruby’ blood oranges. *Proc. First. Intl. Citrus Symp.* 1:271–276.
- Moon, D. G., J.H. Joa, Y.E. Moon, K.C. Seong, C.H. Kim, and Y.K. Ahn. 2011. Plant growth and fruit quality as affected by canopy locations in ‘Shiranuhi’ mandarin. *Hort. Environ. Biotechnol.* 52:443–447.
- Petracek, P.D., D.F. Kelsey, and W. Grierson. 2006. Physiological peel disorders, p. 397–419. In: W.F. Wardowski, W.M. Miller, D.J. Hall, and W. Grierson (eds.). *Fresh citrus fruits*, second edition. Florida Science Source, Inc., Longboat key, Florida.
- Perishable Products Export Control Board (PPECB). 2017. Carrying temperature regimes of perishable produce for sea export official PPECB instructions. 7 November 2017. <<https://ppecb.com/wp-content/uploads/2015/03/HP22-PP04-04-17-Carrying-temperature-regimes-of-perishable-produce-for-sea-export-official-PPECB-instructions-rev-12.pdf>>
- Prins, M.D. 2018. Impact of shade netting on orchard microclimate and the physiology of ‘Nadorcott’ mandarin. Stellenbosch Univ., South Africa. MSc. Thesis. (*submitted*).
- Racsko, J. and L.E. Schrader. 2012. Sunburn of apple fruit: Historical background, recent advances and future perspectives. *Critical Rev. Plant Sci.* 31:455–504.
- Rajapakse, N.C. and Y. Shahak. 2007. Light-quality manipulation by horticulture industry, p. 290–311. In: G.C. Whitelam and K.J. Halliday (eds.) *Light and plant development*. Blackwell Publishing Ltd, UK.

- Reuther, W. 1988. Climate and fruit quality, p. 9–23. In: J.J. Ferguson and W. Wardowski (eds.). Factors affecting fruit quality. Proceedings, Citrus short course. Citrus Research and education center, Lake Alfred, Florida.
- Richardson, A.C., K.B. Marsh, and E.A. MacRae. 2000. Temperature effects on the composition of ‘Satsuma’ mandarins in New Zealand. Proc. Intl. Soc. Citricult. IX Congr. 1:303–307.
- Rodrigo, M., B. Alquézar, E. Alós, J. Lado, and L. Zacarías. 2013. Biochemical bases and molecular regulation of pigmentation in the peel of citrus fruit. *Scientia Hort.* 163:46–62.
- Schrader, L.E., J. Zhang, and W.K. Duplaga. 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Online. Plant Health Progress. 24 April 2017. <<http://www.plantmanagementnetwork.org/pub/php/research/sunburn/>>
- Schrader, L., J. Zhang, and J. Sun. 2003. Environmental stresses that cause sunburn of apple. *Acta Hort.* 618:397–405.
- Shahak, Y., E.E. Gussakovsky, Y. Cohen, S. Lurie, R. Stern, S. Kfir, A. Naor, I. Atzmon, I. Doron, Y. Greenblat-Avron. 2004. ColorNets: A new approach for light manipulation in fruit trees. *Acta Hort.* 636:609–616.
- Shahak, Y., K. Ratner, Y.E. Giller, N. Zur, E. Or, E.E. Gussakovsky, R. Stern, P. Sarig, E. Raban, E. Harcavi, I. Doron, and Y. Greenblat-Avron. 2008. Improving solar energy utilization, productivity and fruit quality in orchards and vineyards by photoselective netting. *Acta Hort.* 772:65–72.
- Singh, K.K. and B.S. Reddy. 2006. Post-harvest physico-mechanical properties of orange peel and fruit. *J. Food Eng.* 73:112–120.
- Sites, J.W. and H.J. Reitz. 1949. The variation in individual Valencia oranges from different locations of tree as a guide to sampling methods and spot picking for quality. Part I. Soluble solids in the juice. *Proc. Amer. Soc. Hort. Sci.* 54:1–9.
- Syvertsen, J.P. and L.G. Albrigo. 1980. Some effects of grapefruit tree canopy position on microclimate, water relations, fruit yield and juice quality. *J. Amer. Soc. Hort. Sci.* 105:454–459 (abstr.).
- Syvertsen, J.P., C. Goñi, and A. Otero. 2003. Fruit load and canopy shading affect leaf characteristics and net gas exchange of ‘Spring’ navel orange trees. *Tree Physiol.* 23:899–906.
- Thomson, W.W., L.N. Lewis, and C.W. Coggins. 1967. The reversion of chromoplasts to chloroplasts in Valencia oranges. *Cytologia* 32:117–124.

- Tietel, Z., E. Lewinsohn, E. Fallik, and R. Porat. 2012. Importance of storage temperatures in maintaining flavor and quality of mandarins. *Postharvest Biol. Technol.* 64:175–182.
- Van Wyk, A.A., M. Huysamer, and G.H. Barry. 2009. Extended low-temperature shipping adversely affects rind colour of ‘Palmer Navel’ sweet orange [*Citrus sinensis* (L.) Osb.] due to carotenoid degradation but can partially be mitigated by optimizing post-shipping holding temperature. *Postharvest Biol. Technol.* 53:109–116.
- Verreyne, J.S., E. Rabe, and K.I. Theron. 2004. Effect of bearing position on fruit quality of mandarin types. *S. Afr. J. Plant Soil.* 21:1–7.
- Wachsmann, Y., N. Zur, Y. Shahak, K. Ratner, Y. Giler, L. Schlizerman, A. Sadka, S. Cohen, V. Garbinshikof, B. Giladi, and M. Faintzak. 2014. Photosensitive anti-hail netting for improved citrus productivity and quality. *Acta Hort.* 1015:169–176.

CHAPTER 2

Literature Review: Light as an important determinant in *Citrus* fruit phenology, development and quality

1. Introduction

Plants depend on light for growth and development (Batschauer, 1999), whereas light is also essential for photosynthesis (Lang et al., 1985). Light quality and quantity influence developmental processes in plants such as germination, growth, maturation, along with playing a major role in determining productivity (Grant, 1997). The presence of photoreceptors in plants allow them to absorb light, which is followed by a specific response (Batschauer, 1999; Grant, 1997). Changes in plant morphology in response to light is referred to as photo-morphogenesis (Rajapakse and Shahak, 2007) and is known to be influenced differently by various spectral qualities i.e. blue, green and red light (Mortensen and Stromme, 1987; Percy, 1989). Mortensen and Stromme (1987) reported that blue light exposure produced a significantly higher plant height and greater total leaf area in *Chrysanthemum* 'Refour' (*Chrysanthemum x morifolium*), compared to the other spectral qualities of green, yellow, red and natural light. Secondary metabolites synthesis, particularly that of flavonoids, is especially influenced by the light environment (Caldwell et al., 1983; Lois, 1994).

Solar radiation is not only essential for photosynthesis, but it also affects the transpiration and temperature of the plants (Lang et al., 1985). However, excess radiation causes heat stress and consequently reduces the CO₂ assimilation, thereby negatively affecting photosynthesis (Jifon and Syvertsen, 2001). In addition to the light intensity exposure, the quality of light in terms of its composition is equally important. Ultraviolet exposure to *Arabidopsis* 'Columbia' (*Arabidopsis thaliana* L.) resulted in early flowering, increased leaf size, leaf death and in some cases plant death (Lois, 1994). Lois and Buchanan (1994) also reported morphological changes in *Arabidopsis* as a result of UV-B exposure. In a review by Mackerness (2000) on plant responses to UV-B stress, high light intensities were again closely associated with a change in the growth and development of plants, which may include significant changes in the pigment composition of the fruit, altered flowering time and even loss in photosynthetic activity.

Such a differential effect of light is clearly seen in the modification of the spectral distribution of light as it penetrates into the tree canopy, resulting in higher light intensities on

the outside canopy compared to the inside (Cronje et al., 2013; Grant, 1997). In *Citrus* species (spp.), differences in fruit quality parameters in relation to canopy position, with emphasis on rind coloration, soluble solid percentages and ascorbic acid content have been reported by several researchers (Cronje et al., 2013; Khalid et al., 2012; Reitz and Sites, 1948; Winston and Miller, 1948). It is, however, not only the light intensity that differs amongst the canopy positions, but also other micro-meteorological elements such as the air temperature and relative humidity (Suzuki et al., 1973).

Strong emphasis is placed in the fruit industry to produce fruit of high nutritive quality, with the flavor and aroma associated with and expected of citrus fruit (Zou et al., 2016). In citrus fruit, juice quality is largely determined by the total soluble solids (TSS) and the sugar to acid ratio (Hodgson, 1967). In addition to the distinctive taste, an attractive rind color is of special importance in the fresh fruit market (Rodrigo et al., 2013) where this quality factor along with a blemish free external appearance is amongst of the main reasons for citrus's popularity with consumers (Zou et al., 2016).

Shade nets are a relative new pre-harvest orchard technique that is implemented in horticultural crops production systems, primarily for the protection from excessive solar radiation, but also for its physical protection against hail, wind and insect damage (Rajapakse and Shahak, 2007; Shahak et al., 2004). Nets do not only effectively reduce the solar radiation (Cohen et al., 2000; Raveh et al., 2003) and therefore reduce sunburn incidence (Lee et al., 2015; Smit, 2007), but also modify the microclimate under the net with regard to temperature, relative humidity and wind speed (Pérez et al., 2006; Wachsmann et al., 2014). An in-depth review on the influence of protective netting on tree physiology and fruit quality of apples has been done by Mupambi et al. (2018). Further, the application of shade nets of color in horticulture was reviewed by Stamps (2009). Shade nets of particular colors (photo-selective nets) have been reported to result in specific light filtrations, which cause a modification of light quality under the nets (Shahak et al., 2004; Shahak, 2008).

The use of shade nets in citriculture has not received in-depth attention in the literature. The aim of this review is therefore to report on light manipulations in fruit production as caused by shade netting. Emphasis is placed on how light quality and quantity may affect citrus external and internal fruit quality parameters, with special reference to the rind pigments and rind color development. Aspects such as defining light quality as relevant to citrus production, and the role thereof in photosynthesis and on the reproductive development of both flower and fruit formation are discussed broadly. Biochemical changes in the rind as driven by light as well environmental damage associated with particular light conditions are highlighted.

2. Characteristics of light

Light has both particle and wave properties. A light particle is known as a photon and contains electromagnetic radiation (light energy). Light energy is delivered in discrete packets of quanta, a term that is used to define the amount of energy in a photon (Pearcy, 1989; Taiz et al., 2015). There is an inverse relationship between the wavelength and the energy content of a photon (Fig. 1). A photon with a wavelength of 700 nm contains 171 $\text{kJ}\cdot\text{mol}^{-1}$ energy, whereas a shorter wavelength (400 nm) contains almost double the amount of energy (299 $\text{kJ}\cdot\text{mol}^{-1}$) (Pearcy, 1989).

The light spectrum is divided into three ranges that are important with regards to growth and development of plants: UV (< 400 nm), visible (400-700 nm) and far-red (700-800 nm) (Fig. 1). The UV spectrum comprises of a further three categories, namely UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (< 280 nm) (Grant, 1997; Rajapakse and Shahak, 2007). The term photosynthetically active radiation (*PAR*) refers to the visible region of sunlight that provides the energy for photosynthesis and is divided into red (600-700 nm), green (500-600 nm) and blue (400-500 nm) light (Pearcy, 1989; Rajapakse and Shahak, 2007). Terminology of light parameters often used in the orchard includes: irradiance of light which is referred to as the incidence of radiant energy on a unit surface from all directions; photosynthetic photon flux density (PPFD) which is the number of photons in the visible waveband (*PAR*), as incident per unit time on a unit surface (Pearcy, 1989); and light quantity which refers to the amount of *PAR* and light quality within the spectral composition of sunlight (Bastías and Corelli-Grappadelli, 2012).

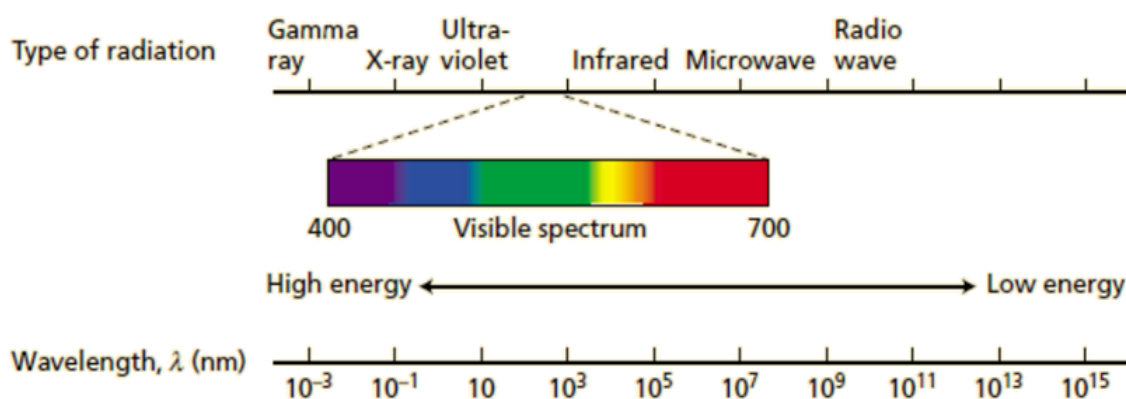
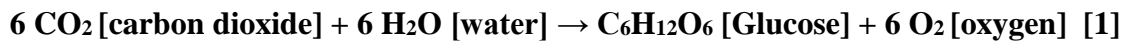


Fig. 1. Electromagnetic spectrum. The types of radiation and the associated wavelength. The energy and wavelength have an inverse relationship (Adapted from Taiz et al., 2015).

3. Light, the driver of photosynthesis

Photosynthesis is the primary source of energy on earth and is directly dependent on light as pigments in the chloroplast absorb the light energy from the sun and convert carbon dioxide in the presence of free water into carbohydrates, with the release of oxygen (Gross, 1987; Lang et al., 1985) (Eq. [1]).



The process of photosynthesis involves two main reactions namely; the photochemical (light reaction) and Calvin cycle (dark reaction), which is responsible for the conversion of light energy to chemical energy. The light reaction is light dependent and the radiant energy from the sun is captured into reduced nicotinamide dinucleotide (NADPH) and adenosine triphosphate (ATP). These two molecules transfer energy due to their reducing power to the dark reaction where the conversion of carbon dioxide to glucose occurs (Gross, 1987; Taiz et al., 2015).

The photosynthetic rate is independent of the energy of the photon (short or long wavelength) absorbed, as excess energy is dissipated either as fluorescence or heat. Therefore the number of photons absorbed in the visible region (400-700 nm) rather than the light quality influences the photosynthetic rate of plants (Mortensen and Stromme, 1987; Pearcy, 1989). High levels of blue and red light enhance photosynthesis and thus the overall productivity in horticultural crops, with little absorption occurring in the green region (Rajapakse and Shahak, 2007).

Citrus leaves are known for their low photosynthetic capacity compared to deciduous fruit, as the CO₂ assimilation rate saturates at fairly low light levels (low PPFD) (Kriedemann, 1968; Syvertsen, 1984). Kriedemann (1968) reported that only 20-25% of the intensity of full sunlight was effective in saturating the photosynthetic rate for both orange [*Citrus sinensis* (L.) Osbeck] and lemon [*C. limonum* (L.)] leaves. Similar results were found in various other studies where maximum net CO₂ assimilation was attained for leaves of different grapefruit (*C. paradisi* Macf.) and orange cultivars which received a relatively low PPFD between 500-800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Jifon and Syvertsen, 2001, 2003; Syvertsen, 1984).

High light intensities exceeding the CO₂ assimilation capacity of a crop can lead to either a decreased or total inhibition of photosynthesis (Apel and Hirt, 2004; Kriedemann, 1968). High or excess irradiance causes heat stress in leaves, which consequently lead to the inactivation of enzymes, photo-inhibition (protective mechanism to avoid photo-damage and photo-oxidation) and finally photo-damage. High light irradiance may thus limit CO₂

assimilation, therefore overall negatively affecting the photosynthetic capacity of the leaves (Jifon and Syvertsen, 2001, 2003; Ribeiro and Machado, 2007). Increased UV-B irradiance and irradiation time have been reported to decrease the photosynthetic activity of radish leaves (*Raphanus sativus*) (Tevini and Iwanzik, 1983). Cohen et al. (2000) found that a reduced radiation (almost half) on ‘Star Ruby’ grapefruit, ‘Murcott’ tangor (*C. reticulata* Blanco hybrid) and ‘Nectar’ mandarin resulted in the photosynthesis rate being two times higher compared to control leaves.

Shade nets have been shown to reduce the PPFD under the net (Cohen et al., 1997; Jifon and Syvertsen, 2001, 2003; Syvertsen et al., 2003). When 50% shading was applied to grapefruit and orange trees, the PPFD was reduced by half, which caused a significant decrease in photo-inhibition and resulted in shaded leaves maintaining a higher CO₂ assimilation rate than full sunlit leaves (Jifon and Syvertsen, 2003). Furthermore, a reduction in PPFD is accompanied by lower leaf temperatures which will enhance the CO₂ assimilation (Cohen et al., 1997; Jifon and Syvertsen, 2001). This is particularly beneficial since citrus leaves in general have a low temperature optimum regarding photosynthesis, with high temperatures known to cause a reduction in photosynthesis (Kriedemann, 1968). Similarly, Syvertsen et al. (2003) reported that shading was effective in increasing the net CO₂ assimilation in ‘Spring’ navel orange leaves on warm days. However, on cooler days in this study the shade treatment had no effect. Therefore, a reduction of PPFD caused by the shade treatment is not solely responsible for the effect on CO₂ assimilation.

Of interest in citrus fruit is that the CO₂ fixation of the flavedo (outside colored portion of the rind) has also been shown to be influenced by light. An illuminated green flavedo of ‘Valencia’ orange had a higher CO₂ fixation (photosynthetic activity) compared to the flavedo where light was excluded (Bean and Todd, 1960). Similar findings were again reported in ‘Nules Clementine’ mandarin by Cronje et al. (2013), where fruit that received light of a higher intensity had a higher CO₂ fixation rate compared to fruit that did not receive direct sunlight.

4. Citrus flower- and fruit development

4.1. Citrus flowering and floral development

Flowering is a critical process ensuring the survival of a plant as it is the start of the reproduction phase that eventually leads to fruit formation and a fruit yield (Kinet, 1993). Factors such as the type of inflorescence, flowering position and flowering time directly affect external fruit quality characteristics such as fruit size and rind color, along with internal quality

parameters as indicated by soluble solid content expressed as °Brix and total acid (TA) content (Ehara et al., 1981).

In *Citrus*, flowering mostly occurs once a year, however, lime and lemon spp. are exceptions and can flower several times a year (Ortiz, 2002). Yet, the phenology and flowering of citrus fruit varies distinctly between species, cultivars and environmental conditions (Davenport, 1990). Endogenous and exogenous factors influencing floral development in *Citrus* have extensively been researched and reviewed by Davenport (1990) and Krajewski and Rabe (1995). Photoperiod, low temperature, water stress, mineral nutrition, carbohydrates and gibberellic acid are some of the known factors involved in the process of citrus flowering (Goldschmidt et al., 1985; Guardiola et al., 1982; Lenz, 1969; Lovatt et al., 1988; Moss, 1976; Southwick and Davenport, 1986). The focus of this review lies primarily on the influence of light on fruit quality development, therefore the other factors influencing flowering will only be discussed as relevant.

Floral induction and differentiation are two important processes that precede visible citrus flowering expression (Davies and Albrigo, 1994). During induction, the meristem is committed to produce an inflorescence rather than a vegetative shoot, normally after an exogenous environmental or endogenous stimulus is perceived (Davenport, 1990). A competent meristem permits the transition of a vegetative bud into a flowering bud (Ortiz, 2002). Generally, *Citrus* is considered as an auto-inductive tree, with no single known inductive stimulus for flowering (Monselise, 1985 cited by Krajewski and Rabe, 1995). However, some stimuli such as photoperiodism, temperature and water status are known to play a significant role to facilitate the switch to a reproductive state (Singh et al., 2004). During floral differentiation, morphological changes occur in the bud (Davenport, 1990), accompanied by the formation of the floral primordia (Kinet, 1993). The onset of differentiation typically occurs in winter (Spiegel-Roy and Goldschmidt, 1996), and flower bud development thereafter continues uninterruptedly towards flowering (Monselise and Halevy, 1964).

Citrus trees have three shoot types: a vegetative shoot and two types of flowering shoots (Goldschmidt et al., 1985). Flowering shoots, or mostly referred to in citrus as inflorescences, are either mixed (leafy inflorescence) or generative (leafless inflorescence) (Davenport, 1990). The generative shoot has one or more flowers, but no leaves present, with flowers developing on the previous season's growth, whereas the mixed shoot types bear either a terminal flower or many single axillary flowers on the new season's growth, also with leaves being present (Davenport, 1990; Davies and Albrigo, 1994). There is, however, a variation in the number of flowers and leaves present on a shoot (Moss, 1970).

The sequence of flower opening initiates terminally, with the apical flower being first, to be followed by the basal and then the subapical buds. The leafy inflorescence is the most productive shoot, with a higher percent of flowers that set fruit, compared to the generative shoot (Jahn, 1973). However, in ‘Tahiti’ lime, fruit set is mainly on generative shoots (Davenport, 1990).

4.1.1. The effect of light on flower initiation and development

Photoperiodism is a term used in horticulture to explain the flowering response of plants in relation to daylength. Leaves as the site of perception record a change in the length of the dark period, and will flower in response to it, depending on the type of plant (Wilkie et al., 2008). Short day plants (SDP) require an extended dark period, and will flower under conditions where the light period is shorter than the critical light period, whereas for long day plants (LDP), flowering will occur if the light period exceeds the critical period, with an accompanying short dark period (Mohr and Schopfer, 1995; Wilkie et al., 2008). In day-neutral plants, flowering will occur irrespective of the photoperiod (Krajewski and Rabe, 1995). In some plant species of long and short-day plants, daylength is known to control floral initiation while in other plant species it is critical for floral development (Kinet, 1993) and can therefore affect fruit production. Examples of such crops depending on photoperiod for a flowering response are southern highbush blueberry (*Vaccinium corymbosum* L.) (Spann et al., 2004) and avocado trees (*Persea americana* Mill.) (Buttrose and Alexander, 1978).

The role of photoperiodism in response to flowering in citrus trees has not been investigated intensively, mainly as citrus is generally considered a day-neutral crop (Krajewski and Rabe, 1995). In a study by Furr et al. (1947) on potted sweet oranges of ‘Parson Brown’ and ‘Valencia’ varieties, it was observed that girdled trees flowered regardless of photoperiod whereas non-girdled trees did not flower under any of the exposed photoperiods. It was thus concluded that photoperiod is not a control factor in citrus flowering. However, in a study conducted on rooted cuttings of ‘Washington navel’ orange, Lenz (1969) described this cultivar as a qualitative SDP, since flowering only occurred under short day conditions of between 8 and 12 hours, but not under a 16 hour photoperiod. Yet, in other studies conducted on the effect of photoperiod on flowering, also again on ‘Washington navel’ orange trees, no direct regulatory role could be found as trees exposed to 8, 12 and 16-hour photoperiods respectively did not show a difference in flowering response (Moss, 1969).

In citrus, changes in the quantity and quality of light may also affect flowering indirectly where photosynthesis impacts on the regulation of carbohydrates in the tree which in turn has

a regulatory role in control of flowering (Goldschmidt et al., 1985; Gross, 1987). Experimental evidence implicates the possible role of carbohydrates in affecting flowering in citrus, especially with carbohydrate accumulation following fruit removal. Fruit removal from a known alternate bearer, ‘Wilking’ mandarin, allowed starch accumulation in the leaves prior to flower bud differentiation the following year. The flowering response correlated positively with the starch levels and resulted in an unexpected medium flowering response in the ‘off’ year (Goldschmidt and Golomb, 1982). Yahata et al. (2006) found a similar response in ‘Okitsu’ wase (*C. unshiu* Marc.) where a higher starch accumulation occurred in the early harvested trees as opposed to the late harvested trees, with a significantly higher flower number in early harvested trees. A similar trend was reported by Garcia-Luis et al. (1995) in ‘Owari’ Satsuma (*C. unshiu* Marc.) a regular bearer, where early fruit removal as opposed to fruit removal at commercial maturity, significantly increased the flower number in the following season. However, although a different flowering response was evident in the two systems, the observed difference in carbohydrate content in the leaves of both trees were surprisingly, relatively small. However, Goldschmidt and Golomb (1982) cautioned that leaves are not the ideal organs to use as an indication for carbohydrate determination.

Contradictory to the research reported above where starch levels correlated well with citrus flowering, various other research showed no clear correlation. García-Luis et al. (1995) proposed that carbohydrate accumulation caused by girdling is not solely responsible for the associated effect on flowering, as different girdling times resulted in different starch levels in the leaves, but a similar flowering response was still observed in ‘Owari’ Satsuma. Goldschmidt et al. (1985) established that the starch levels in ‘Minneola’ (*C. paradisi* Macf. x *C. reticulata* Blanco) trees did not correlate well with an observed flowering response and concluded that carbohydrates in this instance could not be held responsible as a controlling factor in citrus flowering. In ‘Salustiana’ sweet orange the flower numbers differed significantly between ‘on’ and ‘off’ trees, however no difference in the carbohydrate content of the leaves and twigs during flower induction and initiation was observed between the two systems (Monerri et al., 2011). García-Luis et al. (1995) concluded that there was not always a correlation between carbohydrate levels and flower numbers during flower formation. Thus, uncertainty exists as to the direct and indirect role of carbohydrates in citrus flowering (Spiegel-Roy and Goldschmidt, 1996) since there are various other role players responsible in the flowering process (Krajewski and Rabe, 1995).

Influence of shade netting on flowering of fruit trees. Research with respect to the impact of shade netting on the flowering response of different crops has produced conflicting results. Flower numbers and inflorescence per shoot in kiwifruit (*Actinidia deliciosa* cv. Hayward) decreased slightly under photo-selective shade nets (blue, grey, red and white) (Basile et al., 2008). Contradictory to this, Shahak et al. (2004) reported on colored shade nets of different shading factors (pearl, yellow, red and blue 30% and 12% white) that were successfully used for peach trees (*Prunus persica* cv. Hermosa) to enhance flowering, except for the grey color (30%) which did not differ from the control. The researchers ascribed the response to various net types which reduced the *PAR*, as well as a modification of light quality and microclimate, respectively caused by the nets (Basile et al. 2008; Shahak et al., 2004). However, in earlier literature, Garcia-Luis et al. (1995) reported no effect of shading (88% *PAR* intercepted) on the flowering response of both fruited and defruited ‘Owari’ Satsuma trees. The shade net, however, was not permanent and was only applied for a period between 24 (defruited trees), and 66 days (fruited trees), respectively.

4.1.2. Other factors influencing citrus flowering

Flowering in citrus is recognized to be a complex process due to various interrelated factors such as photoperiod, temperature and soil water which may have an impact individually or in combination during field trials at orchard level (Krajewski and Rabe, 1995). However, a more complete understanding of flowering in citrus is becoming evident.

The effect of temperature on flowering was investigated by various researchers where cool temperatures had a promoting effect on flowering in citrus trees (Goldschmidt et al., 1985; Lovatt et al., 1988; Southwick and Davenport, 1986). Lenz (1969) found that lower temperatures of around 24 °C day/19 °C night led to flower initiation in rooted cuttings of ‘Washington’ navel orange as opposed to higher temperatures (30 °C/25 °C). Moss (1970) reported temperature to influence the inflorescence type of ‘Late Valencia’, where low temperature exposure resulted in generative shoots, whereas mixed shoots were found more evident in trees exposed to high temperatures.

Gibberellic acid (GA) is the most widely investigated plant hormone as a possible factor in the control of flowering in citrus trees. Goldschmidt et al. (1985) established a regulatory role for GA on citrus flowering in ‘Shamouti’ orange trees where GA₃ application reduced flowering. Similar findings were reported on ‘Sweet orange’, ‘Satsuma’, ‘Clementine’ (Guardiola et al., 1982) and ‘Shamouti’ orange trees (Monselise and Halevy, 1964). Guardiola et al. (1982) concluded that flowering is a complex phenomenon that requires specific hormonal

and metabolic levels in the bud, in addition to a specific internal state, which may be related to the bud's location on the tree.

Southwick and Davenport (1986) and Lovatt et al. (1988) investigated the effect of water stress on flowering in *Citrus*. It was reported that deficit irrigation in 'Tahiti' lime (*C. latifolia* Tan.) trees resulted in higher flower production (Southwick and Davenport, 1986) and Lovatt et al. (1988) established that the flower intensity (number of flowers/tree) of 'Frost Lisbon' lemon increased with an increase in both duration and severity of water stress in trees. In contrast to these findings, Koshita and Takahara (2004) found that the number of flower buds produced on severe water stressed 'Satsuma' mandarin trees was significantly lower than moderately stressed trees. However, a higher GA_{1/3} content in the leaves of severe stressed trees was measured, which could possibly negatively affect the flower bud production.

When the role of nitrogen in citrus flowering was investigated by Lovatt et al. (1988), urea foliar applications was found to increase the ammonia content in the leaves as well as the flower number per tree, in both 'Frost Lisbon' and 'Washington' navel oranges. This finding was confirmed by Menino et al. (2003) in 'Lane Late navel' orange trees.

Finally, Davies and Albrigo (1994) proposed that the control of citrus flowering is multifaceted, not only because of external factors that may exert some control over flowering, but also as some buds are more easily induced to flower, while other apparently require much more stress to achieve the same state. Due to all the variables that come into play in the control of flowering, the findings are often conflicting (Davenport, 1990).

4.2. Fruit formation

Pollination, fertilization and subsequent seed development are important processes following bloom that prevent ovary- and fruit drop, thereby ensuring fruit set (Kretdorn, 1986). However, in some citrus fruit all these steps are not necessarily an absolute requirement for fruit set as fruit set can occur parthenocarpically (Grierson, 2006).

In citrus fruit, there are three distinct fruit developmental stages namely; cell division (I), cell enlargement (II) and maturation (III), as classified by Bain (1958), that commence from flowering to commercial maturity. Each phase has certain characteristic morphological, anatomical and physiological changes, with growth occurring in a distinctive sigmoidal pattern (El-Otmani et al., 1989). Goldschmidt and Monselise (1977) proposed that the flower number, fruit set percentage and the potential for fruit enlargement, which depends on the number of fruitlets present, can all be considered control points, which will determine the final fruit yield.

4.2.1. Factors influencing fruit set

The final fruit set is determined after the fruit abscission period (Goldschmidt and Monselise, 1977), which is a natural process where the tree adjusts its crop load according to the available carbon source (Bustan et al., 1996). When referring to a fruit as set, it implies that the fruit is expected to remain on the tree until maturity (Ortiz, 2002).

Fruit set is a physiological process which is influenced by an interaction of hormonal and biochemical systems (Goldschmidt, 1999). Iglesias et al. (2007) reviewed the physiological factors that influence fruit set namely: flowering intensity and timing, inflorescence type, pollination with or without parthenocarpy, plant growth regulators and carbohydrates. Fruit set is known to improve on leafy inflorescence compared to generative (leafless) inflorescence (Ehara et al., 1981; Jahn, 1973; Moss et al., 1972).

GA₃ is known to play a distinct role in fruit set. ‘Satsuma’ mandarin, which is highly parthenocarpic, has a higher GA content compared to ‘Clementine’, which has a low fruit set ability in the absence of cross-pollination. It was proposed that ‘Clementine’ mandarins generally have insufficient endogenous GA to ensure adequate fruit set. Fruit abscission was reduced in both cultivars after exogenous GA application (Talon et al., 1992).

Carbohydrate levels are considered important in fruit set. In a study in ‘Satsuma’ mandarin, fruit set increased in response to sucrose supplementation (Iglesias et al., 2003). Schaffer et al. (1985) reported that girdling, which results in an increased allocation of carbohydrates to fruit (Goldschmidt, 1999), produced a positive response with regard to increasing fruit set in ‘Shamouti’ trees. In contrast to this, in the same study by Schaffer et al. (1985), ‘Murcott’ did not respond to girdling, therefore it was suggested that fruit set in this cultivar was not limited by available carbohydrates. Goldschmidt and Golomb (1982) reported on results for alternate-bearing ‘Wilking’ mandarin that during the ‘off’ year, starch levels in the organs were reported to be high, but a distinct decline occurred during the ‘on’ year. This implies that carbohydrate reserves of the previous year are important for the next fruit set and development period. Of interest is a study by Mataa and Tominaga (1998) that established a close relationship between fruit set and assimilate supply in a shading experiment (55% and 70% reduction in light intensity) on ‘Ponkan’ mandarin, where a reduced photosynthesis rate, accompanied by a reduction in fruit set was reported for trees cultivated under shade netting. There is, however, research missing on how shade net influences the fruit set of citrus, and more focus should be placed on this subject.

4.2.2. Fruit structure

Citrus fruit is classified as a hesperidium, which is a type of berry (Baldwin, 1993). Anatomical characteristics associated with hesperidium fruit include a rind, oil glands, an endocarp that consists of fruit segments and juice vesicles, and a central column or axis (Hodgson, 1967).

The fruit morphology of *Citrus* is grouped into two distinct regions, the pericarp that is exterior to the locules and the endocarp that contains the edible portion (pulp) of the fruit (Schneider, 1968; Fig. 2). The pericarp is further classified into the exocarp (flavedo) and mesocarp (albedo). The flavedo refers to the outside, colored section of the rind and contains pigments based in the chloroplast or chromoplast (Baldwin, 1993; Eilati et al., 1969; Meredith and Young, 1969). The flavedo, however, is not only responsible for providing coloration to citrus fruit, but it also produces epicuticular waxes and contains stomata, oil glands and aromatic constituents (Grierson, 2002,2006; Hodgson, 1967). The albedo forms the inside of the rind, and has a spongy texture, with an ivory or pale-yellow color (Ortiz, 2002), however in some cases, it may be tinted such as with red grapefruit (Grierson, 2006).

The pulp segments/locules, is enclosed in a locular membrane, and contains juice vesicles and seeds (Ting and Attaway, 1971; Spiegel-Roy and Goldschmidt, 1996). The membrane is covered by vascular bundles that function as the site of transfer of nutrients to the growing fruit (Ortiz, 2002). Water and solutes from the tree are transported through the vascular system which is confined to the mesocarp (Sinclair and Eny, 1947). The juice contains constituents that contribute to the internal quality of citrus fruit (Hodgson, 1967). Citrus fruit can either be seeded or seedless (Ortiz, 2002), due to some cultivars' ability to set fruit parthenocarpically.

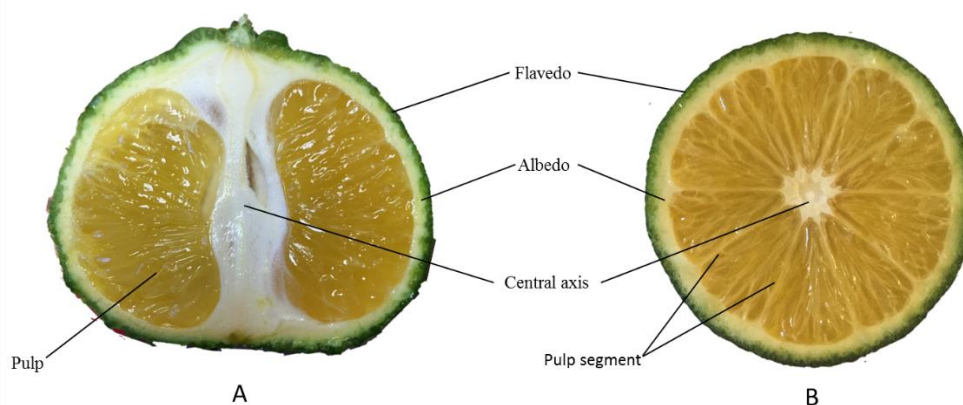


Fig. 2. A longitudinal (A) and cross section (B) of a 'Nadorcott' mandarin citrus fruit. (Adapted from Iglesias et al., 2007).

4.2.3. Fruit development phases

CELL DIVISION. Cell division is the first stage (I) of fruit development that is characterized by the increase in cell numbers of all tissues forming part of the developing fruit, except for the inner cells of the central axis. This stage commences at bloom and continues until mid-December, for ‘Valencia’ orange fruit in the southern hemisphere (SH) (Bain, 1958). During the cell division stage, the rind develops from the ovary wall, and tissues that differentiate into the flavedo and albedo are formed. Fruit size increase is mainly due to the growth of the rind, constituting approximately 95% of the total fruit volume at the end of this stage. An increase in rind volume occurs due to albedo cell enlargement, and because of an increase in the vascular tissue in the albedo (Bain, 1958).

Kuraoka and Kikuchi (1961) and Kikuchi et al. (1964) reported that the increase in peel thickness during the early stage of fruit development for ‘Satsuma’ mandarin and other *Citrus* spp. and varieties is ascribed to cell division in the albedo. This stage is also associated with oil gland development and enlargement in the flavedo as well as an increase in pulp volume (Bain, 1958). The juice sac primordia of ‘Valencia’ orange are considered to develop during the early phase of this stage (Bain, 1958), a finding also supported by Kuraoka and Kikuchi (1961). At the end of the cell division phase the juice sacs constituted up to two-thirds of the pulp segment, with cell division completed in all tissue, except for the flavedo where the process continues throughout the next two stages (Bain, 1958).

CELL ENLARGEMENT. The second phase of fruit development has the longest duration of the three stages, ranging from mid-December to mid-July (SH). This phase entails cell enlargement and differentiation, with rapid morphological, anatomical as well as physiological changes occurring. This phase is also considered as the period of maximum fruit growth (Bain, 1958) and is therefore critical to determine final fruit size, and thus also the economic values of the fruit for the fresh market (Goldschmidt, 1999).

Kikuchi et al. (1964) stated that there is no relation between the duration of the stage and the final fruit size and maturity. During stage II the increase in fruit size is the result of pulp expansion through the increase of juice content in the enlarged juice sacs. The pulp growth caused a reduction in rind thickness, and the albedo experiences anatomical changes to counterbalance the growth in the absence of cell division in the rind tissue, excluding the flavedo. In this phase the characteristic spongy albedo develops. Pectin accumulation occurs in the cell walls of the albedo and the vascular tissue increases. Air spaces develop in the rind tissue, while the oil glands increase in size (Bain, 1958).

At the end of stage II an increase in soluble solids content (SSC) as well as decrease in acids was reported (Bain, 1958). Soluble sugar accumulation begins at the onset of cell enlargement (Mehouachi et al., 1995). In a study by Chen et al. (2002) high amounts of photosynthates were reported to be transferred into ‘Satsuma’ fruit during fruit enlargement, due to juice sacs being a major sink at this time. Stage II is also associated with the onset of rind coloration, as a result of active carotenoid synthesis (Chen et al., 2002; Huff, 1984).

FRUIT MATURATION. Fruit maturation is considered as the final fruit developmental phase (mid-July (SH) until ripe, 7 months), with the reduced morphological, anatomical and physiological changes during this phase distinguishing it from stage II (Bain, 1958). Bain (1958) also stated that the fruit size, fresh weight and moisture content would continue to increase if the fruit were to remain on the tree, with changes mainly occurring in the pulp, due to juice sac enlargement and an increase in width of the central axis. The rind volume increases due to an increase in pulp, but generally with no significant difference in thickness. In the fruit maturation phase the Brix and sugar content remains constant, however a decrease in acid percentage is usually recorded (Bain, 1958). The color change in the flavedo of ‘Valencia’ orange, typically from green to orange, differentiates between stage I and III (Bain, 1958). During the early stages of fruit development the chlorophyll content is high, thereafter as the fruit develops towards maturity, a gradual decrease occurs, with a simultaneous increase in carotenoids (Chen et al., 2002; Eilati et al., 1969).

4.2.4. Fruit development in *Citrus* as affected by light levels

In *Citrus*, the role of light on fruit development is evident from studies where differences in canopy position influenced the growth, weight, size and juice percentage of the fruit. The available *PAR* within the canopy decreases from top to bottom, and again from outside to inside (Cronje et al., 2013). The reduced *PAR* levels at these various positions within the tree canopy are considered to have an influence on fruit development and quality. Ehara et al. (1981) reported that ‘Satsuma’ fruit that developed at the top of the tree were larger. This finding was confirmed by Verreynne et al. (2004) for ‘Satsuma’, ‘Clementine’ and ‘Temple’ tangor, where fruit from the top part of the canopy had a larger fruit diameter compared to the inside, bottom position, yet, the opposite was true for the cultivar ‘Fairchild’. Juice percentage was reported to be the highest for fruit borne on the inside of the canopy, for all the mandarin types included in the study.

Moon et al. (2011) also reported that fruit size and weight of ‘Shiranuhi’ mandarin fruit harvested from the top part of the canopy were significantly higher compared to fruit obtained

from the middle and lower positions. An increased fruit mass was recorded in ‘Nules Clementine’ mandarin fruit that developed on the outside canopy (Cronje et al., 2013). As fruit growth is known to be influenced by canopy position, Cronje et al. (2013) proposed that the higher respiration rate associated with outside fruit which had exposure to higher *PAR* levels, may have resulted in higher pulp and rind growth rates, thereby resulting in increased fruit size. However, Moon et al. (2011) ascribed the difference in fruit growth, in addition to light intensity, also to various other factors including sink strength, hormone balances, fruit load as well as leaf to fruit ratio. Exposed and shaded ‘Campbell’ Valencia fruit had a similar fruit growth pattern until one month after the onset of stage II, whereafter shaded fruit showed a more rapid growth, resulting in exposed fruit having a lower fruit volume compared to the shaded fruit at harvest (Ketchie and Ballard, 1968).

Shade nets, which are known to reduce radiation levels (Iglesias and Alegre, 2006) and also modify the light quality and composition (Shahak, 2008) resulted in contradictory effects with regard to fruit development amongst different studies. Application of high shading nets (75%) on ‘Ponkan’ mandarin trees during the fruit growth stage II, caused a reduction in fruit weight and size, whereas lowered shading of 55% had no effect (Mataa and Tominaga, 1998). In support of this finding, Syvertsen et al. (2003) similarly reported that 50% shade, applied four months before harvest, had no effect on the yield or fruit size of ‘Spring’ navel oranges. However, for ‘Hamlin’ orange, shade nets of 50% applied from April (after bloom) until harvest in the Northern Hemisphere reduced both the yield and fruit weight, whereas for grapefruit the fruit weight was higher, but with no effect on yield. In contrast, late shading (Aug-harvest) significantly increased the grapefruit yield for one season only, ascribing these inconsistent results to water and climatic effects (Jifon and Syvertsen, 2001).

The spectral composition of light in addition to the reduced light intensity produced by shade netting has also been shown to significantly influence fruit growth with regard to size, weight and yield. Shahak et al. (2008) reported that for ‘Golden Delicious’ apples, both the fruit size and yield were enhanced by pearl (30%), red (30%) and white (15%) nets, whereas 30% blue, grey and black nets showed no effect on the yield. In addition, black nets reduced the fruit size, while blue and grey had no effect. In ‘Orri’ mandarin, Wachsmann et al. (2014) reported that red (25%), white (18%) and transparent (13%) nets increased the fruit yield, whereas the yellow (24%) nets had no effect. In this study however, the size was not affected by the change in light intensity and quality. Conflicting results obtained with shade netting strongly suggests that the effect of reduced light intensity on fruit diameter, weight and yield may be highly dependent on the phase of fruit development at the time when the shade is applied (Jifon and

Syvertsen, 2001; Mataa and Tominaga, 1998). In addition, it is important to recognize that reduced light intensity, *PAR* and modifications to light quality as induced by shade nets (Shahak, 2008; Shahak et al., 2004) are most likely not the sole factors influencing fruit development. The impact of shade nets on the microclimate with respect to PPF, water-use efficiency, leaf temperature, and water consumption could all be influential in affecting fruit development (Jifon and Syvertsen, 2001; Wachsmann et al., 2014).

Solar energy (light) is the driving factor in producing assimilates through the photosynthetic process, which in turn is partitioned to developing fruitlets (Goldschmidt and Monselise, 1977). Guardiola (1988) stated that both the supply of metabolites as well as fruit sink strength are determinants of fruitlet growth, with sucrose supplementation enhancing fruit yield in ‘Satsuma’ mandarin (Iglesias et al., 2001). Ehara et al. (1981) reported that larger fruit were more likely to develop from a leafy inflorescence compared to a leafless inflorescence, positively linking the presence of leaves as source of assimilates to the promotion of developing fruit (Goldschmidt and Monselise, 1977). Fruit size is directly influenced by crop load (Goldschmidt and Monselise, 1977; Syvertsen et al., 2003) and inter-fruit competition (Mataa and Tominaga, 1998) as carbohydrate reserves are partitioned between fruitlets, with more reserves being available with a lower number of fruit, thus impacting on fruit growth (Goldschmidt and Monselise, 1977).

5. Impact of light on citrus fruit quality

Citrus fruit mostly attain their quality characteristics through the physiological and biochemical processes occurring during stage II and III of fruit development. The quality traits that refer to physical properties of the fruit include size, shape, rind color, seed number and peelability, whereas other quality characteristics relate to the chemical and nutritional characteristics namely the sugar-, acid- and vitamin C content, but also the flavor and aroma of the fruit (Iglesias et al., 2007).

In *Citrus*, the external quality parameters rely on the outer appearance of the fruit. It includes characteristics like rind coloration, surface texture (smooth/rough), fruit shape and size. The pulp color, juice percentage, juice flavor/taste as determined by the SSC(Brix), acids and SSC to acid ratio (Brix:TA), the rind thickness and firmness and the presence or absence of seeds, all contribute to the internal quality parameters (Hodgson, 1967).

Light penetrating the tree canopy scatters and results in a differential spectral quality and distribution reaching the inside and outside canopy fruit, respectively (Grant, 1997; Spiegel-Roy and Goldschmidt, 1996). Consequently, fruit from different canopy positions have

been shown to differ distinctly in rind color, eating quality and fruit composition (Cronje et al., 2013; Khalid et al., 2012; Reitz and Sites, 1948; Winston and Miller, 1948). Similarly, studies on citrus cultivated under shade (net) treatments, which are known to reduce the *PAR* available to trees under the shade, reported changes in fruit quality aspects, especially rind coloration (Cohen et al., 2000; Jifon and Syvertsen, 2001; Lewis and Coggins, 1964; Wachsmann et al., 2014). Light therefore is an important determinant in citrus fruit quality.

5.1. External fruit quality parameters

Consumer preferences when purchasing fruit are primarily based on the external appearance of fruit. Rind color, smoothness, texture along with the lack of any lesions on the rind are the main attributes that determine the market value of a fruit. Various factors affect the external appearance of citrus fruit, of which climate is the main role player, with genetic potential of the cultivar or vigorous rootstocks, pest and disease pressure and citricultural practices (nutrition, irrigation and pruning) being some of the other important factors (Davies and Albrigo, 1994; Lado et al., 2018).

Direct light exposure to the fruit surface is an important environmental factor influencing rind coloration and appearance of citrus fruit (Cronje et al., 2013; Lado et al., 2015b), with changes occurring as a result of either insufficient or excess radiation (Kays, 1999). Low light levels are associated with poor coloration in fruit, whereas excess solar radiation result in sunburn, which is detrimental to the rind and causes blemishes that affect the marketability of the fruit. Pigments are degraded in the affected area as a result of extended duration of exposure along with high light intensities, and is followed by tissue collapse and eventually cellular death (Kays, 1999).

5.1.1. Effect of light on the morphology and anatomy of plants

Research on how light affects the rind and changes the morphological and anatomical features thereof is lacking in not only citrus fruit, but also other fruit types. In leaves, the cuticle as the outer layer to the epidermis has been observed to be thicker in plants that are exposed to high light intensities, compared to greenhouse-grown plants (Juniper and Jeffree, 1983). This adaptation could be due to an effort to protect the more sun-exposed leaves from excessive moisture loss. In addition, research has shown light to influence wax biosynthesis of leaf surfaces. Wax development was observed on the adaxial surface (exposed side) of pea leaves, when the plants were transferred from dark to light, whilst the development of wax on the abaxial surface (away from light) showed a lag phase (Juniper, 1960). Similarly, Giese (1975) reported the wax content to be lower for leaves grown in the dark compared to light grown

leaves. Sun and shade leaves also exhibit structural and compositional differences, as was shown by Lois and Buchanan (1994) who reported that *Arabidopsis* plants exhibited both thicker and wider leaves when irradiated with UV-B. Similar changes in the wax layer on both tomato and ‘Valencia’ orange fruit has been documented. When Charles (2008) exposed tomato fruit (*Lycopersicon esculentum* Mill. Cv Trust) to UV-C, it resulted in a reduction of epicuticular wax accumulation, an effect that was directly linked to the UV-treatment.

In a study on ‘Valencia’ orange, El-Otmani et al. (1989) investigated the changes in the epicuticular wax during fruit development in relation to fruit position within the canopy. It was reported that the epicuticular wax morphology of the fruit rind differed distinctly between the exposed- and shaded side, from one quadrant of the tree. The amorphous nature of wax on the exposed side of the rind was ascribed to the degree of exposure to a combination of light and temperature. Fruit position also influences the structural changes of the epicuticular wax. El-Otmani et al. (1989) further suggested that maturation of the rind of exposed fruit was more advanced, since the crystalline to amorphous changes in the wax were accelerated on the exposed side of the fruit. Similar to this, McDonald et al. (1993) found that canopy position influenced the wax composition and morphology of ‘Marsh’ grapefruit. In citrus fruit, waxy substances deposited on the epidermis of the fruit, as with leaves, are considered important to prevent water loss (Ting and Attaway, 1971).

Fallahi and Moon (1988) investigated rind thickness in relation to canopy position of mandarin, grapefruit, orange and lemon fruit and recorded a thicker rind for inside canopy fruit. Contradicting this, Khalid et al. (2012) found no difference in rind thickness between outside and inside ‘Kinnow’ mandarin fruit. However, Cronje et al. (2013) established that the respiration rate for outside Clementine fruit was considerably higher than inside fruit, and suggested that an elevated respiration rate could lead to increased growth activities within the rind, resulting in a more developed rind structure. The amount and intensity of light is thus regarded important with regards to the rind characteristics, as can be observed in fruit exposed to higher light intensities as opposed to fruit receiving lower light intensities.

5.1.2. Rind color

PIGMENTS. In *Citrus*, the different species and cultivars each have their own distinct rind color at maturity. The rind color ranges from a pale yellow to an orange red coloration, with some citrus groups having shades of red and/or pink (Hodgson, 1967). The presence of pigments and the concentration thereof in the rind and pulp are responsible for the variety in coloration observed within the different citrus fruits (Gross et al., 1983; Kays, 1999). However,

when fruit development occurs in the dark, not only does the composition of the pigments change, but the fruit can also be completely devoid of pigments (Clijsters, 1975 as cited by Gross, 1987).

The three main classes of pigments that are recognized in citrus fruit are chlorophyll, carotenoids and anthocyanins (Gross, 1987). Chlorophyll is the predominant pigment found in immature and/ or green-colored ripe fruit (Eilati et al., 1969). Carotenoids are responsible for the yellow, red and orange coloration in the rind and pulp of mature citrus fruit (Gross, 1987; Lewis and Coggins, 1964). Anthocyanins are unique to blood oranges and accumulate in both the pulp and rind, providing a purple, pink and dark red coloration (Hodgson, 1967; Meredith and Young, 1969; Rodrigo et al., 2013).

Chlorophyll. Chlorophyll, considered the most important pigment in plants, is localized in the chloroplast, an organelle found in leaves and immature fruit, where it serves as the site of photosynthesis (Gross et al. 1983; Gross, 1987). Chlorophyll functions as part of an antennae light-harvesting complex that is the site where the conversion of light to chemical energy occurs (Gross, 1987). Plants contain chlorophyll a (Chl a) and b (Chl b), with Chl a being more abundant in the rind than Chl b (Meredith and Young, 1969; Yamauchi et al., 1997). Both pigments absorb light in the blue (400-500 nm) and red (600-700 nm) spectrum (Gross, 1987).

The presence of light is strongly linked to the chlorophyll content of the fruit, since light is a prerequisite for chlorophyll synthesis (Gross, 1987; Aschan and Pfanz, 2003). However, under conditions of excess light, the concentration of chlorophyll a and b is lowered in sun damaged rind tissue compared to healthy tissue (Wünsche et al., 2001). The high amount of chlorophyll in unripe fruit has a masking effect on carotenoids that are also present in the chloroplast (Gross, 1987). This was illustrated in ‘Goliath’ Pummelo (*C. grandis* Osbeck), where yellow pigments (carotenoids) were unmasked following the disintegration of chlorophyll, resulting in a yellow colored fruit (Gross et al., 1983).

Carotenoids. *Citrus* with its different species and cultivars are known to produce an extensive diversity of carotenoid types within the genus (Alquézar et al., 2008a; Gross, 1987; Rodrigo et al., 2013). Carotenoids are located in the chloro- and chromoplast plastids, with plastoglobules being the dominant structure in chromoplast, functioning as the site for carotenoid accumulation (Gross et al., 1983; Thomson et al., 1967). The pigment group can be divided into carotenes that consist of a hydrocarbon backbone and xanthophylls that contain oxygen in their structure (Alquézar et al., 2008a, Fig. 3). The carotenoid composition and pattern is characteristic for each development phase and changes as the fruit develops toward

maturity (Gross, 1987; Rodrigo et al., 2004). Colorless, chloroplastic carotenoids which include β -carotene, lutein, violaxanthin and neoxanthin are abundant in immature fruit. However, during color break and progression of the season toward maturity, chloroplastic carotenoids decrease substantially and may even disappear, whilst chromoplastic carotenoids becomes evident due to a *de novo* carotenogenesis that occurs (Gross, 1987; Gross et al., 1983). It is accompanied by the interconversion of chloroplasts in immature fruit to chromoplasts that are present in mature fruit (Thomson et al., 1967).

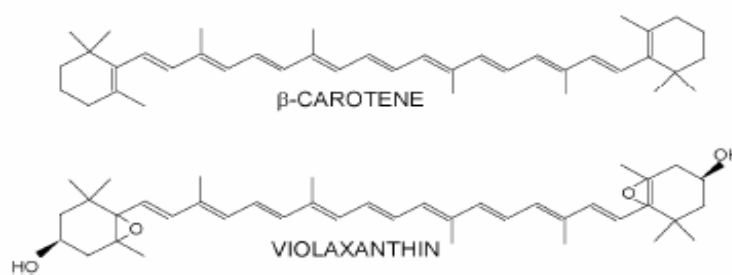


Fig. 3. Chemical structure of β -carotene (carotene) and violaxanthin (xanthophyll) carotenoids (Alqu  zar et al., 2008a).

The carotenoid profile of chromoplastic carotenoids consists mainly of β,β -xanthophylls. Various studies, in different citrus cultivars, showed an increase in β,β -xanthophylls after color break (Al  s et al., 2006; Alquezar et al., 2008b; Eilati et al., 1969; Kato et al., 2004; Rodrigo et al., 2004). The abundant β,β -xanthophylls in mandarin fruit include 9-Z-violaxanthin and β -cryptoxanthin, whilst oranges contain 9-Z-violaxanthin and phytoene (Alquezar et al., 2008b; Kato et al., 2004). Citrus fruit with a yellow, orange and red coloration also contains β -citraurin, a C30-apocarotenoid which is genus specific and restricted to the rind tissue where it provides a reddish tint to the flavedo (Alqu  zar et al., 2008a; Farin et al., 1983; Gross, 1987). Red pigmented grapefruit contains lycopene and carotene as the primary carotenoids in their pulp (Hodgson, 1967; Meredith and Young, 1969). The pulp of the 'Cara Cara', which is a mutation of the 'Navel' orange also contains lycopene, due to a mutation in the carotenogenesis process resulting in lycopene accumulation (Alquezar et al., 2008b).

In citrus, the content and composition of the carotenoids present in a specific cultivar is responsible for the characteristic color of the rind and pulp of the fruit at maturity. Lewis and Coggins (1964) proposed that the ratio of carotene to xanthophyll contributes to the extensive color variation that exists between citrus varieties. Color differences in the juice of three *C. sinensis* cultivars ('Valencia', 'Shamouti' and 'Washington') were associated with the

carotenoid concentration and the ratio of orange (β -cryptoxanthin) to yellow (violaxanthin) pigments. 'Washington' navel orange, which had the lightest color juice, also had the lowest ratio of β -cryptoxanthin to violaxanthin (Gross et al., 1972).

The regulation of carotenoid biosynthesis occurs at a transcriptional and post-transcriptional level, with nuclear genes coding for carotenoid biosynthetic enzymes (Bartley and Scolnik, 1995; Kato et al., 2004), regulating the carotenoid accumulation and composition of the mature fruit (Alquézar et al., 2008a). The carotenoid biosynthesis pathway is schematically presented in figure 4, with geranylgeranyl pyrophosphate (GGPP) being the key precursor of the synthesis pathway (Lado et al., 2015b). Emphasis has been placed on the role of light in the regulation of carotenoid biosynthesis. Raymundo et al. (1976) suggested that light may be a stimulus in the carotenogenesis pathway for tomato fruit, but it is not required for the induction. The carotenoid pattern of three genotypes was identical for fruit produced and ripened in the dark, compared to fruit cultivated under light conditions. However, one genotype did not accumulate lycopene when ripened in the dark, but lycopene was present in dark grown fruit that ripened in the light. In addition, light-grown fruit had a higher β -carotene content compared to dark-grown fruit. This suggests that β -carotene synthesis may be inhibited in the dark, because when dark grown fruit was ripened in the light, a slight increase in the content occurred.

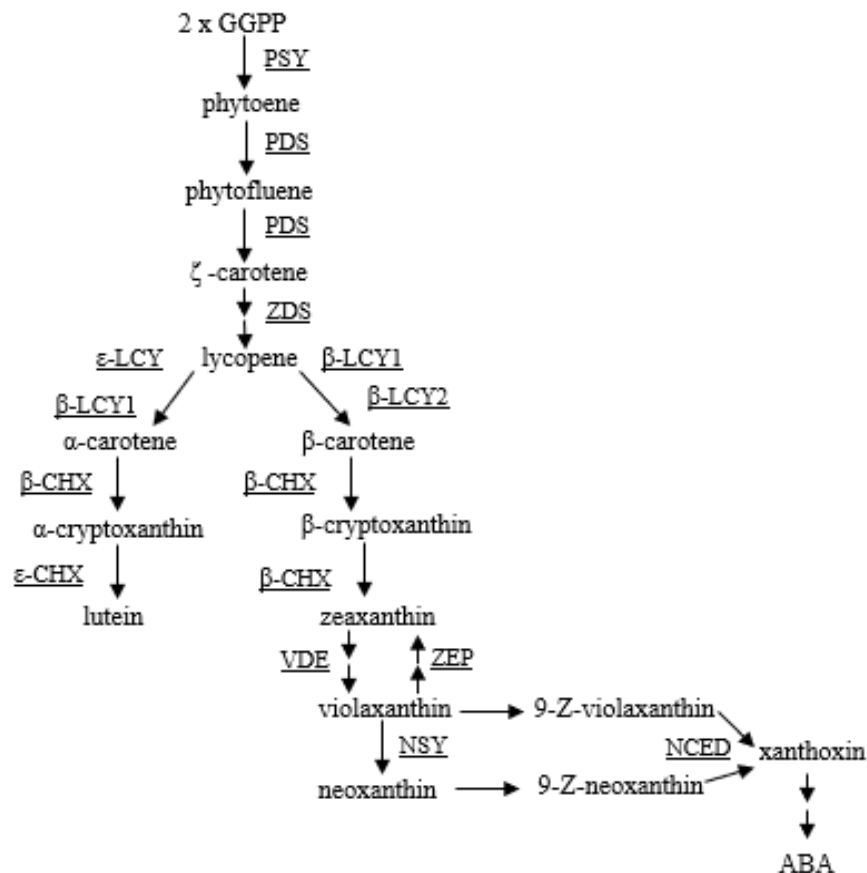


Fig. 4. A diagram of carotenoid biosynthesis in *Citrus* fruit. Words underlined are genes involved in the process. Geranylgeranyl pyrophosphate (GGPP) is the key precursor (Adapted from Lado et al., 2015b).

In a study where citrus callus of four genotypes was exposed to light and dark treatments Gao et al. (2011) established that although light had a regulatory role in the expression of carotenogenesis genes, it did not result in significant changes in the production of carotenoids. Light resulted in the accumulation of carotenoids in ‘Torocco’ blood orange, ‘Bingtangcheng’ and ‘Murcot’ tangor (*C. reticulata* x *C. sinensis*), whereas the opposite effect was achieved for ‘Red Marsh’ grapefruit (*C. paradisi* Macf.). As there was a difference in the expression of carotenogenic genes amongst the genotypes when exposed to light and dark treatments, Rodrigo et al. (2013) proposed that regulation occurs through a complex interaction of various factors affecting the mechanism by which the process occurs.

Red light enhanced β -cryptoxanthin content in ‘Satsuma’ mandarin, thereby increasing the total carotenoid content compared to the control (dark) treatments, whereas blue light induced no effect after six days. The genes involved in the β,β -xanthophyll production was upregulated by red light, whereas with blue light only a temporary effect was noted. In addition

to this, both the blue and red light caused an up regulation of gene expression by the third day of exposure, yet this increase in gene expression did not cause a rapid accumulation of carotenoids, suggesting that another regulatory mechanism is involved (Ma et al., 2012).

Light intensity plays an important role in carotenoid content. ‘Navel’ orange fruit exposed to 5% of normal light, had a 42% reduction in carotene levels compared to fruit that received the full light intensity (Lewis and Coggins, 1964). Cronje et al. (2013) reported that in ‘Nules Clementine’ mandarin the carotenoid concentration was higher for fruit on the outside of the canopy that was exposed to higher *PAR*. However, for ‘Star Ruby’ grapefruit the opposite trend was recorded (Lado et al., 2015b). Fruit that was covered with bags to exclude light had a higher carotenoid content and especially showed an increase in lycopene levels much higher than fruit which was exposed to normal photoperiodic conditions. This result indicates some possible modification to the carotenoid synthesis regulation accompanying the mutation leading to this unique cultivar.

Of interest is that carotenoid accumulation can also occur in the pulp of citrus fruit, thus contributing to the diversity in juice color (Alqu  zar et al., 2008a; Gross et al., 1972). This implies that carotenogenesis occurs inside the fruit, where no light is present, thus light is not essential for the induction of carotenogenesis in fruit (Gross, 1987).

Anthocyanins. Anthocyanins in citrus fruit are predominantly present in the blood oranges, even though some mandarin cultivars are being developed to carry this pigment and characteristic coloration. Rapisarda et al. (1999) reported that in blood orange the high amount of anthocyanins recorded contributed considerably to its high antioxidant activity. The pigment is water soluble and forms part of the flavonoids group (Gross, 1987; Meredith and Young, 1969). The attractive, red coloration of anthocyanins makes it suitable as an addition as a healthy compound to beverages.

Low temperature, maturation and light are some of the important factors regulating anthocyanin production (Gross, 1987; Meredith and Young, 1969). Light is responsible for the activation of enzymes that are involved in anthocyanin biosynthesis (Gross, 1987). Dark grown mustard seedlings (*Sinapis alba* L.) showed an increase in anthocyanin accumulation and consequently anthocyanin levels when they were repeatedly irradiated with either red or far-red light after a 36 hour dark period (Lange et al., 1971). Similarly, continued far-red and red light irradiation was effective to achieve the accumulation of anthocyanins in mustard cotyledons (Beggs et al., 1987). Phenylalanine ammonia-lyase (PAL) activity from the lower epidermis

where most of the anthocyanins were confined correlated positively with the anthocyanin accumulation during the first few hours after induction of continuous far-red light.

COLOR DEVELOPMENT. The rind color of citrus fruit is generally considered as the most important external quality trait (Kays, 1999) and is a primary determinant of consumers acceptance (Lado et al., 2014). Consumers have specific preferences regarding the coloration of mature citrus fruit. The coloration ranges from green for limes, orange for both mandarin and sweet orange, a bright yellow color for lemons and a characteristic pink color for red grapefruit (Alqu  zar et al., 2008a; Rodrigo et al., 2013). Fruit with a good coloration, is known to have a higher market value (Cronje et al., 2013). Therefore, ensuring proper color development, plays an important role in obtaining the right quality standards specified for the different *Citrus* varieties. Rind coloration is also considered an important component of the commercial maturity indexing of citrus fruit (Fig. 5), even though color development is known not to always occur concomitantly with internal ripening (Goodrich, 2000), or a delay in coloration may be sometimes observed.

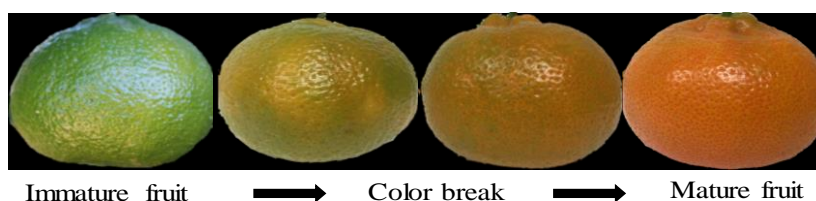


Fig. 5. Rind color development of 'Nadorcott' mandarin fruit.

The difference in color that exists between immature and mature fruit is ascribed to the changes in pigments present in the rind throughout the growing season (Rodrigo et al., 2004). Chlorophyll content in the flavedo of 'Shamouti' orange decreases as the growing season progresses, leaving the peel completely devoid of chlorophyll at maturity. The yellow color becomes visible due to the carotenoid synthesis during the maturation phase (Eilati et al., 1969).

Color development in citrus fruit is associated with ultrastructural changes and interconversions that take place within the two plastid forms: chloroplasts which are characteristic of immature fruit and chromoplasts that are present in mature fruit (Thomson et al., 1967). In the chloroplast during color conversion the grana thylakoids disintegrate which results in the gradual disintegration of chlorophyll, while plastoglobule formation within the chromoplast promotes the accumulation site for carotenoids (Gross et al., 1983). Lewis and Coggins (1964) postulated that carotenoid accumulation is not dependent on the rate of

chlorophyll loss. It was observed that although low light intensity resulted in the early loss of chlorophyll, carotenoid synthesis rate remained similar to that of GA-treated fruit where chlorophyll synthesis was prolonged. In ‘Star Ruby’ grapefruit, however, an association between higher lycopene accumulation and accelerated chromoplast differentiation was reported (Lado et al., 2015b). It was proposed that the accelerated differentiation of the chromoplasts resulted in a higher biosynthetic capacity and sink for carotenoid accumulation, thereby contributing to a better rind coloration.

Goldschmidt (1988) reviewed the factors responsible for the interconversion between chloro- and chromoplasts. This includes environmental factors, mainly temperature as well as light quality and intensity (Rodrigo et al., 2013), in combination with nutritional and hormonal control (Huff, 1983, 1984; Iglesias et al., 2001; Lewis and Coggins, 1964; Fig. 6).

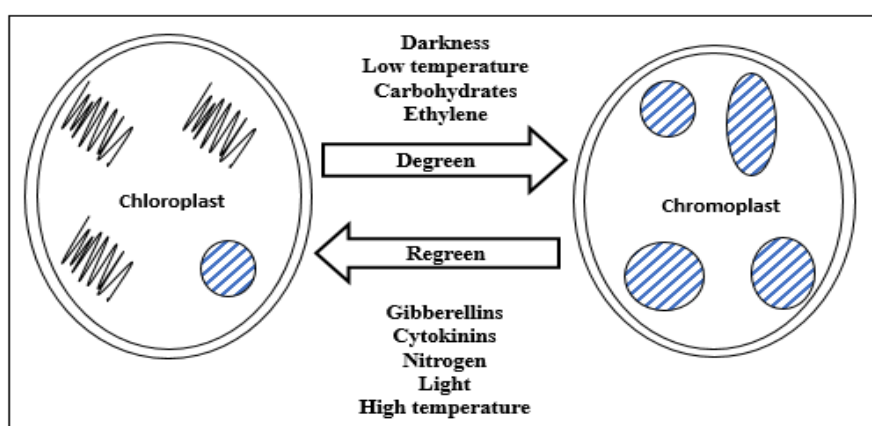


Fig. 6. Schematic representation of the chloroplast-chromoplast interconversion, and the regulatory factors involved (Adapted from Spiegel-Roy and Goldschmidt, 1996).

Light quality and intensity have a strong influence on the peel pigmentation of citrus fruit (Rodrigo et al., 2013). Navel orange fruit that received 5% of the normal light intensity, had approximately a 40% lower carotenoid level than fruit that received the normal intensity, resulting in pale colored fruit (Lewis and Coggins, 1964). The canopy position of fruit (outside vs. inside) also plays an important role in affecting the rind coloration of citrus fruit as the light intensity decreases as it penetrates deeper into the tree canopy (Cronje et al., 2013; Grant, 1997).

Several studies proved that fruit borne on the outside where a higher light intensity is prevalent has better color development compared to fruit from the inside of the canopy. ‘Kinnow’ mandarin and ‘Mihowase’ satsuma fruit harvested from the external canopy displayed better color development than fruit from the inside canopy (Khalid et al., 2012; Tuwana, 1999). ‘Nules Clementine’ fruit located on the outside canopy developed an intense

orange peel color, whereas fruit from the inside displayed a more yellow rind color. This difference was ascribed to the outside canopy fruit that received higher *PAR* levels, thus promoting color development (Cronje et al., 2013). In ‘Ruby Red’ grapefruit and ‘Hamlin’ sweet orange that was exposed to shade treatment a delay in color development was observed. In addition, the shaded grapefruit had a less intense yellow colored rind, compared to the control (Jifon and Syvertsen, 2001).

Contradictory results were found by Syvertsen et al. (2003) where shaded ‘Spring’ navel orange fruit showed a significantly better color development than open fruit. A possible explanation for this report could be that shade treatment that is known for reducing the PPF (radiation), may have caused diffused radiation under the net/cloth which then could have increased the light penetration to the inside of the canopy, resulting in an overall better coloration within the tree.

The effect of light on the pigmentation of ‘Star Ruby’ grapefruit often produces conflicting results, compared to that obtained for ‘sweet orange’ and mandarin fruit, which indicates that the regulatory processes of pigmentation may differ amongst the different citrus species (Rodrigo et al., 2013). Low light intensity and levels were observed to have a more profound effect on enhancing the red coloration of the ‘Star Ruby’ rind (Lado et al., 2015b). Color development was accelerated in fruit that was covered by leaves and other fruit, with the rind having a more intense red color than fruit that was fully exposed to light. Fruit that was covered in a bagging experiment also resulted in having an accelerated color development. The covered fruit developed a red color, whereas non-covered fruit displayed a yellow-orange coloration (Lado et al., 2015b). In blood oranges, intense sunlight exposure can either prevent or destroy anthocyanin development and therefore affect rind coloration. The best rind coloration is generally produced in less or partially shaded fruit (Hodgson, 1967).

Rodrigo et al. (2013) concluded that light alters the accumulation of the main pigments, thereby affecting the rind coloration of citrus fruit, yet the molecular mechanism responsible for regulating the process still needs to be determined.

5.2. Internal fruit quality parameters

The soluble solids content (SSC) of citrus fruit is composed mainly of soluble sugars and organic acids, with a small amount of other constituent’s present (Sinclair, 1961) which includes amino acids, ascorbic acid (Vitamin C), along with secondary metabolites (flavonoids), carotenoids and volatiles all of which are present in relatively small quantities (Davies and Albrigo, 1994).

Since carbohydrates are the major component of the SSC pool, the sugar content of citrus juice is measured with a refractometer and expressed as °Brix (Spiegel-Roy and Goldschmidt, 1996). Similarly, as citric acid is the most abundant organic acid present in citrus, the TA of citrus is also referred to as citric acid percentage (Bean and Todd, 1960; Goodrich, 2000).

During the fruit development phase, as the season progresses, the Brix of the juice increases to reach a constant level at maturity, whilst the acid percentage declines throughout the season, attaining lower levels at maturity (Bain, 1958; Albertini et al., 2006). Citrus juice flavor can range from insipid, sweet, to a sour taste (Hodgson, 1967). The SSC:Acid (SSC:TA) ratio is important in determining the overall juice quality, indicating the balance between sweet and tartness, and serves as a commercial maturity index (Goodrich, 2000; Ting and Attaway, 1971). Sweet fruit has a high SSC to TA ratio, however when this ratio is supra-optimal, the fruit will have an insipid taste. Fruit with low SSC to TA ratio has a sour taste, whereas fruit with a very low SSC:TA ratio are likely to taste tart (Davies and Albrigo, 1994). The SSC to TA ratio varies between locations and *Citrus* varieties, with each market having a specific acceptance standard regarding the SSC to TA ratio (Davies and Albrigo, 1994; Goodrich, 2000).

Citrus fruit is well known for its relatively high content of the secondary metabolite, ascorbic acid, also referred to as Vitamin C (Ting and Attaway, 1971). Vitamin C has many biological functions, including being strongly, positively correlated with high antioxidant activity (Rekha et al., 2012), and the ability to prevent scurvy (Davey et al., 2000).

5.2.1. Soluble Solids Content

The sweetness of citrus juice is determined by carbohydrates which contribute about 75-80% to the soluble component of citrus fruit pulp (Lado et al., 2018). The soluble sugar pool is composed mainly of the monosaccharides glucose and fructose and the oligosaccharide, sucrose (Albertini et al., 2006). Sucrose is then also considered the major translocatable sugar in citrus fruit (Bean and Todd, 1960; Chen et al., 2002; Davies and Albrigo, 1994).

The soluble sugar content of the fruit is highly dependent on photosynthesis as the source for the production of sugars (Jifon and Syvertsen, 2001). During the primary stage of fruit enlargement with the onset of cell enlargement, the leaves translocate most of their photo-assimilates to the fruit, where accumulation occurs in the juice sacs, being the major sink at that time. Thereafter, accumulation occurs throughout the growth and ripening process (Chen et al., 2002; Moon et al., 2011; Yakushiji et al., 1996, 1998). In addition to photosynthates supplied by the leaves, Chen et al. (2002) reported that the relatively high rate of photosynthesis by the

fruit during the onset of fruit enlargement also contributed photosynthates to the juice sacs. As photosynthesis is the main source of sugars to the juice, it is proposed that changes in the light levels in the canopy or under shade net treatments, will necessarily also result in changes in SSC levels of the fruit. In support of this hypothesis, Chen et al. (2002) reported that when fruit of 'Satsuma' mandarin cv. 'Miyagawa wase' was excluded from light by covering the fruit, a lowered total sugar concentration in the juice sacs was measured. In 'Valencia' orange fruit, studies comparing inside and outside canopy fruit concurred with the above finding as the soluble solid percentage decreased towards the inside of the canopy. Outside fruit had the highest sugar percentage (11.08%), followed by canopy fruit embedded within the leaf canopy at 10.01 %, and fruit classified as 'inside fruit', having the lowest SSC at 8.7% (Reitz and Sites, 1948). More recent studies by Khalid et al. (2012) and Verreynne et al. (2004) confirmed this initial report as outside canopy fruit again resulted in higher SSC values. Sites and Reitz (1949) linked the higher percentage soluble solids in outside fruit compared to inside fruit directly to the light levels received at the respective canopy positions. They stated that light and the factors controlling available light levels to be the principle factors affecting and to be highly correlated to the content of soluble solids in citrus juice.

Yet, contradictory to these reports, Tuwana (1999) found no difference in the SSC between outside and inside canopy 'Mihowase' Satsuma fruit. In an older study by Winston and Miller (1948) contradictory results was reported regarding SSC and the relevant canopy position for different citrus cultivars. Both 'Round orange' and 'Dancy' tangerine fruit that was exposed to higher irradiance on an outside canopy position had higher SSC levels than inside fruit. However, for 'Temple' oranges (*C. reticulata* x *C. sinensis*) no significant difference in SSC was recorded between the outside and inside fruit. Contradicting results regarding the SSC differences in fruit between various canopy positions could be ascribed to the canopy density that differs between cultivars, resulting in differences in light penetration in the canopy. The architecture of the canopy will influence the spectral irradiance (Grant, 1997) as in a dense canopy it is easier to distinguish between outside and inside fruit.

In a shade net experiment on 'Marsh seedless' grapefruit (*C. paradisi* Macf.), Cohen et al. (2000) found no difference in the juice sugar content of control and the trees shaded with 30% and 60% reflective screens, respectively. An explanation for this finding is that shade nets will only be detrimental to SSC when light levels are reduced to such an extent that it caused a decline in the rate of photosynthesis. Under conditions of high and excess irradiance, shading may offer protection against photoinhibition, and a possible reduction in photosynthesis (Jifon and Syvertsen, 2001), thereby positively influencing SSC content.

5.2.2. Acidity

Citric acid is considered the predominant acid in citrus fruit juice, although the presence of malic acid has also long been recognized (Clements, 1964). More recently, Albertini et al. (2006) identified seven organic acids in the pulp of several *Citrus* varieties, but with only oxalic, citric and quinic acid showing changes during fruit development. Organic acid metabolism in fruit occurs through the Krebs cycle, with organic acids and/or sugars as precursors (Ulrich, 1970). Accumulation of citric acid in the juice vesicles of citrus fruit could be ascribed to an inhibition of aconitase activity, an enzyme known to interfere with the conversion of citrate to aconitic acid in the Krebs cycle (Baldwin, 1993). The characteristic decrease in TA as the season progresses was, however, linked to a dilution effect as caused by an increase in fruit size and juice content (Ting and Attaway, 1971).

Contradicting results have been reported regarding the acid content of fruit from different canopy positions. Several studies reported no difference between the out- and inside canopy positions for ‘Valencia’ oranges (Reitz and Sites, 1948), ‘Kinnow’ mandarin (Khalid et al., 2012), ‘Mihowase’ satsuma (Tuwana, 1999), ‘round orange’ (Winston and Miller, 1948) or ‘Temple’ tangor (Verreynne et al., 2004). However, some studies have reported differences amongst canopy positions, where inside fruit had a higher acid content as opposed to outside fruit for ‘Dancy’ tangerine (Winston and Miller, 1948), ‘Satsuma’, ‘Clementine’ and ‘Fairchild’ tangor fruit (Verreynne et al., 2004). Contradicting this, Winston and Miller (1948) found a higher acid content for outside ‘Temple’ orange fruit. In an isolated report on the influence of reflective shade screens (30 and 60%) on ‘Marsh seedless’ grapefruit fruit quality, no difference in acidity was reported in the juice acid content for either shaded or non-shaded fruit (Cohen et al., 2000). Based on these contradicting results with regards to the role of light on the acidity of citrus fruit, future research is required on this aspect.

5.2.3. Ascorbic Acid

In a review on the synthesis of ascorbic acid (AsA) in plants by Isherwood and Mapson (1962) it was stated that light has an influence on the ascorbic acid content, although no direct photochemical reaction essential for synthesis was identified. Therefore, photosynthesis is considered to have an indirect role by providing precursor sugars for the formation of AsA, with its synthesis thus dependent on a steady supply of hexose sugars through photosynthesis (Mapson, 1970).

Canopy position and light exposure to a fruit have been shown to have an influence on the AsA content in citrus juice. Reitz and Sites (1948) reported ‘Valencia’ orange fruit borne

on the top and outside of the tree canopy, which was more exposed to light, to have a higher AsA juice content compared to fruit borne on the inside of the canopy. This finding concurred with a report by Winston and Miller (1948) on ‘Dancy’ tangerines, ‘Temple’ oranges and ‘round oranges’. Contradictory to this, Khalid et al. (2012), however found no effect of canopy position on the AsA content of ‘Kinnow’ mandarin, whilst Lado et al. (2015a) reported no difference in AsA content in the pulp of orange, mandarin or grapefruit. This study did, however, conclude that light avoidance resulted in reduced AsA concentration in the flavedo, therefore suggesting that the effect of light on vitamin C may be restricted to the flavedo. Of interest is that Alós et al. (2014) established that the regulation pathways of ascorbic acid differed amongst the various fruit tissues in *Citrus*, with the peel generally having a higher AsA content than the pulp, but with the highest content to be concentrated in the flavedo (Alós et al., 2014; Eaks, 1964). These higher AsA levels in the peel were thus linked to the direct exposure to light (Alós et al., 2014).

To summarize: light is not essential for the synthesis of AsA, but exposure of the fruit during its development to light impacts on the Vitamin C content (Nagy, 1980). Lado et al. (2015a) reported on transcriptional changes in the D-galacturonic acid biosynthetic pathway of AsA in response to light, thereby suggesting that this alternative pathway, in addition also contributes to the AsA pool and has a regulatory role in the concentration of AsA, within the flavedo of citrus fruit.

5.3. Biochemical changes in the rind due to light

5.3.1. Carbohydrates

Carbohydrates are the main constituent in the rind (flavedo and albedo), with free sugars as sucrose, glucose, fructose and traces of xylose and rhamnose making up 50% of the dry weight of orange and grapefruit peel. Pectic substances, hemicellulose and cellulose and other polysaccharides are also present in the rind (flavedo and albedo) (Ting and Deszyck, 1961). The reducing sugars (glucose and fructose) are generally found in higher concentration than sucrose in the flavedo (Holland et al., 2005; Huff, 1984). The albedo itself is rich in monosaccharides and abundant in starch (Ting and Attaway, 1971), whilst starch can also be found in the flavedo, but at lower levels (Holland et al., 2005).

Different exposure to light levels influences the sugar concentration, glucose, fructose and sucrose in the flavedo. Cronje et al. (2013) established that ‘Nules Clementine’ fruit that developed under high light conditions had a higher sucrose concentration in the flavedo, compared to that of fruit that developed under low-light. However, in one season in this study,

although the mean values were higher in outside fruit, it was not statistically different to the inside canopy fruit. Cronje et al. (2013) reported that *PAR* levels in the canopy differed between the outside and inside position and proposed that these levels differentially influenced the chlorophyll concentration of the flavedo, followed by the photosynthetic rate and thereby affecting the carbon-fixation of the fruit accordingly. Contradicting results were, however, reported for ‘Marsh’ grapefruit, which showed no difference in fruit between canopy positions (Purvis, 1980).

An experiment where fruit was covered to exclude light from the fruit surface resulted in lower sugar concentration in the flavedo of both ‘Satsuma’ (Chen et al., 2002) and ‘Nules Clementine’ (Magwaza et al., 2013). Chen et al. (2002) reported that flavedo photosynthesis plays an important role in sugar accumulation in ‘Satsuma’ mandarin, and the preclusion of photosynthesis could therefore explain the reduced sugar concentration that was recorded in the flavedo of covered fruit.

5.3.2. Secondary metabolites

Citrus fruit contain large amounts of flavonoids (Robbins, 1980 cited by Kanes et al., 1993), with flavonols, anthocyanins, flavones, flavanones and also flavanone glycosides as some of the most prominent groups (Maier and Hasegawa, 1970; Ting and Attaway, 1971). The flavonoids are important antioxidants whilst the pigment anthocyanins is well known for its health-promoting function (Schreiner et al., 2013).

The flavonoid composition of citrus fruit, especially within the rind have been well-studied (Kanes et al., 1993; Nogata et al., 2006; Tatum and Berry, 1972). Flavonoid content is usually found to be higher in the rind compared to the rest of the fruit (Ghasemi et al., 2009). In particular, the polymethoxylated flavones have been reported to occur in higher concentrations in the fruit rind than in the juice for oranges (Veldhuis et al., 1970). The most abundant polymethoxyflavones in citrus fruit include tangeretin, heptamethoxyflavone, nobiletin, sinensetin, and quercetogetin (Del Rio et al., 1998; Gaydou et al., 1987). The most important flavanones in citrus fruit includes naringin, hesperidin and neohesperidin (Ortuno et al., 1997). Naringin accumulates particularly in high amounts in grapefruit (Maier and Hasegawa, 1970), whilst hesperidin is more dominant in mandarin and orange fruit (Ortuno et al., 1997).

Flavanones occur in high concentrations in the albedo, with flavone accumulation being more abundant in the flavedo (Kanes et al., 1993). Flavanone synthesis is considered to take place during the early growth stages of fruit development. Naringin, the flavanone component

responsible for the characteristic bitter taste, was high in immature 'Isaac' grapefruit and was shown to decrease towards maturity. The same pattern occurred for hesperidin in 'Galleta' mandarin fruit (Ortuno et al., 1997).

Flavonoid is thought to serve as a protection mechanism in plants against UV-radiation (Schmelzer et al., 1988). Reactive oxygen species (ROS) are formed as a result of high light intensity (UV-B) (Mackerness, 2000). These active forms of oxygen cause the degradation of the plasmamembrane and the cytosolic organelles and consequently cell death, however, flavonoids have high antioxidant activity and are therefore capable of removing damaging oxidants and free radicals (Taiz et al., 2015). Separately or in combination, flavonoids and anthocyanins result in an attenuation in the UV-B radiation (Mackerness, 2000). The antioxidant activity of flavonoids in citrus has also been recognized to have major advantages for human health (Zou et al., 2016).

Arabidopsis has been reported to accumulate flavonoids in response to high light intensity (UV-B) exposure, providing these leaves with their higher flavonoid concentration more resistance to UV-irradiation (Lois, 1994). A study by Lois and Buchanan (1994) supported this hypothesis as an *Arabidopsis* mutant that was incapable of biosynthesizing certain flavonoids showed severe sensitivity to UV-B radiation compared to the wild type and consequently developed severe necrosis in the aerial tissue.

Flavonoid accumulation is regulated by photo-control with phytochrome activity, high intensity blue light and UV exposure as some of the major photo-responses responsible for flavonoid accumulation, in addition to others mentioned (McClure, 1975). Tevini and Iwanzik, (1983) reported an increase in flavonoid content of radish seedlings (*Raphanus sativus*) in response to an increase in UV-B irradiance. Alternatively, in mustard cotyledons, continuous far-red light was effective to promote the accumulation of flavonoids, anthocyanin and quercetin (flavanone). However, continuous red-light was more effective to enable anthocyanin accumulation than quercetin. Quercetin showed a low and weak response to a single red-light pulse, but also continuous red light exposure (Beggs et al., 1987). UV irradiation, with emphasis on irradiation hours, was suggested to modulate the synthesis and accumulation of two important flavonoids, naringin (flavanone) and tangeretin (polymethoxyflavone) in *C. aurantium* fruit. Fruit exposed to irradiation for one hour showed similar levels of flavonoids than control fruit, however, when fruit was irradiated for two hours, the naringin and tangeretin levels were significantly higher in the irradiated fruit compared to the control (Arcas et al., 2000). In parsley leaves (*Petroselinum crispum*) light has also been reported to modulate chalcone synthase, a key enzyme in flavonoid synthesis, which is hypothesized to be light-

induced (Schmelzer et al., 1988). Leaves cultivated under light conditions resulted in accumulating more flavonoids, whilst when a dark period was applied the flavonoid content decreased.

5.4. Environmental damage to the rind

Extreme environmental conditions such as high temperatures and excessive solar radiation generally result in sunburn on fruit surfaces, which either cause a discoloration (yellow to bronze) or in more advanced cases, necrosis of the peel exposed to the sun (Ketchie and Ballard, 1968; Schrader et al., 2001). Racsko and Schrader (2012) ascribed these two environmental factors as the main induction factors of sunburn in apples, with relative humidity, wind velocity and geographic location contributing to the indirect factors. The surface injuries associated with sunburn necessarily lead to a reduction in yield and fruit quality and therefore market value (Piskolczi et al., 2004).

Sunburn in citrus has not been well researched, although it has been established that solar radiation and air temperature are the main inducing factors. Ketchie and Ballard (1968) reported that exposed ‘Campbell Valencia’ orange displayed symptoms of sunburn, as opposed to non-exposed fruit. The average air and core fruit temperature as well as solar radiation were markedly higher during periods coinciding with sunburn incidences. Similarly, Sadamatsu (1981) reported that the sunburn frequency on ‘Satsuma’ mandarin fruit declined with a reduction in sunshine exposure.

Research done on apples (*Malus domestica* Borkh.) reported sunburn to generally occur when the fruit surface temperature exceeds that of the air temperature for extended periods, and during sudden exposure to high irradiance and temperature conditions (Schrader et al. 2003; Wünsche et al., 2001). Schrader et al. (2003) found a correlation between maximum fruit surface temperature and solar radiation as well as maximum air temperature of ‘Fuji’ BC-2 apples. In addition to solar radiation and air temperature, relative humidity, wind speed and tree vigor also influenced fruit surface temperature (Schrader et al., 2001).

Various research has been done on apples under shade net as a method to reduce sunburn, with very limited research on citrus. Gindaba and Wand (2005) reported that 20% black shade net effectively reduced sunburn and fruit temperature on ‘Cripps’ Pink’ and ‘Royal Gala’ apples. Similar findings were reported for ‘Mondial Gala’ apples under black and transparent nets and Iglesias and Alegre (2006) ascribed the reduction in sunburn to the lower radiation on the fruit and fruit temperature. Further in a shade net experiment using 20% black netting on four apple cultivars, ‘Royal Gala’, ‘Braeburn’, ‘Fuji’ and ‘Cripps Pink’, sunburn was reduced

under the nets over two seasons for both 'Braeburn' and 'Fuji', but only in one season for 'Royal Gala'. Smit (2007) ascribed these differences to a reduction in direct sunlight under the net. With regards to citrus under shade net, a lower percentage sunburn was recorded for 'Ponkan' mandarin (*C. reticulata* Blanco) under 20% white shade net (Lee et al., 2015).

6. Conclusion

In this review, it was aimed to discuss the direct or indirect impact of light on citrus fruit quality. Citrus trees can effectively photosynthesize under relatively low *PAR*, thus it may be hypothesized that the implementation of shade nets designed to reduce high light intensity may not necessarily influence carbohydrate production negatively. From current knowledge, it is therefore speculated that citrus flowering will not be influenced directly by shade nets as light has a rather indirect role in the reproductive development of citrus trees through the supply of carbohydrates from photosynthesis.

Light however has shown to have an important effect on the internal fruit quality aspects such as total soluble solids and vitamin C content, based on studies where differences have been found in relation to canopy positions. It is hypothesized that if photosynthesis is to be negatively impacted by shade nets, this negative effect will be transferred to undesirable SSC and ascorbic acid content in the fruit.

Rind coloration, which is considered as the most important external quality trait in citrus fruit, is particularly influenced by light, especially when comparing rind coloration differences in relation to canopy positions. Based on our current understanding color development could be negatively impacted under shade nets with a high shading factor where light levels are greatly reduced. However, as shade nets also cause light diffusion and scattering under the nets it is proposed that light penetration may increase into the tree canopy, possibly resulting in an overall improved coloration of the fruit.

The outcome of the use of shade netting with regard to citrus fruit quality under South African conditions is unknown as contradicting result with regards to quality aspects in relation to canopy positions where different light levels are relevant, have been reported. In studies concerning the use of shade netting in citrus, it is important to consider the whole tree and environment interactions, including the role of rootstocks, tree density, the tree phenology, and presiding temperature when concluding on the possible impact of light on the fruit quality and tree response to cultivation under shade netting.

7. Literature Cited

- Albertini, M., E. Carcouet, O. Pailly, C. Gambotti, F. Luro, and L. Berti. 2006. Changes in organic acids and sugars during early stages of development of acidic and acidless citrus fruit. *J. Agr. Food Chem.* 54:8335–8339.
- Alós, E., M. Cercós, M.J. Rodrigo, L. Zacarías, and M. Talón. 2006. Regulation of color break in citrus fruits: Changes in pigment profiling and gene expression induced by gibberellins and nitrate, two ripening retardants. *J. Agr. Food Chem.* 54:4888–4895.
- Alós, E., M.J. Rodrigo, and L. Zacarías. 2014. Differential transcriptional regulation of L-ascorbic acid content in peel and pulp of citrus fruits during development and maturation. *Planta.* 239:1113–1128.
- Alquézar, B., M.J. Rodrigo, and L. Zacarías. 2008a. Carotenoid biosynthesis and their regulation in citrus fruits. *Tree and For. Sci. Biotechnol.* 2:23–35.
- Alquezar, B., M.J. Rodrigo, and L. Zacarías. 2008b. Regulation of carotenoid biosynthesis during fruit maturation in the red-fleshed orange mutant Cara Cara. *Phytochemistry* 69:1997–2007.
- Apel, K. and H. Hirt. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55:373–399.
- Arcas, M. C., J.M. Botía, A.M. Ortuño, and J.A. Del Río. 2000. UV irradiation alters the levels of flavonoids involved in the defence mechanism of *Citrus aurantium* fruits against *Penicillium digitatum*. *European J. Plant Pathol.* 106:617–622.
- Aschan, G. and H. Pfan. 2003. Non-foliar photosynthesis: A strategy of additional carbon acquisition. *Flora* 198:81–97.
- Bain, J.M. 1958. Morphological, anatomical and physiological changes in the developing fruit of the Valencia orange, *Citrus sinensis* (L.). Osbeck. *Austral. J. Bot.* 6:1–24.
- Baldwin, E.A. 1993. Citrus fruit, p. 108–149. In: G.B. Seymour, J.E. Taylor, and G.A. Tucker (eds.). *Biochemistry of fruit ripening*. Chapman and Hall, London.
- Bartley, G.E. and P.A. Scolnik. 1995. Plant carotenoids: Pigments for photoprotection, visual attraction, and human health. *The Plant Cell.* 7:1027–1038.
- Basile, B., R. Romano, M. Giaccone, E. Barlotti, V.Colonna, C. Cirillo, Y. Shahak, and M. Forlani. 2008. Use of photo-selective nets for hail protection of kiwifruit vines in Southern Italy. *Acta Hort.* 770:185–192.
- Bastías, R.M. and L. Corelli-Grappadelli. 2012. Light quality management in fruit orchards: Physiological and technological aspects. *Chilean. J. Agr. Res.* 72:574–581.

- Batschauer, A. 1999. Light perception in higher plants. *Cell. Mol. Life Sci.* 55:153–166.
- Bean, R.C. and G.W. Todd. 1960. Photosynthesis and respiration in developing fruits: I. $C^{14}O_2$ uptake by young oranges in light and in dark. *Plant Physiol.* 35:425–429.
- Beggs, C. J., K. Kuhn, R. Böcker, and E. Wellmann. 1987. Phytochrome-induced flavonoid biosynthesis in mustard (*Sinapis alba* L.) cotyledons: Enzymatic control and differential regulation of anthocyanin and quercetin formation. *Planta.* 172:121–126.
- Bustan, A., E.E. Goldschmidt, and Y. Erner. 1996. Carbohydrate supply and demand during fruit development in relation to productivity of grapefruit and ‘Murcott’ mandarin. *Acta Hort.* 416:81–88.
- Buttrose, M.S. and D.M. Alexander. 1978. Promotion of floral initiation in “Fuerte” avocado by low temperature and short daylength. *Scientia Hort.* 8:213–217.
- Caldwell, M., R. Robberecht, and S. Flint. 1983. Internal filters: Prospects for UV-acclimation in higher plants. *Physiol. Plant.* 58:445–450.
- Charles, M.T. 2008. Physiological basis of UV-C induced resistance to *Botrytis cinerea* in tomato fruit. II. Modification of fruit surface and changes in fungal colonization. *Postharvest Biol. Technol.* 47:21–26.
- Chen, J.W., S.L. Zhang, L.C. Zhang, Z.Z. Zhao, and J.G. Xu. 2002. Fruit photosynthesis and assimilate translocation and partitioning: Their characteristics and role in sugar accumulation in developing *Citrus unshiu* fruit. *Acta Botanica Sinica* 44:158–163.
- Clements, R.L. 1964. Organic acids in citrus fruits: I. Varietal differences. *J. Food Sci.* 29:276–280.
- Cohen, S., A. Grava, and E.E. Goldschmidt. 2000. Citrus response to radiation load reduction: Water use, photosynthesis, and productivity. *Proc. Intl. Soc. Citricult. IX Congr.* 1:615–618
- Cohen, S., S. Moreshet, L. Le Guillou, J. Simon, and M. Cohen. 1997. Response of citrus trees to modified radiation regime in semi-arid conditions. *J. Expt. Bot.* 48:35–44.
- Cronje, P.J.R., G.H. Barry, and M. Huysamer. 2013. Canopy position affects pigment expression and accumulation of flavonoid carbohydrates of ‘Nules Clementine’ mandarin fruit, thereby affecting rind condition. *J. Amer. Soc. Hort. Sci.* 138:217–224.
- Davenport, T.L. 1990. Citrus flowering. *Hort. Rev.* 12:349–408.
- Davey, M. W., M. Van Montagu, D. Inzé, M. Sanmartin, A. Kanellis, N. Smirnoff, I.J.J. Benzie, J.J. Strain, D. Favell, and J. Fletcher. 2000. Plant L -ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. *J. Sci. Food Agr.* 80:825–860.
- Davies, F.S. and L.G. Albrigo. 1994. *Citrus*. CAB International, Wallingford.

- Del Rio, J. A., M.C. Arcas, O. Benavente-Garcia, and A. Ortuno. 1998. Citrus polymethoxylated flavones can confer resistance against *Phytophthora citrophthora*, *Penicillium digitatum*, and *Geotrichum species*. *J. Agric. Food. Chem.* 46:4423–4428.
- Eaks, I.L. 1964. Ascorbic acid content of citrus during growth and development. *Bot. Gaz.* 125:186–191.
- Ehara, T., T. Nogata, and T. Nakamuta. 1981. Studies on fruit-bearing branches of Satsuma mandarins. *Proc. Int. Soc. Citricult.* 1:209–214.
- Eilati, S.K., S.P. Monselise, and P. Budowski. 1969. Seasonal development of external color and carotenoid content in the peel of ripening ‘Shamouti’ oranges. *J. Amer. Soc. Hort. Sci.* 94:346–348.
- El-Otmani, M., M.L. Arpaia, C.W. Coggins, Jr., J.E. Pehrson, Jr., and N.V. O’Connell. 1989. Developmental changes in ‘Valencia’ orange fruit epicuticular wax in relation to fruit position on the tree. *Scientia Hort.* 41:69–81.
- Fallahi, E. and J.W. Moon, Jr. 1988. Effect of canopy position on quality, photosynthesis and mineral nutrition of four citrus varieties. *Univ. of Arizona Citrus Rpt. P-76:* 5–12.
- Farin, D., R. Ikan, and J. Gross. 1983. The carotenoid pigments in the juice and flavedo of a mandarin hybrid (*Citrus reticulata*) cv Michal during ripening. *Phytochemistry.* 22:403–408.
- Furr, J.R., W.C. Cooper, and P.C. Reece. 1947. An investigation of flower formation in adult and juvenile citrus trees. *Amer. J. Bot.* 34:1–8.
- Gao, H., J. Xu, X. Liu, B. Liu, X. Deng. 2011. Light effect on carotenoids production and expression of carotenogenesis genes in citrus callus of four genotypes. *Acta Physiol. Plant.* 33:2485–2492.
- Garcia-Luis, A., F. Fornes, and J.L. Guardiola. 1995. Leaf carbohydrates and flower formation in Citrus. *J. Amer. Soc. Hort. Sci.* 120:212–227.
- Gaydou, E.M., J.P. Bianchini, and R.P. Randriamiharisoa. 1987. Orange and mandarin peel oils differentiation using polymethoxylated flavone composition. *J. Agr. Food Chem.* 35:525–529.
- Ghasemi, K., Y. Ghasemi, and M.A. Ebrahimzadeh. 2009. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pak. J. Pharm. Sci.*, 22:277–281.
- Giese, B.N. 1975. Effects of light and temperature on the composition of epicuticular wax of barley leaves. *Phytochemistry.* 14:921–929.
- Gindaba, J. and S.J.E. Wand. 2005. Comparative effects of evaporative cooling, kaolin particle film, and shade net on sunburn and fruit quality in apples. *HortScience* 40:592–596.

- Goldschmidt, E.E. 1988. Regulatory aspects of chloro-chromoplast interconversions in senescing *Citrus* fruit peel. *Isr. J. Bot.* 37:123–130.
- Goldschmidt, E.E. 1999. Carbohydrate supply as a critical factor for citrus fruit development and productivity. *HortScience.* 34:1020–1024.
- Goldschmidt, E.E. and A. Golomb. 1982. The carbohydrate balance of alternate-bearing citrus trees and the significance of reserves for flowering and fruiting. *J. Amer. Soc. Hort. Sci.* 107:206–208.
- Goldschmidt, E.E., N. Aschkenazi, Y. Herzano, A.A. Schaffer, and S.P. Monselise. 1985. A role for carbohydrate levels in the control of flowering of citrus. *Scientia Hort.* 26:159–166.
- Goldschmidt, E.E. and S.P. Monselise. 1977. Physiological assumptions toward the development of a citrus fruiting model. *Proc. Int. Soc. Citricult.* 2:668–672.
- Goodrich, R. 2000. Consumer product quality attributes and contribution of citric acid in juices. *Proc. Intl. Soc. Citricult. IX Congr.* 1:639.
- Grant, R.H. 1997. Partitioning of biologically active radiation in plant canopies. *Int. J. Biometeorol.* 40:26–40.
- Grierson, W. 2002. Fruit development, maturation, and ripening, p. 143–159. In: M. Pessarakli (ed.). *Handbook of plant and crop physiology.* Marcel Dekker, Inc., New York.
- Grierson, W. 2006. Anatomy and physiology, p. 1–22. In: W.F. Wardowski, W.M. Miller, D.J. Hall and W. Grierson (eds.). *Fresh citrus fruits.* Florida Science Source, Inc., Florida.
- Gross, J. 1987. *Pigments in fruits.* Academic Press, London.
- Gross, J., Gabai, M., and Lifshitz, A., 1972. A comparative study of the carotenoid pigments in juice of Shamouti, Valencia and Washington oranges, three varieties of *Citrus sinensis*. *Phytochemistry.* 11:303–308.
- Gross, J., R. Timberg, and M. Graef. 1983. Pigment and ultrastructural changes in the developing pummelo citrus grandis ‘Goliath’. *Bot. Gaz.* 144:401–406.
- Guardiola, J., C. Monerri, and M. Agusti. 1982. The inhibitory effect of gibberellic acid on flowering in *Citrus*. *Physiol. Plant.* 55:136–142.
- Guardiola, J.L. 1988. Factors limiting productivity in citrus: A physiological approach. *Proc. 6th Int. Citrus Congress* 1:381–394.
- Hodgson, R.W. 1967. Horticultural varieties of citrus, p. 431–591. In: W. Reuther, H.J. Webber, and L.D. Batchelor (eds.). *The citrus industry, Vol 1.* University of California, USA.
- Holland, N., H.C. Menezes, and M.T. Lafuente. 2005. Carbohydrate metabolism as related to high-temperature conditioning and peel disorders occurring during storage of citrus fruit. *J. Agr. Food Chem.* 53:8790–8796.

- Huff, A. 1983. Nutritional control of regreening and degreening in citrus peel segments. *Plant Physiol.* 73:243–249.
- Huff, A. 1984. Sugar regulation of plastid interconversions in epicarp of citrus fruit. *Plant Physiol.* 76:307–312.
- Iglesias, D. J., F.R. Tadeo, F. Legaz, E. Primo-Millo, and M. Talon. 2001. In vivo sucrose stimulation of colour change in citrus fruit epicarps: Interactions between nutritional and hormonal signals. *Physiol. Plant.* 112:244–250.
- Iglesias, D.J., F.R. Tadeo, E. Primo-Millo, and M. Talon. 2003. Fruit set dependence on carbohydrate availability in citrus trees. *Tree Physiol.* 23:199–204.
- Iglesias, D.J., M. Cercós, J.M. Colmenero-Flores, M.A. Naranjo, G. Ríos, E. Carrera, O. Ruiz-Rivero, I. Lliso, R. Morillon, F.R. Tadeo, and M. Talon. 2007. Physiology of citrus fruiting. *Braz. J. Plant Physiol.* 19:333–362.
- Iglesias, I. and S. Alegre. 2006. The effect of anti-hail nets on fruit protection, radiation, temperature, quality and profitability of ‘Mondial Gala’ apples. *J. Appl. Hort.* 8:91–100.
- Isherwood, F.A. and L.W. Mapson. 1962. Ascorbic acid metabolism in plants: Part II. biosynthesis. *Annu. Rev. Plant Physiol.* 13:329–350.
- Jahn, O.L. 1973. Inflorescence types and fruiting patterns in Hamlin and Valencia oranges and Marsh grapefruit. *Amer. J. Bot.* 60:663–670.
- Jifon, J.L. and J.P. Syvertsen. 2001. Effects of moderate shade on citrus leaf gas exchange, fruit yield and quality. *Proc. Fla. State Hort. Soc.* 114:177–181.
- Jifon, J.L. and J.P. Syvertsen. 2003. Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves. *Tree physiol.* 23:119–27.
- Juniper, B.E. 1960. Growth, development, and effect of the environment on the ultra-structure of plant surfaces. *J. Linn. Soc.* 56:413–419.
- Juniper, B.E. and C.E. Jeffree. 1983. *Plant surfaces*. Edward Arnold, London.
- Kanes, K., B. Tisserat, M. Berhow, and C. Vandercook. 1993. Phenolic composition of various tissues of rutaceae species. *Phytochemistry.* 32:967–974.
- Kato, M., Y. Ikoma, H. Matsumoto, M. Sugiura, H. Hyodo, and M. Yano. 2004. Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit. *Plant Physiol.* 134:824–837.
- Kays, S.J. 1999. Preharvest factors affecting appearance. *Postharvest Biol. Technol.* 15:233–247.

- Ketchie, D.O. and A.L. Ballard. 1968. Environments which cause heat injury to 'Valencia' oranges. Proc. Amer. Soc. Hort. Sci. 93:166–172.
- Khalid, S., A.U. Malik, B.A. Saleem, A.S. Khan, M.S. Khalid, and M. Amin. 2012. Tree age and canopy position affect rind quality, fruit quality and rind nutrient content of 'Kinnow' mandarin (*Citrus nobilis* Lour × *Citrus deliciosa* Tenora). Scientia Hort. 135:137–144.
- Kikuchi, T., K. Kadoya, and T. Kuraoka. 1964. Morphological studies on the development of citrus fruits. II. Differences in certain species and varieties. J. Jpn. Soc. Hort. Sci. 33:8 (abstr.).
- Kinet, J.M. 1993. Environmental, chemical and genetic control of flowering. Hort. Rev. 15:279–334.
- Koshita, Y. and T. Takahara. 2004. Effect of water stress on flower-bud formation and plant hormone content of Satsuma mandarin (*Citrus unshiu* Marc.). Scientia Hort. 99:301–307.
- Krajewski, A.J. and E. Rabe. 1995. Citrus flowering: A critical evaluation. J. Hort. Sci. 70:357–374.
- Krezdorn, A.H. 1986. Flowering and fruit set of citrus, p. 1–14. In: J.J. Ferguson (ed.). Citrus flowering, fruit set and development (citrus short course). Fruit crops Dept, IFAS, University of Florida.
- Kriedemann, P.E. 1968. Some photosynthetic characteristics of citrus leaves. Aust. J. Biol. Sci. 21:895–905.
- Kuraoka, T. and T. Kikuchi. 1961. Morphological studies on the development of citrus fruits I. Satsuma orange. J. Jpn. Soc. Hort. Sci. 30:189 (abstr.).
- Lado, J., E. Alós, M.J. Rodrigo, and L. Zacarías. 2015a. Light avoidance reduces ascorbic acid accumulation in the peel of *Citrus* fruit. Plant Science. 231:138–147.
- Lado, J., P. Cronje, B. Alquézar, A. Page, M. Manzi, A. Gómez-Cadenas, A.D. Stead, L. Zacarías, and M.J. Rodrigo. 2015b. Fruit shading enhances peel color, carotenes accumulation and chromoplast differentiation in red grapefruit. Physiol. Plant. 154:469–484.
- Lado, J., G. Gambetta, and L. Zacarias. 2018. Key determinants of citrus fruit quality: Metabolites and main changes during maturation. Scientia Hort. 233:238–248.
- Lado, J., M.J. Rodrigo, and L. Zacarías. 2014. Maturity indicators and citrus fruit quality. Stewart Post. Rev. 2(2):1–6.
- Lang, A.R.G., X. Yueqin, and J.M. Norman. 1985. Crop structure and the penetration of direct sunlight. Agr. For. Meteorol. 35:83–101.
- Lange, H., W. Shropshire, Jr., and H. Mohr. 1971. An analysis of phytochrome-mediated anthocyanin synthesis. Plant Physiol. 47:649–655.

- Lee, T.C., P.J. Zhong, and P.T. Chang. 2015. The effects of preharvest shading and postharvest storage temperatures on the quality of 'Ponkan' (*Citrus reticulata* Blanco) mandarin fruits. *Scientia Hort.* 188:57–65.
- Lenz, F. 1969. Effects of day length and temperature on the vegetative and reproductive growth of 'Washington Navel' orange. *Proc. First Intl. Citrus Symp.* 1:333–338.
- Lewis, L.N. and C.W. Coggins. 1964. The inhibition of carotenoid accumulation in Navel oranges by gibberellin A₃, as measured by thin layer chromatography. *Plant and Cell Physiol.* 5:457–463.
- Lois, R. 1994. Accumulation of UV-absorbing flavonoids induced by UV-B radiation in *Arabidopsis thaliana* L. *Planta.* 194:498–503.
- Lois, R. and B.B. Buchanan. 1994. Severe sensitivity to ultraviolet radiation in an *Arabidopsis* mutant deficient in flavonoid accumulation. *Planta.* 194:504–509.
- Lovatt, C.J., Y. Zheng, and K.D. Hake. 1988. Demonstration of a change in nitrogen metabolism influencing flower initiation in *Citrus*. *Isr. J. Bot.* 37:181–188.
- Ma, G., L. Zhang, M. Kato, K. Yamawaki, Y. Kiriwa, M. Yahata, Y. Ikoma, and H. Matsumoto. 2012. Effect of blue and red LED light irradiation on β -Cryptoxanthin accumulation in the flavedo of citrus fruits. *J. Agr. Food Chem.* 60:197–201.
- Mackerness, S.A.H. 2000. Plant responses to ultraviolet-B (UV-B: 280 – 320 nm) stress: What are the key regulators? *Plant Growth Regulat.* 32:27–39.
- Magwaza, L. S., U.L. Opara, P.J.R. Cronje, S. Landahl, and L.A. Terry. 2013. Canopy position affects rind biochemical profile of 'Nules Clementine' mandarin fruit during postharvest storage. *Postharvest Biol. Technol.* 86:300–308.
- Maier, V.P. and S. Hasegawa. 1970. L-phenylalanine ammonia-lyase activity and naringenin glycoside accumulation in developing grapefruit. *Phytochemistry* 9:139–144.
- Mapson, L.W. 1970. Vitamins in fruits, p. 369-384. In: A.C. Hulme (ed.). *The biochemistry of fruits and their products.* Vol 1. Academic Press INC., London.
- Mataa, M., and S. Tominaga. 1998. Effects of shading stage and level on fruit set and development, leaf carbohydrates and photosynthesis in Ponkan (*Citrus reticulata* Blanco). *Jpn. J. Trop.Agr.* 42:103–110.
- McClure, J.W. 1975. Physiology and functions of flavonoids, p. 970–1055. In: J.B. Harborne, T.J. Mabry and H. Mabry (eds.). *The flavonoids.* Chapman and Hall Ltd., London.
- McDonald, R.E., H.E. Nordby, and T.G. McCollum. 1993. Epicuticular wax morphology and composition are related to grapefruit chilling injury. 28:311–312.

- Mehouachi, J., D. Serna, S. Zaragoza, M. Agusti, M. Talon, and E. Primo-Millo. 1995. Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of *Citrus unshiu*. *Plant Sci.* 107:189–197.
- Menino, M. R., C. Carranca, A. De Varennes, V.V. d’Almeida, and J. Baeta. 2003. Tree size and flowering intensity as affected by nitrogen fertilization in non-bearing orange trees grown under Mediterranean conditions. *J. Plant Physiol.*, 160:1435–1440.
- Meredith, F.I. and R.H. Young. 1969. Effect of temperature on pigment development in Red Blush grapefruit and Ruby blood oranges. *Proc. First. Intl. Citrus Symp.* 1:271–276.
- Mohr, H. and P. Schopfer. 1995. *Plant physiology*. Springer-Verlag, Berlin Heidelberg.
- Monerri, C., A. Fortunato-Almeida, R.V. Molina, S.G. Nebauer, A. García-Luis, and J.L. Guardiola. 2011. Relation of carbohydrate reserves with the forthcoming crop, flower formation and photosynthetic rate, in the alternate bearing ‘Salustiana’ sweet orange (*Citrus sinensis* L.). *Scientia Hort.* 129:71–78.
- Monselise, S.P. and A.H. Halevy. 1964. Chemical inhibition and promotion of citrus flower bud induction. *Am. Soc. Hort. Sci.* 84:141–146.
- Moon, D. G., J.H. Joa, Y.E. Moon, K.C. Seong, C.H. Kim, and Y.K. Ahn. 2011. Plant growth and fruit quality as affected by canopy locations in ‘Shiranuhi’ mandarin. *Hort. Environ. Biotechnol.* 52:443–447.
- Mortensen, L.M. and E. Stromme. 1987. Effects of light quality on some greenhouse crops. *Scientia Hort.* 33:27–36.
- Moss, G. 1969. Influence of temperature and photoperiod on flower induction and inflorescence development in sweet orange (*Citrus sinensis* L. Osbeck). *J. Hort. Sci.* 44:311–320.
- Moss, G.I. 1970. Chemical control of flower development in sweet orange (*Citrus sinensis*). *Aust. J. Agr. Res.* 21:233–242.
- Moss, G.I. 1976. Temperature effects on flower initiation in sweet orange (*Citrus sinensis*). *Aust. J. Agr. Res.* 27:399–407.
- Mupambi, G., B.M. Anthony, D.R. Layne, S. Musacchi, S. Serra, T. Schmidt, and L.A. Kalcsits. 2018. The influence of protective netting on tree physiology and fruit quality of apple: A review. *Scientia Hort.* 236:60–72.
- Nagy, S. 1980. Vitamin C contents of citrus fruit and their products: A review. *J. Agr. Food Chem.* 28:8–18.
- Nogata, Y., K. Sakamoto, H. Shiratsuchi, T. Ishii, M. Yano, and H. Ohta. 2006. Flavonoid composition of fruit tissues of citrus species. *Bioscience. Biotechnol. Biochem.* 70:178–192.

- Ortiz, J.M. 2002. Botany: Taxonomy, morphology and physiology of fruits, leaves and flowers, p.16–35. In: G. Dugo and A.D. Giacomo (eds.). *Citrus: The genus Citrus*. Taylor & Francis, New York.
- Ortuno, A., I. Reynaldo, M.D. Fuster, J. Botia, D.G. Puig, F. Sabater, A.G. Lidón, I.Porrás, J.A. Del Río. 1997. Citrus cultivars with high flavonoid contents in the fruits. *Scientia Hort.* 68:231–236.
- Pearcy, R.W. 1989. Radiation and light measurements, p. 97–116. In: R.W. Pearcy, J.R. Ehleringer, H.A. Mooney, and P.W. Rundel (eds.). *Plant physiological ecology: Field methods and instrumentation*. Chapman and Hall, London.
- Pérez, M., B.M. Plaza, S. Jiménez, M.T. Lao, J. Barbero, and J.L. Bosch. 2006. The radiation spectrum through ornamental net houses and its impact on the climate generated. *Acta Hort.* 719:631–636.
- Piskolczi, M., C. Varga, J. Racsco. 2004. A review of the meteorological causes of sunburn injury on the surface of apple fruit (*Malus domestica* Borkh.). *J. Fruit Ornament. Plant Res.* 12:245–252.
- Purvis, A.C. 1980. Influence of canopy depth on susceptibility of ‘Marsh’ grapefruit to chilling injury. *HortScience.* 15:731–733.
- Racsco, J. and L.E. Schrader. 2012. Sunburn of apple fruit: Historical background, recent advances and future perspectives. *Critical Rev. Plant Sci.* 31:455–504.
- Rajapakse, N.C. and Y. Shahak. 2007. Light-quality manipulation by horticulture industry, p. 290–311. In: G.C. Whitelam and K.J. Halliday (eds.) *Light and plant development*. Blackwell Publishing Ltd, UK.
- Rapisarda, P., A. Tomaino, R.L. Cascio, F. Bonina, A. De Pasquale, and A. Saija. 1999. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *J. Agric. Food. Chem.* 47:4718–4723.
- Raveh, E., S. Cohen, T. Raz, D. Yakir, A. Grava, and E.E Goldschmidt. 2003. Increased growth of young citrus trees under reduced radiation load in a semi-arid climate. *J. Expt. Bot.* 54:365–373.
- Raymundo, L.C., C.O. Chichester, and K.L. Simpson. 1976. Light-dependent carotenoid synthesis in the tomato fruit. *J. Agric. Food. Chem.* 24:59–64.
- Reitz, H.J. and J.W. Sites. 1948. Relationship between position on the tree and analysis of citrus fruit with special reference to sampling and meeting internal grades. *Proc. Florida State Hort. Soc.* 54:80–90.

- Rekha, C., G. Poornima, M. Manasa, V. Abhipsa, J. P. Devi, H.T.V. Kumar, and T.R.P. Kekuda. 2012. Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chem. Sci. Trans.* 1:303–310.
- Ribeiro, R.V. and E.C. Machado. 2007. Some aspects of citrus ecophysiology in subtropical climates: Re-visiting photosynthesis under natural conditions. *Braz. J. Plant Physiol.* 19:393–411.
- Rodrigo, M., B. Alquézar, E. Alós, J. Lado, and L. Zacarías. 2013. Biochemical bases and molecular regulation of pigmentation in the peel of citrus fruit. *Scientia Hort.* 163:46–62.
- Rodrigo, M., J.F.Marco, and L. Zacarías. 2004. Biochemical and molecular analysis of carotenoid biosynthesis in flavedo of orange (*Citrus sinensis* L.) during fruit development and maturation. *J. Agric. Food Chem.* 52:6724–6731.
- Sadamatsu, M. 1981. Occurrence of sun scald in early Satsuma mandarin and its control. *Proc. Int. Soc. Citricult.* 1:205–207.
- Schaffer, A.A., E.E. Goldschmidt, R. Goren, and D. Galili. 1985. Fruit set and carbohydrate status in alternate and nonalternate bearing *Citrus* cultivars. *J. Amer. Soc. Hort. Sci.* 110:574–578.
- Schmelzer, E., W. Jahnen, and K. Hahlbrock. 1988. *In situ* localization of light-induced chalcone synthase mRNA, chalcone synthase, and flavonoid end products in epidermal cells of parsley leaves. *Proc. Natl. Acad. Sci.* 85:2989–2993.
- Schneider, H. 1968. The anatomy of citrus, p. 1–85. In: W. Reuther, L.D. Batchelor, and H.J. Webber (eds.). *The citrus industry*, Vol 2. University of California Press, Berkeley.
- Schrader, L. E., J. Zhang, and W.K. Duplaga. 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Online. *Plant Health Progress*. 24 April 2017. <<http://www.plantmanagementnetwork.org/pub/php/research/sunburn/>>
- Schrader, L., J. Zhang, and J. Sun. 2003. Environmental stresses that cause sunburn of apple. *Acta Hort.* 618:397–405.
- Schreiner, M., M. Korn., M. Stenger, L. Holzgreve, and M. Altmann. 2013. Current understanding and use of quality characteristics of horticulture products. *Scientia Hort.* 163:63–69.
- Shahak, Y. 2008. Photo-selective netting for improved performance of horticultural crops: A review of ornamental and vegetable studies carried out in Israel. *Acta Hort.* 770:161–168.
- Shahak, Y., E.E. Gussakovsky, Y. Cohen, S. Lurie, R. Stern, S. Kfir, A. Naor, I. Atzmon, I. Doron, Y. Greenblat-Avron. 2004. ColorNets: A new approach for light manipulation in fruit trees. *Acta Hort.* 636:609–616.

- Shahak, Y., K. Ratner, Y.E. Giller, N. Zur, E. Or, E.E. Gussakovsky, R. Stern, P. Sarig, E. Raban, E. Harcavi, I. Doron, and Y. Greenblat-Avron. 2008. Improving solar energy utilization, productivity and fruit quality in orchards and vineyards by photosensitive netting. *Acta Hort.*772:65–72.
- Sinclair, W.B. 1961. Principal juice constituents, p. 131–160. In: W.B. Sinclair (ed.). *The orange: Its biochemistry and physiology*. University of California, California, USA
- Sinclair, W.B. and D.M. Eny. 1947. Ether-soluble organic acids and buffer properties of citrus peels. *Bot. Gaz.* 108:398–407.
- Singh, R., R. Srivastava, and K.K. Mishra. 2004. Stress physiology and growth regulators, p. 424–443. In: S. Singh, V.J. Shivankar, A.K. Srivastava, and I.P. Singh (eds.). *Advances in citriculture*. Jagmander book agency, New Delhi.
- Sites, J.W. and H.J. Reitz. 1949. The variation in individual Valencia oranges from different locations of tree as a guide to sampling methods and spot picking for quality. Part I. Soluble solids in the juice. *Proc. Amer. Soc. Hort. Sci.* 54:1–9.
- Smit, A. 2007. Apple tree and fruit responses to shade netting. MS Thesis, SU, South Africa.
- Southwick, S.M. and T.L. Davenport. 1986. Characterization of water stress and low temperature effects on flower induction in citrus. *Plant Physiol.*81:26–29.
- Spann, T.M., J.G. Williamson, and R.L. Darnell. 2004. Photoperiod and temperature effects on growth and carbohydrate storage in Southern Highbush blueberry interspecific hybrid. *J. Amer. Soc. Hort. Sci.* 129:294–298.
- Spiegel-Roy, P. and E.E. Goldschmidt. 1996. *Biology of citrus*. Cambridge University Press, Cambridge.
- Stamps, R.H. 2009. Use of colored shade netting in horticulture. *HortScience.* 44:239–241.
- Suzuki, T., S. Okamoto and T. Seki. 1973. Effects of micro-meteorological elements and positions in the tree crown on the development of shoots, leaves and fruits of satsuma mandarin. *J. Jpn. Soc. Hort. Sci.* 42:201–209 (abstr.).
- Syvertsen, J.P., C. Goñi, and A. Otero. 2003. Fruit load and canopy shading affect leaf characteristics and net gas exchange of ‘Spring’ navel orange trees. *Tree Physiol.* 23:899–906.
- Syvertsen, J.P.1984. Light Acclimation in citrus leaves. II: CO₂ assimilation and light, water, and nitrogen use efficiency. *J. Amer. Soc. Hort. Sci.* 109:812–817.
- Taiz, L., E. Zeiger, I.M. Moller, and A. Murphy. 2015. *Plant physiology and development*. 6th ed. Sinauer Associates, Inc. Sunderland, Massachusetts, U.S.A.

- Talon, M., L. Zacarias, and E. Primo-Millo. 1992. Gibberellins and parthenocarpic ability in developing ovaries of seedless mandarins. *Plant Physiol.* 99:1575–1581
- Tatum, J.H. and R.E. Berry. 1972. Six new flavonoids from *Citrus*. *Phytochemistry* 11:2283–2288.
- Tevini, M. and W. Iwanzik. 1983. Inhibition of photosynthetic activity by UV-B radiation in radish seedlings. *Physiol. Plant.* 58:395 (abstr.).
- Thomson, W.W., L.N. Lewis, and C.W. Coggins. 1967. The reversion of chromoplasts to chloroplasts in Valencia oranges. *Cytologia* 32:117–124.
- Ting, S. V. and E.J. Deszyck. 1961. The carbohydrates in the peel of oranges and grapefruit. *J. Food Sci.* 26:146–152.
- Ting, S.V. and J.A. Attaway. 1971. Citrus fruits, p. 107–169. In: A.C. Hulme (ed.). *The biochemistry of fruits and their products*, Vol 2. Academic Press INC, London.
- Tuwana, S.W. 1999. Factors affecting fruit size and quality in citrus species. MS Thesis, U.S., South Africa.
- Ulrich, R. 1970. Organic acids, p. 89–118. In: A.C. Hulme (ed.). *The biochemistry of fruits and their products*, Vol 1. Academic Press INC, London.
- Veldhuis, M.K., L.J. Swift, and W.C. Scott. 1970. Fully-methoxylated flavones in Florida orange Juices. *J. Agr. Food Chem.* 18:590–592.
- Verreynne, J.S., E. Rabe, and K.I. Theron. 2004. Effect of bearing position on fruit quality of mandarin types. *S. Afr. J. Plant Soil.* 21:1–7.
- Wachsmann, Y., N. Zur, Y. Shahak, K. Ratner, Y. Giler, L. Schlizerman, A. Sadka, S. Cohen, V. Garbinshikof, B. Giladi, and M. Faintzak. 2014. Photosensitive anti-hail netting for improved citrus productivity and quality. *Acta Hort.* 1015:169–176.
- Wilkie, J.D., M. Sedgley, and T. Olesen. 2008. Regulation of floral initiation in horticultural trees. *J. Expt. Bot.* 59:3215–3228.
- Winston, J.R. and E.V. Miller. 1948. Vitamin C content and juice quality of exposed and shaded citrus fruits. *J. Food Sci.* 13:456–460.
- Wünsche, J.N., D.H. Greer, J.W. Palmer, A. Lang, and T. McChie. 2001. Sunburn: The cost of a high light environment. *Acta Hort.* 557:349–356.
- Yahata, D., K. Matsumoto, and K. Ushijima. 2006. The effect of the time of fruit harvest on flower formation and carbohydrate contents. *J. Japan. Soc. Hort. Sci.* 75:32–37.
- Yakushiji, H., H. Nonami, T. Fukuyama, S. Ono, N. Takagi, and Y. Hashimoto. 1996. Sugar accumulation enhanced by osmoregulation in Satsuma mandarin fruit. *J. Amer. Soc. Hort. Sci.* 121:466–472.

- Yakushiji, H., K. Morinaga, and H. Nonami. 1998. Sugar accumulation and partitioning in Satsuma mandarin tree tissues and fruit in response to drought stress. *J. Amer. Soc. Hort. Sci.* 123:719–726.
- Yamauchi, N., Y. Akiyama, S. Kako, F. Hashinaga. 1997. Chlorophyll degradation in Wase satsuma mandarin (*Citrus unshiu* Marc.) fruit with on-tree maturation and ethylene treatment. *Scientia Hort.* 71:35–42.
- Zou, Z., W. Xi, Y. Hu, C. Nie, and Z. Zhou. 2016. Antioxidant activity of *Citrus* fruits. *Food Chem.* 196:885–896.

CHAPTER 3

The influence of 20% white shade netting on ‘Nadorcott’ mandarin fruit development and quality parameters

ABSTRACT

Consumers’ acceptance of citrus fruit is strongly based on fruit quality, which is characterized by an acceptable size and rind coloration, along with a blemish-free rind and good taste, as determined by the Brix to acid ratio. High temperature and light intensity are known to negatively influence fruit quality, therefore shade nets have been implemented as a strategy to ameliorate negative climatic events. A main objective is to reduce excessive light intensity in order to limit sunburn, which is currently considered a major cause of losses in South African citrus orchards. However, reduced light may alter rind coloration and affect the soluble solids content (SSC) of the fruit. The aim of this study was to determine the influence of 20% white shade netting on the quality variables of ‘Nadorcott’ mandarin fruit (*Citrus reticulata*), a key South African citrus export product. The study was conducted over two seasons in Citrusdal, where monthly recordings from January to June included that of fruit size and rind color, in addition to destructive sampling for the determination of SSC, citric acid percentage, flavedo chlorophyll and carotenoids concentration along with total rind dry matter percentage and the incidence of sunburn at harvest. In the second season, pulp coloration, fruit surface temperature and the incidence of gumming were also recorded. Results indicate that the nets promoted fruit size throughout the development period, with larger fruit being harvested under netting compared to non-shaded fruit in the second season. Rind color was not affected at any point of fruit development. At harvest, chlorophyll and carotenoid concentration, SSC, citric acid percentage, SSC to acid ratio and pulp color were comparable, except in the second season when a higher carotenoid concentration was recorded in shaded fruit. Shade netting significantly reduced sunburn incidence but did not impact on fruit surface temperature or fruit maturation. The use of shade net therefore shows significant potential to reduce sunburn percentage without any detrimental impact on fruit size or quality.

ADDITIONAL INDEX WORDS: *Citrus reticulata*, light intensity, Brix, fruit size, rind color

Introduction

Citrus fruit development follows a typical sigmoidal curve with three distinct phases viz. cell division (I), cell enlargement (II) and fruit maturation (III) (Bain, 1958). The last two stages are of particular importance in attaining the quality characteristics that determine the market value of fruit. Stage II is considered as the critical fruit growth period as it coincides with soluble sugar accumulation, a decrease in acidity and the onset of rind coloration. These changes occur towards the end of the phase and continue throughout fruit maturation (stage III) (Albertini et al., 2006; Bain, 1958; Mehouchi et al., 1995).

In citrus fruit, quality parameters such as fruit size and external appearance of the fruit, including the degree of rind coloration, and the absence of blemishes are important determinants of consumers' preferences and determine largely the initial purchasing decision. Furthermore, the soluble solids content (SSC) to acid ratio, which is an important determinant of the taste and used as a maturity index of citrus fruit, is the most important internal quality parameter that determines continued purchasing (Hodgson, 1967; Ting and Attaway, 1971).

In citriculture, the emphasis is placed on producing high volumes of good quality fruit. However, it is evident from studies on citrus fruit where canopy positions are compared with regard to their impact on fruit quality, that environmental factors such as temperature and light intensity influence fruit size (Ehara et al., 1981; Moon et al., 2011; Verreyne et al., 2004), acidity (Moon et al., 2011; Verreyne et al., 2004; Winston and Miller, 1948), soluble solids content (SSC) (Khalid et al., 2012; Reitz and Sites, 1948; Sites and Reitz, 1949) and rind color (Cronje et al., 2013; Khalid et al., 2012). The influence of the canopy positions was mainly due to a change in light spectral quality as it penetrates into the canopy, resulting in reduced photosynthetic active radiation (*PAR*) levels inside the canopy (Cronje et al., 2013; Grant, 1997). However, micro-climatic factors, such as air temperature and relative humidity, also vary between canopy positions and may affect fruit quality (Barry et al., 2000; Suzuki et al., 1973).

The SSC in citrus fruit is the result of the translocation to and accumulation of leaf photosynthates in the juice sacs (Chen et al., 2002; Yakushiji et al., 1998), therefore light and other environmental factors that influence photosynthesis play an important role in the SSC of fruit. Sites and Reitz (1949) established a correlation with SSC in fruit pulp and the amount of light received. This relationship is also evident from studies that reported a higher SSC for outside canopy fruit (Fallahi and Moon, 1988; Khalid et al., 2012; Verreyne et al., 2004).

Reduced light and low temperature are known to influence the chloro-chromoplast conversion associated with color development (Goldschmidt, 1988; Thomson et al., 1967). The characteristic green color of immature fruit is due to the abundant chlorophyll pigment located in the chloroplast (Eilati et al., 1969), while carotenoids which provide the yellow-orange coloration of mature citrus fruit is present in the chromoplast (Gross, 1987). The rate of chlorophyll production is reduced at low light intensity (Lewis and Coggins, 1964), with enhanced chlorophyll degradation under cool temperatures (Meredith and Young, 1969), whilst for carotenoids, light promotes the carotenoid levels (Lewis and Coggins, 1964) and results in improved rind coloration (Cronje et al., 2013). In addition to these environmental factors there are other aspects within the tree system that influence the chloro-chromoplast conversion, such as the presence of sucrose in the rind which enhances degreening, whilst high nitrogen content may cause a regreening (Huff, 1984), along with gibberellic acid that causes chlorophyll synthesis (Lewis and Coggins, 1964). Lado et al. (2015) proposed that an accelerated chromoplast differentiation would support earlier carotenoid accumulation and therefore promote better rind coloration.

In contrast to the low light levels, excessive solar radiation levels alone or in combination with high air temperatures have been shown to cause sunburn (Ketchie and Ballard, 1968; Wünsche et al., 2001), which results in the yellow rind discoloration or in some cases necrotic tissue, thereby affecting the rind appearance and reducing the fruit value (Racsko and Schrader, 2012).

Extreme environmental stress conditions are difficult to predict and control. Therefore, shade nets, a relatively new introduction within pre-harvest cultural technology, is now being implemented in orchards as protection against such climatic events that negatively affect the appearance of fruit such as excess sunlight, wind and hail damage and/or that can have the additional benefit of protection against insects (Rajapakse and Shahak, 2007). Furthermore, shade nets reduce the photosynthetic photon flux (PPF) (Lee et al., 2015), causing a reduction in photo-inhibition under excessive light levels and thus increase the photosynthetic rate (Jifon and Syvertsen, 2003; Syvertsen et al., 2003). In addition to offering protection, shade nets also alter the microclimate by increasing the relative humidity and reducing the maximum air temperature (Iglesias and Alegre, 2006; Wachsmann, 2014). As a result, the use of shade netting has been reported to be effective in reducing sunburn on both apples (Gindaba and Wand, 2005; Iglesias and Alegre, 2006) and citrus (Lee et al., 2015). However, the impact of netting on fruit quality has not been as clear as its documented success in reducing sunburn.

Rind coloration was enhanced by shade net for ‘Spring Navel’ orange [*Citrus sinensis* (L.) Osbeck] (Syvertsen et al., 2003) and ‘Top Red’ apple (*Malus domestica* Borkh.) (Shahak et al., 2004), but for ‘Cripps’ pink’ and ‘Royal Gala’ apple coloration was negatively affected (Gindaba and Wand, 2005). The influence of shade nets on fruit size also resulted in contrasting reports for citrus and apples, where no impact was evident for ‘Spring Navel’ oranges (Syvertsen et al., 2003) as compared to apples albeit positive or negative (Shahak et al., 2004, 2008). With reference to the sugar acid ratio, shade nets enhance the ratio for ‘Orri’ mandarin (Wachsmann, 2014), but with no effect found in ‘Spring Navel’ orange (Syvertsen et al., 2003), or a reduction in ‘Ruby Red’ grapefruit (Jifon and Syvertsen, 2001). These contradicting findings could be the result of different shade factors and/or the time of application during fruit development as well as different cultivars used. In a review by Mupambi et al. (2018) on the influence of shade netting on the tree physiology and fruit quality of apple fruit, they concluded that the use of shade nets will aid as a buffer to climatic extremes i.e. intense heat, light and wind stress, in addition to protecting the crop from sunburn, wind and hail damage.

To date the influence of shade nets for the entire fruit development period on the fruit quality of mandarin has not received in-depth attention. The aim of this study was to determine the influence of 20% white permanent shade netting on the quality aspects critical for fruit export of ‘Nadorcott’ mandarin (*C. reticulata* Blanco). These factors include fruit developmental changes viz. fruit diameter, rind color, internal quality parameters (SSC, citric acid percentage and the ratio), pigment expression, and in addition, the incidences of gumming and sunburn.

Materials and Methods

SITE LOCATION AND PLANT MATERIAL. The experiments were carried out in a commercial orchard of ‘Nadorcott’ mandarin trees on Carizzo citrange (*Poncirus trifoliata* x *C. sinensis*) in Citrusdal (-32.542140, 19.011877), Western Cape Province, South Africa. The orchard was planted in 2012, with a tree spacing of 5.5 m between and 2.5 m within rows, in a north to south row orientation. The orchard management followed standard citriculture practices for both treatments, with regards to irrigation and nutrition, as well as pest and disease management. Trials were conducted over two consecutive seasons, during 2015/2016 (season 1) and 2016/2017 (season 2).

EXPERIMENTAL LAYOUT AND TREATMENTS. The experimental layout was a randomised complete block design (RCBD), where each experimental block was divided into two plots of 75 m x 25 m, with the treatments being randomly allocated to the plots within a

block and replicated four times per treatment ($n = 4$). The treatments consisted of either the shade netting or the control (no netting), where the shade plots were covered by permanent 20% white shade netting (Plusnet, 13 Bussing Road, Aureus, Randfontein, 1759, Gauteng, South Africa) after flowering in Sept. 2015 of the first season. The shade net structure was not fully enclosed on the sides (halfway down). In order to compensate for a border effect, trees in the first 10 m at either side of the rows and the first two rows from the sides of each plot were excluded from data collection.

DATA COLLECTION AND FRUIT SAMPLING. *Monthly orchard-based measurements.* For each treatment replicate per block, two trees of uniform size, health and crop load from adjacent rows, facing each other, were used as an experimental unit on which monthly repeated recordings of fruit diameter and rind color were conducted. This selection procedure was chosen to reduce variation and counteract any possible missing plots due to tree die back. In the first season, 10 fruit were randomly tagged on the eastern side of one tree, with another 10 fruit that were selected on the western side of the corresponding tree from the adjacent row. The fruit were selected mid-way of the tree canopy height, $\pm 1-1.5$ m from ground level, within the first 20 cm of the outer canopy boundary, to include only fruit exposed to direct light. In the second season, the number of fruit selected was increased to 15 per tree, with an additional 20 fruit per tree that were tagged for fruit surface temperature measurements. Fruit were tagged in the first week of Jan. 2016 and 2017, following physiological fruit drop in Dec. 2015 and 2016, respectively. Measurements for both seasons were conducted from January until June, approximately two weeks prior to commercial harvest. Fruit rind color measurements were collected with a chroma meter (CR-400, Konica Minolta Sensing, Inc., Japan) from pre-marked areas on the sun-exposed side of each fruit and expressed by means of the Hunter *a/b* ratio. Fruit diameter was determined with an Electronic Fruit Size Measure and Data Logger (GÜSS Manufacturing (Pty) Ltd., Strand, South Africa).

Fruit surface temperature measurements. The fruit surface temperature (FST) was recorded by means of a Hybrid Infrared Thermometer (ST642, Sentry Optronics Corp., Ban-Ciao, Taipei, Taiwan) at a fixed distance of 18 cm from the sun-exposed side of the fruit. One measurement per fruit was recorded between 1300 -1500 HR on seven, hot days, selected based on predictions by the Southern Hemisphere weather service (<https://weather.com>) of days to reach a maximum of above 30 °C. In order to account for differences in ambient air temperature that may occur over the measurement period, measurements alternated per replicate between the control and shade net treatment.

Internal quality parameters and flavedo removal. In the first season, a total of 10 trees per replicate for each treatment were selected as experimental units. A replication consisted of five trees from one row on which fruit were sampled from the eastern side (row 1) of the canopy along with an additional five trees from the adjacent row from which fruit were sampled on the western side (row 2) of the canopy, from the same canopy position as described above. A total of 10 fruit (two per tree) were sampled per replicate from row 1 and 2, respectively. To summarize: two respective samples of 10 fruit each per treatment replicate were collected. Sampling occurred at monthly intervals from January until two weeks prior to commercial harvest, to determine the internal quality and collect flavedo for biochemical analysis. Fruit were sampled to be representative of the fruit that were monitored within that month for fruit size and rind color. During the second season, only three experimental units per row were selected and an additional five fruit per row for each replicate were sampled to determine rind dry mass percentage and pulp color. Quality analyses were done on each of the two respective samples per replicate.

Flavedo removal for pigment analysis. The flavedo was removed from the 10 fruit (east and west, respectively) per treatment replicate with a lemon zester and pooled accordingly before being frozen in liquid nitrogen. Thereafter, it was freeze dried (Christ Beta 1-8 LD, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 2 d before milled to a fine powder with an analytical grinder (Yellow line, A10, IKA-Werke, Staufen, Germany) and stored at -80 °C until rind pigment analysis.

Internal fruit quality parameters. To measure internal quality parameters, following flavedo removal, the fruit was cut in half along the longitudinal plane (from stem-to-calyx end) for the extraction of juice using a citrus juicer (8-SA10, Sunkist®, Chicago, USA). The juice was strained through a muslin cloth to remove the pulp particles whereafter the soluble solids content (SSC), measured as °Brix, was determined with a digital refractometer (PR-32 Palette, ATAGO CO, Tokyo, Japan). From the extracted juice, a 50 mL sample was used to determine the citric acid content, with a potentiometric titrator (888 Titrand, Metrohm, Switzerland), using Tiamo™ software. Due to citric acid being the major component of organic acids within citrus, the percentage citric acid was also used in calculating the SSC to acid ratio (°Brix/citric acid percentage).

GUMMING INCIDENCE. The incidence of gumming was scored during the second season for fruit sampled in June (for internal quality analyses), prior to juice extraction. The score was based on a visual score system of the prevalence of brown droplets in the central axis, ranging

from 0 = no incidence, 1 = moderate to small drops, to where 2 = severe incidence (Fig. 1). The gumming index and gumming percentage were calculated according to Eq. [1] and Eq. [2].

$$\text{Gumming index} = \frac{\sum [\text{Number of fruit in scoring category} \times \text{Scoring category (0-2)}]}{\text{Total of fruit per rep/sample}} \quad [1]$$

$$\text{Gumming percentage} = \frac{(\text{Sum of fruit scored as class 1-2}) \times 100}{\text{Total fruit}} \quad [2]$$

RIND DRY MASS AND PULP COLOR. Dry mass was determined by carefully dissecting a 10 x 20 mm rind section and weighing it before and directly after being oven dried for three days at 70 °C. The percentage dry mass (g) was calculated as [(Dry weight/ Fresh weight) x 100)]. Data were collected individually for each fruit. In the first season 10 fruit per sample for each replicate were used, while in the second season it was reduced to only five fruit. From the same fruit, the pulp color was recorded during the second season by making a cross section along the equator of the fruit, whereafter pulp color was recorded in the same pulp segment of each fruit with a chroma meter (CR-400, Konica Minolta Sensing, Inc., Japan). In the first season the pulp color was recorded before the juice extraction from the fruit subjected for internal quality.

RIND PIGMENT DETERMINATION. The chlorophyll and carotenoid concentration in the flavedo were determined spectrophotometrically. One extraction per flavedo sample (two samples in total i.e. east and west) from each treatment replicate were performed in diminished light conditions to limit direct light exposure. A sample of 0.050 – 0.055 g freeze-dried and milled flavedo was weighed into a Kimax tube before adding 2 mL absolute ethanol (99.9% v/v) [Merck (Pty) Ltd, Modderfontein, Gauteng, South Africa] and vortexed until the solution was well dispersed. The tubes were placed in a shaker (KS 500, IKA-Werke GmbH&Co. KG, Staufen, Germany) for 30 min at a speed of 265 rpm, whereafter the tube was centrifuged (5810 R, Eppendorf AG, Hamburg, Germany) for 5 min at 4000 g_n and 4 °C. The alcoholic supernatant was transferred to a savant vial that was closed to prevent evaporation. The ethanol extraction procedure was repeated, with the alcoholic phases subsequently pooled. Thereafter, 2 mL n-hexane ($\geq 97.0\%$ v/v) [Sigma-Aldrich, Co., St. Louis, Missouri, United States] containing butylated hydroxytoluene (BHT) ($100 \text{ mg} \cdot \text{L}^{-1}$) [Sigma-Aldrich Co., St. Louis, Missouri, United States] was added to the pellet and the tube was vortexed, before a 15 min shake was applied at 265 rpm. The tube was then centrifuged, whereafter the extraction solution was transferred to

the savant tube containing the ethanol fraction, which was closed to prevent evaporation. The hexane extraction was repeated twice, following the first extraction.

The extraction was dried under vacuum using a speedvac concentrator (SC210A, Thermo scientific, Asheville, United States), set at low – medium temperature. The dried extracts were reconstituted with 10 mL pure acetone (100% v/v) [Merck (Pty) Ltd, Modderfontein, Gauteng, South Africa]. The acetone volume was, however, increased up to 16 mL, as rind color development progressed. The pigment-containing solution was then transferred to microtubes and centrifuged (5417 R, Eppendorf AG, Hamburg, Germany) for 7 min, 10000 g_n and 4 °C, before being transferred to a UV-specific cuvette. The absorbance was measured with a spectrophotometer (Cary 60 UV-Vis, Agilent technologies, Santa Clara, United States) at 470, 644, and 662 nm wavelengths respectively, with 100% acetone as the blank solution. The concentrations of chlorophyll a (C_a), chlorophyll b (C_b) and carotenoids (C_{x+c}), expressed as $\mu\text{g}\cdot\text{mL}^{-1}$, were determined through substituting the absorbance values within equations based on Lichtenthaler (1987) for 100% acetone (v/v).

$$C_a = 11.24A_{661.6} - 2.04A_{644.8} \quad [3]$$

$$C_b = 20.13A_{644.8} - 4.19A_{661.6} \quad [4]$$

$$C_{x+c} = (1000A_{470} - 1.90C_a - 63.14C_b)/214 \quad [5]$$

Concentration values were converted to express data as $\mu\text{g}\cdot\text{g}^{-1}$ dry weight (DW) for chlorophyll a (C_{Ca}), chlorophyll b (C_{Cb}) and carotenoids (C_{car}) using the following equations, where vf represents the final volume of acetone in L and the unit for flavedo mass in g:

$$C_{Ca} = (C_a \times vf \times 1000) / \text{flavedo mass} \quad [6]$$

$$C_{Cb} = (C_b \times vf \times 1000) / \text{flavedo mass} \quad [7]$$

$$C_{car} = (C_{x+c} \times vf \times 1000) / \text{flavedo mass} \quad [8]$$

SUNBURN INCIDENCE EVALUATION. Sunburn incidence was graded based on a visual scoring scale according to severity, ranging from 0 = no sunburn incidence, 1 = moderate to light yellow coloration to 2 = severe, bright yellow coloration, leathery and necrotic appearance (Fig. 2A-F). In the first season sunburn was graded when all the fruit had reached full color development, prior to harvest. Two adjacent trees, uniform in size, health and crop load were selected per treatment replicate, and 100 fruit on the eastern side of each tree, from the middle canopy position, approximately 1 m above the ground level, were randomly selected for scoring. For the second season, the 40 fruit used for FST measurements were graded before color break, on 27 Apr. 2017. Evaluation at a earlier stage was considered to provide a more accurate indication of sunburn, without the interference of masking of some symptoms due to

color development, as was experienced in the first season. The sunburn index and percentage, were calculated using the same equation as provided previously for gumming Eq. [1] and Eq. [2].

STATISTICAL ANALYSIS. Mixed model repeated measures analysis of variance (ANOVA) was performed using Statistica 13's VEPAC module (TIBCO Software Inc., 2017). "Treatment", "Month" and "Side (canopy)" were included as fixed effects and "block nested in treatment" as a random effect. For post hoc testing, the Fisher least significant difference (LSD) post hoc test was used. Sunburn and gumming incidence were analysed by means of a one-way ANOVA. Significant differences were determined at $P \leq 0.05$ (5% significant level).

Initial analyses were performed to determine whether an interaction between Side (east and west, respectively) and Treatment existed, and when no interaction was obtained, the two respective samples (east and west) per replicate were pooled. A significant interaction occurred between Treatment, Side and Month for citric acid percentage, the sugar to acid ratio and the rind color, which consequently led to a Side and Month interaction. However, this side effect was inconsistent and only occurred in certain months, only during the first season. Therefore, due to this inconsistency the Side effect is ascribed to normal variation between samples, allowing for the Side effect identities to be pooled for each replicate. Furthermore, in some cases the data are presented in figures where both Treatments are shown, and although no interaction was evident in some cases, this was done merely to indicate the two trends followed by the two treatments.

Results

FRUIT GROWTH. The fruit development pattern as estimated by fruit diameter was not influenced by shade netting, irrespective of the season, with no significant interaction occurring between Treatment and Month (Table 1). Fruit diameter increased significantly from January to June, in both seasons (Fig. 3A and B). No significant differences in the fruit diameter between the shade net and control fruit was recorded over the development period during the first season (Table 1). However, in the second season, the average fruit diameter over the development period for the shaded fruit was consistently, significantly larger, with a diameter of 53.7 mm compared to 51.6 mm for the control (Fig. 3B). The final fruit diameter for the shaded fruit was larger than that of control fruit ($P = 0.001$) at 68.8 mm compared to 66.2 mm, respectively.

RIND DRY MASS. The rind dry mass percentage followed an inverse trend compared to fruit diameter, in both seasons, as a significant decrease was recorded as fruit growth progressed throughout the season (Fig. 3C and D). No significant interaction between Treatment and

Month for rind dry mass occurred in 2016 (Table 1). The dry mass over the development period decreased significantly from February (data first recorded) until June, except for a lag phase at the start and into autumn, between April and May. A decrease of 7.2% in rind dry mass was recorded over the season. In addition, no difference between the two respective treatments over the development period were evident in the first season ($P = 0.637$).

A significant interaction occurred between Treatment and Month in the 2017 season (Fig. 3D). During this season, control fruit showed a significant decrease in rind dry mass between the consecutive months until May, whereas for shaded fruit the decrease was only evident until March, and then again later, between April and May. A decrease of 7.8% in dry mass for the control fruit rind and 9.2% for rind of shaded fruit was reported from January to June (Fig. 3D). The only difference between treatments was noted in January ($P = 0.0123$) and March ($P = 0.0034$), where shade net produced fruit at first had a higher percentage dry mass and then later a lower percentage than the control fruit. However, at the end of the growing season no significant difference between control and shaded fruit was recorded.

RIND COLOR DEVELOPMENT AND PIGMENT CONTENT. *Rind color.* No significant interaction was evident for rind color between Treatment and Month, regardless of the season (Table 2). Shade net did not influence rind color development negatively as rind color development patterns were similar between control and shaded fruit (Table 2), with no significant difference detected between the treatments, over both seasons (Fig. 4A and B). A trend was recorded where the hunter a/b ratio was negative at the onset of the season, depicting a green color, and as the season progressed a positive ratio was attained by June, resembling a yellow-orange rind color (Fig. 4A).

During the first season the monthly development showed a decrease in the hunter a/b ratio from January to March, whereafter the ratio increased significantly towards June (Table 2). From the significant increase in the ratio between April and June, it was evident that color break occurred within that time period, however due to a loss of data for May 2016 (indicated by parallel lines in Fig. 4A) it was difficult to conclude exactly when, but it was accepted that it occurred within the May sampling period.

During the second season the two treatments followed the same trend in color development (Fig. 4B), however with a slower progression compared to the first season, since the ratio remained constant during the first three months, only thereafter increasing significantly until June, by 1.03 units from the onset of the season (Table 2).

Color break was delayed during the second season, only to occur between May and June (Fig. 4B). Although the ratios between the two seasons were not statistically compared, a

slightly lower ratio in June 2017 compared to June 2016 is evident. In addition, it was also observed in the 2017 season in general, that not all the fruit in the orchard was fully colored, as was to be expected around harvest.

Pigment content. A significant interaction exists between Treatment and Month for the chlorophyll concentration during the first, but not second season (Fig. 4C and D, respectively). For carotenoid content, a significant interaction between Treatment and Month was not obtained in the first season, where such an interaction only emerged in the second season (Fig. 4E and F). Chlorophyll breakdown and carotenoid synthesis patterns followed that recorded for color development (Fig. 4A and B), with chlorophyll content decreasing (Fig. 4C and D) in contrast to the increase in carotenoid content for both treatments, over the two seasons (Fig. 4E and F).

Chlorophyll concentration. The shade net treatment significantly influenced the chlorophyll concentration in January and February during the first season, resulting in a higher concentration in January and a lower concentration in February for shaded fruit compared to control fruit (Fig. 4C). The reasons for the reduction in chlorophyll content for Feb. 2016 may possibly be the result of an experimental error during lab analyses, and may possibly be the reason for the observed interaction between Treatment and Month ($P = 0.001$), as no other treatments differences occurred later during fruit development until harvest.

The chlorophyll content for the control fruit remained constant throughout the first three months of development, whereafter a significant reduction from $1920 \mu\text{g}\cdot\text{g}^{-1}$ DW in January to $91 \mu\text{g}\cdot\text{g}^{-1}$ DW in June occurred, leaving the fruit almost completely devoid of the pigment (Fig. 4C). For the shaded fruit, following the fluctuation described earlier until March, a significant decrease was recorded until May, whereafter the content did not differ significantly until June, just prior to harvest (Fig. 4C). A chlorophyll content of $2404 \mu\text{g}\cdot\text{g}^{-1}$ DW was recorded in January, with a significantly lower content of $171 \mu\text{g}\cdot\text{g}^{-1}$ DW reported in June 2016.

During the second season, on average over the whole development period, an increased chlorophyll content of $109 \mu\text{g}\cdot\text{g}^{-1}$ DW was reported for shaded fruit compared to that of fruit produced under control conditions (Table 2). A slight increase in chlorophyll content between January and February was recorded with shade net showing a higher trend, though not significantly so, as to result in an interaction between Treatment and Month (Fig. 4D). Further, from February onwards the chlorophyll concentration of both control and shaded fruit significantly decreased, from $2122 \mu\text{g}\cdot\text{g}^{-1}$ DW to $225 \mu\text{g}\cdot\text{g}^{-1}$ DW, as was reported for June 2017 (Table 2).

Carotenoid concentration. The carotenoid concentration was not significantly influenced by shade netting in the first season (Table 2; Fig. 4E). However, in the second season, differences occurred in January and June, respectively, that resulted in shade net produced fruit containing a higher carotenoid concentration during those months than control fruit (Fig. 4F) at $71 \mu\text{g}\cdot\text{g}^{-1}$ DW and $137 \mu\text{g}\cdot\text{g}^{-1}$ DW, respectively. These differences were most likely also the cause of the significant interaction that was obtained between Treatment and Month (Fig. 4F). A distinct increase in the carotenoid content occurred from January to June, irrespective of the season or treatment (Fig. 4E and F, Table 2).

In 2016, the carotenoid content showed a slight decrease between January and February whereafter the concentration increased significantly until the end of the season, except between March and April when no significant increase occurred (Table 2). During this season the carotenoid concentration increased from $602 \mu\text{g}\cdot\text{g}^{-1}$ DW at the onset of the season to $1688 \mu\text{g}\cdot\text{g}^{-1}$ DW at the final measurement (Table 2).

In Jan. 2017, the carotenoid concentration was $579 \mu\text{g}\cdot\text{g}^{-1}$ DW for shaded fruit compared to $508 \mu\text{g}\cdot\text{g}^{-1}$ DW for control fruit (Fig. 4F). At the final measurement just prior to harvest, the shaded fruit recorded a carotenoid concentration of $985 \mu\text{g}\cdot\text{g}^{-1}$ DW compared to $848 \mu\text{g}\cdot\text{g}^{-1}$ DW for control fruit. During the development of control fruit, carotenoid levels remained relatively similar, until a significant increase between May and June, except for some fluctuations between February and March (Fig. 4F). For fruit grown under shade netting, the carotenoid synthesis remained constant during the first two months, followed by a significant decrease in March, whereafter the carotenoid concentration recovered in April and May to attain similar levels to those recorded in January and February, before a significant increase in concentration towards June (Fig. 4F).

INTERNAL QUALITY PARAMETERS. Soluble solids content. No interaction occurred for the soluble solids content between Treatment and Month, either in 2016 ($P = 0.945$) or 2017 ($P = 0.817$), with both treatments impacting on SSC in a similar trend over the developmental period (Fig. 5A and B). In addition, the soluble solids content of shaded fruit was not significantly affected compared to that recorded for the control, during fruit development irrespective of the season (2016: $P = 0.850$; 2017: $P = 0.482$) or just prior to harvest in June for both seasons (2016: $P = 0.672$; 2017: $P = 0.449$). Furthermore, with regards to the month main effects in the first season, the SSC value increased significantly from February throughout the fruit development until the final measurement date, prior to harvest (Fig. 5A), whereas during the second season this distinct increase was only noted from April onwards (Fig. 5B). For both seasons, a significant decrease occurred from January to February, with no significant

difference in Brix value between January and April, (Fig. 5A and B). The final values attained were recorded as 12.8 and 10.6 °Brix for the first and second season, respectively.

Citric acid percentage. No interaction occurred between Treatment and Month for citric acid percentage, either in 2016 ($P = 0.690$) or 2017 ($P = 0.559$). In addition, no differences between the two respective treatments were recorded over the developmental period (2016: $P = 0.696$; 2017: $P = 0.151$) or in June, prior to harvest, for both seasons (2016: $P = 0.824$; 2017: $P = 0.862$). However, a significant decline throughout fruit development was recorded for both seasons over time (Fig. 5C and D). During the first season the citric acid content of 7.02% in January significantly decreased to 1.23% in June, whereas for the second season a value of 5.59% in January decreased significantly until May, reaching a level of 1.27%, whereafter it remained relatively constant.

SSC (°Brix) to citric acid percentage ratio. No interaction was detected between Treatment and Month for the Brix to citric acid percentage ratio, either during the 2016 ($P = 0.538$) or the 2017 ($P = 0.081$) season. The Brix to citric acid percentage ratio was not differently influenced for fruit grown under shade netting, compared to that of fruit grown without shading, over the fruit development period (2016: $P = 0.621$, 2017: $P = 0.135$), right up to two weeks prior to harvest (2016: $P = 0.482$; 2017: $P = 0.961$) as no treatment differences occurred. During the first season, the ratio increased significantly from 1.5 at the onset of the season to 10.5 in June, while during the second season, the ratio increase occurred only from February onwards when the value increased from 1.69 to 8.46 in June (Fig. 5E and F).

Pulp color. The development of the pulp coloration (only measured in the second season) followed similar trends for both the control and shaded fruit, with no significant interaction obtained between Treatment and Month (Fig. 6). The pulp coloration increased significantly for both treatments from a negative ratio in Jan. 2017, that indicates a green-yellow color, to a positive ratio in June 2017 resembling a yellow pulp color ($P < 0.001$). There was a trend of shaded fruit having a higher hunter a/b ratio, which consequently resulted in shaded fruit having a higher average over the development period, however only significant at 10% confidence interval (Fig. 6) ($P = 0.066$).

GUMMING INDEX AND PERCENTAGE. Gumming incidence was not influenced by 20% white shade netting (Table 3), even though a lower index value (an indication of severity) was recorded for shaded fruit compared to the control ($P = 0.581$). Similarly, a lower, but not significant percentage gumming was noted in shaded fruit (Table 3).

SUNBURN INCIDENCE. The presence of shade netting effectively reduced the sunburn index (a low index indicates a low severity) as well as the percentage incidence, in both seasons

(Table 4). A sunburn index of 0.37 was recorded for the control fruit, with 0.07 assigned to shaded fruit in the first season, whereas the indices of 0.28 and 0.05 for control and shaded fruit were recorded in 2017. In both seasons the 20% white shade netting significantly reduced the percentage of sunburned fruit with 18% and 16% in the respective seasons (Table 4).

FRUIT SURFACE TEMPERATURE MEASUREMENTS. No significant interaction emerged between Treatment and Date of fruit surface temperature measurements (Table 5). With regards to the main effects, there was a significant difference between the measurement dates (data not shown), but with no significant difference between treatments, with approximately 39 °C as fruit surface temperature for both treatments (Table 5).

Discussion

FRUIT GROWTH. The shade net treatment did not influence the fruit development pattern, regardless of the season, with no fruit size difference reported in the first season. However, during the second season the average fruit diameter for shaded-fruit was increased by 3.9% (Table 1), resulting in these fruits being 2.6 mm larger at the end of the season. Bain (1958) identified stage II as the period of maximum fruit growth, due to cell enlargement of mainly the pulp segments, whereafter fruit size can continue to increase if the fruit remains on the tree. The average fruit diameter for the two treatments (control and shade net) increased with 26% and 31% for the first and second season, respectively during stage II (January – April), with a continued increase during the maturation phase (stage III; April-June) of 31% in the first season and 25% during the second season. Results from the 2016 season are in accordance with findings from Wachsmann et al. (2014) on ‘Orri’ mandarin where no influence of shade netting regarding fruit size was reported for 18% white, 25% red, 24% yellow or 13% transparent netting. Furthermore, research done on apples (*Malus domestica* Borkh.) showed no influence of 20% black nets on the fruit size of ‘Royal Gala’ and ‘Cripps Pink’ (Gindaba and Wand, 2005) or for ‘Mondial Gala’ grown under black and crystal netting, respectively (Iglesias and Alegre, 2006).

Shade nets or screens are known to reduce solar radiation (Jifon and Syvertsen, 2001; Lee et al., 2015; Mataa and Tominaga, 1998) and light intensity that can also differ amongst canopy positions, with a reduction in light levels as it penetrates deeper into the canopy (Cronje et al., 2013). In addition to this, it has been shown by Verreyne et al. (2004) that light intensity influences fruit size of mandarin types, with greater fruit size reported for outside canopy ‘Clementine’, ‘Temple’ tangor and ‘Satsuma’ mandarin fruit, but with opposite results reported for ‘Fairchild’. The results from the first season in the current study indicate that the reduction

of light intensity (17-18%) (Addendum A: Fig. 1; Prins, 2018) imposed by the shade net did not affect the fruit growth negatively.

The influence of shade net on fruit growth was, however, more evident in the second season, resulting in an overall larger average fruit diameter compared to control fruit which concurs with Shahak et al. (2008) where 15% white shade netting resulted in larger fruit for 'Golden Delicious' and 'Top Red' apples.

The general reduction in photosynthetic photon flux (PPF) caused by shade nets, may result in higher photosynthesis (Cohen et al., 2000; Syvertsen et al., 2003) due to a decrease in photo-inhibition which is associated with high light and leaf temperatures (Jifon and Syvertsen, 2003). The inconsistent results obtained in our study regarding fruit size between seasons can therefore not directly be ascribed to the role of photosynthesis in providing carbohydrates to drive fruit development (Goldschmidt and Golomb, 1982; Goldschmidt and Monselise, 1977). Syvertsen et al. (2003) reported a higher photosynthesis rate under the 50% shade cloth, yet fruit size of 'Spring Navel' orange was not affected. Moon et al. (2011) suggest that fruit growth is not only dependent on light intensity, but aspects such as sink strength, hormone balance, fruit load and leaf to fruit ratio all being influential. When fewer fruit are present on a tree, more assimilates are made available for development which in turn will lead to larger fruit (Goldschmidt and Monselise, 1977). Therefore, the crop load might have played a significant role in causing the observed inconsistency between seasons. Possible climatic differences between the two seasons might also be responsible for producing fruit size differences, since high heat and humidity are known to lead to large fruit (Hodgson, 1967) (Addendum A: Fig. 2 and 3; Prins, 2018). However, Reuther (1988) proposed air temperature to have a broad influence on fruit growth and that heat accumulation could be used as a better indicator to relate to fruit growth (Addendum A: Fig. 4; Prins, 2018).

RIND DRY MASS PERCENTAGE. The rind dry mass percentage followed the same trend for both treatments and seasons, decreasing as the fruit diameter increased. Bain (1958) reported that citrus fruit rind becomes thinner during stage II, with little change occurring during maturation. In addition, the moisture content was also found to increase within the rind for the majority of stage II, with little change thereafter, which subsequently implies that the dry mass percentage decreases up until the end of stage II. In the current study, the only difference between the two treatments occurred during the second season, but with inconsistent results, where shaded fruit recorded higher rind dry mass in January, but with lower rind dry mass in March, compared to control fruit. This discrepancy may possibly be ascribed to a low water content in the rind in January, but with an increased water content in March, for control fruit,

but mostly these results remain inexplicable. Findings from Fallahi and Moon (1988) showed that the external canopy fruit (exposed to higher light intensity) of mandarin, grapefruit and orange cultivars had a higher rind dry mass percentage, however with no difference in lemon fruit, which could possibly indicate a lower water content and more structural carbohydrates in the rind of external sun exposed fruit.

RIND COLOR DEVELOPMENT. Rind color, critical in determining market value, was not negatively affected by the shade net treatment in either season. A similar color development pattern was seen for both treatments, with the hunter *a/b* ratio increasing from March, attaining a positive ratio in June when an orange rind coloration is observed. Color development generally occurs during the end of the cell enlargement phase (Bain, 1958; Rodrigo et al., 2004). In this study however, in both seasons, color change from green to yellow-orange only commenced between May and June, which concurs with stage III of fruit development in ‘Nadorcott’ mandarin. The chlorophyll concentration and breakdown thereof followed almost a similar trend for both treatments in both seasons, with a high concentration present at the onset of the season and a drastic decrease towards the end of the season. Furthermore, the carotenoid concentration displays an inverse trend to that of the chlorophyll, where low levels are initially present at the onset of the season, with carotenogenesis only occurring during the later stages of the season to increase the concentration that resulted in the elevated concentration recorded at the end of the season, for both treatments. These trends in terms of the rind color development, observed to occur simultaneously with changes in pigment concentration during fruit development, concur with findings of Rodrigo et al. (2004) and Alquezar et al. (2008).

Shade netting influenced the chlorophyll concentration by resulting in a high concentration in shade net-grown fruit in January during the first season, to be followed by overall higher concentration over the entire development period in the second season, possibly because of a higher concentration at the onset of the season in shaded fruit, compared to control fruit. A positive relation between chlorophyll content and fruit rind photosynthesis is reported for ‘Satsuma’ mandarin by Chen et al. (2002) and for carbohydrate synthesis in ‘Nules clementine’ mandarin rind (Cronje et al., 2013). Although carbohydrates are known to promote the chloro-chromoplast conversion, leading to rind coloration (Goldschmidt, 1988; Huff, 1984), the rind color of fruit grown under the shade net was not enhanced, which thereby indicates that the higher chlorophyll content at the onset of the season did not contribute to the positive cascade associated with increased photosynthesis, carbohydrate and color conversion (Fig. 4A and B).

The carotenoid concentration only differed between treatments in January and June 2017, where shaded fruit recorded a higher concentration in both months. The higher concentration in June, however, did not impact on the hunter *a/b* ratio. Color development was delayed in the second season, which is supported by the observation in the orchard during the last measurement prior to harvest, that not all the fruit fully experienced a complete color conversion as was noted in the 2016 season. In addition to the delay in color development, it can be proposed that the differences in the carotenoid concentration might be of no biological significance at full color development, as no color differences occurred in the 2016 season between treatments at full color development, shortly before harvest.

These results, however, do not concur with other studies where shade net treatment affected the rind coloration, either positively or negatively. Findings from Syvertsen et al. (2003) and Otero et al. (2011) reported that the rind coloration of ‘Spring’ navel orange was improved by a 50% shade cloth. In contrast to this, Jifon and Syvertsen (2001) found that rind coloration was delayed for ‘Ruby Red’ grapefruit and ‘Hamlin’ orange when exposed to a 50% reflective shade screen. The shade percentage factor and type of netting product, along with time of application and the tree age (since architecture of the tree changes with age and results in a denser canopy), are all suspected to play a role in the extent to which the particular shade net may impact on fruit color. In addition, climatic conditions also influence the shade net effect, which is evident from findings by Gindaba and Wand (2005), where high temperatures within the one season only, along with the light reduction (caused by the shade net), reduced the color development under the crystal net during that particular season. Although light intensity is important for rind coloration, as seen in studies where outside fruit had a better color through exposure to higher PPF levels (Cronje et al., 2013; Hamadziripi et al., 2014; Khalid et al., 2012), a reduction in light intensity is also known to reduce carotenoid levels (Hamadziripi et al., 2014; Lewis and Coggins, 1964). However, in this study, the reduced light levels and the expected climatic alterations associated with shade nets were not detrimental to the rind coloration or pigment composition (Addendum A: Fig. 1 and 2; Prins, 2018).

SUNBURN INCIDENCE AND PERCENTAGE. The reduction in light as ameliorated by the shade netting was effective in reducing the percentage and incidence of sunburn on the shaded fruit in both seasons, as sunburn is strongly linked to high sunlight exposure of fruit (Ketchie and Ballard, 1968; Sadamatsu, 1981). The reduction in sunburn incidence under the shade netting concurs with findings by Lee et al. (2015) for ‘Ponkan’ mandarin, as well as with studies on apples by Iglesias and Alegre (2006), Smit (2007) and Gindaba and Wand (2005). Both Lee et al. (2015) and Gindaba and Wand (2005) reported that shade netting significantly reduced

fruit surface temperatures. This, however, was not found in the current study, as no differences in the fruit surface temperatures between shaded and control fruit were measured. Schrader (2003) reported the fruit surface temperature for 'Fuji' BC-2 apples to be correlated with the maximum daily air temp, and mean solar radiation as prevalent between 1100-1700 HR. Of interest is an observation by Smit (2007) that although 20% black shade net did not reduce the air temperature greatly, the fruit surface temperature of studied apple cultivars was, however, significantly reduced. From results obtained in the current study, it can be postulated that light intensity in this instance had a greater influence on sunburn, than elevated fruit surface temperatures. The reduced light intensity (17 -18%) (Addendum A: Fig 1.; Prins, 2018), caused by the shade nets was effective in reducing the sunburn percentage, yet some incidence still occurred, therefore it can be concluded that the light intensity was not reduced to such an extent that it influenced the fruit surface temperature.

INTERNAL FRUIT QUALITY. In terms of export value, in addition to the external appearance, the taste of the fruit as expressed by the SSC, acids and the SSC to acid ratio, is also of the utmost importance. The internal quality development of shaded fruit was not affected, irrespective of the season. The SSC to citric acid ratio increased throughout the development, as the SSC content increased, and the citric acid content decreased with fruit development towards maturity, as expected. This unaffected internal quality status reported in our study is in agreement with findings of Cohen et al. (2000), where the sugar and acids in 'Marsh' grapefruit juice were not influenced by the application of either 30 or 60% shade. However, both concurring and contradicting findings to the current study were reported by Jifon and Syvertsen (2001). In their study, shade screens (50%) applied either early (after the bloom-peak 'May-June drop') or continued (after bloom to harvest) or late (only in August to harvest) did not affect the Brix of 'Hamlin' sweet orange, whereas continued shade application decreased the °Brix/acid ratio. In 'Ruby Red' grapefruit late applied shade had no effect on the Brix or its SSC to acid ratio in the one season, while in the other season both late and continued applied shaded reduced the Brix and its associated ratio with the acids. Similarly, Otero et al. (2011) also reported that the use of 50% shading cloth on 'Spring' navel oranges, reduced the total soluble solids, but without affecting the ratio. In addition, Wachsmann et al. (2014) documented that 'Orri' mandarin under 18% white shade net had significantly higher sugar to acid ratios, therefore reaching commercial maturity earlier, due to the desired reduced acid levels.

From the above findings, it is evident that no consistent trend exists as to the direct influence of shading on the SSC. Again, as was discussed with regard to the reduction of light,

the season may play a significant role in the SSC content obtained and the effect of shade net thereon. This was also evident in a study by Jifon and Syvertsen (2001) where, similar to our study, inconsistent results during two consecutive seasons were reported. In the current study, the reduced light was reported not to be detrimental in reducing the SSC content, despite studies that have shown that fruit that are exposed to more light generally have a higher SSC compared to fruit receiving less light (Khalid et al., 2012; Sites and Reitz, 1949; Verreyne et al., 2004).

During the development of pulp color in the second season, the hunter *a/b* ratio consistently increased to reach a positive value at the end of the season. Fruit grown under shade netting showed a trend of a higher average ratio over the development period ($P = 0.066$).

In addition to the challenge of managing above mentioned internal quality parameters, growers of ‘Nadorcott’ mandarin are currently facing a new disorder in citriculture, known as gumming where brown droplets develop on the central axis inside the fruit, and in severe cases render the fruit cosmetically unacceptable to the consumer. However, to date no published research is available as to what the exact cause of this disorder may be, although a condition of water stress affecting vascular development during fruit development is suspected to play a role. In these findings, gumming incidence was 48.8% and 45.7% for the control- and shade net- produced fruit respectively, highlighting the complexity of identifying a causal factor of this disorder.

To conclude, the 20% white shade netting had a positive influence on the fruit size in the second season resulting in a higher average fruit size. The shade net was effective in reducing the percentage of sunburn, without affecting the rind coloration. Furthermore, the maturity of the fruit grown under the shade net was not influenced, therefore, the internal quality at harvest was not affected. It is, however, important to note that the effect of shading may be influenced by the climatic conditions in particular, with changes being more evident on warm, sunny days as opposed to cooler days (Iglesias and Alegre, 2006; Syvertsen et al., 2003). In addition to this, orchard factors and cultivars used, are important aspects to consider in determining the effect of the shade net on production and fruit quality, since tree architecture and bearing positions are known to affect the amount of light penetrating into the canopy before reaching inside canopy fruit.

The ‘Nadorcott’ orchards used in the current study were predominantly young trees, with less dense canopies than would have been the case in more mature orchards. Therefore, adequate light was able to penetrate into the canopy, and thus with the reduced light not being detrimental at this particular stage. Furthermore, ‘Nadorcott’ mandarin is also considered a tip bearer, which means that more fruit are exposed to light than would be the case for other citrus

crops. Therefore, when developing management strategies involving shade netting in particular, it is important to consider the possible interaction that climate, tree architecture, cultivar selection and age of the crop may have on fruit quality.

Literature Cited

- Albertini, M., E. Carcouet, O. Pailly, C. Gambotti, F. Luro, and L. Berti. 2006. Changes in organic acids and sugars during early stages of development of acidic and acidless citrus fruit. *J. Agr. Food Chem.* 54:8335–8339.
- Alquezar, B., M.J. Rodrigo, and L. Zacarías. 2008. Regulation of carotenoid biosynthesis during fruit maturation in the red-fleshed orange mutant Cara Cara. *Phytochemistry* 69:1997–2007.
- Bain, J.M. 1958. Morphological, anatomical and physiological changes in the developing fruit of the Valencia orange, *Citrus sinensis* (L.). Osbeck. *Austral. J. Bot.* 6:1–24.
- Barry, G.H., W.S. Castle, and F.S. Davies. 2000. Juice quality of ‘Valencia’ sweet orange among citrus-producing regions in Florida and between canopy positions. *Proc. Intl. Soc. Citricult. IX Congr.*1: 308–314.
- Chen, J.W., S.L. Zhang, L.C. Zhang, Z.Z. Zhao, and J.G. Xu. 2002. Fruit photosynthesis and assimilate translocation and partitioning: Their characteristics and role in sugar accumulation in developing *Citrus unshiu* fruit. *Acta Botanica Sinica* 44:158–163.
- Cohen, S., A. Grava, and E.E. Goldschmidt. 2000. Citrus response to radiation load reduction: Water use, photosynthesis, and productivity. *Proc. Intl. Soc. Citricult. IX Congr.*1:615–618.
- Cronje, P.J.R., G.H. Barry, and M. Huysamer. 2013. Canopy position affects pigment expression and accumulation of flavedo carbohydrates of ‘Nules Clementine’ mandarin fruit, thereby affecting rind condition. *J. Amer. Soc. Hort. Sci.* 138:217–224.
- Ehara, T., T. Nogata, and T. Nakamuta. 1981. Studies on fruit-bearing branches of Satsuma mandarins. *Proc. Int. Soc. Citricult.* 1:209–214.
- Eilati, S.K., S.P. Monselise, and P. Budowski. 1969. Seasonal development of external color and carotenoid content in the peel of ripening ‘Shamouti’ oranges. *J. Amer. Soc. Hort. Sci.* 94:346–348.
- Fallahi, E. and J.W. Moon, Jr. 1988. Effect of canopy position on quality, photosynthesis and mineral nutrition of four citrus varieties. *Univ. of Arizona Citrus Rpt. P-76:* 5–12. 28 September 2017. < <http://hdl.handle.net/10150/215697/>>.
- Gindaba, J. and S.J.E. Wand. 2005. Comparative effects of evaporative cooling, kaolin particle film, and shade net on sunburn and fruit quality in apples. *HortScience* 40:592–596.

- Goldschmidt, E.E. 1988. Regulatory aspects of chloro-chromoplast interconversions in senescing *Citrus* fruit peel. *Isr. J. Bot.* 37:123–130.
- Goldschmidt, E.E. and A. Golomb. 1982. The carbohydrate balance of alternate-bearing citrus trees and the significance of reserves for flowering and fruiting. *J. Amer. Soc. Hort. Sci.* 107:206–208.
- Goldschmidt., E.E. and S.P. Monselise. 1977. Physiological assumptions toward the development of a citrus fruiting model. *Proc. Int. Soc. Citricult.* 2:668–672.
- Grant, R.H. 1997. Partitioning of biologically active radiation in plant canopies. *Int. J. Biometeorol.* 40:26–40.
- Gross, J. 1987. *Pigments in fruits*. Academic Press, London.
- Hamadziripi, E.T., K.I. Theron, M. Muller, and W.J. Steyn. 2014. Apple compositional and peel color differences resulting from canopy microclimate affect consumer preference for eating quality and appearance. *HortScience* 49:384–392.
- Hodgson, R.W. 1967. Horticultural varieties of citrus, p. 431–591. In: W. Reuther, H.J. Webber, and L.D. Batchelor (eds.). *The citrus industry*, Vol 1. University of California, USA.
- Huff, A. 1984. Sugar regulation of plastid interconversions in epicarp of citrus fruit. *Plant Physiol.* 76:307–312.
- Iglesias, I. and S. Alegre. 2006. The effect of anti-hail nets on fruit protection, radiation, temperature, quality and profitability of ‘Mondial Gala’ apples. *J. Appl. Hort.* 8:91–100.
- Jifon, J.L. and J.P. Syvertsen. 2001. Effects of moderate shade on citrus leaf gas exchange, fruit yield and quality. *Proc. Fla. State Hort. Soc.* 114:177–181.
- Jifon, J.L. and J.P. Syvertsen. 2003. Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves. *Tree physiol.* 23:119–127.
- Ketchie, D.O. and A.L. Ballard. 1968. Environments which cause heat injury to ‘Valencia’ oranges. *Proc. Amer. Soc. Hort. Sci.* 93:166–172.
- Khalid, S., A.U. Malik, B.A. Saleem, A.S. Khan, M.S. Khalid, and M. Amin. 2012. Tree age and canopy position affect rind quality, fruit quality and rind nutrient content of ‘Kinnow’ mandarin (*Citrus nobilis* Lour × *Citrus deliciosa* Tenora). *Scientia Hort.* 135:137–144.
- Lado, J., P. Cronje, B. Alquézar, A. Page, M. Manzi, A. Gómez-Cadenas, A.D. Stead, L. Zacarías, and M.J. Rodrigo. 2015. Fruit shading enhances peel color, carotenes accumulation and chromoplast differentiation in red grapefruit. *Physiol. Plant.* 154:469–484.
- Lee, T.C., P.J. Zhong, and P.T. Chang. 2015. The effects of preharvest shading and postharvest storage temperatures on the quality of ‘Ponkan’ (*Citrus reticulata* Blanco) mandarin fruits. *Scientia Hort.* 188:57–65.

- Lewis, L.N. and C.W. Coggins. 1964. The inhibition of carotenoid accumulation in Navel oranges by gibberellin A₃, as measured by thin layer chromatography. *Plant and Cell Physiol.* 5:457–463.
- Lichtenthaler, H.K. 1987. [34] Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology* 148:350–382.
- Mataa, M., and S. Tominaga. 1998. Effects of shading stage and level on fruit set and development, leaf carbohydrates and photosynthesis in Ponkan (*Citrus reticulata* Blanco). *Jpn. J. Trop.Agr.* 42:103–110.
- Mehouachi, J., D. Serna, S. Zaragoza, M. Agusti, M. Talon, and E. Primo-Millo. 1995. Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of *Citrus unshiu*. *Plant Sci.* 107:189–197.
- Meredith, F.I. and R.H. Young. 1969. Effect of temperature on pigment development in Red Blush grapefruit and Ruby blood oranges. *Proc. First. Intl. Citrus Symp.* 1:271–276.
- Moon, D. G., J.H. Joa, Y.E. Moon, K.C. Seong, C.H. Kim, and Y.K. Ahn. 2011. Plant growth and fruit quality as affected by canopy locations in ‘Shiranuhi’ mandarin. *Hort. Environ. Biotechnol.* 52:443–447.
- Mupambi, G., B.M. Anthony, D.R. Layne, S. Musacchi, S. Serra, T. Schmidt, and L.A. Kalcsits. 2018. The influence of protective netting on tree physiology and fruit quality of apple: A review. *Scientia Hort.* 236:60–72.
- Otero, A., C. Goni, J.L. Jifon, and J.P. Syvertsen. 2011. High temperature effects on citrus orange leaf gas exchange, flowering, fruit quality and yield. *Acta Hort.* 903:1069–1076.
- Prins, M.D. 2018. Impact of shade netting on orchard microclimate and the physiology of ‘Nadorcott’ mandarin. Stellenbosch Univ., South Africa. MSc. Thesis. (*submitted*).
- Racsko, J. and L.E. Schrader. 2012. Sunburn of apple fruit: Historical background, recent advances and future perspectives. *Critical Rev. Plant Sci.* 31:455–504.
- Rajapakse, N.C. and Y. Shahak. 2007. Light-quality manipulation by horticulture industry, p. 290–311. In: G.C. Whitelam and K.J. Halliday(eds.) *Light and plant development*. Blackwell Publishing Ltd, UK.
- Reitz, H.J. and J.W. Sites. 1948. Relationship between position on the tree and analysis of citrus fruit with special reference to sampling and meeting internal grades. *Proc. Florida State Hort. Soc.* 54:80–90.
- Reuther, W. 1988. Climate and fruit quality, p. 9–23. In: J.J. Ferguson and W. Wardowski (eds.). *Factors affecting fruit quality*. Proceedings, Citrus short course. Citrus Research and education center, Lake Alfred, Florida.

- Rodrigo, M., J.F. Marco, and L. Zacarías. 2004. Biochemical and molecular analysis of carotenoid biosynthesis in flavedo of orange (*Citrus sinensis* L.) during fruit development and maturation. *J. Agric. Food Chem.* 52:6724–6731.
- Sadamatsu, M. 1981. Occurrence of sun scald in early Satsuma mandarin and its control. *Proc. Int. Soc. Citricult.* 1:205–207.
- Schrader, L. E., J. Zhang, and J. Sun. 2003. Environmental stresses that cause sunburn of apple. *Acta Hort.* 618:397–405.
- Shahak, Y., E.E. Gussakovsky, Y. Cohen, S. Lurie, R. Stern, S. Kfir, A. Naor, I. Atzmon, I. Doron, Y. Greenblat-Avron. 2004. ColorNets: A new approach for light manipulation in fruit trees. *Acta Hort.* 636:609–616.
- Shahak, Y., K.Ratner, Y.E. Giller, N. Zur, E. Or, E.E. Gussakovsky, R. Stern, P. Sarig, E. Raban, E. Harcavi, I. Doron, and Y. Greenblat-Avron. 2008. Improving solar energy utilization, productivity and fruit quality in orchards and vineyards by photoselective netting. *Acta Hort.* 772:65–72.
- Sites, J.W. and H.J. Reitz. 1949. The variation in individual Valencia oranges from different locations of tree as a guide to sampling methods and spot picking for quality. Part I. Soluble solids in the juice. *Proc. Amer. Soc. Hort. Sci.* 54:1–9.
- Smit, A. 2007. Apple tree and fruit responses to shade netting. MS Thesis, SU, South Africa.
- Suzuki, T., S. Okamoto, and T. Seki. 1973. Effects of micro-meteorological elements and positions in the tree crown on the development of shoots, leaves and fruits of satsuma mandarin. *J. Jpn. Soc. Hort. Sci.* 42:201–209 (abstr.).
- Syvetsen, J.P., C. Goñi, and A. Otero. 2003. Fruit load and canopy shading affect leaf characteristics and net gas exchange of ‘Spring’ navel orange trees. *Tree Physiol.* 23:899–906.
- Thomson, W.W., L.N. Lewis, and C.W. Coggins. 1967. The reversion of chromoplasts to chloroplasts in Valencia oranges. *Cytologia* 32:117–124.
- Ting, S.V. and J.A. Attaway. 1971. Citrus fruits, p. 107–169. In: A.C. Hulme (ed.). *The biochemistry of fruits and their products*, Vol 2. Academic Press INC, London.
- Verreyne, J.S., E. Rabe, and K.I. Theron. 2004. Effect of bearing position on fruit quality of mandarin types. *S. Afr. J. Plant Soil* 21:1–7.
- Wachsmann, Y., N. Zur, Y. Shahak, K. Ratner, Y. Giler, L. Schlizerman, A. Sadka, S. Cohen, V. Garbinshikof, B. Giladi, and M. Faintzak. 2014. Photoselective anti-hail netting for improved citrus productivity and quality. *Acta Hort.* 1015:169–176.

- Winston, J.R. and E.V. Miller. 1948. Vitamin C content and juice quality of exposed and shaded citrus fruits. *J. Food Sci.* 13:456–460.
- Wünsche, J.N., D.H. Greer, J.W. Palmer, A. Lang, and T. McChie. 2001. Sunburn: The cost of a high light environment. *Acta Hort.* 557:349–356.
- Yakushiji, H., K. Morinaga, and H. Nonami. 1998. Sugar accumulation and partitioning in Satsuma mandarin tree tissues and fruit in response to drought stress. *J. Amer. Soc. Hort. Sci.* 123:719–726.

Tables

Table 1. The influence of 20% white netting on fruit diameter (mm) and rind dry mass accumulation (%) as recorded during the development period of ‘Nadorcott’ mandarin fruit over two consecutive seasons, in 2016 and 2017. Data where no interaction between main effects was evident, are presented.

Season	2016		2017
	Fruit diameter (mm)	Rind dry mass (%)	Fruit diameter (mm)
<u>Treatment</u>			
Control (no net)	48.1 ^{NS}	27.7 ^{NS}	51.6 b
Shade net	48.8	27.4	53.7 a
<u>Month^z</u>			
Jan.	31.6 f ^y	- ^x	34.6 f
Feb.	40.6 e	32.0 a	43.1 e
Mar.	42.6 d	28.2 b	50.3 d
Apr.	55.1 c	26.3 c	57.6 c
May	59.3 b	26.5 c	63.1 b
June	61.6 a	24.8 d	67.5 a
<u>P-value</u>			
Treatment	0.648	0.637	0.034
Month	< 0.001	< 0.001	< 0.001
Treatment x Month	0.678	0.098	0.775

^{NS} Denotes non-significant difference at 5% significant level.

^z Values reported are for the two treatments (control and shade net) combined.

^y Different letters denote significant difference between means within a column for each main effect at $P \leq 0.05$ according to Fisher's LSD test.

^x Data not recorded.

Table 2. The influence of 20% white shade net on the rind color development and pigment concentration of chlorophyll and carotenoids ($\mu\text{g}\cdot\text{g}^{-1}$ DW) of 'Nadorcott' mandarin fruit during the 2016 and 2017 season. Data where no interaction between main effects was evident, are presented.

Season	2016		2017	
	Rind color (Hunter <i>a/b</i>)	Carotenoid concentration ($\mu\text{g}\cdot\text{g}^{-1}$ DW)	Rind color (Hunter <i>a/b</i>)	Chlorophyll concentration ($\mu\text{g}\cdot\text{g}^{-1}$ DW)
<u>Treatment</u>				
Control	-0.35 ^{NS}	910 ^{NS}	-0.59 ^{NS}	1323 b
Shade net	-0.34	887	-0.59	1432 a
<u>Month^z</u>				
Jan.	-0.62 c ^y	602 d	-0.84 d	1927 b
Feb.	-0.74 d	499 e	-0.84 d	2122 a
Mar.	-0.71 d	712 c	-0.83 d	1774 c
Apr.	-0.49 b	790 c	-0.72 c	1439 d
May	- ^x	1102 b	-0.51 b	777 e
June	0.87 a	1688 a	0.19 a	225 f
<u>P-value</u>				
Treatment	0.697	0.437	0.908	0.029
Month	< 0.001	< 0.001	< 0.001	< 0.001
Treatment x Month	0.320	0.191	0.775	0.086

^{NS} Denotes non-significant difference at 5% significant level.

^z Values reported are the average for the two treatments, control and shade net.

^y Different letters denote significant difference between means within a column for each main effect at $P \leq 0.05$ according to Fisher's LSD test.

^x Data not recorded.

Table 3. The incidence of gumming expressed as an index and percentage (%) on ‘Nadorcott’ mandarin fruit grown under 20% white shade net compared to a control (fruit grown without shade net). Scores were applied in June 2017, two weeks prior to harvest and the values are the mean of four replications.

Treatment	Index ^z	Percentage ^y (%)
Open	0.74 ^{NS}	48.8 ^{NS}
Shade net	0.65	45.7
<i>P</i> -value	0.581	0.734

^z Severity is based on scale from 0 to 2 (0 = no incidence, 1 = moderate incidence, small droplets, 2 = severe incidence and browning of droplets. A higher index indicates a more severe incidence of gumming.

^y Percentage (%) of fruit scored between 1 and 2.

^{NS} Denotes non-significant difference between means within a column according to Fisher's LSD test at $P \leq 0.05$.

Table 4. The influence of 20% white shade net application on the sunburn index and percentage (%) of 'Nadorcott' mandarin fruit. Values are the mean of four replications.

	Treatment	Index ^z	Percentage ^y (%)
2016 ^w	Control	0.37 a ^x	23.7 a
	Shade net	0.07 b	5.75 b
	<i>P</i> -value	<i>0.005</i>	<i>0.002</i>
2017 ^v	Control	0.28 a	20.8 a
	Shade net	0.05 b	5.00 b
	<i>P</i> -value	<i>0.014</i>	<i>0.031</i>

^z Degree of severity scale 0-2. 0 = no sunburn, 1 = moderate, 2 = severe.

^y Percentage of fruit scored 1-2.

^x Different letters denote significant difference between means within a column for each season at $P \leq 0.05$ according to Fisher's LSD test.

^w 200 fruit per replicate scored on the eastern side of the tree after color break.

^v 40 fruit per replicate scored (20 on the eastern and 20 on the western side) before color break.

Table 5. The influence of 20% white shade net on the fruit surface temperature (°C) of ‘Nadorcott’ mandarin fruit measured during the second season (2017). Values are the means of four replications.

Treatment	Fruit surface temperature ^z (°C)
Control	39.4 ^{NS}
Shade net	39.5
<i>P</i> -value	
Treatment	0.786
Date	< 0.001
Treatment x Date	0.619

^z Measurements were recorded with a temperature gun on selected warm days where maximum temperatures of above 30 °C were reached.

^{NS} Non-significant differences between treatments at $P \leq 0.05$ according to Fisher's LSD test.

Figures

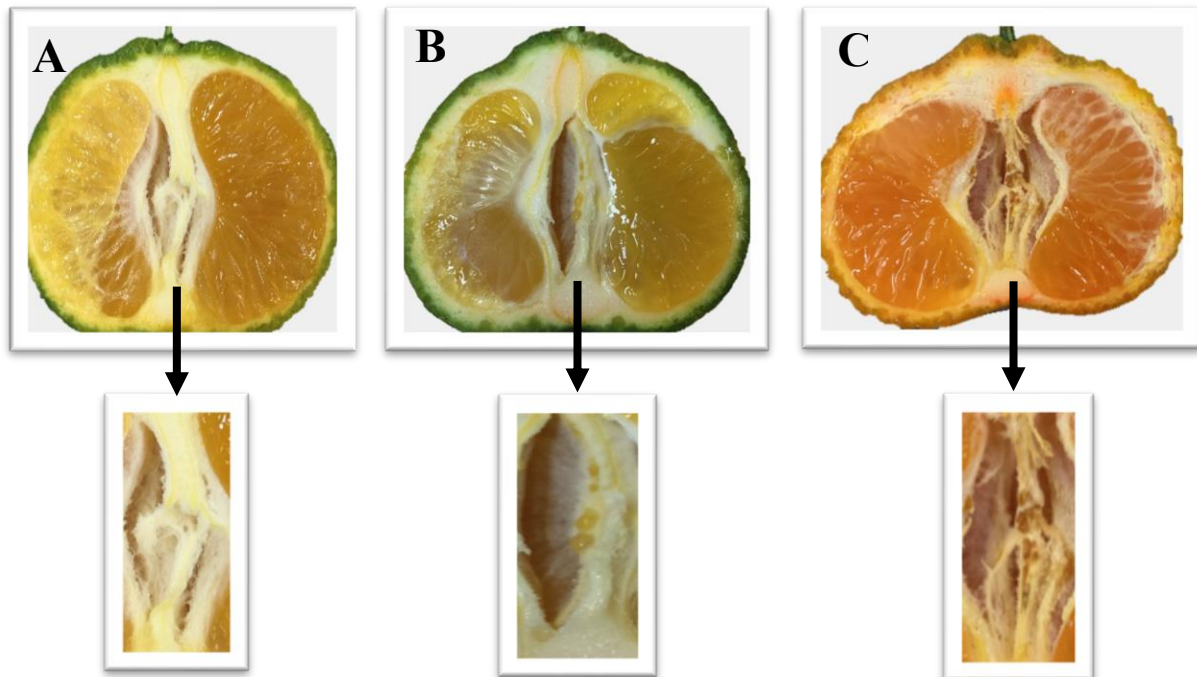


Fig. 1. A photographic representation of the scoring system used to calculate the gumming incidence in a study on 'Nadorcott' mandarin assessing the influence of shade netting on fruit quality. The scoring scale ranged from 0 = no incidence of gumming (**A**), 1 = moderate incidence and small droplets (**B**), to where 2 = severe incidence and browning of droplets (**C**).

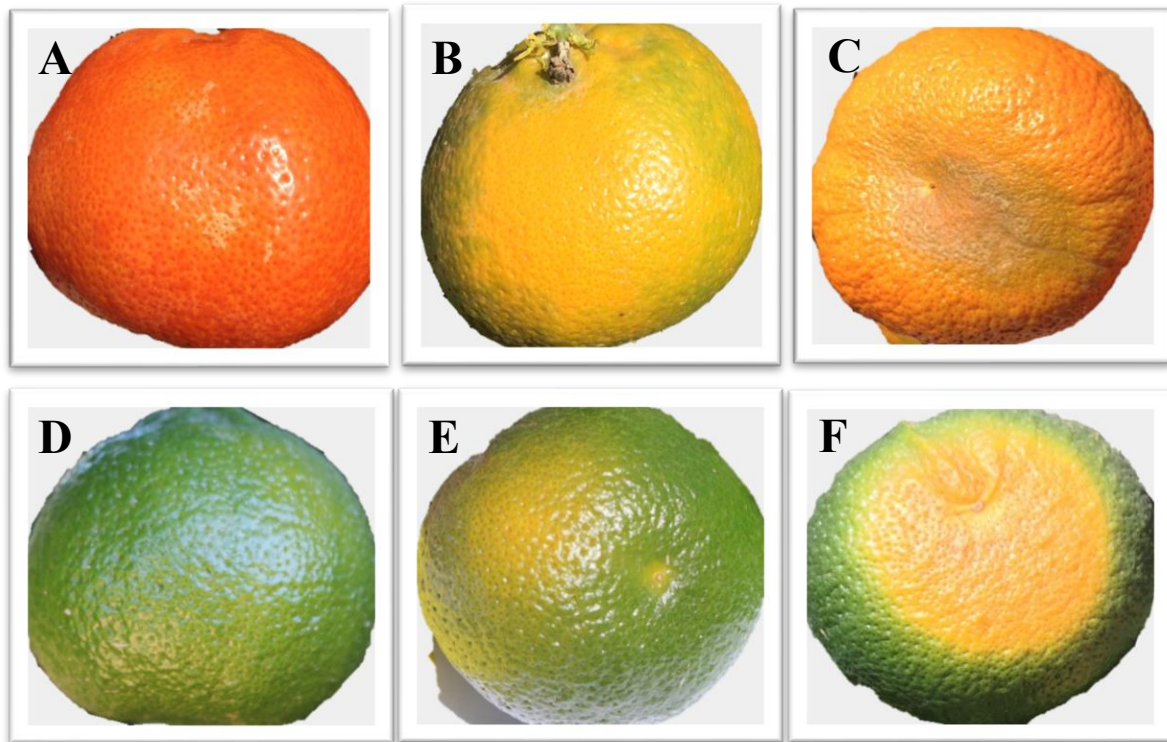


Fig. 2. A photographic representation of the visual scoring system used to assess sunburn incidence in a study on 'Nadorcott' mandarin assessing the influence of shade netting on fruit quality during the 2016 season (**A-C**) and 2017 season (**D-F**) respectively. Fruit were scored at full color development in the 2016 season. Scores ranged from 0 = no incidence (**A**), 1 = moderate incidence, yellow coloration (**B**), to where 2 = severe incidence, leathery rind appearance and in some cases necrotic tissue (**C**). During the 2017 season, grading was done prior to color break, and ranged from 0 = no incidence (**D**), 1 = moderate incidence, light yellow coloration on the rind (**E**) to where 2 = severe incidence with a bright yellow coloration and in some cases leathery rind texture (**F**).

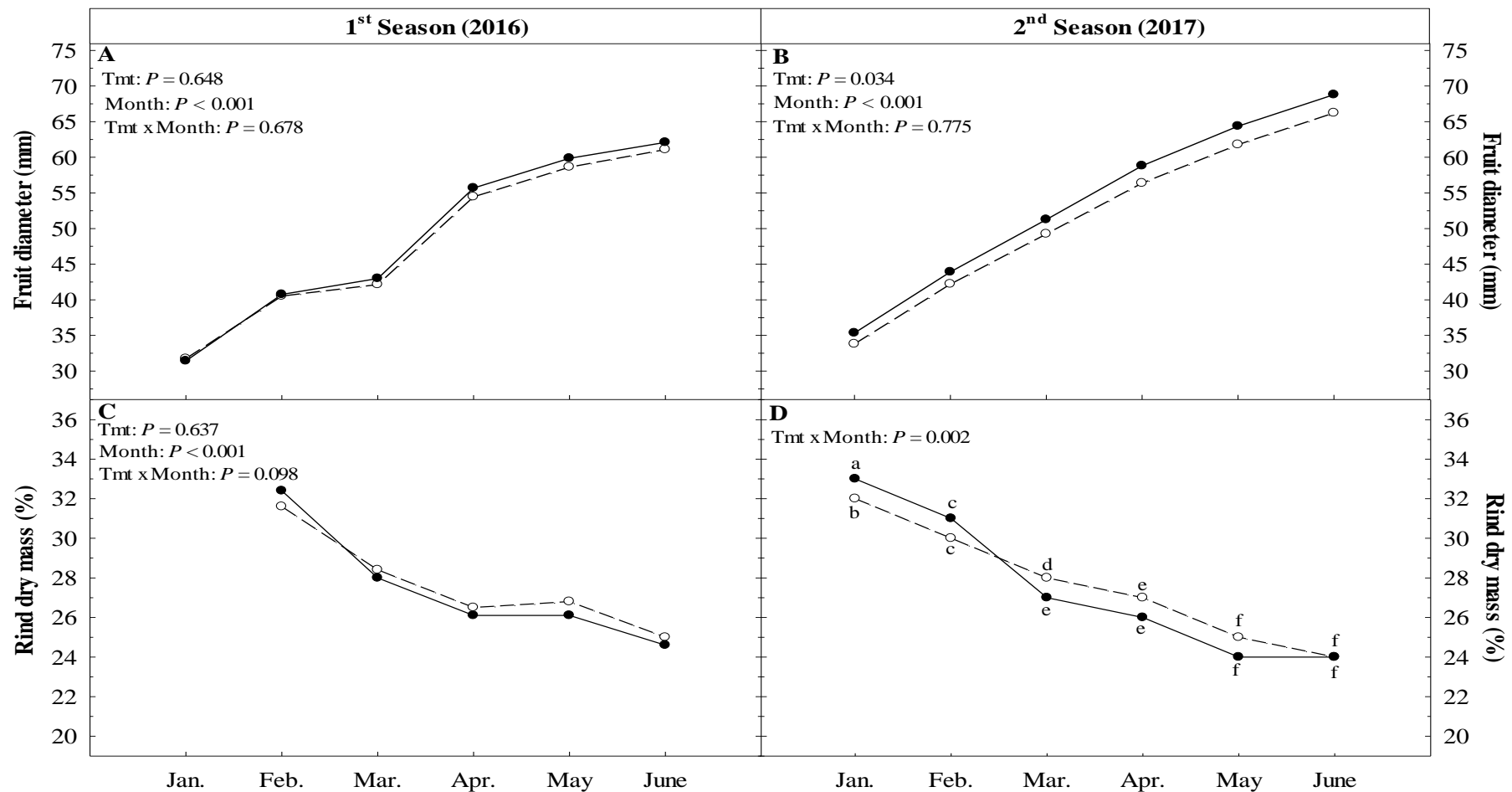


Fig. 3. Fruit size (mm) increase (A-B) and rind dry mass percentage (C-D) of 'Nadorcott' mandarin fruit grown under 20% white shade net (solid line ●) compared to a control (no shade net, broken line ○) over two consecutive seasons. The main effects, Treatment (Tmt), Month and the interaction between Treatment and Month (Tmt x Month) are indicated on the graph. Different letters denote significant differences between means at 5% significant level according to Fisher's LSD test on figures where the interaction is significant. Values are the means of four replications.

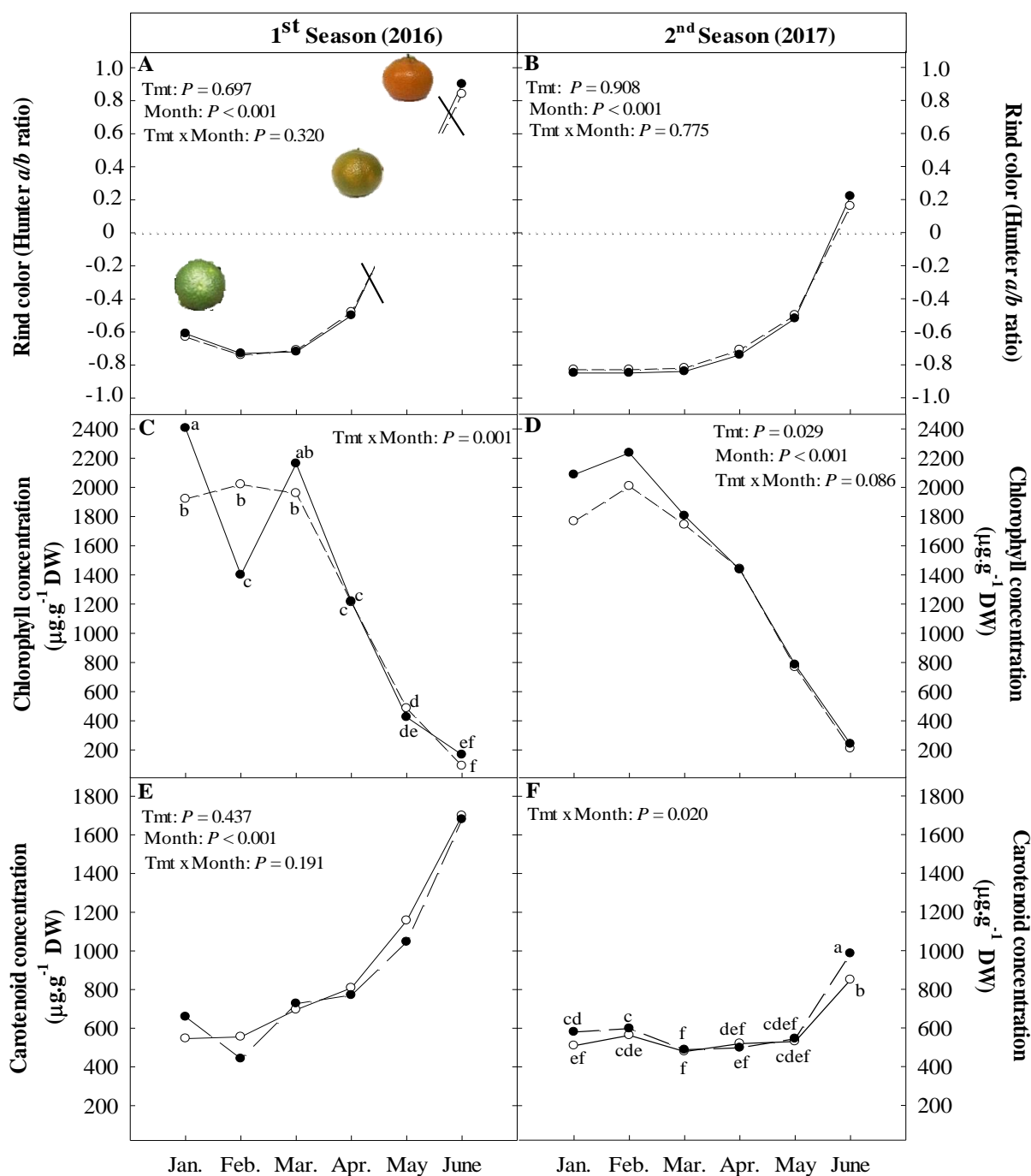


Fig. 4. Changes in rind color development (A-B) and that of chlorophyll (C-D) and carotenoid content (E-F) of 'Nadorcott' mandarin fruit grown under 20% white shade net (solid line ●) and control (no net, broken line ○) over two consecutive seasons. The P -values depict the main effects, Treatment (Tmt), Month and the interaction between Tmt x Month. Values reported are the means of four replications. Different letters at each point denote significant differences between means ($P \leq 0.05$) where the interaction was significant. Mean separation was done by means of Fisher's LSD test. Parallel bars in A indicate a missing value. The dotted line through the 0.0 axis indicates where color break occurs and where the hunter a/b ratio changed from a negative to positive value.

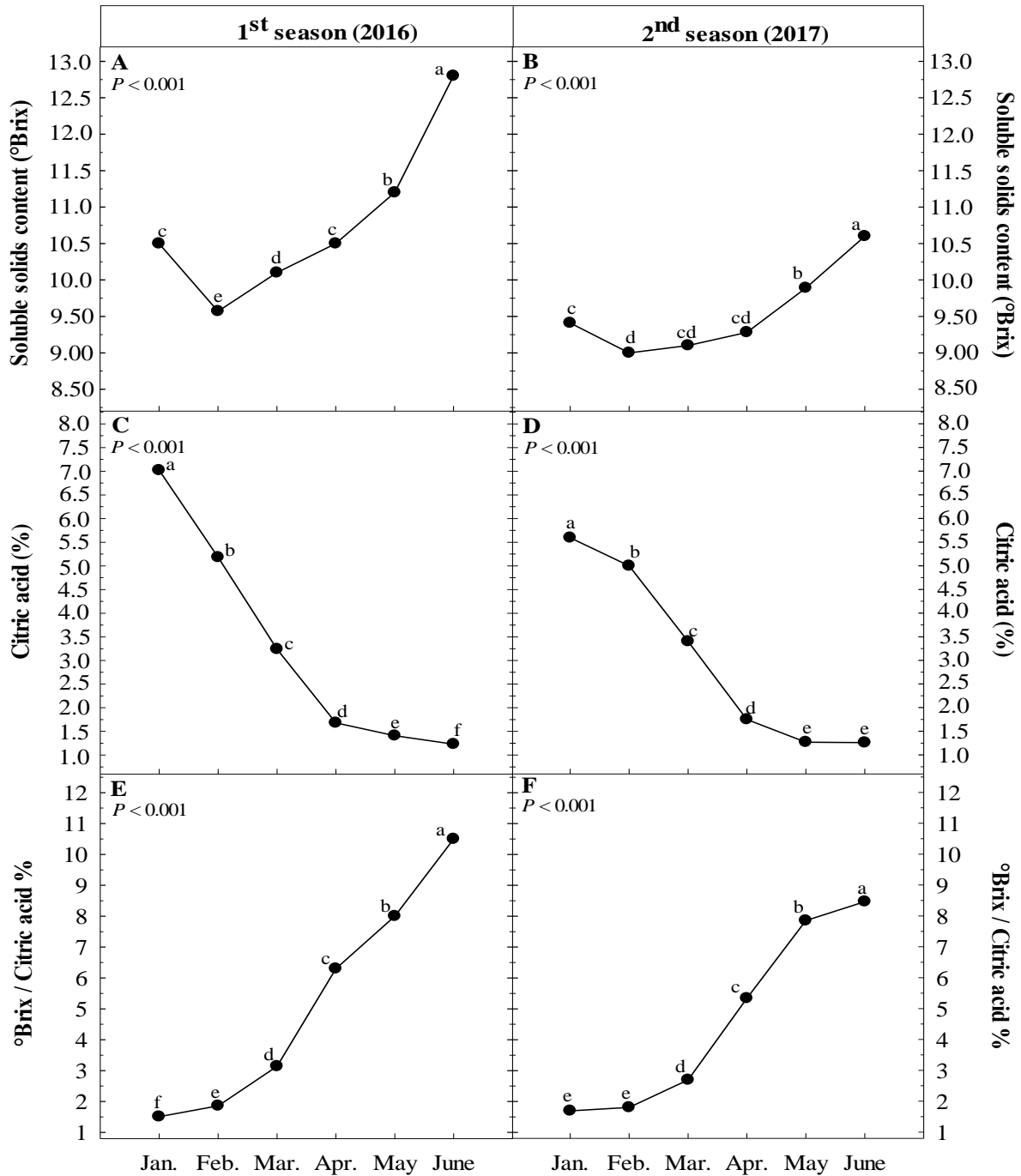


Fig. 5. Seasonal development of °Brix (A-B), citric acid percentage (C-D) and the °Brix:citric acid percentage ratio (E-F) of the combined control and 20% white shade net treatments for 'Nadorcott' mandarin fruit during the 2016 and 2017 seasons, respectively. The P -value for the Month main effect is indicated on each graph. Different letters denote significant differences between means at 5% significant level according to Fisher's LSD test.

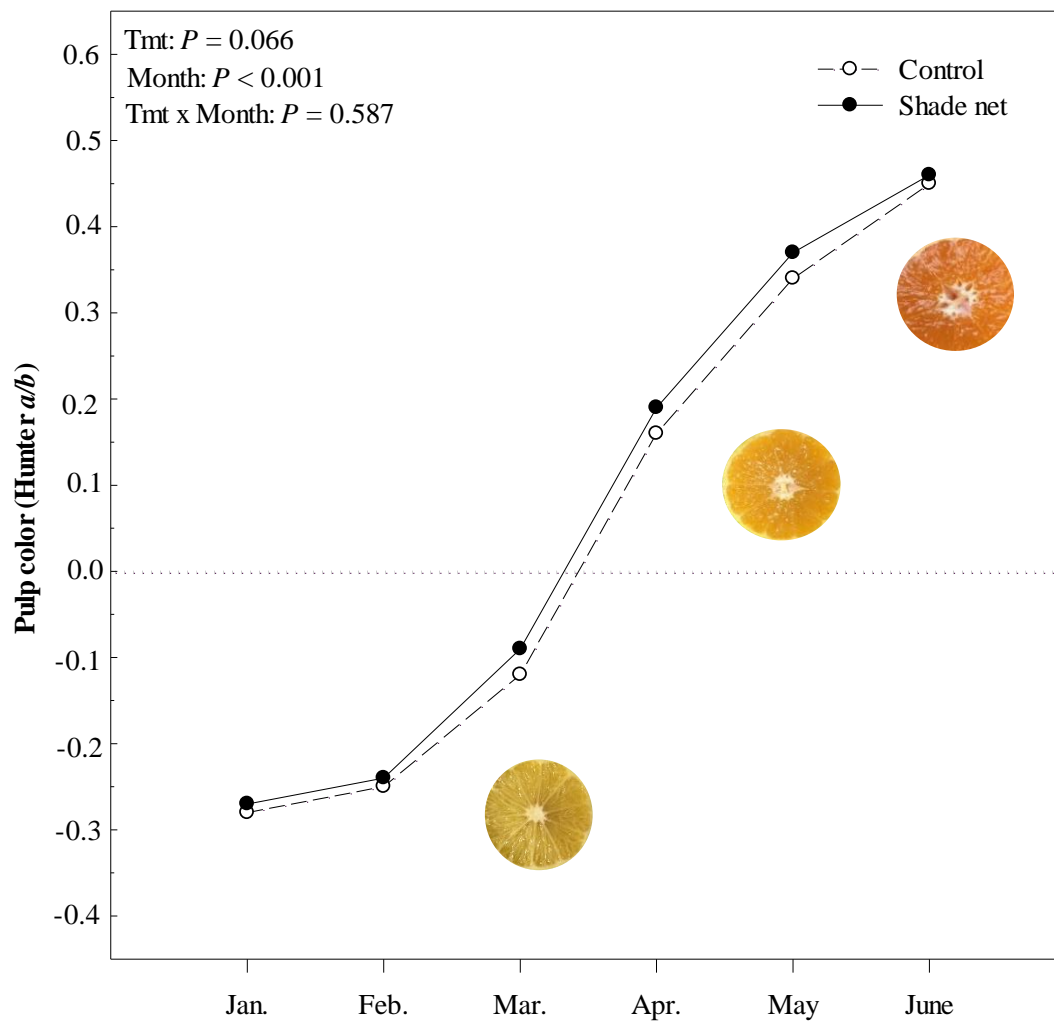


Fig. 6. Monthly development of pulp coloration of 'Nadorcott' mandarin fruit grown under 20% white shade net, in comparison to control fruit grown without shade netting in the second season (2017). The dotted line through the 0.0 axis indicates where color break occurs and where the hunter a/b ratio changed from negative to positive. The P -values on the graph depict the main effect of Treatments (Tmt), Month and the Treatment and Month interaction (Tmt x Month), which in this case is non-significant at the 5% confidence interval. Values are the mean of four replications. The fruit indicated on the graph represents the pulp coloration at the corresponding developmental phase.

CHAPTER 4

Cold storage behavior of ‘Nadorcott’ mandarin fruit as affected by preharvest shading

ABSTRACT

During postharvest cold storage the fruit are submitted to physical and biochemical changes which may result in incidences of physiological rind disorders such as chilling injury and rind staining which consequently affect the marketability of the fruit. Preharvest conditions are known to affect postharvest fruit quality and the effect of shade netting, which is used to protect fruit against excessive sunlight, on internal and external quality parameters during postharvest are unknown. The aim of this study was to determine if 20% white shade net will have a negative influence on the quality of ‘Nadorcott’ mandarin (*Citrus reticulata* Blanco) fruit during storage at -0.6 and 4 °C for 34 days. Fruit was harvested during two consecutive seasons from an orchard in Citrusdal, South Africa, but did not receive any postharvest treatments. Fruit was evaluated prior to storage and after a 7 day shelf life period following cold storage at either -0.6 or 4 °C for 14, 27 and 34 days, respectively for changes in rind color, pulp color, rind carotenoids, soluble solids content (SSC), citric acid content and SSC: citric acid ratio. Fruit weight loss during storage as well as the incidence of rind physiological disorders (staining) were recorded. Shade net had no effect on the storage behavior of the fruit as no difference occurred between shade net and control (treatments). However, a storage duration effect was evident in some internal and external quality parameters viz. weight loss and carotenoid concentration increased over the storage duration for the two respective temperatures. Different trends were evident in the rind color between the two seasons at 4 °C, whilst for fruit stored at -0.6 °C, a decline in color was recorded in 2016, but not in 2017. Staining only occurred in the first season after 34 days storage, at both storage temperatures. Inconsistency with regards to the storage duration effect on the SSC and acid content were evident between seasons at both temperatures. Storage duration had a significant effect on the SSC to acid ratio, regardless of the storage temperature or season, resulting in a higher ratio evident for fruit in cold storage. The preharvest application of 20% white shade net did not negatively influence the cold storage behavior of ‘Nadorcott’ fruit, however, due to some contrasting results that were obtained between the two seasons, it can be proposed that the condition of the fruit at harvest plays a significant role in the postharvest behavior of the fruit during cold storage.

ADDITIONAL INDEX WORDS: white shade net, *Citrus reticulata*, storage temperature, °Brix: citric acid ratio, carotenoids, staining.

Introduction

South Africa is ranked as the 10th largest fresh citrus producer in the world, with soft citrus accounting for 16% of the production area, of which ‘Nadorcott’ (*Citrus reticulata* Blanco.) is the most widely planted mandarin. South Africa exports 76% of its production (990 749 ton), while 18% is used for processing and the remaining volume of fruit being consumed locally, which results in South Africa being the second largest citrus exporter (CGA, 2017).

In addition to pre-harvest production conditions, the storage life and quality of citrus fruit are affected by the handling and environmental factors that the fruit is exposed to during the post-harvest chain (El-Otmani et al., 2011). During postharvest handling, the fruit continues with its essential metabolic processes as are active during fruit development. Of all these various physiological and biochemical changes that occur within the fruit, respiration is considered a critical driver as it affects the postharvest potential of the fruit either directly or indirectly through changes of the biochemical composition of the fruit (Biale, 1961). The respiration rate of citrus fruit decreases with development towards maturity, with a relatively low rate in mature fruit (Aharoni, 1968; Eaks, 1970), which finally results in associated changes in both the biochemical and physical properties of the fruit after harvest (Ting and Attaway, 1971).

Physical changes are ascribed to moisture loss which causes fruit softening and shriveling of the peel and consequently accelerates fruit deterioration (Ben-Yehoshua, 1969). The biochemical changes involved in most instances are losses in organic acids and sugars when used as substrates in the respiration process, providing energy to the fruit to maintain cellular functions (Miller, 1946; Tucker, 1993) and consequently affecting the taste (Kader, 1992). Cold storage and shipping conditions are important to ensure that the fruit maintains its good quality; i.e. no rind physiological disorders and defects along with a good internal- and nutritional quality, when it reaches the consumer in targeted export markets (El-Otmani et al., 2011). High relative humidity (RH) (85-95%) and low temperature conditions are critical to control the fruit deterioration rate and thereby ensuring that the keeping qualities of the fruit are maintained for longer through preventing excessive moisture loss (Alferez et al., 2003; Ben-Yehoshua, 1969). Furthermore, when fruit is subjected to cold storage (i.e. 2 to 8 °C) the rate of biochemical changes are reduced and in addition the development of decay i.e. blue and

green mold caused by *Penicillium* spp. is retarded or prevented, which in turn prolongs the fruits storage life (Miller, 1946; Tietel et al., 2012).

There are, however, phytosanitary protocols that need to be met in order to allow shipment of citrus fruit from South Africa to certain countries (PPECB, 2012). Currently soft citrus can either be shipped between -0.6 °C (chilling) and 4 °C (non-chilling temperature) with the temperature and cold storage duration (days) being dependent on the export market (PPECB, 2017a,b).

During the cold chain, the fruit experiences certain gradual physiological changes. ‘Washington Navel’ (*C. sinensis* (L.) Osbeck) oranges which were stored at 4 °C for an extended storage time of five months showed an improvement in the rind color, along with an increase in the soluble solids content (SSC), sugars and weight loss percentage, while the citric acid content decreased (Çandir et al., 2013). ‘Or’ and ‘Odem’ mandarins stored at 2, 5 and 8 °C showed no increase in SSC, but the acids decreased, which consequently led to an increase in the SSC to acid ratio during storage (Tietel et al., 2012). In addition, these changes differ between cultivars or varieties. The physiological changes of citrus fruit stored at 15 °C and 95% RH have shown contrasting changes in the °Brix, citric acid and sugars (sucrose, glucose and fructose) content amongst different citrus varieties, ‘Hamlin’ orange, ‘Marsh’ grapefruit (*C. paradisi* Macf.) and ‘Robinson’ tangerine (*C. tangerina*) (Echeverria and Ismail, 1987). Furthermore, studies by Matsumoto et al. (2009) and Carmona et al. (2012) reported that the carotenoid content increased more rapidly during storage at higher temperatures (12 to 20 °C) as opposed to lower temperatures (2 to 5 °C) for both ‘Navelina’ orange and ‘Satsuma’ mandarin (*C. unshiu* Marc.). Matsumoto et al. (2009) proposed that citrus fruit carotenoid biosynthesis is temperature sensitive. In addition to this, Van Wyk et al. (2009) found that the rind coloration was enhanced (more orange) and carotenoid content increased in ‘Palmer Navel’ sweet oranges shipped at 4.5 °C as opposed to -0.6 °C. The same effect was seen in a study by Tietel et al. (2012) who reported that “mandarin fruit stored at 8 °C developed a better rind color than fruit stored at 2 and 5 °C”.

Continuous exposure to low temperature (0 to 10 °C), however, may result in chilling injury (CI) in citrus fruit which is considered a physiological disorder (Bassal and El-Hamahmy, 2011; Petracek et al., 2006; Purvis and Yelenosky, 1993; Rodov et al., 1995) that is characterized by a localized discoloration of the rind and results in depressions in the fruit surface (Cohen et al., 1994). CI incidence is determined by various factors i.e. cultivar, species, season and maturity index (Strano et al., 2017).

Rind disorders also develop at non-chilling temperatures, and they have been reviewed by Lafuente and Zacarías (2006), with rind staining or rind-breakdown (RB), generally referred to as ‘postharvest non-chilling peel pitting’, being one such disorder. It is generally characterized by depressions in the flavedo, which becomes dry and turns brown and black (Alfárez et al., 2003). These postharvest physiological rind disorders affect the fruit’s external appearance, thereby reducing the marketability (El-Otmani et al., 2011). Cronje et al. (2011), however, proposed that the fruit sensitivity to RB is determined during preharvest conditions, with symptom development occurring only after a signal which is triggered during postharvest handling.

Studies have reported that canopy positions, which are known to receive different light levels (Cronje et al., 2013) have a marked influence on the post-harvest physiological disorders i.e. CI in ‘Marsh’ grapefruit (McDonald et al., 1993), and rind breakdown in ‘Nules Clementine’ mandarin (Cronje et al., 2011). Furthermore, it has been reported that light levels influence the carbohydrate content (glucose, sucrose and fructose) in the rind with generally higher carbohydrate levels in the rind of outside canopy ‘Nules Clementine’ fruit (Cronje et al., 2013), even though in one season no differences were found. A study on ‘Marsh’ grapefruit, showed no difference between canopy positions (Purvis, 1980). In addition to this, fruit covering studies where light is excluded from the fruit also reported a lower carbohydrate content in the flavedo of both ‘Miyagawa wase’ ‘Satsuma’ mandarin (Chen et al., 2002) and ‘Nules Clementine’ (Magwaza et al., 2013).

Production of citrus fruit under shade net is a relatively new orchard technique used to protect fruit against rind blemishes such as sunburn through light interception (Iglesias and Alegre, 2006; Lee et al., 2015) and wind damage (Wachsmann et al., 2014). In addition, shade nets have been reported to influence the external and internal quality parameters of citrus fruit in both positive and negative ways. Syvertsen et al. (2003) reported that rind color of ‘Spring’ navel orange was enhanced by shade net, while Jifon and Syvertsen (2001) reported a delay in rind color expression in ‘Ruby Red’ grapefruit and ‘Hamlin’ orange fruit. In terms of internal quality, shade nets enhanced the sugar to acid ratio of the juice in ‘Orri’ mandarin (Wachsmann et al., 2014), with no effect in ‘Spring Navel’ orange (Syvertsen et al., 2003) and a reduction in ‘Ruby Red’ grapefruit and ‘Hamlin’ sweet orange (Jifon and Syvertsen, 2001). Cohen et al. (2000) reported that sugars and acids in ‘Marsh’ grapefruit were not influenced by shade netting, while in ‘Hamlin’ oranges the Brix was reduced in the presence of shade nets (Jifon and Syvertsen, 2001).

The postharvest potential of citrus fruit is determined by the rind and its ability to resist microbial attack, and physiological disorder development in addition to retard aging (Biale, 1961). In addition, Cronje et al. (2011, 2013) proposed that a well-developed rind, with higher carbohydrate- and carotenoid content and good rind color, prior to postharvest storage, will result in a good physiological condition of the rind during storage. Van Wyk et al. (2009) proposed that the initial rind color is important in determining the final quality after cold storage. Based on this it can also be postulated that a fruit of good internal quality at harvest will have a better storage behavior since some changes are evident during cold storage. However, to date there is no available literature as to what the effect of preharvest shade net is on the postharvest storage potential of citrus fruit at various long-term cold storage temperatures.

The aim of this study was to determine if 20% white shade netting would negatively influence the external and internal quality parameters of 'Nadorcott' mandarin fruit during long term cold storage at -0.6 and 4 °C. Aspects such as changes in rind and pulp color, internal quality parameters (Brix, citric acid percentage and the sugar to acid ratio), carotenoid concentration and fruit weight loss over the storage duration at these temperatures was evaluated in addition to the incidence of physiological disorders such as chilling injury and staining.

Materials and Methods

PLANT MATERIAL AND SITE LOCATION. Fruit were obtained from 'Nadorcott' mandarin (*Citrus reticulata* Blanco) trees, grafted on 'Carrizo citrange' (*Poncirus trifoliata* x *C. sinensis*) rootstock within a commercial orchard in Citrusdal (-32.542140, 19.011877), Western Cape Province, South Africa. Fruit were harvested over two consecutive seasons, on 7 July 2016 (first season) and 10 July 2017 (second season). Full orchard details are supplied in Chapter 3 (p. 59).

EXPERIMENTAL DESIGN AND TREATMENTS. In brief, a randomized complete block design with four replicates per treatment ($n = 4$) was used. The two treatments were shade netting (20% white shade net) and the control (no shade net). Three adjacent trees per block were selected, uniform in size, health and crop load, which served as sampling units per replicate for each treatment. A detailed experimental layout is provided in Chapter 3 (p. 59).

FRUIT SAMPLING AND MEASUREMENTS. Fruit was harvested from a central position within the canopy at approximately 1-1.5 m from the ground level, and 0-20 cm from the outside

part into the canopy, on the eastern side of the tree. Fruits were all uniform in color and free of rind blemishes, with a fruit diameter between 55-74 mm. A total of 280 fruit per treatment from each block were sampled in the first season, and 140 fruit per treatment from each block in the second season.

In the first season, 40 fruit per block replicate of each treatment were randomly allocated to be stored at either 4 °C or -0.6 °C for 14, 27 or 34 d, respectively. The remaining 40 fruit of each replicate were subjected to immediate quality analysis on day 0 (at harvest) with no exposure to cold storage. The same process was repeated during the second season, however, the replicate number was reduced to 20 fruit per replicate due to low variation observed in the first season.

To increase potential susceptibility to rind pitting and staining fruit were dehydrated at 25-30 °C for 3 d, in both seasons before cold storage. Thereafter the fruit were rehydrated for 1 d at room temperature by placing wet paper towels in each berry tray and covering it with plastic, to achieve approximately 100% RH. Fruit did not receive any of the postharvest treatments that are commercial practice for citrus fruit such as the application of wax or thiabendazole prior to cold storage.

During the second season weight loss of the fruit over the cold storage period was calculated using the following formula:

$$\% \text{ Weight loss} = \frac{[(\text{Initial fruit weight}) - (\text{Fruit weight after storage}) \times 100]}{(\text{Initial fruit weight})}$$

Following cold storage at the respective storage regimes, fruit were subjected to a 7d shelf life period at 20-22 °C, whereafter fruit were evaluated for internal and external quality parameters.

EXTERNAL AND INTERNAL QUALITY ANALYSIS. External quality assessment included the determination of rind color on the most vivid colored side of the fruit by means of a chroma meter (CR-400, Konica Minolta Sensing, Inc., Japan), immediately prior to the removal of the flavedo for preparation for rind pigment analyses (Chapter 3, p. 61).

Analyses of internal quality parameters included measurements of pulp color with a chroma meter (CR-400, Konica Minolta Sensing, Inc., Japan) on one pulp segment as well as the determination of SSC, expressed as °Brix, and citric acid content, from an extracted juice sample. The ratio of SSC to citric acid percentage was also calculated. Full details with regards to measurements are provided in Chapter 3 (p. 61). The rind and pulp color was expressed as the Hunter *a/b* ratio, by dividing the Hunter *a* by the Hunter *b* value.

POSTHARVEST RIND DISORDERS. Fruit was rated for detected incidences of staining following the respective storage days and shelf life period, based on a visual rating system where 0 = no incidence; and 3 = severe (Fig. 1A-D). The staining incidence was calculated as a staining index to provide an estimate of severity and a staining percentage, by using the equations below:

$$\text{Staining index} = \frac{\sum [\text{Number of fruit in rating category} \times \text{Rating category (0-3)}]}{\text{Total of fruit per rep}}$$

$$\text{Staining \%} = \frac{(\text{Sum of fruit scored 1 -3}) \times 100}{\text{Total fruit per rep}}$$

RIND PIGMENT ANALYSIS. The rind pigments, chlorophyll and carotenoids, were extracted from the flavedo and analyzed as described in Chapter 3 (p. 62). Two extractions per replicate were done in the first season and were increased to three extractions per replicate in the second season. Briefly, 0.030 g dried and milled flavedo samples were weighed off in a Kimax tube and extracted twice with absolute ethanol (99.9% v/v) (Merck (Pty) Ltd, Modderfontein, Gauteng, South Africa) and three times with n-hexane ($\geq 97.0\%$ v/v) [Sigma-Aldrich, Co., St. Louis, Missouri, United States], containing butylated hydroxytoluene (BHT) ($100 \text{ mg}\cdot\text{L}^{-1}$) [Sigma-Aldrich Co., St. Louis, Missouri, United States]. The extractions were dried under vacuum by means of a speedvac concentrator (SC210A, Thermo scientific, Asheville, United States) whereafter they were reconstituted with 16 mL pure acetone (100% v/v) [Merck (Pty) Ltd, Modderfontein, Gauteng, South Africa]. The absorbance at 470, 644, and 662 nm wavelengths were measured (Cary 60 UV-Vis, Agilent technologies, Santa Clara, United States) and the concentrations of chlorophyll a, chlorophyll b and carotenoids were determined by substituting the absorbance values into the equations shown in Chapter 3 (p. 63), Eq. [3] – [5]. Results were expressed as microgram per gram dry weight, based on Eq. [6] – [8] (Chapter 3, p. 63).

STATISTICAL ANALYSIS. Statistical analyses were done by means of Statistica 13's VEPAC module (TIBICO Software Inc., 2017). Mixed model was used to take into account repeated measures that were done on the same blocks over time. Treatment (control and shade net) and Storage duration (days) were entered as fixed effects, and block nested in Treatment as a random effect. For cases where there were technical reps within a block (e.g. fruit coming from the same block were individually measured i.e. rind color), block*Storage duration nested in Treatment was further added as random effect. Fisher Least Significant Difference (LSD) tests were used for Post hoc testing.

Normal probability plots were inspected to check the normality of the data, and for cases where there were prominent deviations from normality, Box-Cox transformations were performed. The staining index and percentage were analyzed by a one-way analysis of variance (ANOVA). Significant differences were determined at $P \leq 0.05$ (5% significance level).

Results

WEIGHT LOSS. In the 2016/2017 season, no interaction occurred between Treatment (control and shade net) and Storage duration at 4 °C ($P = 0.253$) and -0.6 °C ($P = 0.376$), as the weight loss recorded for fruit grown under the shade net was not differently influenced by the cold storage duration compared to the control fruit at either 4 or -0.6 °C.

The percentage weight loss recorded in fruit stored at 4 °C, however, increased significantly over the storage period (Fig. 2A). Data obtained for weight loss at -0.6 °C storage was not normally distributed, however, the effects from the transformed and untransformed data remained the same, except for a significant difference between 14 and 27 d of storage occurring in the transformed data, which was not evident in the untransformed data. The untransformed data are reported to demonstrate the percentage weight loss at -0.6 °C over the storage period. Weight loss was observed to increase significantly between 27 and 34 d of cold storage, with no difference between 14 and 27 d (Fig. 2B). Furthermore, averages for the control and shade net grown fruit stored at either 4 °C ($P = 0.617$) or -0.6 °C ($P = 0.539$) were reported to be comparable over the entire storage duration period.

RIND COLORATION. 4 °C cold storage. There was no interaction between Treatment and Storage duration in either the first ($P = 0.134$) or second season ($P = 0.330$), indicating that the two treatments followed the same pattern regarding rind color development with storage. With regards to the rind coloration changes over the storage duration, there was a contrasting trend evident between the two seasons (Fig. 3A and B). In the first season, the Hunter *a/b* ratio decreased significantly between 14 and 27 d of storage whereafter it remained constant (Fig. 3A). This resulted in fruit stored at 27 and 34 d having a significantly lower ratio, compared to fruit at harvest and 14 d, which did not differ from each other (Fig. 3A). During the second season, fruit that received cold storage, had a significantly higher Hunter *a/b* ratio compared to harvest. The ratio increased significantly between harvest and 14 d of storage, thereafter no further increase occurred (Fig. 3B). A higher ratio is indicative of a better rind color (more orange).

Furthermore, in the first season, no differences occurred between the respective averages over the storage duration for the two treatments ($P = 0.658$). However, in the second

season and although the difference between the treatments were not significant ($P = 0.059$), shade net-grown fruit had a slightly higher Hunter a/b ratio of 1.32 compared to 1.27 for the control, possibly indicating a better rind coloration.

-0.6 °C cold storage. No interaction occurred between Treatment and Storage duration during the first ($P = 0.837$) and second season ($P = 0.518$) with regards to rind color development. In addition to this, storage duration influenced the rind color significantly during the first season only (Fig. 4A and B).

The Hunter a/b ratio decreased significantly after harvest (0 d storage) until 27 d after which the ratio remained constant during the rest of the storage duration in the first season (Fig. 4A). In the second season there was no significant effect at the 5% confidence interval on rind color over the storage duration ($P = 0.051$), however, there was a distinct trend indicating a possible effect on the ratio at 14 d of storage, followed by a steady decrease occurring thereafter (Fig. 4B).

Furthermore, a significant difference in the average a/b ratios over the storage duration between treatments was evident during the first season ($P = 0.041$), with shade net attaining a higher ratio (1.22) compared to the control (1.20), whilst in the second season no differences were found between the two treatments ($P = 0.107$).

CHLOROPHYLL CONCENTRATION. The chlorophyll concentration measured for the fruit samples that received cold storage at either 4 or -0.6 °C, was very low, with the highest concentration measured being 115 $\mu\text{g}\cdot\text{g}^{-1}$ DW, which could possibly be an outlier. Since this concentration is approximately only 6% of that measured in a green immature fruit (1959 $\mu\text{g}\cdot\text{g}^{-1}$ DW) the data were not reported.

CAROTENOID CONCENTRATION. Cold storage at 4 °C. The carotenoid concentration of fruit grown under shade net was not influenced differently compared to the control over storage duration, irrespective of the season. No interaction occurred between Treatment and Storage duration during 2016 ($P = 0.094$) or 2017 ($P = 0.374$). The carotenoid concentration was, however, significantly influenced by the storage duration (Fig. 3C and D).

In both seasons, cold storage resulted in a higher concentration of carotenoids compared to fruit at harvest (0 d storage). In the first season, the carotenoid content increased significantly from harvest until 14 d, whereafter there was a slight decrease towards 27 d, with no differences reported thereafter (Fig. 3C). The same pattern was observed during the second season, with an

increase in the concentration between harvest and 14 d, with no further differences occurring throughout the rest of the storage duration (Fig. 3D).

Furthermore, the average concentration of carotenoids over the storage duration during the first season was 2322 $\mu\text{g}\cdot\text{g}^{-1}$ DW for the control and 2264 $\mu\text{g}\cdot\text{g}^{-1}$ DW for the shade net, which did not differ ($P = 0.511$). In the second season, although the two treatments did not differ, there is an indication that the shade net = 1978 $\mu\text{g}\cdot\text{g}^{-1}$ DW had a higher carotenoid concentration compared to the control = 1829 $\mu\text{g}\cdot\text{g}^{-1}$ DW as the P -value was significant at the 10% confidence level ($P = 0.059$).

Cold storage at $-0.6\text{ }^{\circ}\text{C}$. No interaction occurred between Treatment and Storage duration during either the 2016 ($P = 0.472$) or the 2017 season ($P = 0.852$), indicating that cold storage had a similar effect on both treatments with regards to carotenoid content. Furthermore, Storage duration had a significant influence on the carotenoid content of the fruit, irrespective of the season (Fig 4C and D).

Fruit stored for 14, 27 and 34 d contained a significantly higher content of carotenoids compared to fruit at harvest during both seasons (Fig. 4C and D). In the first season a significant increase occurred between harvest and 14 d, whereafter the concentration decreased slightly until 27 d, with no differences thereafter for the rest of the storage duration (Fig. 4C). In the second season, the content increased significantly until 14 d, after which the carotenoid content remained stable the rest of the storage duration (Fig. 4D).

The average carotenoid content over the whole storage duration did not differ between the control (2207 $\mu\text{g}\cdot\text{g}^{-1}$ DW) and shade net (2289 $\mu\text{g}\cdot\text{g}^{-1}$ DW) during the first season ($P = 0.171$). The same trend was reported for the second season ($P = 0.148$) where comparable concentrations of 1690 $\mu\text{g}\cdot\text{g}^{-1}$ DW for the control and 1824 $\mu\text{g}\cdot\text{g}^{-1}$ DW for the shade net produced fruit were recorded.

INTERNAL FRUIT QUALITY PARAMETERS. $4\text{ }^{\circ}\text{C}$ cold storage. SSC ($^{\circ}\text{Brix}$). No interaction occurred between Treatment and Storage duration during either the 2016 ($P = 0.144$) or the 2017 season ($P = 0.877$). This indicates that the shade net did not differently influence the SSC during storage compared to the control. The Storage duration, however, did significantly influence the SSC during the first season (Fig. 5A), with no apparent effect in the second season (Fig. 5B).

The SSC of the three storage periods (14, 27 and 34 d) was significantly higher compared to fruit at harvest that did not receive cold storage (Fig. 5A). Furthermore SSC of the fruit at 34 d was significantly higher than that of fruit at 14 d, but no significant difference

occurred between 14 and 27 d or between 27 and 34 d. No significant influence (at the 5% confidence level) of storage duration during the second season ($P = 0.071$) was reported, however, there was a slight trend indicating a possible effect of storage on the SSC (Fig. 5B).

No significant differences occurred between the respective averages over the storage duration for fruit grown with or without shade net, regardless of the season. In the first season a value of 14.0 °Brix for the control and 13.6 °Brix for the shade net was attained, while in the second season the control had a value of 12.2 °Brix compared to 11.9 °Brix recorded for fruit grown under shade netting.

Citric acid percentage. No interaction existed between Treatment and Storage duration in either the first ($P = 0.998$) or second ($P = 0.460$) season, implicating that fruit exposed to shade netting was not affected differently by cold storage when compared with control fruit. In addition to this, the storage duration did significantly influence the citric acid percentage during the 2017 season, but with no effect reported in 2016 (Fig. 5C and D).

The citric acid content remained constant throughout the storage duration in the first season (Fig. 5C), whereas in the second season, a significant decrease was evident between harvest and 14 d, whereafter no further differences in values were recorded for the remaining storage duration (Fig. 5D).

The average citric acid percentage reported for fruit grown under shade net, did not differ from that of the control, over the whole storage duration, for either season. In the first season, an average of 1.34% was recorded for the control compared to 1.27% citric acid obtained for the shade net grown fruit, while an average citric acid content of 1.13% was reported for the control fruit compared to 1.08% citric acid measured in shade net grown fruit in the second season.

SSC to citric acid ratio. The SSC to citric acid ratio for fruit grown under shade net was not differently influenced by the storage duration compared to that reported for control fruit, since no interaction was evident between Treatment and Storage duration in either of the seasons at $P > 0.05$. The storage duration over the 34 d period at 4 °C, however, significantly influenced the sugar to acid ratio during both seasons.

The ratio for fruit that received cold storage was significantly higher compared to fruit at harvest, irrespective of the season (Fig. 5E and F). In the first season, the ratio increased significantly between harvest and 14 d, with no differences occurring thereafter, except for fruit stored for 34 d having a higher ratio compared to fruit stored for only 14 d (Fig. 5E). The same effect was observed during the second season, although the ratio did not differ for the remaining

storage duration after the significant increase occurred between harvest and 14 d of cold storage (Fig. 5F).

Furthermore, no significant differences occurred between the averages over the storage duration for fruit grown with or without shade net during either the first (shade net = 13.6, control = 14.0) or the second season (shade net = 11.1, control = 10.8), respectively.

-0.6 °C cold storage. SSC (°Brix). A significant interaction occurred between Treatment and Storage duration during the first season ($P = 0.034$), with no interaction evident in the second season ($P = 0.757$) (Fig. 6A and B). Although no differences occurred between fruit grown with or without shade net at each respective storage day and harvest, the interaction was caused by the steeper increase between harvest and 14 d of storage in the control fruit as compared to the shade net fruit, which also showed a significant increase between the two storage periods, but not to the same extent (Fig. 6A). In addition to this, the SSC as recorded for the three cold storage durations was significantly higher compared to that reported for fruit at harvest, both for the control and shade net-produced fruit. SSC values, however, did not differ in fruit once exposed to cold over the entire storage period.

However, no storage effect was seen on the SSC of fruit from the 2017 season (Fig. 6B). In addition to this, the respective means for SSC over the storage duration for the control and shade net did not differ ($P = 0.584$) and average values of 12.0 °Brix and 11.7 °Brix were recorded for the control and shade net, respectively.

Citric acid percentage. No interaction occurred between Treatment and Storage duration for the two respective seasons ($P > 0.05$), indicating that storage duration influenced the citric acid percentage of the control and shade net grown fruit similarly. The citric acid content was not influenced by Storage duration in the first season (Fig. 6C). However, during the second season the citric acid percentage decreased significantly from harvest until 14 d of storage, whereafter no further differences were seen for the rest of the storage duration (Fig. 6D).

Furthermore, no difference occurred between the averages over the storage duration for the control and shade net grown fruit respectively in the first ($P = 0.278$) or second season ($P = 0.567$). The control attained an average of 1.37% citric acid compared to 1.24% recorded for the fruit produced under shade netting in 2016, while in 2017 the average citric acid content reported was 1.12% and 1.07% for control and shade net grown fruit, respectively.

SSC to citric acid ratio. The SSC to citric acid ratio of fruit grown under shade net was not influenced differently by cold storage duration compared to the control in either of the seasons, as no interaction was evident between Treatment and Storage duration ($P > 0.05$). Storage duration, however, did have a significant effect on the ratio during both seasons. The ratio was significantly elevated in fruit that received cold storage compared to fruit at harvest (Fig. 6E and F).

In the first season, the ratio increased significantly from harvest until 27 d of cold storage, with no difference between 27 and 34 d storage duration (Fig. 6E), while in the second season the ratio increased significantly between harvest and 14 d of cold storage whereafter no further differences were recorded for the remaining storage duration (Fig. 6F).

The ratio averages over the storage duration for the two respective treatments did not differ significantly, irrespective of the season. In the first season the values recorded were 10.5 and 10.8 for the control and shade net grown fruit respectively, while in the second season a ratio of 10.8 was recorded for the control and 11.1 for fruit produced under shade netting.

PULP COLORATION. 4 °C cold storage. Cold storage duration did not have a different effect on the pulp coloration of shade net grown fruit compared to the control, irrespective of the season, since no significant interaction occurred between Treatment and Storage duration as main effects. In addition to this, Storage duration did influence the pulp color, but only in the first season (Fig. 7A and B). Fruit that received cold storage had a significantly higher Hunter *a/b* ratio compared to fruit at harvest, which indicates a more yellow-orange coloration. The ratio increased significantly from harvest until 27 d, whereafter a slight decrease occurred towards 34 d of storage. However, no difference was noticed in the ratio between 14 and 34 d of storage (Fig. 7A).

There were no differences recorded between the means over the storage duration for the control and shade net respectively, regardless of the season. A Hunter *a/b* ratio of 0.50 was measured for both treatments in the first season, while in the second season both treatments attained a ratio of 0.47, respectively.

-0.6 °C cold storage. No interaction occurred between Treatment and Storage duration in the first ($P = 0.686$) or second ($P = 0.374$) season. In addition to this, Storage duration had a significant effect on the pulp color in the first season (Fig. 7C) but had no influence on the fruit from the second season (Fig. 7D).

Fruit that received cold storage had a significantly higher Hunter *a/b* ratio compared to fruit at harvest. The ratio increased steeply between harvest and 14 d of storage, whereafter a

significant decrease occurred between 14 and 27 d, followed by a significant increase between the latter and 34 d. The highest ratio was attained at 14 d, followed by 34 and 27 d of storage duration, respectively (Fig. 7C).

Furthermore, the mean values over the storage duration for fruit grown with and without shade net did not differ from each other during either the first or second season, respectively. An *a/b* ratio of 0.49 was recorded for both the control and shade net in the first season, while in the second season the control had a ratio of 0.46 compared to 0.47 measured for the shade net produced fruit.

POSTHARVEST RIND DISORDER: STAINING. Staining only occurred during the 2016 season in fruit stored for 34 d at both storage temperatures (4 and -0.6 °C) (Table 1), with no staining reported for shorter storage durations, irrespective of storage temperature (data not shown). In addition to this, no differences were found between fruit grown with or without shading, regardless of the storage temperature (Table 1).

Discussion

The production and delivery of high quality fruit in the market remain key factors in ensuring competitiveness in the fresh fruit industry, thus driving producers to incorporate new technology such as shade netting to realize these goals. The impact of shade netting on fruit development and its effect on the postharvest potential of citrus fruit are largely unknown. Results from the current study considering the impact of 20% white shade netting on ‘Nadorcott’ mandarin fruit when subjected to extended periods of postharvest cold storage, however, reported no negative effects on either internal or external fruit quality.

Moisture loss in citrus fruit during postharvest handling and storage is mainly ascribed to the process of water loss through the rind, causing fruit softening and shriveling of the rind. Water-based waxing as a postharvest treatment is generally applied to reduce water loss in citrus fruit during storage (Ben-Yehoshua, 1969; Tietel et al., 2012). In this study, fruit were deliberately not treated with waxes in order to clearly demonstrate the possible impact of preharvest cultivation conditions such as exposure to shade netting versus open cultivation (no shade netting) on moisture loss in fruit postharvest. Our results showed that preharvest shading did not affect weight loss of fruit during long-term cold storage, irrespective of whether the storage temperatures was at 4 or -0.6 °C. However, an increase in weight loss at the lower storage temperature was reported with an 8.9% weight loss in fruit stored at 4 °C compared to 11.9% weight loss when fruit was kept at -0.6 °C for an extended period. This higher percentage

weight loss at $-0.6\text{ }^{\circ}\text{C}$ could be ascribed to a higher degree of moisture loss due to the lower moisture content known to be a characteristic of colder air (Kays and Paull, 2004).

The results concur with both Cohen et al. (1994) in ‘Marsh’ grapefruit and ‘Villa franca’ lemon (*C. limon* L. Burm.f.) as well as with that of Çandir et al. (2013) in ‘Washington Navel’ who reported that weight loss increased during cold storage. However, the weight loss reported was not to the same extent as was recorded in our study, mostly as the fruit in the current study was only evaluated after an additional 7 d shelf life period at 20 to 22 °C which then exacerbated the extent of weight loss. Such an extended shelf period is known to influence weight loss as reported by Cohen et al. (1994) in grapefruit and lemon fruit after being transferred from cold storage to a shelf life of three days at 20 °C. Tietel et al. (2012) reported contradicting findings to the current study in reported reduced weight loss. This could possibly be ascribed to the presence of a wax application on ‘Or’ and ‘Odem’ mandarins which would have prevented excessive weight loss, whereas no wax application was made in the current study. However, it is important to note that none of these aspects in itself resulted in any difference in fruit weight loss between the shade net and control treatments.

Rind color to a large extent determines consumers’ acceptance. In *Citrus*, carotenoids are mainly responsible for the characteristic orange rind coloration (Gross, 1987; Lado et al., 2014). In the current study, rind coloration as quantified by the Hunter *a/b* ratio, was not affected by the shade netting treatment when subjected to either of the two cold storage temperatures. However, shade net-produced fruit had a higher average *a/b* ratio over the storage period (0-34 d) in the first season when stored at $-0.6\text{ }^{\circ}\text{C}$, although no visual difference could be detected (personal observation). Seasonal variation in color development was seen as illustrated by the different Hunter *a/b* ratios of fruit stored at 4 °C. In 2016, the *a/b* ratio decreased after 14 d of cold storage, whereas in the second season an increase in *a/b* ratio values occurred. In addition, the starting point at harvest was lower in 2017 compared to 2016. The ratio of fruit exposed to $-0.6\text{ }^{\circ}\text{C}$ decreased in the first season, with no cold storage effect evident in the second season. However, there was a trend for fruit stored for 34 d of having a lower ratio in general. Although numerical differences existed in the *a/b* ratio, no visible differences were observed and therefore was considered to be of little or no commercial importance.

The shade netting had no effect on the carotenoid content throughout fruit development as was determined at harvest, with similarly no different response between control and shade net produced fruit when subjected to cold storage at either 4 or $-0.6\text{ }^{\circ}\text{C}$, regardless of the season. However, storage duration did have a significant effect on the carotenoid content of both

treatments, to result in a general increase in carotenoid content, which corresponds with the changes in the colorimeter measurements.

The difference in rind coloration development between the two seasons might be explained by a delay in rind carotenoid synthesis in 2017 (Chapter 3: Fig. 4E and F, p. 88). This observed delay resulted in the flavedo not attaining the same level of coloration as in 2016. Therefore, the full coloration could only be achieved during cold storage. This was indeed the case and was confirmed by an increase in the carotenoids concentration measured. Carmona et al. (2012) reported that when 'Navelina' oranges were harvested at the breaker stage (-0.11 Hunter *a/b* ratio, not fully colored) and stored at 2 °C it resulted in a slight increase in the rind color, as opposed to full colored fruit, which showed no change in color. Furthermore, the total carotenoid content was not always directly related to color as differences in the ratio of orange and yellow carotenoids impacted on the actual flavedo coloration (Carmona et al., 2012; Gross et al., 1972). It is of interest to note that although the Hunter *a/b* ratio (color) decreased in 2016 at both temperatures during storage, the carotenoid content increased, and therefore it can be a possible change in the different carotenoid species present, not directly contributing to the rind color.

Cold storage is known to influence rind coloration. Tietel et al. (2012) reported 'Or' and 'Odem' mandarin fruit exposed to 2 and 5 °C to develop a paler rind color compared to fruit stored at 8 °C, whereas Carmona et al. (2012) found no change in the rind color and carotenoid content of full colored 'Navelina' orange fruit stored at 2 °C compared to fruit kept at 12 °C. In our study, in the first season when fruit was stored at 4 °C, results were in accordance to previous reports showing a reduction in rind color. Results from our study where fruit was stored at -0.6 °C in the first season are also in agreement with a previous study by Van Wyk et al. (2009) on 'Palmer Navel' orange but is contradicting to that reported by Cronje et al. (2011) for 'Nules Clementine' where an improvement of rind coloration was observed during storage. The increase in carotenoid concentration for fruit in cold storage as recorded in our study followed similar trends, as reported for 'Nules Clementine' stored at both -0.5 and 7.5 °C (Cronje et al., 2011) and for 'Satsuma' mandarins stored at 5 °C (Matsumoto et al., 2009), whereas Van Wyk et al. (2009) reported contradicting findings for 'Palmer Navel' at -0.6 °C, but results for fruit which was stored at 4.5 °C concurred with our findings in the current study.

The citrus fruit carotenoid biosynthesis is considered temperature sensitive (Matsumoto et al., 2009). In addition, different carotenoids are known to be present in the various commercial citrus varieties. An interaction of these factors may result in different storage behavior of the fruit, offering an explanation for the contradicting reports obtained from studies

on the different cultivars (Alquézar et al., 2008), also as Rodrigo et al. (2013) proposed that the regulatory process of pigmentation in *Citrus* fruit differs amongst species.

The lack of postharvest physiological disorders is, jointly with rind color, responsible for determining the appearance and thus consequently the value of the fruit. The incidence of the uncommon postharvest disorder of rind staining of ‘Nadorcott’ mandarin (Fig. 1) occurred in fruit at both cold storage temperatures. Yet, there was no difference when fruit from the shade netting and the control treatment were compared. The highest staining incidence occurred in the first season and is considered indicative of the impact of macroclimatic factors in determining fruit rind sensitivity during fruit development (Cronje et al., 2011; Ting and Attaway, 1971). The absence of any negative impact of the shade netting with regard to inducing postharvest disorders in ‘Nadorcott’ mandarin is of great commercial significance and is considered a key result from this study.

The taste and flavor of mandarin fruit, which is determined by the pulp SSC, acid content and the sugar:acid ratio (Tietel et al., 2012) is important in shaping consumers’ perception of quality. Pre-harvest shade netting did not influence either the SSC, citric acid content or the ratio thereof during storage at either 4 or -0.6 °C. The SSC: citric acid ratio increased during both seasons when fruit were subjected to extended periods of cold storage, due to the SSC increase and acid decrease. There is limited research on how mandarin fruit respond to cold storage at chilling temperatures <0 °C, but the observed increase in the ratio reported in our study due to a decrease in acid content could be beneficial in producing mandarins with more acceptable quality for export markets after shipment at these often mandatory ultra-low temperature regimes. Mature citrus fruit has a low respiration rate (Eaks, 1970), however, respiration does occur during storage, although at a lower rate compared to fruit not exposed to cold storage (Cronje et al., 2011). In addition, acids have the tendency to decrease much faster than sugars under storage (Grierson, 2006). During postharvest storage there are however, changes that occur in the individual sugars of the juice that directly affect the Brix content (glucose, fructose and sucrose) as reported by Echeverria and Ismail (1987). The increase in SSC during the first season can possibly be ascribed to the known changes in these individual sugars, although they were not measured in this study. Even though some internal quality parameter changes did occur during cold storage, it was still well within the range set as export standards for late mandarins, where a minimum of 12 °Brix, acid of 0.85% and sugar:acid ratio of 10 is allowed (DAFF, 2016). There was, however, a sub-minimum value for SSC at 11.8 recorded, in some storage days during the 2017 season, at both storage temperatures. However, this value is still considered close to acceptable standards.

As with rind coloration and the reported disorder incidences, the values measured to quantify internal quality also differed between seasons, indicating the likely impact of macroclimatic effects prevalent between seasons, yet these pre-harvest conditions were not altered by the shade netting to such an extent to result in any difference in internal fruit quality. In addition, it was concluded by various researchers that the unique genetics of each mandarin variety plays a significant role in how the fruit respond to low temperatures during storage (Echeverria and Ismail, 1987; Obenland et al., 2011; Tietel et al., 2012).

To conclude: ‘Nadorcott’ mandarin fruit developing under a permanent 20% white shade netting did not differ from fruit of the control treatment (no shade net) in their postharvest storage potential with regard to color, the expression of physiological disorders or with respect to internal quality at either 4 or -0.6 °C. However, changes in weight loss, rind and pulp color, carotenoid content and internal quality parameters (SSC, citric acid percentage and sugar:acid ratio) during cold storage were reported for both treatments. Due to the differences in these parameters observed between seasons it can be postulated that in some of the characteristics, the condition of the fruit at harvest as influenced by developmental conditions, can possibly influence the corresponding postharvest response. It is also important to note that the fruit used in the current study did not receive any postharvest treatments such as wax- or thiabendazole applications, therefore the storage effect may differ during commercial storage conditions.

Literature cited

- Aharoni, Y. 1968. Respiration of oranges and grapefruits harvested at different stages of development. *Plant Physiol.* 43:99–102.
- Alfárez, F., M. Agustí, and L. Zacarías. 2003. Postharvest rind staining in ‘Navel’ oranges is aggravated by changes in storage relative humidity: Effect on respiration, ethylene production and water potential. *Postharvest Biol. Technol.* 28:143–152.
- Alquézar, B., M.J. Rodrigo, and L. Zacarías. 2008. Carotenoid biosynthesis and their regulation in citrus fruits. *Tree and For. Sci. Biotechnol.* 2:23–35.
- Ben-Yehoshua, S. 1969. Gas exchange, transpiration, and the commercial deterioration in storage of orange fruit. *J. Amer. Soc. Hort. Sci.* 94:524–528.
- Bassal, M. and M. El-Hamahmy. 2011. Hot water dip and preconditioning treatments to reduce chilling injury and maintain postharvest quality of Navel and Valencia oranges during cold quarantine. *Postharvest Biol. Technol.* 60:186–191.
- Biale, J.B. 1961. Postharvest Physiology and Chemistry, p. 96–130. In: W.B. Sinclair (ed.). *The orange: Its biochemistry and physiology*. University of California, California, USA.

- Çandir, E., M. Kamiloğlu, D. Üstün, and G.T. Kendir. 2013. Comparison postharvest quality of conventionally and organically grown ‘Washington Navel’ oranges. *J. App. Bot. Food Quality*. 86:59–65.
- Carmona, L., L. Zacarías, and M.J. Rodrigo. 2012. Stimulation of coloration and carotenoid biosynthesis during postharvest storage of ‘Navelina’ orange fruit at 12 °C. *Postharvest Biol. Technol.* 74:108–117.
- Chen, J.W., S.L. Zhang, L.C. Zhang, Z.Z. Zhao, and J.G. Xu. 2002. Fruit photosynthesis and assimilate translocation and partitioning: Their characteristics and role in sugar accumulation in developing *Citrus unshiu* fruit. *Acta Botanica Sinica* 44:158–163.
- Citrus Growers Association of Southern Africa (CGA). 2017. Key Industry Statistics 2017. 7 November 2017. < <http://www.citrusresourcewarehouse.org.za/home/document-home/information/cga-key-industry-statistics/4589-cga-key-industry-statistics-2017>
- Cohen, E., B. Shapiro, Y. Shalom, and J.D. Klein. 1994. Water loss: A nondestructive indicator of enhanced cell membrane permeability of chilling-injured *Citrus* fruit. *J. Amer. Soc. Hort. Sci.* 119:983–986.
- Cohen, S., A. Grava, and E.E. Goldschmidt. 2000. Citrus response to radiation load reduction: Water use, photosynthesis, and productivity. *Proc. Intl. Soc. Citricult. IX Congr.* 1:615–618.
- Cronje, P.J.R., G.H. Barry, and M. Huysamer. 2011. Postharvest rind breakdown of ‘Nules Clementine’ mandarin is influenced by ethylene application, storage temperature and storage duration. *Postharvest Biol. Technol.* 60:192–201.
- Cronje, P.J.R., G.H. Barry, and M. Huysamer. 2013. Canopy position affects pigment expression and accumulation of flavonoid carbohydrates of ‘Nules Clementine’ mandarin fruit, thereby affecting rind condition. *J. Amer. Soc. Hort. Sci.* 138:217–224.
- Department of Agriculture, Forestry and Fisheries (DAFF). 2016. Export standards and requirements: Part 2 soft citrus. 24 November 2017. <[http://www.nda.agric.za/daaDev/sideMenu/foodSafety/doc/Citrus\(\(PART%20Soft%20Citrus\)%20\(2016\).doc](http://www.nda.agric.za/daaDev/sideMenu/foodSafety/doc/Citrus((PART%20Soft%20Citrus)%20(2016).doc)>.
- Eaks, I.L. 1970. Respiratory response, ethylene production and response to ethylene of citrus fruit during ontogeny. *Plant Physiol.* 45:334–338.
- Echeverria, E.D. and M. Ismail. 1987. Changes in sugars and acids of citrus fruits during storage. *Proc. Fla. State Hort. Soc.* 100:50–52.
- El-Otmani, M., A. Ait-Oubahou, and L. Zacarías. 2011. *Citrus spp.*: Orange, mandarin, tangerine, clementine, grapefruit, pomelo, lemon and lime, p. 437–514. In: E.M. Yahia (ed.).

- Postharvest biology and technology of tropical and subtropical fruits. Vol 2. Woodhead Publishing, Cambridge, UK.
- Grierson, W. 2006. Anatomy and physiology, p. 1–22. In: W.F. Wardowski, W.M. Miller, D.J. Hall, and W. Grierson (eds.). Fresh citrus fruits, second edition. Florida Science Source, Inc., Longboat key, Florida.
- Gross, J. 1987. Pigments in fruits. Academic Press, London.
- Gross, J., M. Gabai, and A. Lifshitz. 1972. A comparative study of the carotenoid pigments in juice of Shamouti, Valencia and Washington oranges, three varieties of *Citrus sinensis*. *Phytochemistry* 11:303–308.
- Iglesias, I. and S. Alegre. 2006. The effect of anti-hail nets on fruit protection, radiation, temperature, quality and profitability of ‘Mondial Gala’ apples. *J. Appl. Hort.* 8:91–100.
- Jifon, J.L. and J.P. Syvertsen. 2001. Effects of moderate shade on citrus leaf gas exchange, fruit yield and quality. *Proc. Fla. State Hort. Soc.* 114:177–181.
- Kader, A.A. 1992. Postharvest biology and technology: An overview, p. 15–20. In: A.A. Kader (ed.) Postharvest technology of horticultural crops, publication 3311. The regents of the university of California, Division of agriculture and natural resources, Oakland, California.
- Kays, S.J. and R.E. Paull. 2004. Postharvest Biology. Exon Press, Athens, Georgia. USA.
- Lado, J., M.J. Rodrigo, and L. Zacarías. 2014. Maturity indicators and citrus fruit quality. *Stewart Postharvest Rev.* 2(2):1–6.
- Lafuente, M.T. and L. Zacarías. 2006. Postharvest physiological disorders in citrus fruit. *Stewart Postharvest Rev.* 1:1–9.
- Lee, T.C., P.J. Zhong, and P.T. Chang. 2015. The effects of preharvest shading and postharvest storage temperatures on the quality of ‘Ponkan’ (*Citrus reticulata* Blanco) mandarin fruits. *Scientia Hort.* 188:57–65.
- Magwaza, L. S., U.L. Opara, P.J.R. Cronje, S. Landahl, and L.A. Terry. 2013. Canopy position affects rind biochemical profile of ‘Nules Clementine’ mandarin fruit during postharvest storage. *Postharvest Biol. Technol.* 86:300–308.
- Matsumoto, H., Y. Ikoma, M. Kato, N. Nakajima, and Y. Hasegawa. 2009. Effect of postharvest temperature and ethylene on carotenoid accumulation in the flavedo and juice sacs of Satsuma mandarin (*Citrus unshiu* Marc.) fruit. *J. Agric. Food Chem.* 57:4724–4732.
- McDonald, R.E., H.E. Nordby, and T.G. McCollum. 1993. Epicuticular wax morphology and composition are related to grapefruit chilling injury. *HortScience* 28:311–312.
- Miller, E.V. 1946. Physiology of citrus fruits in storage. *Bot. Rev.* 12(7):393–423.

- Obenland, D., S. Collin, B. Mackey, J. Sievert, and M.L. Arpaia. 2011. Storage temperature and time influences sensory quality of mandarins by altering soluble solids, acidity and aroma volatile composition. *Postharvest Biol. Technol.* 59:187–193.
- Petracek, P.D., D.F. Kelsey, and W. Grierson. 2006. Physiological peel disorders, p. 397–419. In: W.F. Wardowski, W.M. Miller, D.J. Hall, and W. Grierson (eds.). *Fresh citrus fruits*, second edition. Florida Science Source, Inc., Longboat key, Florida.
- Perishable Products Export Control Board (PPECB). 2012. Procedure for in-transit cold treatment to eradicate Codling moth in citrus, grapes, nectarines, peaches and plums shipped from South African ports to the United States of America. 7 November 2017. < <https://ppecb.com/wp-content/uploads/2015/03/HP05U-PROCEDURE-FOR-INTRANSIT-COLD-TREATMENT-TO-ERADICATE-CODLING-MOTH-IN-CITRUS-GRAPES-NECTARINES-PEACHES-AND-PLUMS-SHIPPED-FROM-SOUTH-AFRICAN-PORTS-TO-THE-UNITED-STATES-OF.pdf>>
- Perishable Products Export Control Board (PPECB). 2017a. Carrying temperature regimes of perishable produce for sea export official PPECB instructions. 7 November 2017. < <https://ppecb.com/wp-content/uploads/2015/03/HP22-PP04-04-17-Carrying-temperature-regimes-of-perishable-produce-for-sea-export-official-PPECB-instructions-rev-12.pdf>>
- Perishable Products Export Control Board (PPECB). 2017b. Cold treatment container protocols. 7 November. < <https://ppecb.com/wp-content/uploads/2015/03/Cold-Treatment-Protocols-Rev-68-23102017.pdf>>
- Purvis, A.C. 1980. Influence of canopy depth on susceptibility of ‘Marsh’ grapefruit to chilling injury. *HortScience* 15:731–733.
- Purvis, A.C. and G. Yelenosky. 1993. Inducible chilling injury to grapefruit on trees. *HortTechnology* 3:68–69.
- Rodov, V., S. Ben-Yehoshua, R. Albagli, and D.Q. Fang. 1995. Reducing chilling injury and decay of stored citrus fruit by hot water dips. *Postharvest Biol. Technol.* 5:119–127.
- Rodrigo, M., B. Alquézar, E. Alós, J. Lado, and L. Zacarías. 2013. Biochemical bases and molecular regulation of pigmentation in the peel of citrus fruit. *Scientia Hort.* 163:46–62.
- Strano, M.C., G. Altieri, N. Admane, F. Genovese, and G.C. Di Renzo. 2017. Advances in citrus postharvest management: Diseases, cold storage and quality evaluation, p. 139–159. In: H. Gill and H. Garg (eds.). *Citrus pathology*. InTech, Croatia, EU.
- Syvertsen, J.P., C. Goñi, and A. Otero. 2003. Fruit load and canopy shading affect leaf characteristics and net gas exchange of ‘Spring’ navel orange trees. *Tree Physiol.* 23:899–906.

- Tietel, Z., E. Lewinsohn, E. Fallik, and R. Porat. 2012. Importance of storage temperatures in maintaining flavor and quality of mandarins. *Postharvest Biol. Technol.* 64:175–182.
- Ting, S.V. and J.A. Attaway. 1971. Citrus fruits, p. 107–169. In: A.C. Hulme (ed.). *The biochemistry of fruits and their products*, Vol 2. Academic Press INC, London.
- Tucker, G.A. 1993. Introduction, p. 1–51. In: G.B. Seymour, J.E. Taylor, and G.A. Tucker (eds.). *Biochemistry of fruit ripening*. Chapman & Hall, Boundary Row, London.
- Van Wyk, A.A., M. Huysamer, and G.H. Barry. 2009. Extended low-temperature shipping adversely affects rind colour of ‘Palmer Navel’ sweet orange [*Citrus sinensis* (L.) Osb.] due to carotenoid degradation but can partially be mitigated by optimizing post-shipping holding temperature. *Postharvest Biol. Technol.* 53:109–116.
- Wachsmann, Y., N. Zur, Y. Shahak, K. Ratner, Y. Giler, L. Schlizerman, A. Sadka, S. Cohen, V. Garbinshikof, B. Giladi, and M. Faintzak. 2014. Photosensitive anti-hail netting for improved citrus productivity and quality. *Acta Hort.* 1015:169–176.

Tables

Table 1. The staining index and staining percentage of ‘Nadorcott’ mandarin fruit grown with or without 20% white shade netting in the 2016 season, after cold storage at either 4 or -0.6 °C for a 34 day duration. Values reported are the means of four replications.

Storage temperature	4 °C		-0.6 °C	
	Index ^z	Percentage ^y (%)	Index ^z	Percentage ^y (%)
Control	0.36 ^{NS}	26.9 ^{NS}	0.03 ^{NS}	3.25 ^{NS}
Shade net	0.4	26.9	0.04	4.75
<i>P</i> -value	<i>0.829</i>	<i>1.000</i>	<i>0.722</i>	<i>0.680</i>

^z Severity based on scale from 0-3 (0 = no incidence, 1 = slight, 2 = moderate, 3 = severe).

A higher index indicates more severe incidences of staining.

^y Percentage of fruit scored 1-3.

^{NS} Denotes non-significant difference between means within a column according to Fisher's LSD test at $P \leq 0.05$.

Figures



Fig.1. Rind staining incidence of 'Nadorcott' mandarin fruit as scored on a color chart according to an increasing severity (0 to 3, left to right), following cold storage at either 4 or -0.6 °C for a period of 14, 27 and 34 days respectively, during the 2016 and 2017 seasons.

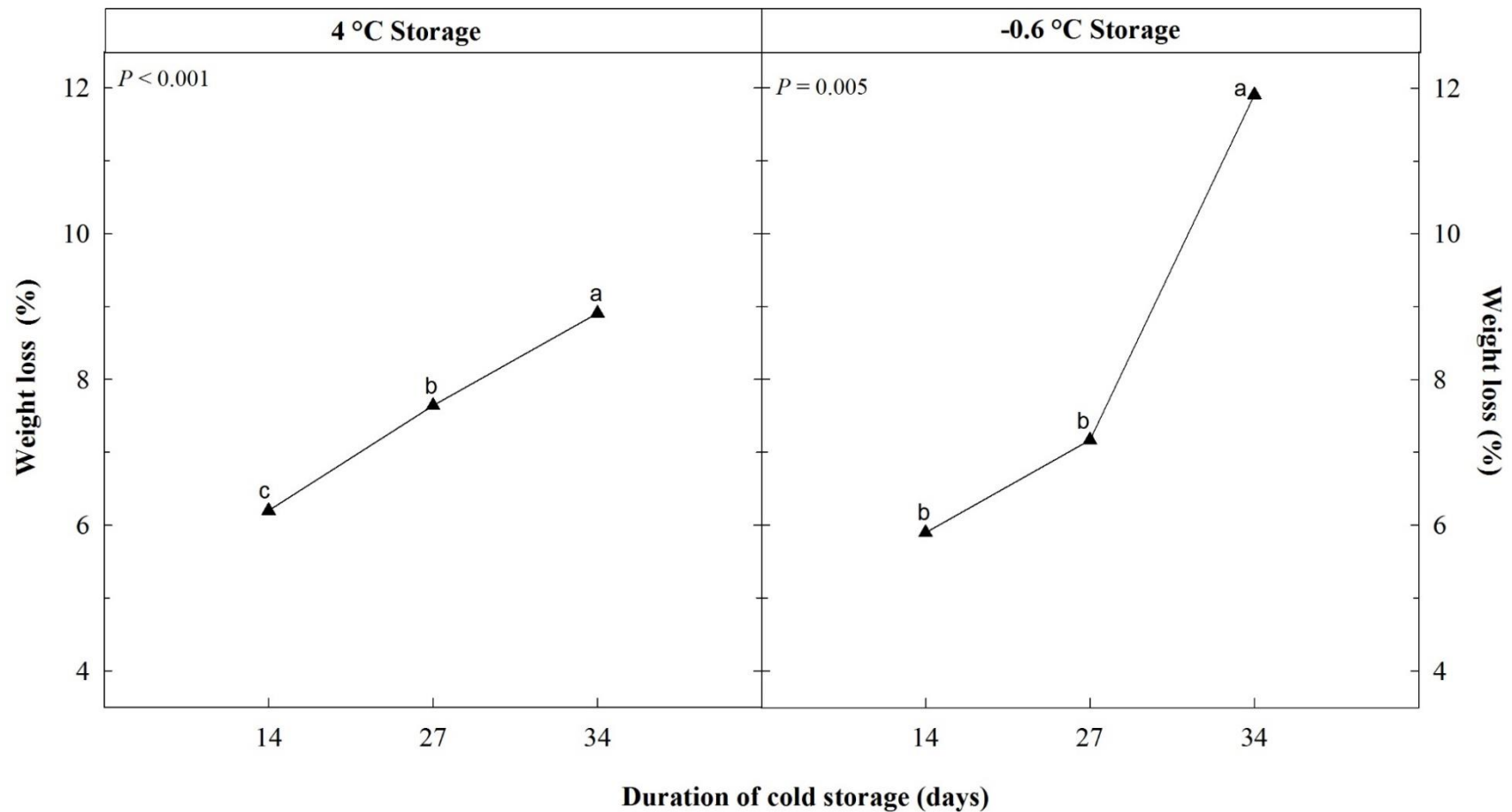


Fig. 2. The influence of cold storage duration at 4 °C (A) and -0.6 °C (B) on the weight loss percentage of 'Nadorcott' mandarin fruit following a storage period of 14, 27 and 34 d after a 7 d shelf life period. Values reported are the combined means of the two treatments, control and shade netting (20% white), as no interaction between Storage duration and Treatment was evident as well as no significant treatment effect. The P -value obtained for Storage duration (main effect) is indicated. Means with different letters differs significantly at $P \leq 0.05$ determined by Fisher's LSD test.

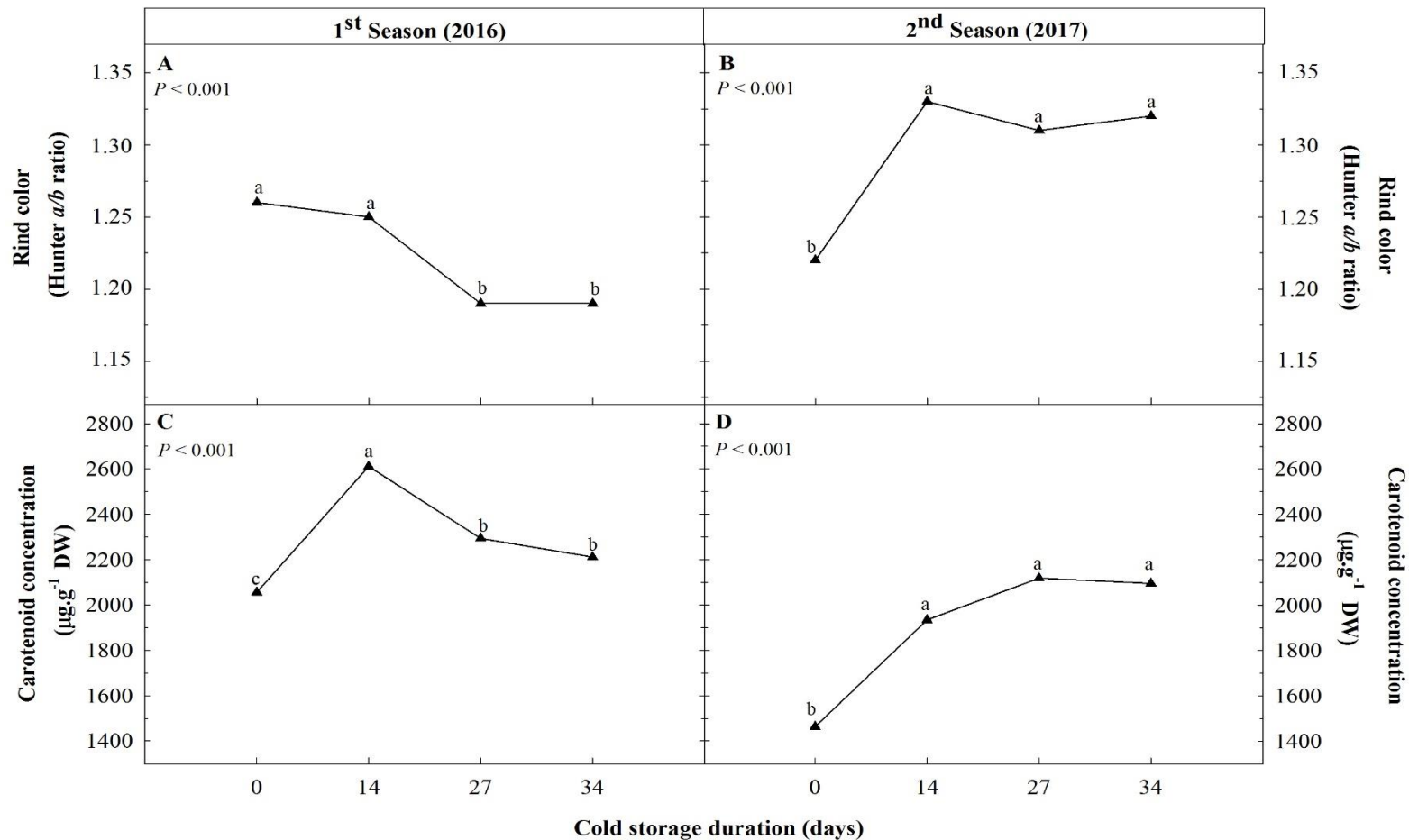


Fig. 3. The effect of 4 °C cold storage duration of 14, 27 and 34 d on the rind color (A-B) and carotenoid (C-D) concentration of ‘Nadorcott’ mandarin fruit evaluated at harvest (0 d) and after a 7 d shelf life period following storage during the two consecutive seasons. The values reported are the means of the two treatments, control and shade net (20% white), as no interaction between Treatment and Storage duration was evident as well as no significant treatment effect. The storage duration (main effect) P -value is indicated on the graph. Different letters on each graph indicate significant differences at 5% level ($P \leq 0.05$) according to Fisher’s LSD test.

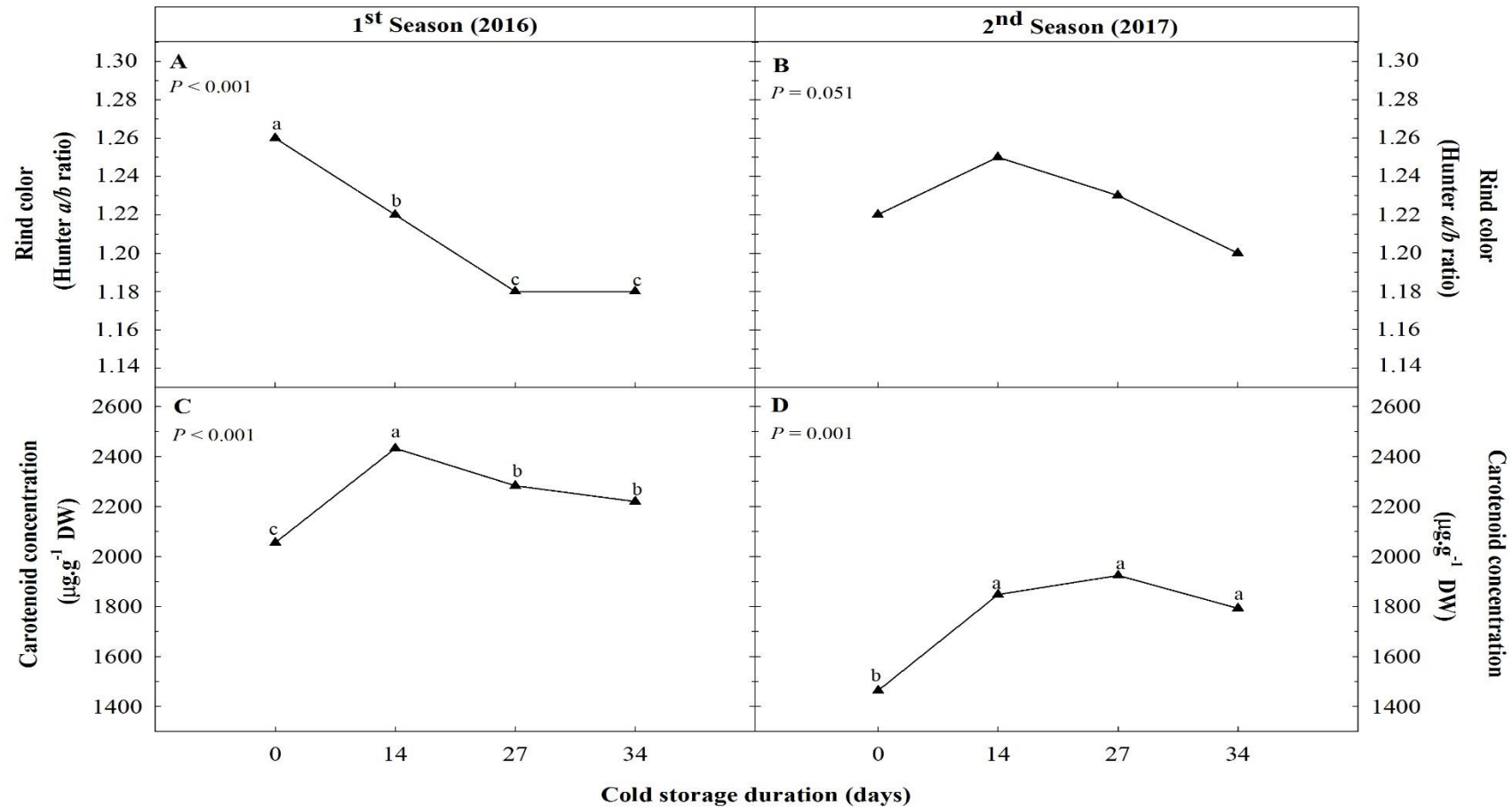


Fig. 4. Changes in the rind color (A-B) and carotenoid (C-D) concentration of ‘Nadorcott’ mandarin fruit stored at $-0.6\text{ }^{\circ}\text{C}$ for a storage duration of 14, 27 and 34 d followed by a 7 d shelf life period. Fruit was also evaluated at harvest (0 d). Data from the two respective seasons are shown. Values reported are the combined mean for the two treatments, control and shade net (20% white), since no interaction between Treatment and Storage duration was evident. Storage duration (main effect) *P*-value is indicated on each graph. Different letters on each graph denote significant differences according to Fisher’s LSD test at $P \leq 0.05$.

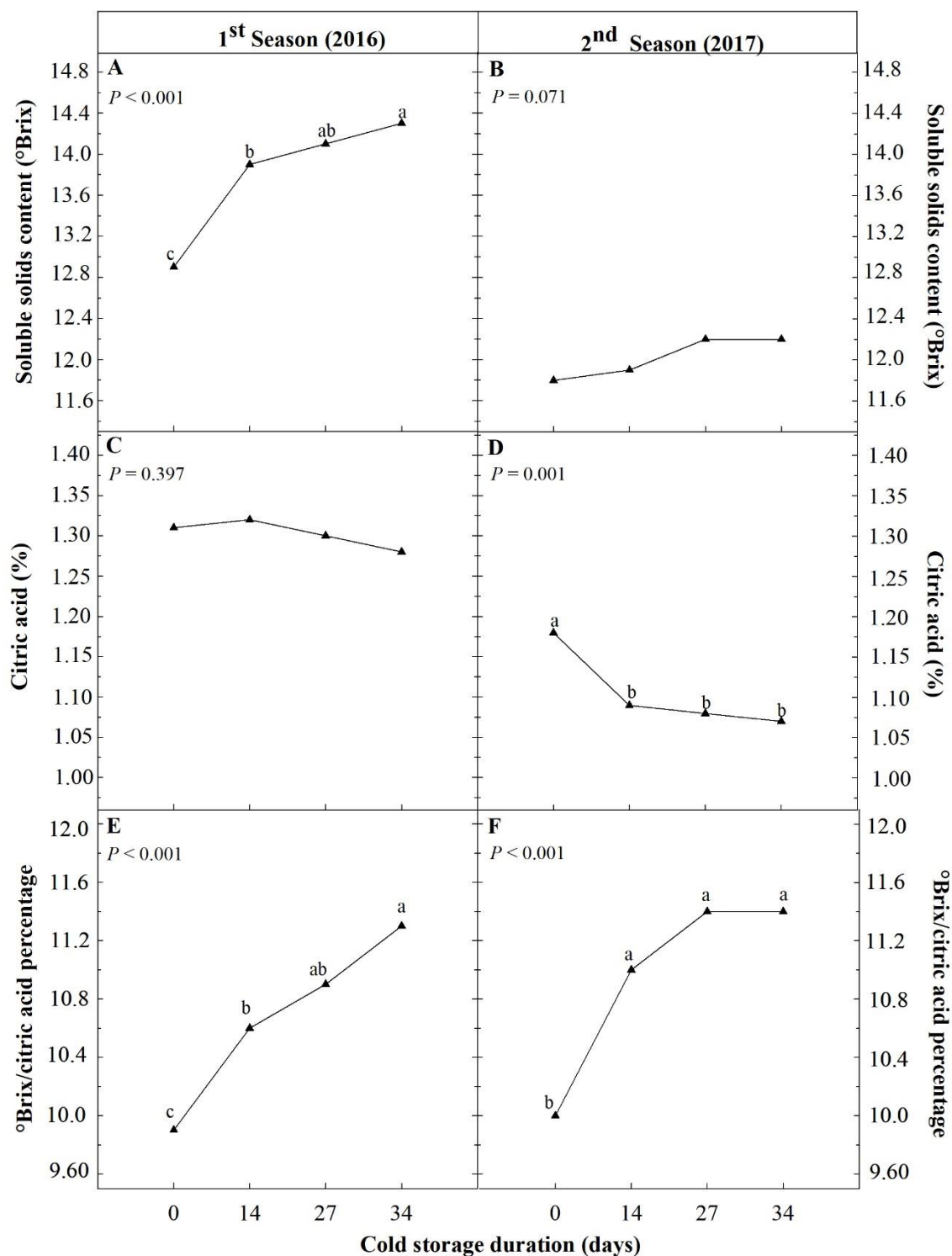


Fig. 5. Changes during cold storage duration of internal quality parameters: SSC (A-B), citric acid content (C-D) and °Brix to citric acid ratio (E-F) of 'Nadorcott' mandarin fruit as affected by 4 °C cold storage at harvest (day 0) and after a 7 d shelf life period following cold storage at 14, 27 and 34 d respectively for two consecutive seasons. Values reported are the combined mean for the two treatments, control and shade net (20% white). The *P*-value of Storage duration (main effect) is indicated on each graph. Different letters on each graph denote significant differences at $P \leq 0.05$ according to Fisher's LSD test.

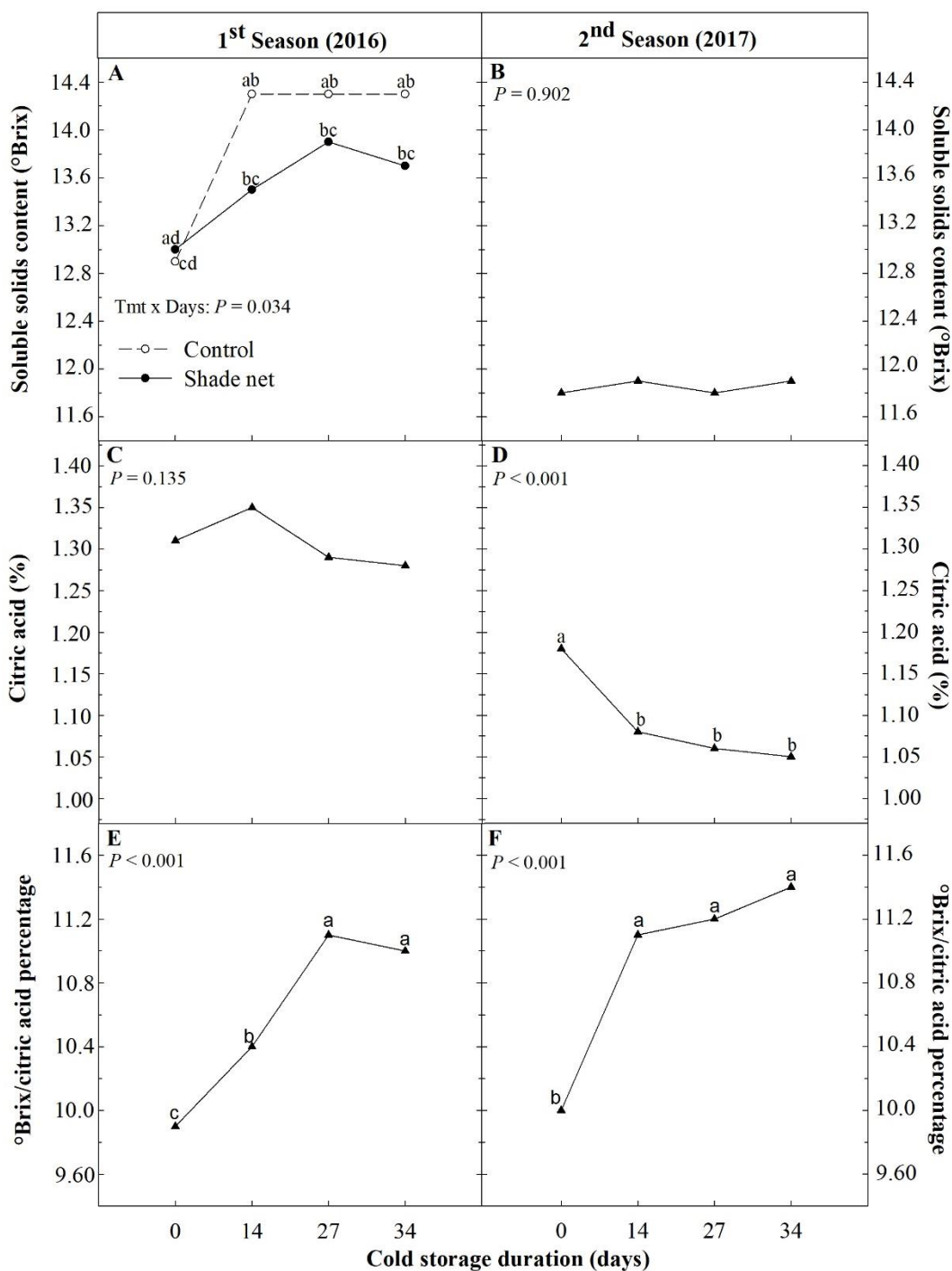


Fig. 6. The effect of cold sterilization ($-0.6\text{ }^{\circ}\text{C}$) on the internal quality parameters: SSC (**A-B**), citric acid content (**C-D**) and the °Brix to citric acid ratio (**E-F**) of 'Nadorcott' mandarin fruit after evaluation at harvest (0 d), as well as after a 7 d shelf life period for fruit that received 14, 27 and 34 d of cold storage respectively. Values reported in **A** are the means of the two respective treatments ($n = 4$), control and shade net (20% white), with the P -value of the interaction between Treatment (Tmt) and Storage duration (day) indicated on the graph. The values reported in **B-F**, are the combined mean of the two treatments, with the P -value of the Storage duration (main effect) indicated on the graph. Means with different letters differ significantly at $P \leq 0.05$ according to Fisher's LSD test.

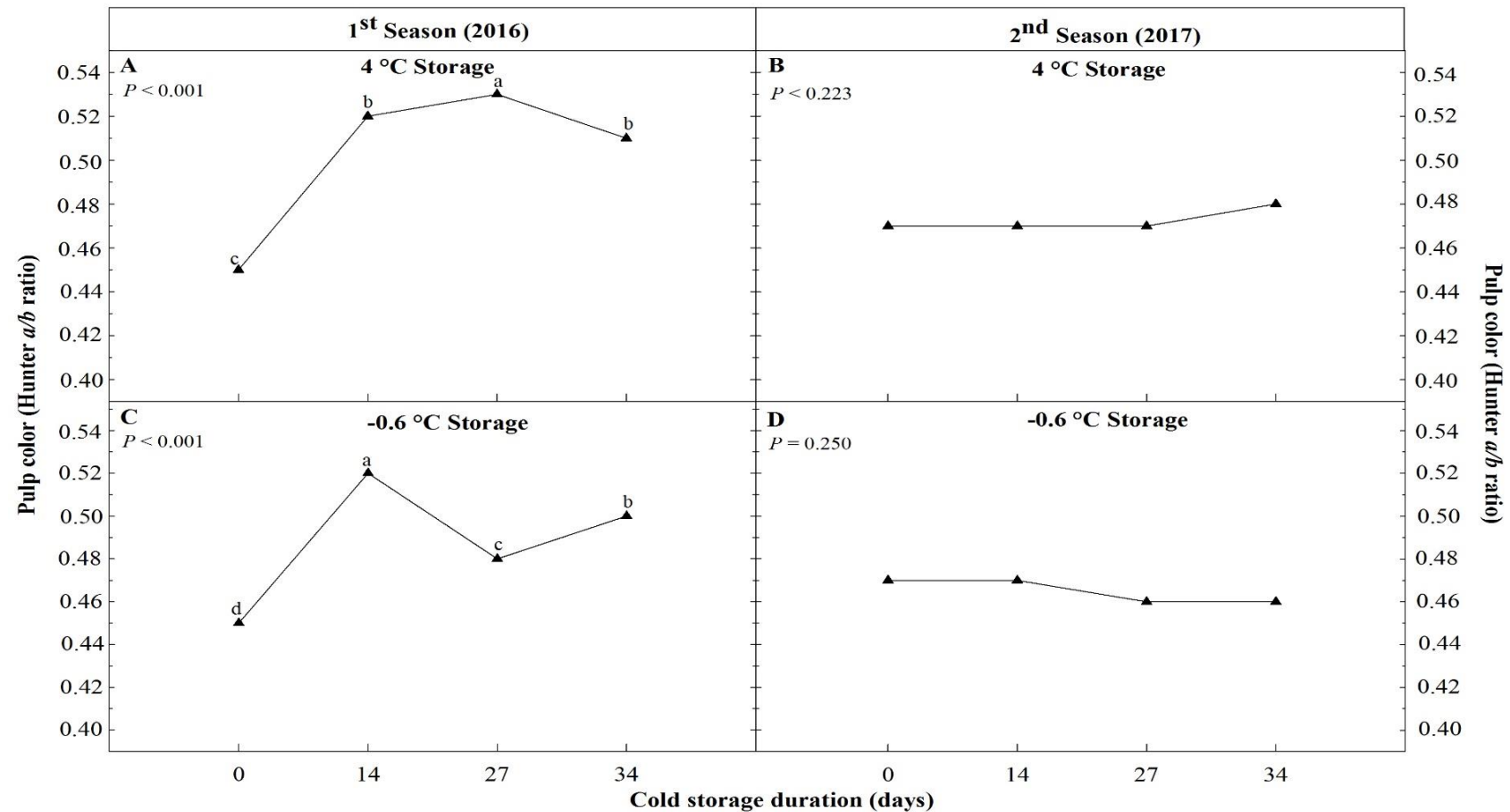


Fig. 7. Pulp coloration changes over the cold storage duration of ‘Nadorcott’ mandarin fruit stored at 4 °C (A-B) and -0.6 °C (C-D) respectively during the two respective seasons, 2016 and 2017. Fruit was evaluated after a 7d shelf life period following the respective storage days (14, 27 and 34), while 0 d represents harvest. The values reported are the combined mean of the two treatments, control and shade net (20% white). The *P*-value of Storage duration (main effect) is indicated on each graph. Different letters on each graph denote a significant difference between means according to Fisher’s LSD test at $P \leq 0.05$.

CHAPTER 5

Rind physical properties of shade net produced ‘Nadorcott’ mandarin at harvest and following long-term cold storage

ABSTRACT

Postharvest handling processes can be deleterious to citrus fruit rind quality. During fruit growth and development, light is known to play an important role in the rind formation and composition. However, it is unclear whether and what the effect of shade netting would be on the rind physical properties with regard to rind strength, firmness and resistance against puncture. The aim of this study was to determine the effect of permanent 20% white shade netting on the rind strength and fruit firmness of ‘Nadorcott’ mandarin fruit at harvest and after long-term cold storage of 34 d at -0.6 or 4 °C. It was also aimed to provide some threshold values which the fruit can be exposed to before damage is inflicted on the rind. The studied quality parameters were assessed by means of a texture analyzer by determining the peak cutting force, resistance against puncture and fruit compression. Conflicting results were obtained when rind strength at harvest was shown not to be influenced by shade net production conditions when using the rind cutting test, whereas when determined by the fruit puncture test, control fruit required a higher force ($P = 0.020$). In general, shade netting had no consistent, negative effect on the rind strength at harvest, whilst in one of the two seasons the shade netted fruit was reported to be even firmer at harvest than control fruit ($P = 0.021$). Exposure to shade netting did not influence the rate of fruit moisture loss or rind moisture content during cold storage. Yet, cold storage affected the rind strength of both treatments, by requiring increased cutting and puncturing force during the first 14 days of cold storage, thus possibly affecting the ease of peeling. There was however, some indication that shade net-produced fruit may be more susceptible to deformation and puncturing, based on the observed trend where control fruit required a higher average cutting and puncturing force over the storage duration, in comparison to fruit exposed to shade netting. Of interest is that a more rapid decline in fruit firmness within the first 14 days of storage was reported for shade net-produced fruit in the first season at 173 N·mm⁻¹ compared to the recorded 67 N·mm⁻¹ for control fruit. This paper is a first documentation of the possible impact of shade netting on the physical rind properties of citrus fruit when subjected to long-term cold storage, whilst also providing a first-time estimation of the threshold force required before damage is inflicted on ‘Nadorcott’ fruit.

ADDITIONAL INDEX WORDS: *Citrus reticulata*, firmness, rind strength, texture analyzing, moisture loss, temperature

Introduction

Citrus fruit are invariably subjected to various types of physical handling actions, starting at picking and continuing throughout the postharvest cold chain. Fruit external quality can be compromised either through the cumulative impact of a chain of suboptimal handling procedures (Davies and Albrigo, 1994), or even because of just one incorrect handling protocol within the value chain. The postharvest storage potential, and value, of citrus fruit is primarily dependent by the rind condition (Biale, 1961). The rind serves as the primary protective barrier against damage and prevents excessive moisture loss, both during fruit development as well as throughout postharvest handling (El-Otmani et al., 2011). An intact and physiologically active rind will result in firmer, more resilient fruit, with a greater ability to withstand handling stresses, resulting in reduced incidences of rind injuries.

The citrus fruit rind (pericarp) is divided into three main morphological sections: the flavedo (exocarp), which is the outer layer responsible for the coloration; the albedo (mesocarp), the inner white spongy textured (Ortiz, 2002) part of the rind in most citrus fruit (Schneider, 1968); and the endocarp which is the edible portion of the fruit, consisting of a membrane containing juice vesicles and fruit segments (pulp) (Hodgson, 1967). Most critical in terms of rind condition and appearance is the flavedo as it contains oil glands, stomata and a cuticle (Grierson, 2006), where the cuticle wax layers serve as a protective cover for the fruit (El-Otmani et al., 1989).

Light levels are well-known to influence plant structure and morphology, as plants exposed to high light intensities generally appear to have both thicker (Juniper and Jeffree, 1983) and wider leaves (Lois and Buchanan, 1994). Within a citrus tree canopy, light level differs, with higher light levels recorded externally compared to the internal canopy (Cronje et al., 2013). As a result of this variation in light levels, Fallahi and Moon (1988) reported fruit from the internal canopy of ‘Kinnow’ mandarin (*Citrus nobilis* Lour x *C. deliciosa* Tenora), ‘Redblush’ grapefruit (*C. paradisi* MacFad.), ‘Valencia’ orange [*C. sinensis* (L.) Osbeck] and ‘Lisbon’ lemon [*C. limon* (L.) Burm. f.] to have a thicker rind than fruit from the external canopy position. Khalid et al. (2012), however, found no difference in rind thickness of ‘Kinnow’ mandarin between the two contrasting canopy positions.

Shade netting is designed to lower light levels reaching the fruit (Iglesias and Alegre, 2006; Jifon and Syvertsen, 2003) and is seen as an effective tool to improve fruit appearance, by reducing sunburn damage (Gindaba and Wand, 2005; Lee et al., 2015) as well as offering protection against wind, hail damage and insects (pollinators) (Rajapakse and Shahak, 2007). However, despite an increasing number of studies on deciduous fruit (Mupambi et al., 2018), the impact of shade netting on citrus fruit rind structure at harvest, together with its possible impact on postharvest storage potential, is still undocumented.

During postharvest handling and cold storage of citrus fruit, physical and biochemical changes occur in the rind as a result of an interaction between the environment and handling practices. One such critical change to occur in citrus fruit, primarily in the rind during postharvest cold storage, is moisture loss. Moisture loss typically results in shriveling which consequently reduces the firmness of the fruit (Ben-Yehoshua, 1969; Çandir et al., 2013; Cohen et al., 1994), as was reported by Singh and Reddy (2006) where the firmness of ‘Nagpur’ mandarin (*C. reticulata* Blanco) decreased with prolonged storage.

The effect that shade netting may have on rind strength, fruit firmness and postharvest storage potential of ‘Nadorcott’ mandarin fruit has not been documented. Therefore, it is unknown whether the harvesting and/or pack house procedures should be further adapted to minimize rind damage when handling fruit grown under shade nets as compared to existing protocols. The aim of this study was thus to generate knowledge on the effect of 20% white shade netting on the rind strength and fruit firmness of ‘Nadorcott’ mandarin fruit at harvest as well as during long-term cold storage at either -0.6 or 4 °C. In addition, the threshold forces before damage is inflicted on the fruit will be determined and will therefore be of use in possibly adapting current harvest and packhouse procedures of fruit grown without shade netting. Findings from this study would make a valuable contribution when considering the need for different postharvest handling and storage protocols for shade net produced citrus fruit.

Materials and Methods

PLANT MATERIAL AND EXPERIMENTAL LAYOUT. Fruit of ‘Nadorcott’ mandarin trees (*Citrus reticulata* Blanco) grafted on Carrizo citrange (*Poncirus trifoliata* x *C. sinensis*) rootstock from a commercial orchard in Citrusdal (-32.542140, 19.011877), Western Cape Province, South Africa were used in this trial which was conducted over two consecutive seasons (2016 and 2017). Full orchard details and experimental layout are supplied in Chapter 3(p. 59). A randomized complete block experimental design with two treatments, namely 20% white shade netting (Plusnet, 13 Bussing Road, Aureus, Randfontein, 1759, Gauteng, South

Africa) and a control (no shade net), replicated four times, was followed. To represent each replicate, four adjacent trees, uniform in size, health and crop load were selected for fruit sampling.

FRUIT SAMPLING AND COLD STORAGE ALLOCATION. From each tree within a replicate (four in total per replicate) 42 fruit, free of rind blemishes, uniform in color and size (51-71 mm), were sampled from sun-exposed fruit positions, within the first 20 cm of the canopy, at a mid-canopy position ($\pm 1-1.5$ m from ground level), on the eastern side of the tree. In total, 168 fruit per replicate were harvested at commercial maturity on 13 July 2016 (first season) and 10 July 2017 (second season), respectively.

For the rind evaluation, the 168 fruit were divided into batches of eight fruit each, whereafter, the fruit lots were randomly allocated to three different tests which included rind cutting, fruit compression and fruit puncturing. Tests were performed on day 0 (no cold storage, one day after harvest) as well as following a 7 d shelf life period at 20-22 °C, on completion of the 4 or -0.6 °C cold storage periods (PPECB, 2017) of 14, 24 and 34 d, respectively. Fruit were allocated to their cold storage regime prior to being placed into cold storage. No postharvest treatments such as fungicides or wax application which are routinely used postharvest in citrus fruit, were applied during this trial.

PHYSICO-MECHANICAL PROPERTIES OF THE RIND AND FRUIT. All three tests were performed by means of a texture analyzer (TA.XT.plus, Stable Micro Systems, Godalming, UK) which carried a 294 N load cell and was calibrated with a 10 kg weight. A heavy-duty platform (HDP/90) was fixed to the texture analyzer. The settings that was set up for each respective test is indicated in Table 1. Tests were performed for each fruit separately, whereafter results were pooled per replicate for a respective test and evaluation day.

THE RIND CUTTING TEST (FIG. 1A). A rind piece of 20 x 20 mm was dissected from the equatorial region of each fruit and placed on the platform. A blade was fixed to the probe carrier, with the knife edge (HDP/BS blade set) positioned 10 mm above the rind piece, immediately prior to the cutting test. Two rind pieces per fruit were used and the results pooled. The force required to cut through the rind was reported by means of the rind peak cutting force (N) (Fig. 1D).

FRUIT PUNCTURE TEST (FIG. 1B). A 2 mm cylindrical probe was attached to the probe carrier. The fruit was placed on a stand with the stem-calyx axis in the horizontal position on the platform to ensure that the fruit was stationary. The probe was positioned approximately 5 mm above the fruit surface, whereafter the peak force (N) required to puncture the fruit was recorded (Fig. 1E).

FRUIT COMPRESSION TEST (FIG. 1C). The fruit was placed on a stand with the stem-calyx axis perpendicular to the platform ensure stability. A 100 mm platen probe was fitted to the probe carrier and moved approximately 5 mm above the fruit surface. Thereafter, the peak force (N) and area under the graph ($\text{N}\cdot\text{mm}^{-1}$) was documented as it is considered as a reliable indicator of the fruit firmness (Fig. 1F). Fruit was compressed for a certain distance based on a 20% strain setting on the texture analyzer (Table 1).

RIND MOISTURE CONTENT. The same fruit which was assigned to the cutting test during the second season was also used for determining rind moisture content at the day of rind analysis. After pieces of rind were removed for the cutting tests, an additional 20 x 20 mm piece was carefully dissected from the equator of each of the four fruit per replicate and the fresh weight was recorded. The rind piece was oven dried at 70 °C for 3 d, whereafter the dry weight was recorded, and the moisture content was calculated using the following equation:

$$\% \text{ Moisture content} = [(\text{Fresh weight} - \text{dry weight}) \times 100] / (\text{Fresh weight}).$$

FRUIT WEIGHT LOSS. In the second season, the weight loss of fruit during storage was determined by weighing four fruit each per replicate ($n = 4$) from the samples assigned to the compression test prior to cold storage. Following cold storage plus a 7 d shelf life period, the weight of each fruit was recorded again before the compression test was performed, and the weight loss percentage was calculated using the following equation:

$$\% \text{ Weight loss} = [(\text{Weight before storage} - \text{Weight after storage}) \times 100] / (\text{Weight before storage}).$$

STATISTICAL ANALYSES. Statistical analyses were done by means of the Statistica 13 VEPAC module (TIBCO Software Inc., 2017). A mixed model analysis was used to take into account repeated measures that were done on the same blocks over time. Treatment (control and shade net) and Storage duration (days) were entered as fixed effects, and block nested in Treatment as a random effect. For cases where there were technical reps within a block (e.g. fruits coming from the same block were individually measured), block x Storage duration nested in Treatment was further added as a random effect. Fisher Least Significant Difference (LSD) tests were used for post hoc testing. Significant differences were determined at $P \leq 0.05$ (5% Significant level).

For analyses done on day 0 only to compare treatments, one-way analysis of variance (ANOVA) was used. Normality was checked by inspecting normal probability plots, and the Levene's test was performed to assess the assumption of homogeneity of variance.

Results

AT HARVEST

RIND CUTTING. The peak force required to cut through a dissected rind piece did not differ between fruit grown with or without shade netting, regardless of the season (Table 2).

FRUIT PUNCTURE. The control fruit which was grown without a shade net, required a significantly higher force to puncture through the rind (6.39 N) compared to fruit from the shade net (5.61 N) during the first season. However, in the second season no differences in the peak force between the control and shade net fruit were detected (Table 2).

FRUIT COMPRESSION. Force. There was no significant difference between treatments with regards to the force required to apply a 20% strain on the fruit, during both seasons (Table 2).

Area (force/distance). Shade net grown fruit had a significantly higher area value compared to the control, with a difference of $94 \text{ N}\cdot\text{mm}^{-1}$ measured between the two treatments. This result indicates the shade net fruit to be significantly firmer at harvest than control fruit (Table 2), however this measurement was not recorded in the second season.

POSTHARVEST COLD STORAGE EFFECT

RIND CUTTING

4 °C cold storage. Similar trends were observed for the two treatments over the cold storage duration at a non-chilling temperature, with no interaction between Treatment and Storage duration evident in 2016 ($P = 0.237$) or 2017 ($P = 0.731$). However, the storage duration had a significant effect on cutting force, resulting in a higher cutting force required for fruit in cold storage compared to fruit at harvest, regardless of the season (Fig. 2A and B). During both seasons, the force increased significantly between harvest and 14 d of storage (first season = 6.4 N, second season = 7.4 N), whereafter it remained constant.

In the first season, the average force required to cut the rind when considered over the whole storage duration of the control fruit (28.7 N) was slightly higher than that required for shade net- produced fruit (26.1 N), although not significant at the 5% confidence level ($P = 0.053$). The same trend was reported during the second season where the control again required a slightly higher force for rind cutting (29.1 N), however this was again not significant compared to shade net grown fruit at the 5% confidence level (26.3 N) ($P = 0.064$).

-0.6 °C cold storage. An interaction between Treatment and Storage duration was evident in the first season ($P = 0.016$) but not in the second season ($P = 0.291$) (Fig. 2C and D). In the first season, both treatments showed a significant increase between harvest and 14 d with

regards to the cutting force, however, the increase for the control (8.9 N) was more marked compared to the shade net (4.3 N) (Fig. 2C). This resulted in the control fruit requiring a significantly higher cutting force as opposed to the shade net grown fruit, at each of the respective storage days (14, 24 and 34), but with no difference recorded between treatments at harvest (Fig. 2C). However, following the significant increase in the first 14 d of storage for both treatments, the force remained constant for the rest of the storage period.

In the second season, no significant interaction occurred between Treatment and Storage duration, thus the required rind cutting force equally increased between harvest and the first 14 d for both treatments, whereafter the value remained stable throughout the remainder of the storage period (Fig. 2D). Shade net thus had no significant effect at the 5% confidence level on the cutting force over the storage duration between the two treatments (shade net = 26.3 N; control = 29.0 N) ($P = 0.084$).

FRUIT PUNCTURE

4 °C cold storage. No interaction occurred between Treatment and Storage duration during the first ($P = 0.562$) and second ($P = 0.059$) season, with both treatments following a similar trend. In the first season, Storage duration did not have a significant influence at 5% confidence level on the peak force required to puncture the fruit, although the increasing trend with storage duration was significant at the 10% confidence level (Fig. 3A). In the second season, the peak force was significantly influenced by cold storage, with values increasing from harvest to 14 d of cold storage, whereafter it remained constant for the remainder of the storage duration (Fig. 3B).

Analysis of the average values recorded over the storage duration indicate a significantly higher force required to puncture the control fruit compared to that required to puncture shade net-produced fruit, regardless of the season. In the first season, a peak force of 6.76 N for the control fruit and that of 5.95 N for the shade net-produced fruit was recorded ($P = 0.008$), whilst in the second season 7.01 N was required for control fruit, with 6.35 N required for the shade net grown fruit ($P = 0.027$).

-0.6 °C cold storage. No interaction occurred between Treatment and Storage duration in the first ($P = 0.119$) or second season ($P = 0.154$). Shade net produced fruit was thus not affected differently compared to control fruit with regards to storage duration and the influence thereof on the peak puncture force. The storage duration, however, did have a significant effect on the peak puncture force required, regardless of the season, with a significantly higher puncture force that was necessary for fruit in cold storage, compared to force required directly

after harvest (Fig. 3C and D). Consistently, the highest increase was evident within the first 14 d of storage, with no or slight increases thereafter.

As was observed with fruit stored at 4 °C, shade netting did impact on the rind quality, as the average force required for puncturing over the cold storage duration for the control was higher compared to that required for shade net exposed fruit, both in the first (control = 7.21 N, shade net = 6.20 N) ($P = 0.001$) and second season (control = 7.34 N, shade net = 6.73 N) ($P = 0.032$).

FRUIT COMPRESSION: FORCE

4 °C cold storage. A significant interaction emerged in the first season between Treatment and Storage duration with regards to the force required to apply a 20% strain on the fruit, while no interaction was evident in the second season ($P = 0.271$) (Fig. 4A and B). A sharp decrease in the force required to compress shade net fruit (21.9 N) was recorded during the first 14 d of cold storage, compared to the force required for compression of control fruit which did not decline to the same extent (11.2 N) (Fig. 4A). The compression force differed between control and shade net produced fruit at harvest, where the shade net fruit required a significantly higher force to apply a 20% strain than that required by control fruit. Thereafter, throughout cold storage, both the control and shade net grown fruit required a significantly lower force to compress the fruit. Furthermore, for the shade net grown fruit, no difference in the force occurred between 14 and 24 d or between 24 and 34 d of storage, however, fruit stored for the full duration of 34 d required a significantly lower force to compress the fruit compared to the force that was required after only 14 d of storage (Fig. 4A). For the control fruit, the force continued to decrease from 14 d until 24 d, with no further difference occurring in the remainder of the storage duration.

In the second season, Storage duration again had a significant effect on the force required to compress the fruit, as a significant decrease between 14 and 24 d of storage was recorded, whereafter no further significant decrease was recorded for the last storage period (Fig. 4B). Significant treatment differences were evident, as the shade net produced fruit required on average a lower compression force (41.8 N) as opposed to that necessary for compression of the control fruit (44.9 N) ($P = 0.021$).

-0.6 °C cold storage. A significant interaction between Treatment and Storage duration was recorded, but only in the first season. Similar to observations at the 4 °C storage temperature, a sharper decline in the force between harvest and 14 d of cold storage was noted for the shade net fruit (21.6 N) in comparison to the compression force required for the control fruit (10.6 N) for the same period (Fig. 4C). The difference required in compression force that

was evident between Treatments at harvest, dissipated where compression values throughout cold storage were comparable between control and shade net grown fruit. Furthermore, the compression forces remain stable for shade net produced fruit after the initial 14 d of cold storage, whereas for the control fruit, the force did not differ between 14 and 24 d, but decreased significantly between 24 and 34 d of storage duration (Fig. 4C). In the first season, cold storage clearly affected fruit firmness as fruit from both treatments required a significantly lower force for compression of the fruit with a 20% applied strain, compared to that required when the fruit was evaluated at harvest.

In the second season, this reduction in fruit firmness with cold storage could not be confirmed as data were only recorded after 14 d of cold storage. In this season, however, Storage duration had no effect on the force required for compression, and thus per implication on fruit firmness (Fig. 4D). Furthermore, no treatment differences occurred with control and shade netting fruit requiring comparable compression forces (control = 46.3 N, shade net = 44.0 N) ($P = 0.191$).

FRUIT COMPRESSION: AREA (FORCE/DISTANCE)

4 °C cold storage. An interaction ($P = 0.002$) was obtained between Treatment and Storage duration during the first season (Fig. 5A), but not for the second season ($P = 0.642$) (Fig 5A and 5B). In the first season, shade net grown fruit produced a significantly greater area value at harvest, whereafter no further differences between treatments were evident over the entire storage duration. For both treatments, the area value was significantly lower as a result of cold storage, with the greatest decline observed during the first 14 d (Fig. 5A). This observed decrease in area values was much more distinct ($173 \text{ N}\cdot\text{mm}^{-1}$) in the shade net produced fruit compared to control fruit ($67 \text{ N}\cdot\text{mm}^{-1}$). Following 14 d of cold storage fruit firmness remained stable for shade net produced fruit for the entire storage duration whereas control fruit declined further in fruit firmness between 24 and 34 d, in the final phase of cold storage (Fig. 5A).

In the second season, the Storage duration significantly influenced the area parameter as the area value decreased significantly between 14 and 24 d of storage, but with no further decrease evident for the remaining storage duration (Fig. 5B). The area parameter for the control fruit, was higher ($234 \text{ N}\cdot\text{mm}^{-1}$) than that required for the shade net grown fruit which recorded an average value of $217 \text{ N}\cdot\text{mm}^{-1}$, suggesting control fruit to be firmer over the storage duration ($P = 0.032$).

-0.6 °C cold storage. In the first season, shade net grown fruit stored at a chilling temperature followed a different pattern compared to control fruit, due to a significant interaction between Treatment and Storage duration regarding the area parameter (Fig. 5C).

The only difference between the Treatments occurred at harvest, where shade net produced fruit produced a larger area value, implying a firmer fruit. Further, both treatments produced a sharp decline in the area value between harvest and 14 d of cold storage, but with the shade net grown fruit displaying a sharper decrease of $173 \text{ N}\cdot\text{mm}^{-1}$ compared to that of $69 \text{ N}\cdot\text{mm}^{-1}$ recorded for the control fruit. No further decrease occurred during the remaining storage duration for the shade net grown fruit. The area value recorded for the control fruit however, did not differ between 14 and 24 d of storage, but a further decrease occurred between 24 and 34 d which resulted in fruit stored for 34 d to record the lowest fruit firmness documented through the cold storage period.

In the second season, no interaction occurred between Treatment and Storage duration, implicating that both treatments followed the same pattern for the area parameter with regards to the Storage duration. In addition, the Storage duration also had no significant effect on the area value produced (Fig. 5D). Furthermore, the area value for the control and shade net grown fruit did not differ between Treatments ($P = 0.394$), with recorded averages of $241 \text{ N}\cdot\text{mm}^{-1}$ for the control and $232 \text{ N}\cdot\text{mm}^{-1}$ for that of the shade net exposed fruit.

FRUIT WEIGHT LOSS

4 °C cold storage. No interaction occurred between Treatment and Storage duration ($P = 0.514$), indicating that the fruit grown under shade net was not differently affected for percentage fruit weight loss by the storage duration compared to the control fruit. Increased Storage duration had a significant effect on percentage fruit weight loss with a difference of 2.5% recorded between 14 and 34 d of cold storage, with fruit stored for 34 d having the highest percentage of weight loss (Fig. 6A). However, the mean weight loss over the storage duration for fruit grown under shade net was calculated at 10.8%, which did not differ from the 10.9% weight loss reported for the control fruit ($P = 0.879$).

-0.6 °C cold storage. No interaction was evident between Treatment and Storage duration ($P = 0.134$) for percentage fruit weight loss at the chilling temperature of -0.6 °C . As with the non-chilling temperature, the weight loss of the fruit (shade net and control) was influenced by the Storage duration, with a significant increase of 3.1% occurring between 14 and 34 d, with fruit stored for 34d again having the highest percentage of weight loss (Fig. 6B). Yet again, the average percentage weight loss over the storage duration did not significantly differ between the shade net grown (10.7%) and control fruit (11.5%) ($P = 0.105$).

RIND MOISTURE CONTENT

4 °C cold storage. A similar trend was noted for both shade net grown and control fruit with regards to the percentage rind moisture content as affected by cold storage duration at a non-chilling temperature, as no interaction was evident between the Treatment and Storage duration ($P = 0.435$). The Storage duration significantly influenced the rind moisture content (Fig. 7A). There was a significant decrease of 3.2% between harvest and 14 d, whereafter no further decrease occurred for the rest of the storage duration (Fig. 7A). In addition, no difference ($P = 0.194$) between the average percentage moisture content over the storage duration for the two treatments occurred (shade net = 72.7%; control = 71.5%).

-0.6 °C cold storage. No interaction between Treatment and Storage duration for the percentage moisture content of the rind was evident when fruit was stored at a chilling temperature ($P = 0.603$). Storage duration, however, significantly reduced the rind percentage moisture content (Fig. 7B). The percentage moisture content declined significantly between 0 and 14 d (3.5%), with no decrease noted between 14 and 24 d, but was followed by a significant decrease between 24 and 34 d. A decrease of 5.3% moisture in the fruit rind was recorded between harvest and following 34 d of cold storage. However, in terms of the means over the storage duration for the two treatments, no differences ($P = 0.145$) were evident for shade net (72%) and control (71.2%) fruit.

Discussion

Physical properties of ‘Nadorcott’ mandarin rind were shown to be inconsistently influenced by shade netting, with results based on tests conducted to determine the force required for cutting, puncturing and compression, when performed on fruit at harvest and after a period of extended postharvest cold storage at non-chilling and chilling temperatures. Results reported from the current study are a first documentation of the possible impact on the physical rind properties of citrus fruit produced under shade netting.

From harvest until the fruit is packed into cartons for shipment to their various export markets, the fruit is exposed to a range of physical handling impacts (Davies and Albrigo, 1994). In *Citrus*, the rind serves as a protective layer of the fruit (El-Otmani et al., 2011), thus a strong rind is associated with a firmer fruit which is able to tolerate impact better than fruit with a softer rind. However, a strong rind may also negatively affect the ease with which fruit can be peeled, which is an important criterion in consumer buying behavior.

The rind cutting, and fruit puncture tests were performed to determine the rind strength of the fruit and the effect of shade netting thereon. In addition, these tests provided some

indication of the ease of peeling that would be associated with the fruit, whereas the puncture test revealed some information on the threshold force required before rind puncturing would occur. Results which reported no difference in the cutting force required between Treatments at harvest suggested that the rind strength and consequently the ease of peeling was not affected by shade net during the growth and development of 'Nadorcott' fruit. However, inconsistency was noted, as in one season control fruit required a higher puncture force, indicating this treatment to be more tolerant to a higher force applied while on the tree than would be expected for shade net produced fruit. However, if it is argued that, in practice, fruit on the tree would not likely be subjected to such a force (5-6 N = 510-612 g), fruit produced under shade netting would be considered not to be negatively influenced and thus the recorded difference would not be of commercial importance.

The results from the current study also provide an estimation of the force the fruit is likely to withstand before being harvested, since damage to fruit can occur while still on the tree. This may happen either through neighboring branches, or during the initial stages of harvest, involving picking scissors or when a stem punctures fruit, which then produces an entrance wound to pathogens, thereby causing decay (Baldwin, 1993).

The compression test is generally performed to determine the static load the fruit can withstand (Singh and Reddy, 2006), as well as to provide some indication of the fruit firmness. The force applied during a 20% strain compression did not differ between treatments at harvest. However, the area value (force:distance) which is a more complete indication of the firmness, was higher for shade net produced fruit, possibly indicating firmer fruit. As it is incorrect and presumptuous to draw conclusions and make recommendations based on one season's data, it is proposed that both shade net produced- and control fruit should be handled using the same harvest and pack house protocols as the force exerted on the fruit during these processes has not been conclusively established. Thus, the focus of a subsequent study should be to obtain a clearer understanding of the single and accumulative forces to which fruit is subjected when placed in plastic bins during harvest as well as throughout handling procedures in the pack house and pack lines, in order to evaluate the suitability of current protocols for shade net produced fruit.

The export potential of citrus fruit during postharvest is primarily dependent on the rind quality (Biale, 1961). Ben-Yehoshua (1969) reported that the greatest moisture loss during postharvest storage occurred in the rind that consequently resulted in fruit becoming softer and less firm. Changes in the percentage fruit weight loss and percentage rind moisture content during cold storage was determined in the current study, in order to relate these findings to

results obtained from the three respective tests over the storage duration, whilst linking the effect of cold storage duration to the firmness or the force required for cutting of the rind.

Shade netting had no effect on the percentage fruit weight loss and percentage rind moisture content as was recorded over the cold storage duration. Percentage weight loss, however, did increase significantly over the storage duration, irrespective of storage temperature, following a similar trend for both treatments. Similar results were reported by Cohen et al. (1994) for grapefruit and lemon fruit and by Çandir et al. (2013) for 'Navel' orange. The percentage rind moisture content of both treatments was lower in fruit that received cold storage (-0.6 and 4 °C), compared to fruit at harvest. Singh and Reddy (2006) reported a 3.7% weight loss in the peel of 'Nagpur' mandarin stored at 7 °C for 10 days, which is in agreement with the current study where a decrease of 3.2% and 3.5% occurred at 4 and -0.6 °C, respectively after 14 days. The highest percentage weight/ moisture loss of fruit/rind during the storage duration occurred within the first 14 days, with very little loss for the remaining period, therefore indicating dehydration of the rind to be a complex process.

The peak cutting, and puncture force required for shade net grown fruit was not influenced differently by cold storage duration compared to that required for control fruit in most instances, as both treatments followed the same trend in the majority of tests. The only exception was in the case where a higher cutting force was required for control fruit in 2016 when stored at -0.6 °C. Control fruit recorded a more marked increase in force required to cut through the rind compared to shade net grown fruit within the first 14 days of cold storage, a trend that was retained throughout the storage duration. The role of different percentage rind moisture content between shade net grown and control fruit to explain these results could only be speculated on as it was not recorded during the first season. In general, the lower force required for cutting of the rind of shaded fruit could possibly be considered beneficial as it may largely facilitate the ease of peeling, although this factor was not tested in the current study. Alternatively, the lower force can again result in the rind of shaded fruit being softer and thus being more prone to either or both pre-and postharvest damage.

Cold storage in general, resulted in a higher peak force required to either cut or puncture fruit compared to fruit at harvest, irrespective of treatments. The most distinct increase in the peak force over the storage duration occurred between harvest and the first 14 days of cold storage. The higher peak force as required for cutting and puncture of cold-stored fruit can be brought in relation to the observed percentage rind moisture loss which was recorded to be the most severe during the first 14 days of storage. This change in rind texture was also noted both through touch and visual appearance, as the rind at this stage felt and appeared to be "leathery",

which explains the recorded increase required in cutting/puncture force of the fruit rind observed with cold storage.

Most results obtained in the current study, indicated shaded fruit to require a lower force to either cut the rind ($P < 0.10$) or puncture the fruit ($P \leq 0.05$) compared to that required by control fruit over the storage duration (0-34 days). These results can possibly be interpreted as being beneficial through facilitating the ease of peeling, however, the implicated rind strength of shaded fruit may alternatively suggest that these fruit may be punctured more readily, which may provide an entrance wound for pathogens, which invariably will reduce shelf life. Different light levels received by the control fruit compared to the shade netted fruit during preharvest production conditions may presumably play a role in determining rind strength, based on findings from Juniper and Jeffree (1983) and Lois and Buchanan (1994) where high light intensities resulted in leaves with thicker cuticles. Unexpectedly, treatment differences cannot be ascribed to differences in percentage rind moisture content, since no such differences were evident between treatments. As comparative reports in literature are lacking on the extent of the force that is required during peeling, or with a stem puncture, it is difficult to judge whether the values reported in this study are of commercial importance.

When considering fruit firmness in the first season as determined over the cold storage duration using the 20% compression test, shade net produced fruit was more affected by cold storage during the first 14 days of cold storage compared to control fruit. Shaded fruit displayed a steep decline in firmness during this period and retained this reduced firmness throughout the storage period, resulting in similar firmness values than were reported for control fruit during the cold storage period of 14-34 days. This suggests control fruit to be more suitable for cold storage than shaded fruit. However, during the second season, both treatments followed a similar trend over the storage duration (14-34 days). Control fruit, however, required a higher compression force, and consequently had an increased firmness value over the storage duration of 14-34 days, compared to shade net produced fruit when stored at 4 °C, but no difference between treatments was detected at -0.6 °C. It is proposed that the rind of control fruit offered more resistance to compression compared to that of shade net produced fruit, a suggestion that concurs with the puncture results during cold storage, although only evident in one season.

In addition, irrespective of the temperature, cold storage exposure resulted in both treatments being less firm compared to fruit at harvest. This decrease in parameters estimating firmness, especially during the first 14 days of cold storage, can be ascribed to the percentage weight loss and rind moisture content, that were most severe during this period. Olmo et al. (2000) stated fruit water content to be a determinant of its firmness. In addition, weight loss and

firmness were positively correlated in ‘Valencia’ and ‘Lane-late’ oranges. Similarly, Singh and Reddy (2006) also reported a decreasing trend in firmness of ‘Nagpur’ mandarin over a storage period of ten days at 7 °C.

The significant decrease in the firmness of the fruit following cold storage should be a serious consideration in handling protocols post cold storage as fruit might be more inclined to deformation, which would invariably affect its appearance. For instance, fruit at the bottom of a palette or box, may also be deformed due to the force exerted on it by the other fruit or by packaging. However, the threshold force required to induce permanent deformation is yet unknown and urgently requires further study and quantification.

The current understanding obtained from literature on the effect of climatic factors on the rind strength of citrus fruit remains incomplete, with even a greater need to relate how shade nets may alter these climatic factors such as relative humidity and maximum air temperature in orchards (Gindaba and Wand, 2005; Wachsmann et al., 2014). This study offers a first documentation of the interaction of fruit produced on a 20% light reduction and the impact on rind physical aspects imposed on these fruit during postharvest cold storage.

Based on results from this study it is concluded that ‘Nadorcott’ mandarin fruit from 20% white shade netting does not warrant special handling protocols at harvest as inconsistent results occurred between the two treatments analyzed at harvest. Furthermore, the percentage fruit weight loss and rind moisture content were not influenced by shade netting. However, it was confirmed that cold storage duration did result in accelerated moisture loss which consequently contributed to the increase in peak cutting and puncture force, and in addition caused a loss in fruit firmness, in both the control and shade net grown fruit. This storage effect may negatively affect fruit quality due to reduced firmness compared to that at harvest, in addition to a reduced ease of peeling. Improved fruit firmness and presumed storability for control fruit compared to shade net produced fruit also required further exploration and confirmation.

This paper therefore provides baseline information on the physical rind properties of ‘Nadorcott’ mandarin fruit grown under 20% white shade net against no shade net exposed fruit. In addition, it provides the threshold force the fruit can be exposed to before damage is applied to the rind, and therefore can make a contribution to adapting the harvest or packhouse process.

Literature Cited

- Baldwin, E.A. 1993. Citrus fruit, p. 108-149. In: G.B. Seymour, J.E. Taylor, and G.A. Tucker (eds.). *Biochemistry of fruit ripening*. Chapman and Hall, London.
- Ben-Yehoshua, S. 1969. Gas exchange, transpiration, and the commercial deterioration in storage of orange fruit. *J. Amer. Soc. Hort. Sci.* 94:524–528.
- Biale, J.B. 1961. Postharvest physiology and chemistry, p. 96–130. In: W.B. Sinclair (ed.). *The orange: Its biochemistry and physiology*. University of California, California, USA.
- Çandır, E., M. Kamiloğlu, D. Üstün, and G.T. Kendir. 2013. Comparison postharvest quality of conventionally and organically grown ‘Washington Navel’ oranges. *J. App. Bot. Food Quality* 86:59–65.
- Cohen, E., B. Shapiro, Y. Shalom, and J.D. Klein. 1994. Water loss: A nondestructive indicator of enhanced cell membrane permeability of chilling-injured *Citrus* fruit. *J. Amer. Soc. Hort. Sci.* 119:983–986.
- Cronje, P.J.R., G.H. Barry, and M. Huysamer. 2013. Canopy position affects pigment expression and accumulation of flavonoid carbohydrates of ‘Nules Clementine’ mandarin fruit, thereby affecting rind condition. *J. Amer. Soc. Hort. Sci.* 138:217–224.
- Davies, F.S. and L.G. Albrigo. 1994. *Citrus*. CAB International, Wallingford.
- El-Otmani, M., A. Ait-Oubahou, and L. Zacarías. 2011. *Citrus spp.*: Orange, mandarin, tangerine, clementine, grapefruit, pomelo, lemon and lime, p. 437–514. In: E.M. Yahia (ed.). *Postharvest biology and technology of tropical and subtropical fruits*. Vol 2. Woodhead Publishing, Cambridge, UK.
- El-Otmani, M., M.L. Arpaia, C.W. Coggins, Jr., J.E. Pehrson, Jr., and N.V. O’Connell. 1989. Developmental changes in ‘Valencia’ orange fruit epicuticular wax in relation to fruit position on the tree. *Scientia Hort.* 41:69–81.
- Fallahi, E. and J.W. Moon, Jr. 1988. Effect of canopy position on quality, photosynthesis and mineral nutrition of four citrus varieties. *Univ. of Arizona Citrus Rpt. P-76*: 5–12. 28 September 2017. < <http://hdl.handle.net/10150/215697/>>.
- Gindaba, J. and S.J.E. Wand. 2005. Comparative effects of evaporative cooling, kaolin particle film, and shade net on sunburn and fruit quality in apples. *HortScience* 40:592–596.
- Grierson, W. 2006. Anatomy and physiology, p. 1–22. In: W.F. Wardowski, W.M. Miller, D.J. Hall, and W. Grierson (eds.). *Fresh citrus fruits*, second edition. Florida Science Source, Inc., Longboat key, Florida.

- Hodgson, R.W. 1967. Horticultural varieties of citrus, p. 431–591. In: W. Reuther, H.J. Webber, and L.D. Batchelor (eds.). The citrus industry, Vol 1. University of California, USA.
- Iglesias, I. and S. Alegre. 2006. The effect of anti-hail nets on fruit protection, radiation, temperature, quality and profitability of ‘Mondial Gala’ apples. *J. Appl. Hort.* 8:91–100.
- Jifon, J.L. and J.P. Syvertsen. 2003. Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves. *Tree physiol.* 23:119–27.
- Juniper, B.E. and C.E. Jeffree. 1983. Plant surfaces. Edward Arnold, London.
- Khalid, S., A.U. Malik, B.A. Saleem, A.S. Khan, M.S. Khalid, and M. Amin. 2012. Tree age and canopy position affect rind quality, fruit quality and rind nutrient content of ‘Kinnow’ mandarin (*Citrus nobilis* Lour × *Citrus deliciosa* Tenora). *Scientia Hort.* 135:137–144.
- Lee, T.C., P.J. Zhong, and P.T. Chang. 2015. The effects of preharvest shading and postharvest storage temperatures on the quality of ‘Ponkan’ (*Citrus reticulata* Blanco) mandarin fruits. *Scientia Hort.* 188:57–65.
- Lois, R. and B.B. Buchanan. 1994. Severe sensitivity to ultraviolet radiation in an *Arabidopsis* mutant deficient in flavonoid accumulation. *Planta* 194:504–509.
- Mupambi, G., B.M. Anthony, D.R. Layne, S. Musacchi, S. Serra, T. Schmidt, and L.A. Kalcsits. 2018. The influence of protective netting on tree physiology and fruit quality of apple: A review. *Scientia Hort.* 236:60–72.
- Olmo, M., A. Nadas, and J.M. García. 2000. Nondestructive methods to evaluate maturity level of oranges. *J. Food Sci.* 65:365–369.
- Ortiz, J.M. 2002. Botany: Taxonomy, morphology and physiology of fruits, leaves and flowers, p.16-35. In: G. Dugo and A.D. Giacomo (eds.). *Citrus: The genus Citrus*. Taylor & Francis, New York.
- Perishable Products Export Control Board (PPECB). 2017. Carrying temperature regimes of perishable produce for sea export official PPECB instructions. 7 November 2017. <<https://ppecb.com/wp-content/uploads/2015/03/HP22-PP04-04-17-Carrying-temperature-regimes-of-perishable-produce-for-sea-export-official-PPECB-instructions-rev-12.pdf>>
- Rajapakse, N.C. and Y. Shahak. 2007. Light-quality manipulation by horticulture industry, p. 290-311. In: G.C. Whitelam and K.J. Halliday (eds.) *Light and plant development*. Blackwell Publishing Ltd, UK.
- Schneider, H. 1968. The anatomy of citrus, p. 1-85. In: W. Reuther, L.D. Batchelor, and H.J. Webber (eds.). The citrus industry, Vol 2. University of California Press, Berkeley.
- Singh, K.K. and B.S. Reddy. 2006. Post-harvest physico-mechanical properties of orange peel and fruit. *J. Food Eng.* 73:112–120.

Wachsmann, Y., N. Zur, Y. Shahak, K. Ratner, Y. Giler, L. Schlizerman, A. Sadka, S. Cohen, V. Garbinshikof, B. Giladi, and M. Faintzak. 2014. Photosensitive anti-hail netting for improved citrus productivity and quality. *Acta Hort.* 1015:169–176.

Tables

Table 1. Texture analyzer tests and their associated parameters (TA.XT.plus, Stable Micro Systems, Godalming, UK) as performed for rind cutting, fruit puncture and fruit compression to evaluate rind strength of ‘Nadorcott’ mandarin fruit. A 294 N load cell was installed, with the system calibrated with a 10 kg weight and a heavy-duty platform (HDP/90) fitted to the texture analyzer.

Parameters	Test mode	Speed (mm·s ⁻¹)		Target mode			Advanced options
		Pre-test	Test	Strain (%)	Distance (mm)	Trigger force (N)	Stop plot at
Rind cutting	Compression	1.5	1	100	-	0.049	-
Fruit puncture	Compression	1.5	1	-	15	0.049	-
Fruit compression	Compression	1.5	1	20	-	0.049	Target position

Table 2. The influence of 20% white shade net on the rind properties of ‘Nadorcott’ mandarin fruit at harvest presented for two consecutive seasons, 2016 and 2017, as determined by means of three texture analyzer tests namely rind cutting, fruit puncture and fruit compression, performed at a speed of $1 \text{ mm}\cdot\text{s}^{-1}$ and at a trigger force of 0.049 N. Values reported are the means of four replications.

Season	Treatments	Rind cutting ^z	Fruit puncture ^y	Fruit compression ^x	
		Peak force (N)	Peak force (N)	Force (N) ^w	Area ($\text{N}\cdot\text{mm}^{-1}$)
2016	Control	23.6 ^{NS}	6.39 a ^v	58 ^{NS}	301 b
	Shade net	22.7	5.61 b	66	395 a
	<i>P</i> -value	0.528	0.020	0.133	0.021
2017	Control	23.8 ^{NS}	6.04 ^{NS}	69.8 ^{NS}	- ^u
	Shade net	21.7	6.05	68.5	-
	<i>P</i> -value	0.144	0.982	0.602	-

^z A HDP/BS blade set was used to cut a dissected rind piece at 100% strain.

^y A 2 mm cylindrical probe was used to puncture 15 mm into the fruit.

^x A 100 mm platen probe compressed fruit at 20% strain.

^w Tests performed in 2017 had a different parameter set than in 2016.

^v Different letters denote significant differences between means within a column in each respective season according to Fisher's LSD test at $P \leq 0.05$.

^u Test not performed.

^{NS} Indicates non-significant differences between treatment means at a 5% confidence level.

Figures

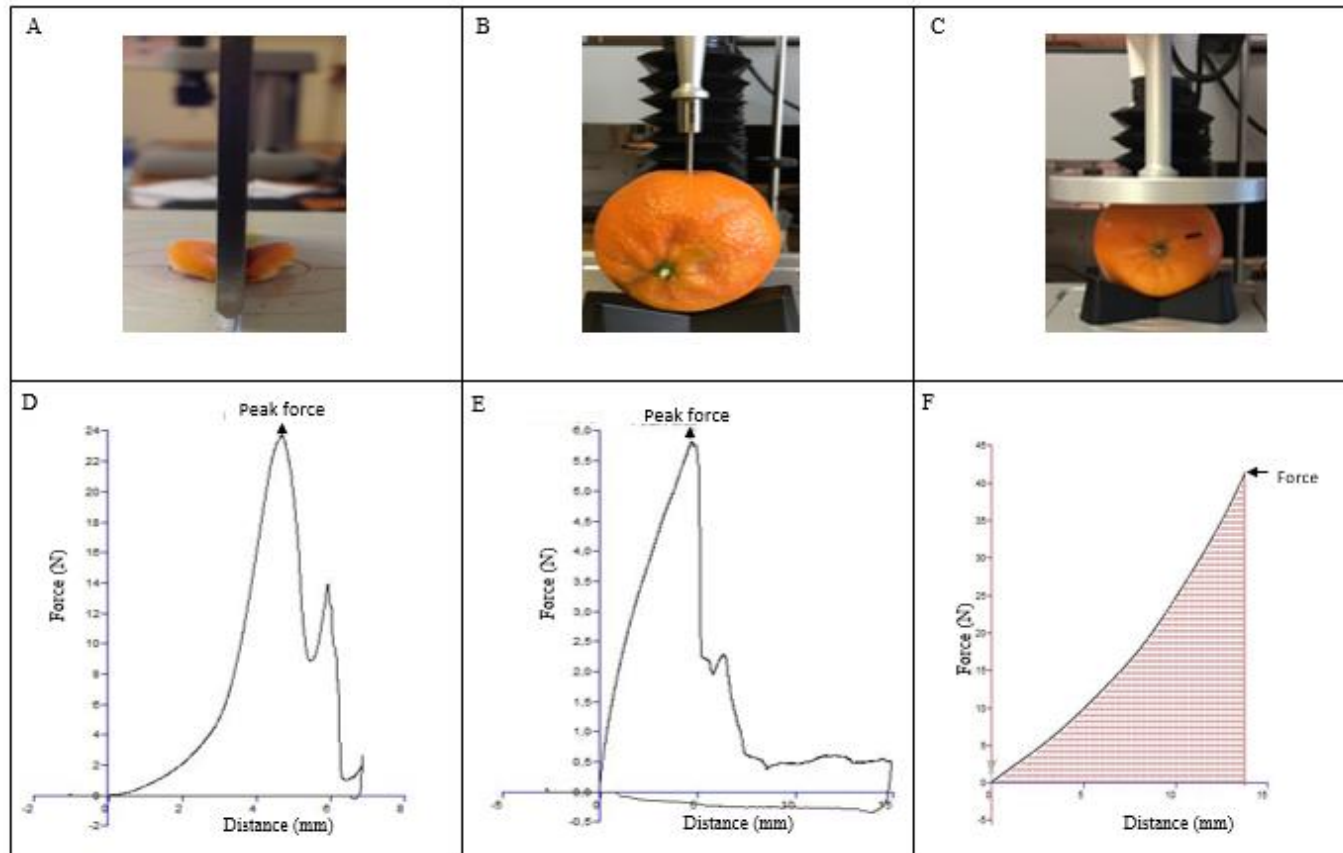


Fig 1. Pictures and graphs of the three respective rind tests performed on ‘Nadorcott’ mandarin fruit to evaluate rind quality and fruit firmness. **A-C** indicate the different probes used for the rind cutting, fruit puncture and fruit compression test respectively. **D-F** represent typical graphs generated for the respective tests: cutting (**D**), puncture (**E**) and compression (**F**). The peak force indicated in **D** and **E** is considered the maximum force required to cut and puncture through the rind of the fruit. The graph as illustrated in **F** was generated after a 20% strain was applied during the compression test, with the area below the curve indicating the firmness of the fruit in $\text{N}\cdot\text{mm}^{-1}$.

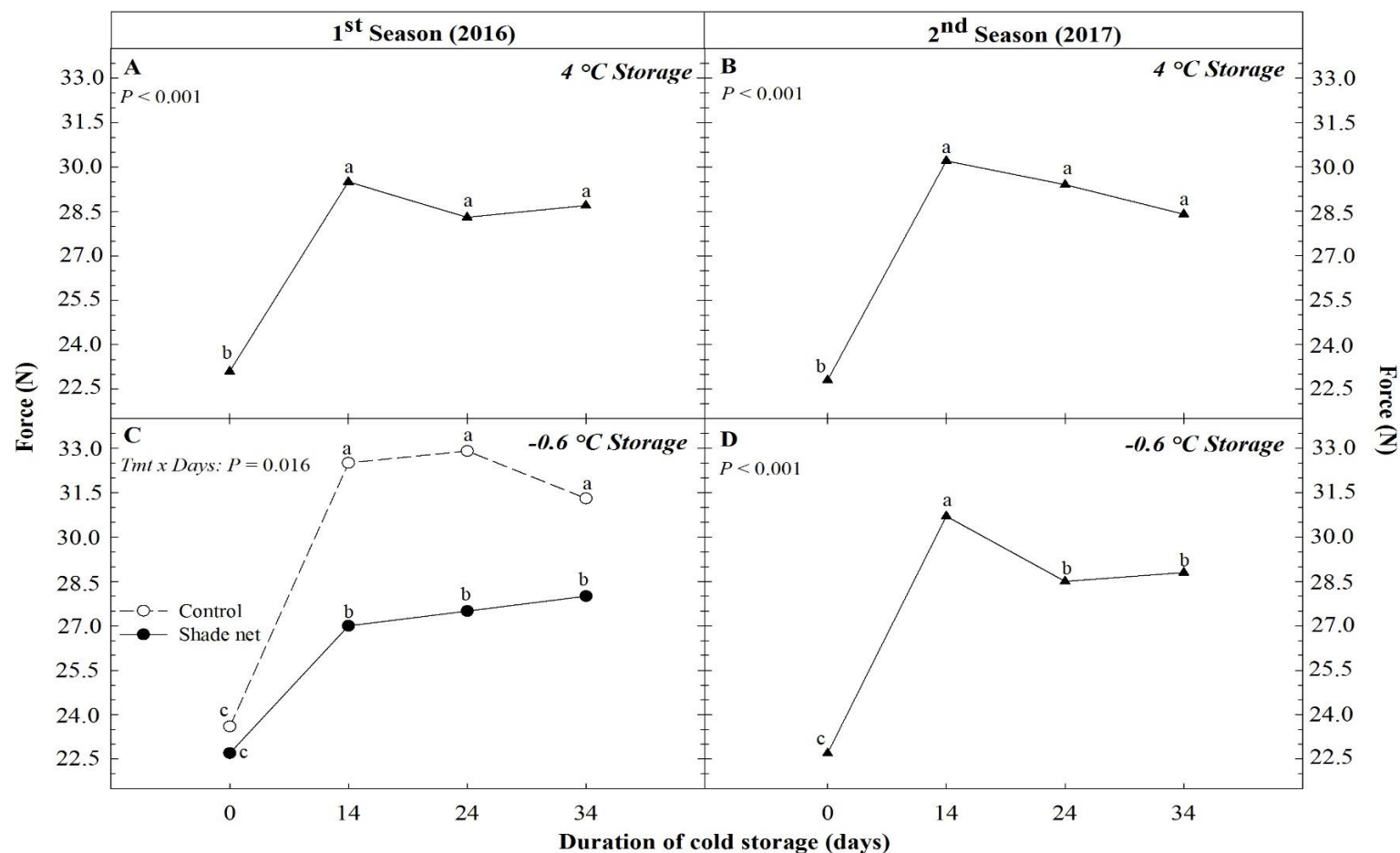


Fig. 2. The peak force required to cut through a dissected rind piece (20 x 20 mm) using a HDP/BS blade set when evaluating the effect of shade netting on rind quality of 'Nadorcott' mandarin fruit after cold storage at either 4 °C (**A** and **B**) or -0.6 °C (**C** and **D**), over two consecutive seasons (2016 and 2017). Tests were performed at harvest (day 0) and after a 7 d shelf life period following a storage period of 14, 24 and 34 d, respectively. Values reported in **A**, **B** and **D** are the means of the two treatments, control and shade net (20% white) combined, with the P -value for the main effect, Storage duration (days), indicated on the graph. Values reported in **C** are the means of four replications per treatment and the P -value for the interaction between Treatment (Tmt) and Storage duration (days) is indicated on the graph. Means with different letters denote a significant difference at $P \leq 0.05$ according to Fisher's LSD test.

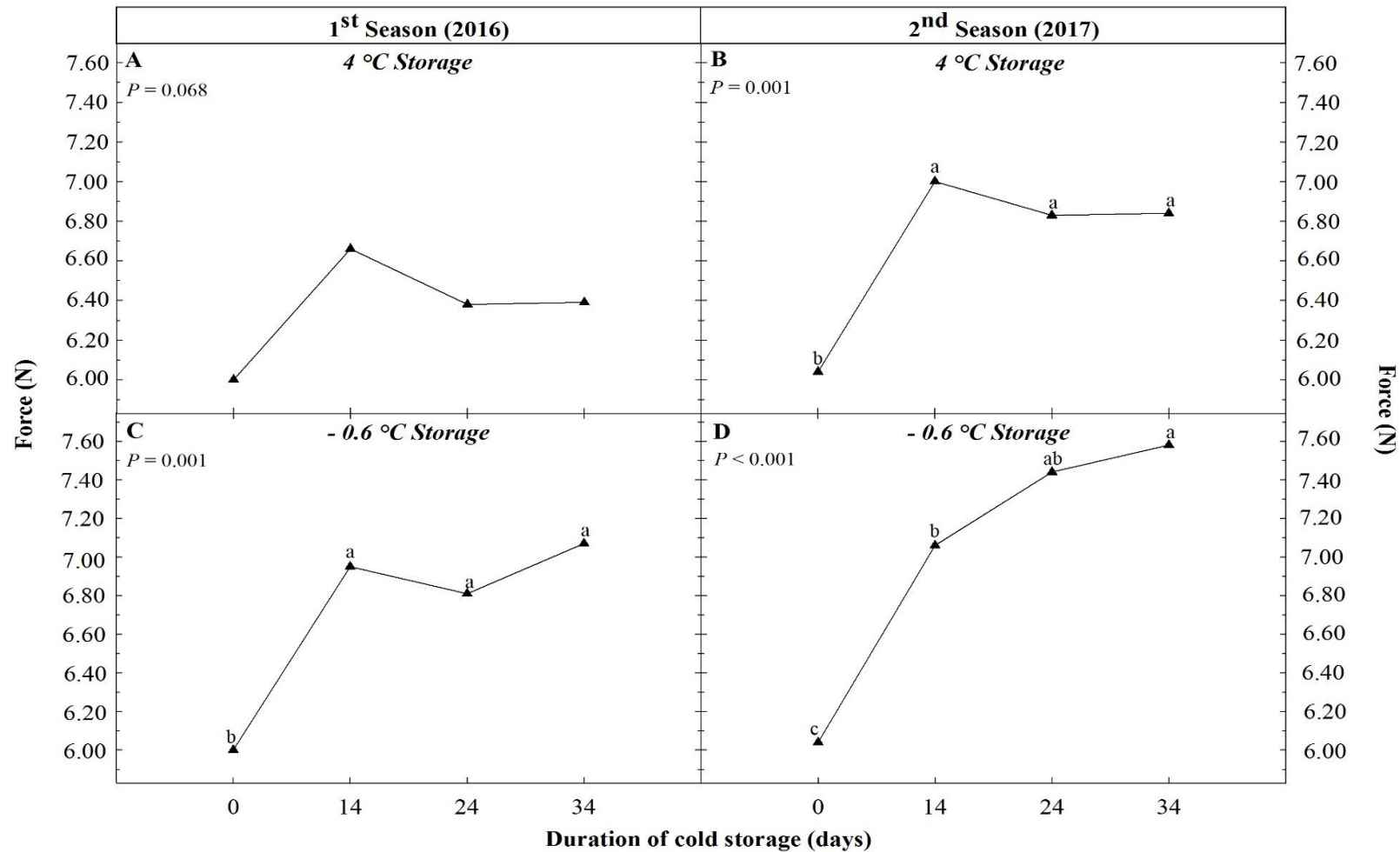


Fig. 3. The influence of cold storage duration at either 4 °C (A and B) or -0.6 °C (C and D) for two consecutive seasons on the peak force required for a 2 mm cylindrical probe to puncture through the rind of 'Nadorcott' mandarin fruit. Tests were performed at harvest prior to cold storage (0 d), and after a 7 d shelf life period following a cold storage duration of 14, 24 and 34 d, respectively. Values reported in each graph are the combined mean for the treatments, control and shade net (20% white), with the P -value of the main effect, Storage duration provided. Different letters denote a significant difference between means determined by Fisher's LSD at $P \leq 0.05$.

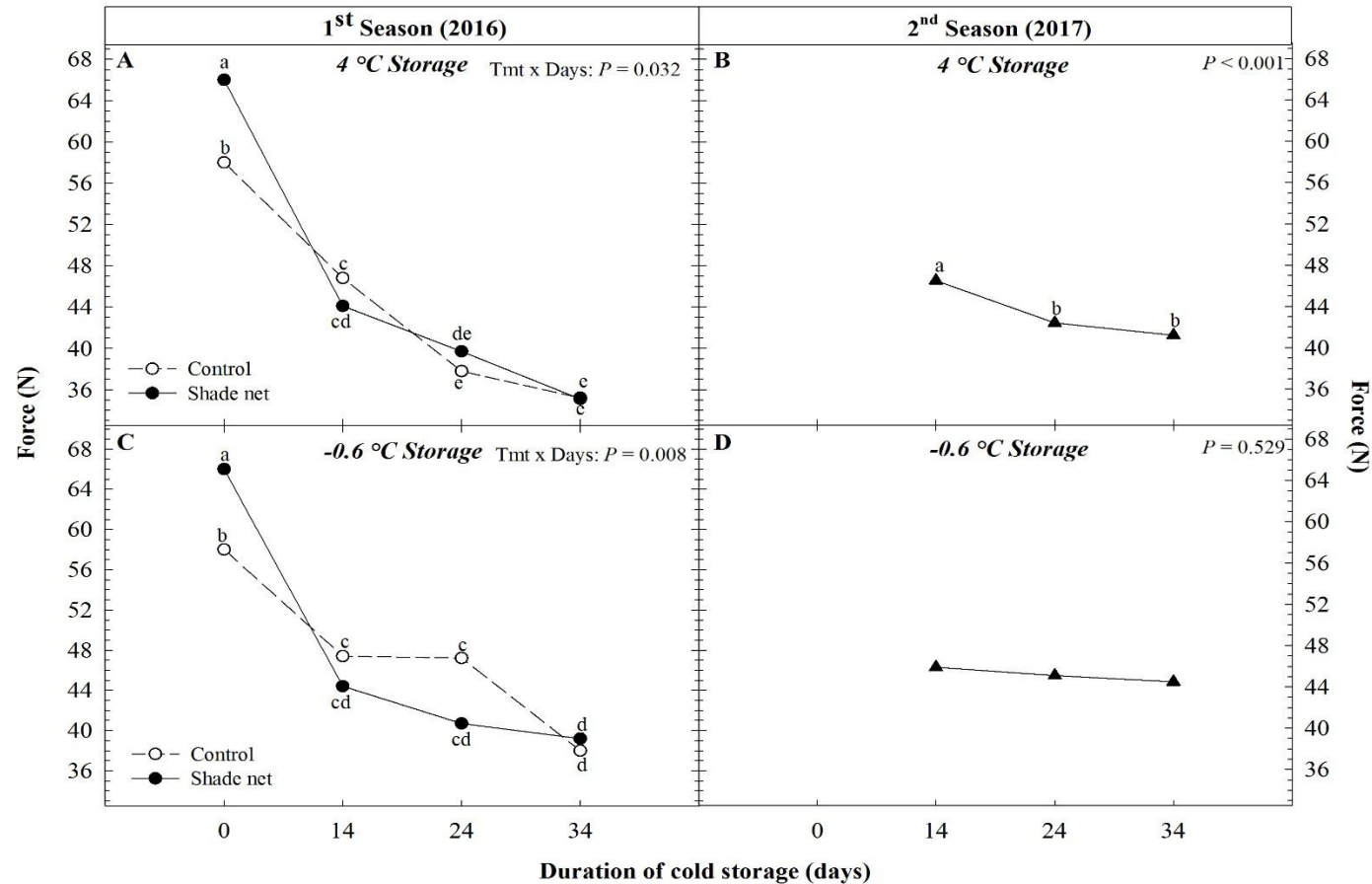


Fig. 4. Changes in the force required to compress 'Nadorcott' mandarin fruit with a 20% strain added following cold storage at non-chilling (4 °C) (**A and B**) and chilling (-0.6 °C) (**C and D**) temperatures during the 2016 and 2017 season. Tests were performed at harvest prior to cold storage (0 d) and after the fruit received a 7 d shelf life period following cold storage at the respective storage days (14, 24 and 34 d, respectively). No data were, however, collected at harvest in the second season. Values reported in **A** and **C** are the means of four replications per treatment, [control and shade net (20% white) produced fruit], and the P -value of the interaction between Treatment (Tmt) and Storage duration (days). No interaction occurred in **B** and **D** therefore the combined mean for the two treatments are reported at each evaluation day. Means with different letters denote a significant difference at $P \leq 0.05$ as determined by Fisher's LSD test.

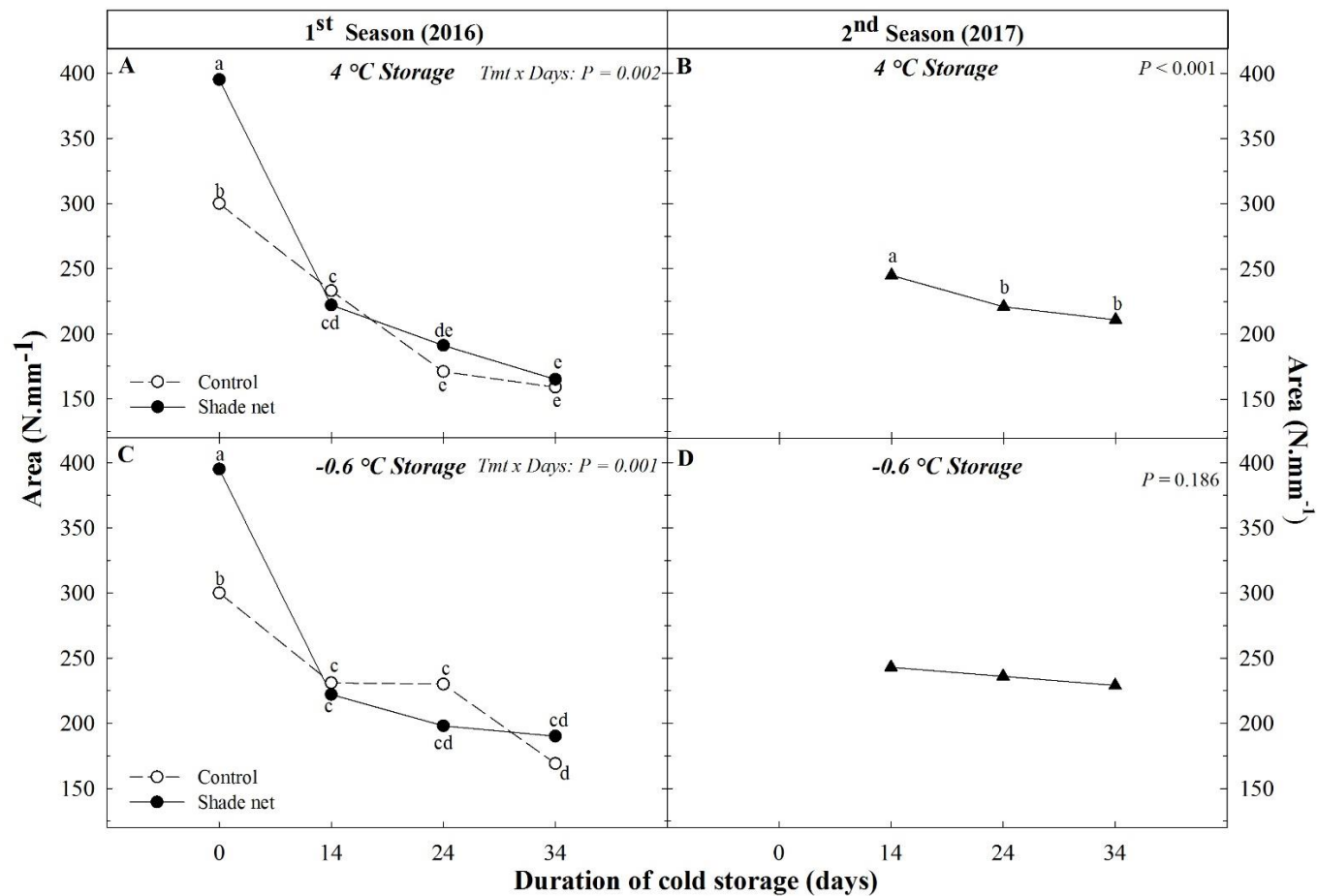


Fig. 5. The influence of cold storage duration at non-chilling (4 °C) (A and B) and chilling (-0.6 °C) (C and D) temperatures on the firmness of 'Nadorcott' mandarin fruit as determined by a fruit compression test using a 100 mm platen probe. Tests were performed at harvest (0 d storage) and after a 7 d shelf life period following cold storage of 14, 24 and 34 d respectively. No data were collected at harvest during the 2017 season. Values reported for A and C are the means of four replications per treatment, control and shade net (20% white), with the *P*-value of the interaction between Treatment (Tmt) and Storage duration (days) indicated. In B and D, values reported are the means of the treatments combined and the *P*-value of the main effect, Storage duration, is presented. Different letters denote a significant difference between means according to Fisher's LSD test at $P \leq 0.05$.

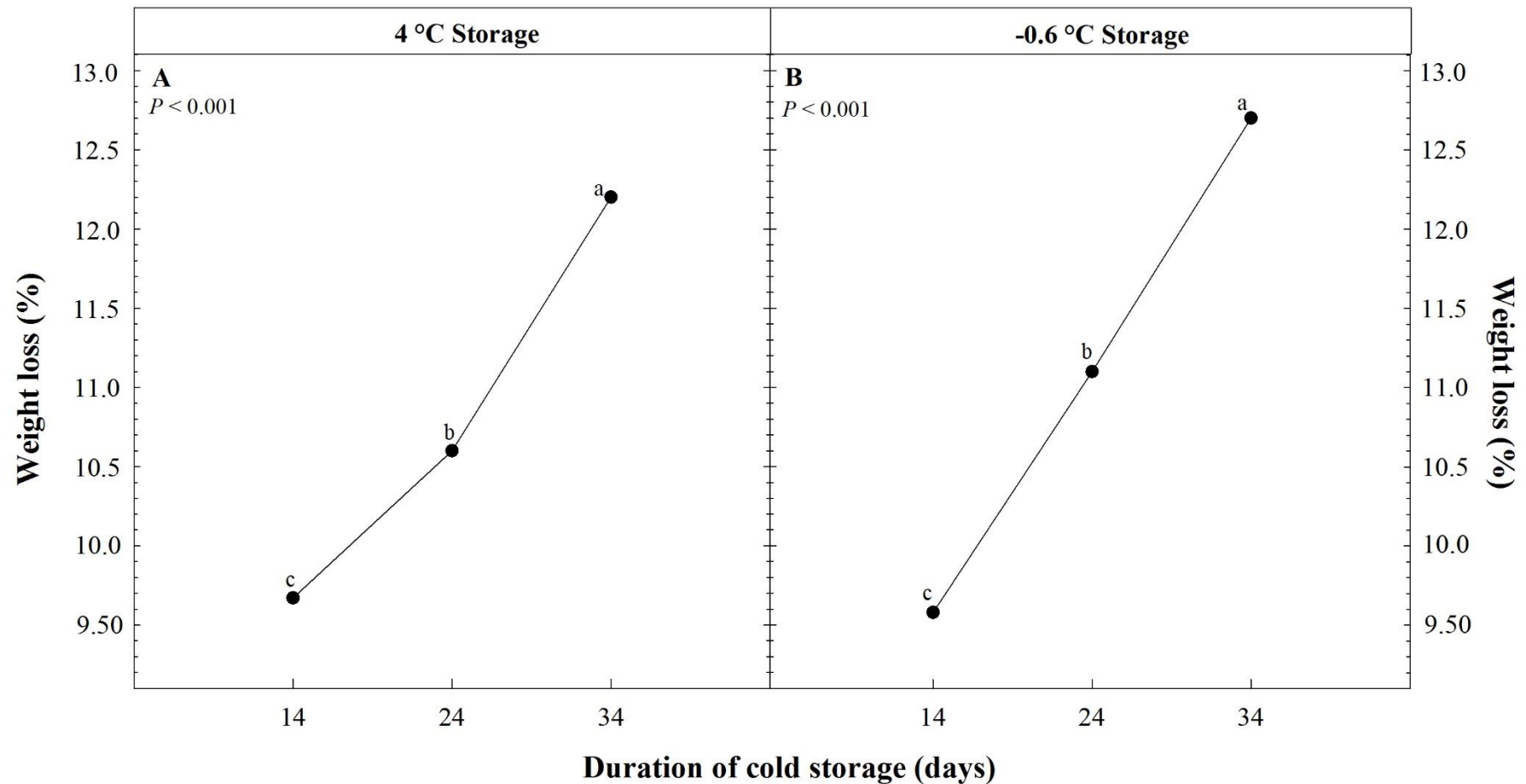


Fig. 6. The percentage weight loss of 'Nadorcott' mandarin fruit with cold storage at either 4 (A) or -0.6 °C (B) for a period of 14, 24 and 34 d, respectively as recorded during the 2017 season. Fruit was weighed prior to storage and again after a 7 d shelf life period following the respective storage days. Values reported are the combined mean for the two treatments, control and shade net (20% white). The P -value of the main effect (Storage duration) is indicated. Means with different letters denote a significant difference at 5% level as determined by Fisher's LSD test ($P \leq 0.05$).

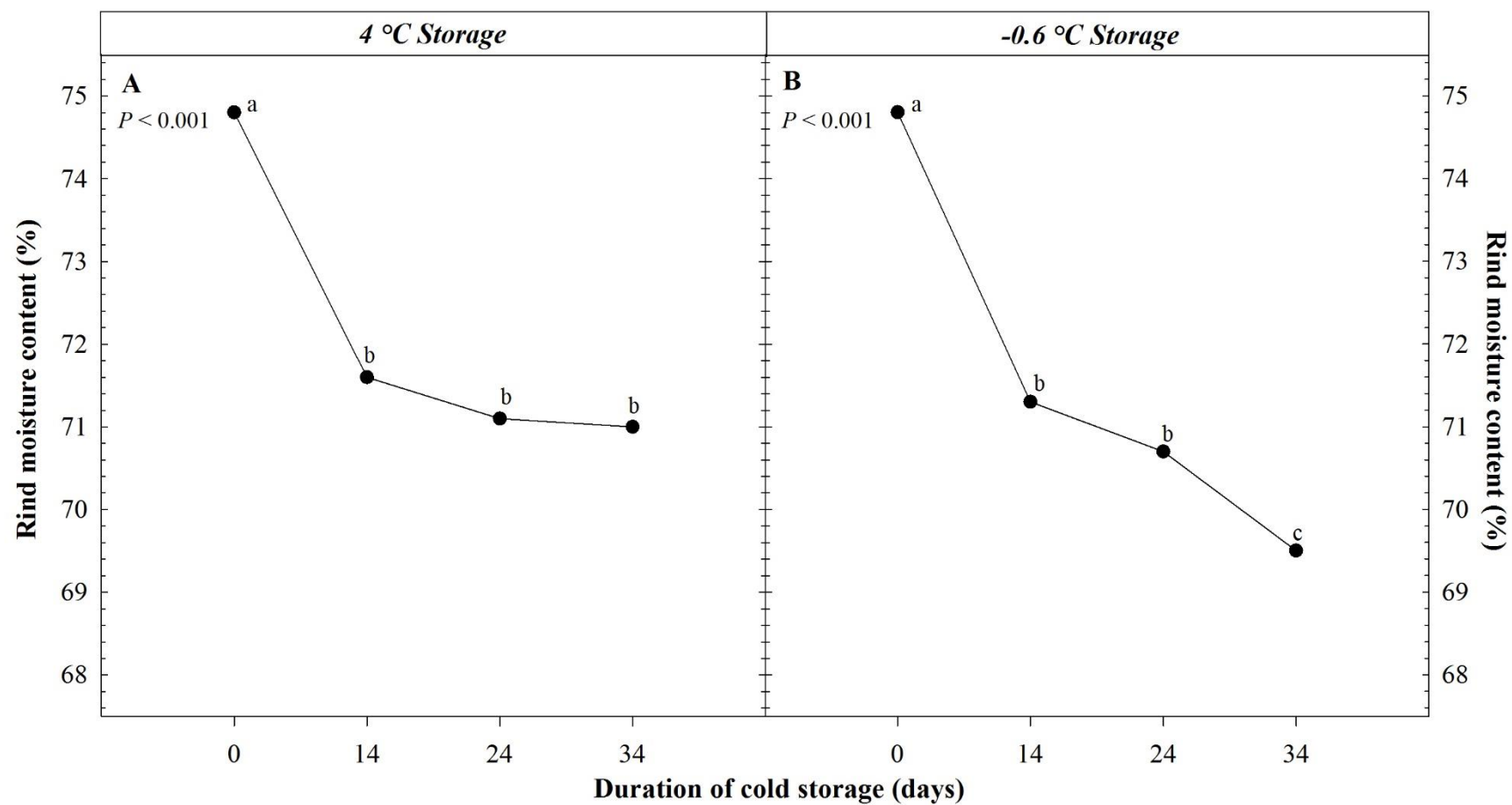


Fig 7. Changes in the percentage moisture content of the rind of 'Nadorcott' mandarin fruit that received cold storage at non-chilling (4 °C) (A) and chilling (-0.6 °C) (B) temperatures for 14, 24 and 34 d, respectively. The moisture content was determined at harvest (no storage, 0 d) and following a 7 d shelf life period after the respective cold storage durations. Values reported are the combined mean for the two treatments, control and shade net (20% white). The P -value of the main effect, storage duration, is indicated. Different letters denote a significant difference between means as determined by Fisher's LSD test at $P \leq 0.05$.

CHAPTER 6

General Discussion and Conclusion

A main focus for a southern African citrus grower is to produce high volumes of fruit of export quality, at the lowest production costs. With shade nets being the latest technology used to reduce sunburn, wind and hail damage, the question to resolve is whether the costs associated with shade nets will sufficiently benefit fruit quality in order to be a sound return on investment.

Various studies on the effect of shade net in fruit crops have been conducted, however, few reports could be sourced that concentrated on the impact of permanent shade net structures on tree phenology to include all stages from flower to harvest and even less with a special emphasis on citrus fruit. Results from the few available studies on citrus were also inconsistent with regards to how shade nets may influence both internal and external fruit quality parameters, except for sunburn incidence which was effectively reduced in all cases (Jifon and Syvertsen, 2001; Lee et al., 2015). Even though the postharvest shelf life of fruit that was exposed to shade netting was investigated by Lee et al. (2015), the study did not include low temperature long-term storage regimes i.e. 4 and -0.6 °C as is often required for citrus exported from South Africa.

To date, no literature is available on how shade nets influence the rind strength and firmness of citrus fruit and for mandarin in particular, therefore results from this study could significantly contribute to the understanding of the threshold force required for damage to be inflicted on the rind. This study also provides a guideline threshold value of fruit firmness which could serve as a precautionary alert indicator to pack houses that handling practices of both shaded and non-shaded 'Nadorcott' mandarin fruit should be adapted to prevent rind damage. This research chapter is also a first documentation of the effect of shade netting on the physical rind properties of citrus fruit.

The current study provides insightful information on the influence of shade nets on fruit development as well as on the postharvest behavior of untreated (no wax or fungicides application) shaded and non-shaded 'Nadorcott' mandarin fruit in the Citrusdal region of South Africa. The results aim to serve as a basis for future comparative studies where the entire complexity of the system, including the production area where the research was conducted (subtropical vs. Mediterranean), the tree age and the shade factor, should be considered.

In the first experiment, 'Nadorcott' fruit quality was not negatively affected by 20% white shade netting, with no negative impact on the processes involved in determining quality, as fruit size, rind coloration and internal quality development were similar for both control and shaded fruit. Inconsistent results with regards to the fruit size between seasons was ascribed to crop load as a possible factor (Moon et al., 2011), since Brown (2018) reported crop load differences, although not statistically significant, between treatments monitored on the same experimental site during the two seasons. Fruit size differences could also be due to climatic variances between seasons, with the fruit exposed to shade netting responding differently to the prevailing environmental factors, especially since the effect of climate on the delay of fruit maturation and rind coloration was noted in both treatments during the second season, compared to the first season. An observation was made in the second season that fruit in the orchard in general was not fully colored as would be expected at that stage, close to harvest. Of interest in this study is that the effect of the microclimate on quality parameters was not altered by shade netting. It would be interesting to determine whether, in a subsequent season, similar results could be obtained or whether inconsistency would occur.

The shade net was effective in reducing the sunburn percentage, yet of importance is that the fruit surface temperature did not differ between the two treatments. Future studies should be conducted with more frequent recordings of both air temperature and light intensity in addition to fruit surface temperature, in order to provide a better understanding as to how shade nets provide effective protection against sunburn, if not necessarily affecting fruit surface temperatures (Racsko and Schrader, 2012).

An important consideration for this study is that the trees on which the experiment were carried out were young at approximately 4-5 years, thus fruit were sampled from the outside canopy position (± 20 cm within the canopy) due to the open architecture of such young trees. In addition, 'Nadorcott' is considered a tip bearer which results in fruit being more exposed to the outer part of the canopy, with generally more adequate light levels compared to non-tip bearers. However, as the tree develops a denser canopy, light penetration will consequently be reduced and may produce different results than were obtained in this study on young trees (Grant, 1997). Therefore, including fruit which are located inside the canopy (> 20 cm) would also assist in determining how shade netting will influence fruit from a dense canopy. What should also be considered is a full analysis of the differences in rind blemishes between the two treatments at harvest examined by the Perishable Product Export Control Board (PPECB) to get a holistic idea on how shade net influences the external appearance of the fruit.

Results from the second experiment showed that the storage behavior of the shade net exposed fruit was not differently influenced by cold storage compared to the control fruit at either 4 or -0.6 °C, over a storage period of 34 days. Both the control and shade netted fruit followed similar changing patterns in quality parameters over the storage duration with regards to weight loss, rind- and pulp coloration, internal quality parameters (SSC, citric acid %, and the SSC:Citric acid % ratio) and rind carotenoid content. However, all parameters recorded for both treatments remained well within the range of export standards (DAFF, 2016).

The storage duration effect on quality parameters varied between seasons for both treatments in some instances, which indicates that the shade net was not effective in altering the impact that macroclimatic factors possibly had on these parameters. A treatment effect was evident on the rind coloration over the storage duration in one instance, based on the Hunter *a/b* ratio measured by a chroma meter, however, visually no difference was detected. In future studies, additional data based on a color chart should be included to highlight possible visual differences. In addition, a minimum of four sections along the equator should be used for recordings for color and not only the most vivid side as was done in this experiment.

Preharvest shade netting did not influence the incidence of rind staining that only occurred in the first season, on both the control and shade netted fruit, irrespective of the storage temperature. These results suggest that the use of shade nets as protection against sunburn is unlikely to affect the postharvest behavior of untreated fruit (no wax treatments or fungicides applied), and the fruit of the same quality as control fruit will reach the export markets. It would be of interest to repeat the experiment, but then to include fruit treated with commercial waxing and fungicide.

In the third research paper the use of netting had no consistent effect on the physical rind properties at harvest. The lack of research on the forces that are exerted in practice when a fruit stem or dry branches puncture the rind, makes it difficult to interpret whether a recorded difference between the two treatments is of commercial importance. Therefore, further research is required to determine if the harvesting process needs to be adapted when handling shade-netted fruit. Furthermore, shade netted fruit was reported to be firmer in the first season, however, due to a technical error the comparative results in the second season could not be recorded. A consecutive season's data, if with similar results, would have provided some important insights as to whether shade-net exposed fruit can possibly handle a higher force when placed in the bins, as well as throughout the pack house processes.

Fruit was also subjected to cold storage at either 4 or -0.6 °C for 34 days to determine how storage influenced the peak rind cutting and puncture force as well as the firmness of the

control and shaded fruit. Overall shade netting influenced the peak force of the cutting and puncture over the storage duration (0-34 days), resulting in shaded fruit requiring a significantly lower peak force. However, these results may either be positive or negative. The lower force, results in a lower threshold before damage is inflicted on the fruit during or after cold storage, but it can possibly be advantageous regarding the ease of peeling. That being said, a lower cutting or puncture force during cold storage are actually required in order to assure the ease of peeling when the fruit reach the export markets. It would be of value to conduct tests to determine what force is required to peel the fruit and if the differences found are of commercial value. The recorded treatment differences were also not related to the rind moisture content, since no differences were evident for this parameter between the two treatments over the storage duration.

With regards to the firmness and force studied during a 20% compression test, shade net exposed fruit was more influenced by cold storage, showing a much more rapid decline in these parameters within the first 14 days of cold storage compared to the control. Shade net exposed fruit did not attain a consistent higher firmness throughout the storage, resulting in comparable firmness between treatments in the first season. It would be expected, that if it was not for the missing data of day 0 evaluation, that the same interaction effect of treatment and storage could have been evident, which warrants a consecutive season's data as confirmation. However, in 2017 the control fruit handled cold storage better, by displaying a higher average firmness over the storage duration, and possibly resulting in less deformation when packed in a carton. In addition, this treatment effect could not be ascribed to weight loss, since no difference occurred between treatments. Further investigation, possibly on a cellular level through microscopic studies on the rind histology, would be useful to provide insights on how shade netting caused differences in the firmness and rind strength. However, it is important to document the exact forces fruit are exposed to during harvest, throughout packhouse process and following cold storage, in order to determine if shade netted fruit should be handled differently compared to control fruit.

To conclude, 20% white preharvest permanent shade netting on 'Nadorcott' mandarin fruit in the Citrusdal region of South Africa was effective in reducing the sunburn incidence of fruit grown under the net, which in turn led to a higher packout percentage, without negatively influencing the external and internal quality parameters. The shade nets also had no influence on the fruit development (size, rind color, internal quality), with similar trends observed over the season for both treatments. Furthermore, the quality of shade netted fruit when subjected to cold storage at either 4 or -0.6 °C was not negatively influenced, with no effect of the shade net

evident on the incidence of the physiological disorder, staining, during cold storage. The rind cutting, puncture and fruit compression test results serve as a guideline value of the threshold force that can be applied before damage is inflicted on ‘Nadorcott’ mandarin fruit. It may also be of value in adapting the harvest- and packhouse procedures to ensure that a minimum force is subjected on the fruit at all times. However, further investigation is needed in order to draw clear conclusions regarding the effect of shade netting on the rind properties of ‘Nadorcott’ fruit.

Recommendations that emanate from this study are to ensure the correct percentage of shade netting is used for a specific environment, depending on climate, location and cultivar. With emphasis on cultivar, the tree architecture should be considered as it is likely to influence light penetration into the deeper canopy. In addition, research should be conducted on cultivars which generally struggle to attain a good rind color, to determine the possible impact of shade netting on carotenoid synthesis. The external appearance of fruit is also influenced by pests and diseases, therefore a detailed study should be done on the pest and disease pressures under the shade net, as well as a full classification of external blemishes occurring on the fruit as caused by these factors.

The use of shade nets shows promising results and is highly recommended on high value cultivars such as ‘Nadorcott’ mandarin and ‘Cambria’ Navel orange which are prone to sunburn. This new technology seems to be the way forward in providing blemish free fruit, however, there are many unanswered questions such as pest and disease pressures under the shade net as well as how factors such as various cultivars and geographic location influences the effect shade nets have on fruit quality during both pre- and post-harvest conditions. Research on shade netting on *Citrus* in South Africa is in its infant stages, and further research is needed to aid in these unanswered questions.

Literature cited

- Brown, R. 2018. Effect of permanent shade netting on ‘Nadorcott’ mandarin tree phenology and productivity. Stellenbosch Univ., South Africa. MSc. Thesis (*submitted*).
- Department of Agriculture, Forestry and Fisheries (DAFF). 2016. Export standards and requirements: Part 2 soft citrus. 24 November 2017. <[http://www.nda.agric.za/daaDev/sideMenu/foodSafety/doc/Citrus\(\(PART%20Soft%20Citrus\)%20\(2016\).doc](http://www.nda.agric.za/daaDev/sideMenu/foodSafety/doc/Citrus((PART%20Soft%20Citrus)%20(2016).doc)>.
- Grant, R.H. 1997. Partitioning of biologically active radiation in plant canopies. *Int. J. Biometeorol.* 40:26–40.

- Jifon, J.L. and J.P. Syvertsen. 2001. Effects of moderate shade on citrus leaf gas exchange, fruit yield and quality. *Proc. Fla. State Hort. Soc.* 114:177–181.
- Lee, T.C., P.J. Zhong, and P.T. Chang. 2015. The effects of preharvest shading and postharvest storage temperatures on the quality of ‘Ponkan’ (*Citrus reticulata* Blanco) mandarin fruits. *Scientia Hort.* 188:57–65.
- Moon, D. G., J.H. Joa, Y.E. Moon, K.C. Seong, C.H. Kim, and Y.K. Ahn. 2011. Plant growth and fruit quality as affected by canopy locations in ‘Shiranuhi’ mandarin. *Hort. Environ. Biotechnol.* 52:443–447.
- Racsko, J. and L.E. Schrader. 2012. Sunburn of apple fruit: Historical background, recent advances and future perspectives. *Critical Rev. Plant Sci.* 31:455–504.

Addendum A

[Figures from Prins, M.D. 2018. Impact of shade netting on orchard microclimate and the physiology of 'Nadorcott' mandarin. Stellenbosch Univ., South Africa. MSc. Thesis. (submitted)].

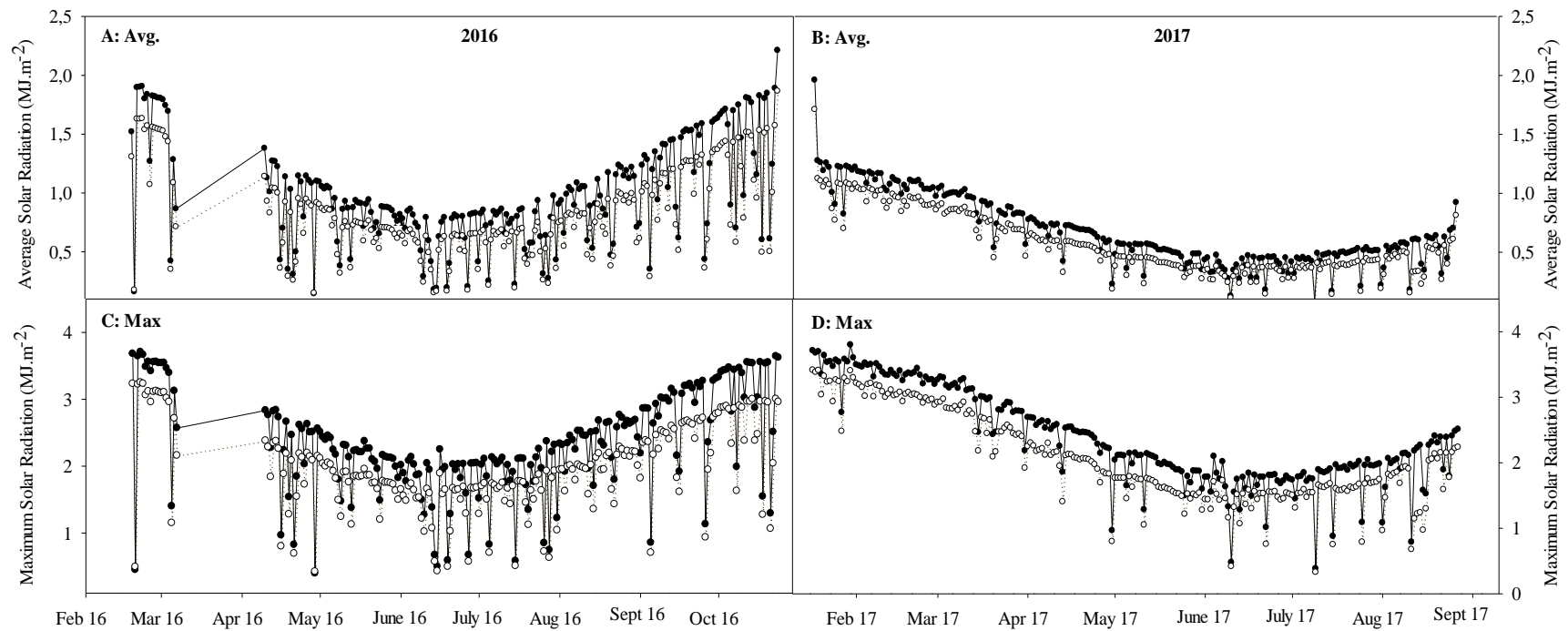


Fig. 1. The effect of 20% white shade net on the average (A and B) and maximum (C and D) solar radiation in a 'Nadorcott' mandarin orchard based in Citrusdal measured at 4 m height. (○ = Shade net; ● = Control).

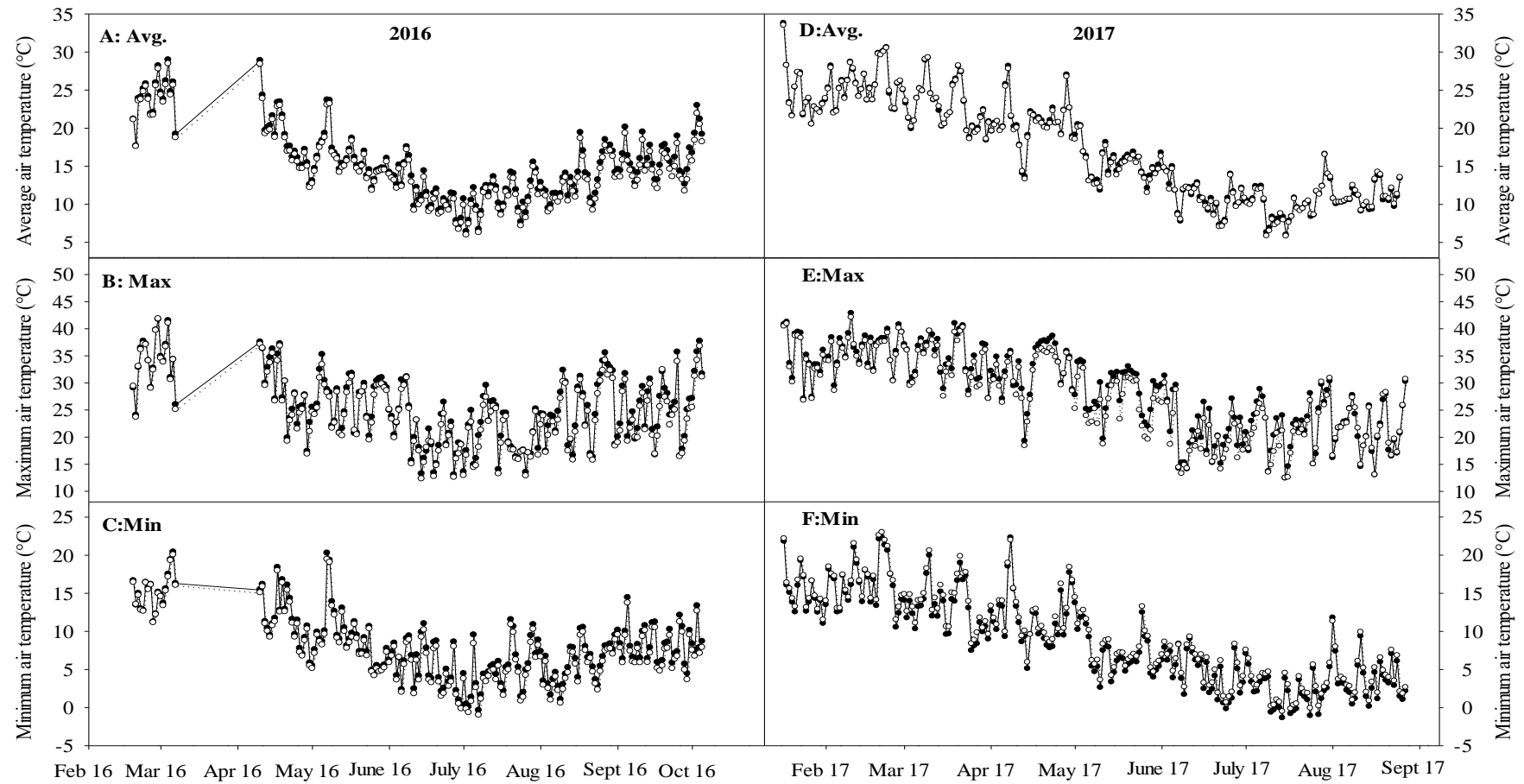


Fig. 2. The effect of 20% white shade net on the average (A and B), maximum (C and D) and minimum (E and F) temperatures in a ‘Nadorcott’ mandarin orchard based in Citrusdal measured at 4 m height above canopy for 2016 and 2017. (○ = Shade net; ● = Control).

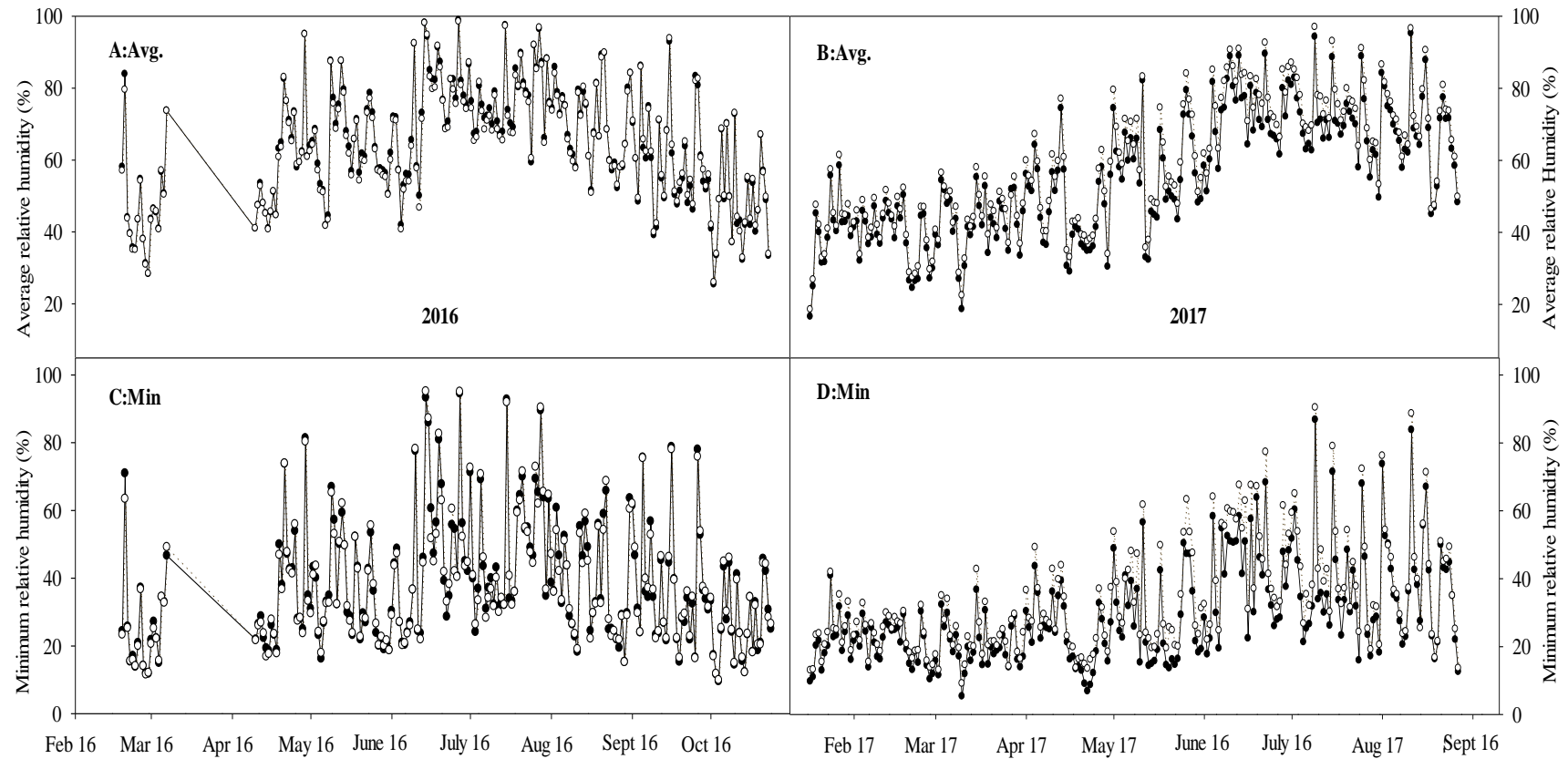


Fig. 3 The effect of 20% white shade net on daily average (A and B) and minimum (C and D) relative humidity (%) in a 'Nadorcott mandarin' orchard in Citrusdal measured 4 m above canopy from (○ = Shade net, ● = Control).

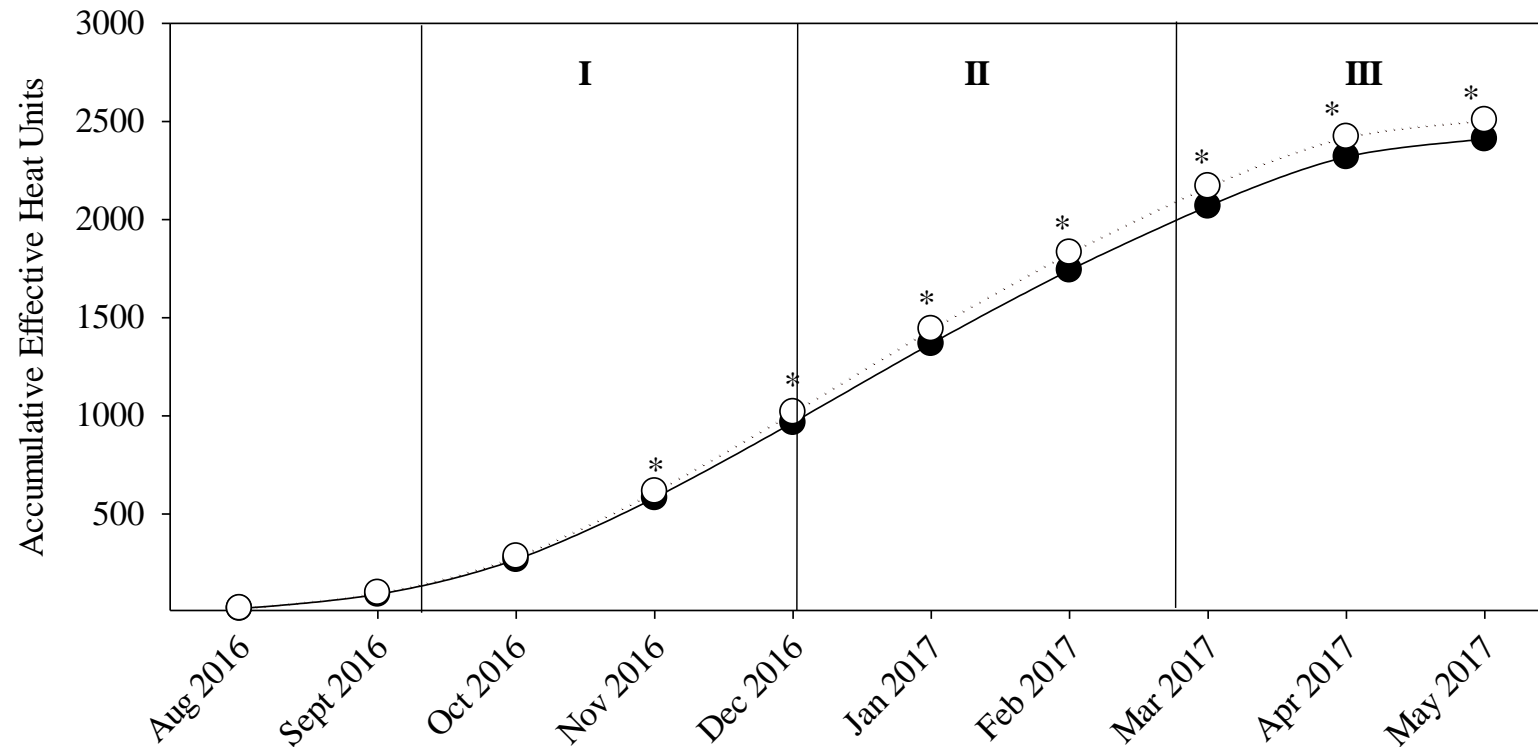


Fig. 4 The effect of 20% white shade netting on within tree canopy accumulative effective heat units during critical fruit growth stages I, II and III in a 'Nadorcott' mandarin orchard in Citrusdal (\circ = Shade net; \bullet = Control). * indicates mean values ($n = 4$) within a month differing significantly at $P < 0.05$. Month*Treatment = $P < 0.0001$.