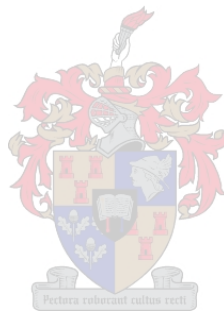

Water relations and sunburn in apple fruit

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature:

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Abstract

Sunburn in apple is a major problem in South Africa with losses of up to 50% of total yield. The primary objective of this study was to investigate the relationship between tree water relations and sunburn development under South African conditions. Cultural practices that may affect sunburn development through modification of plant water status and the effect of microclimate on fruit peel anatomy was also studied.

Induced water stress increased sunburn necrosis and incidence in ‘Granny Smith’ and sunburn severity in ‘Cripps’ Pink’ apple. Although not very pronounced, chlorophyll fluorescence pointed to photochemical changes within the peel under water stress. Water stress influenced ascorbic acid levels in peel, indicating the occurrence of oxidative stress. Leaf net carbon assimilation was reduced due to lower stomatal conductance and possibly also photoinhibition.

Pulsing irrigation reduced the incidence of sunburn in ‘Golden Delicious’ and ‘Granny Smith’ compared to an under irrigated control. The reduction in sunburn might be attributed to either the improved plant water status, which reduced tree stress compared to the under irrigated control, or the comparatively more vegetative growth resulting in shading of fruit. Canopy ambient temperature was lower under pulsing irrigation and plant water status was improved.

Foliar S-ABA (active form of abscisic acid) application had no effect on sunburn incidence and severity under South African conditions over three growing seasons. The main apple growing regions in South Africa (33-34° S latitude) experience high radiation, while temperatures often exceed 40 °C during the growing season. S-ABA application resulted in lower stomatal conductance with a consequent decrease in gas exchange and reduction in fruit size. Tree water status in terms of stem water potential was improved due to the reduction in transpiration.

Fruit transpiration and water loss was higher in young compared to mature fruit, which seemingly corresponds with increasing susceptibility of fruit to sunburn during fruit growth. Stomata are present in young fruit and are under regulatory control, which makes them more effective for transpiration. In mature fruit, the predominant lenticel type was partially open and not effective for transpiration. Lenticel density and morphology varied considerably within and between cultivars, between different canopy positions, and between production areas. Although fruit transpiration and water loss decreased over the growing season, it did

not always follow the same pattern as fruit surface morphology. In addition, the contribution of transpiration and water loss to the fruit heat balance is negligible. Hence, differences in fruit surface morphology may not explain differences in cultivar and fruit sunburn sensitivity.

In conclusion, this study provides insight into ecophysiological and biochemical changes that occur under water stress and that might have a bearing on sunburn development in apple fruit. The study provides further proof of the link between plant water status and sunburn development and shows that good management of plant water status through irrigation is instrumental to controlling sunburn in a warm production region.

Opsomming

Sonbrand is 'n ernstige probleem in Suid-Afrika met verliese van tot 50% van totale produksie wat ondervind word. Die primêre doelwit van hierdie studie was om die verband tussen boom waterverwantskappe en sonbrand onder Suid-Afrikaanse kondisies te ondersoek. Verbouingspraktyke wat sonbrand ontwikkeling mag affekteer deur die modifikasie van die plantwaterstatus asook die effek van makro- en mikroklimaat op die vrugskil anatomie is ook ondersoek.

Geïnduseerde waterstres het die graad van sonbrand in 'Granny Smith' en 'Cripps' Pink' appels vererger. Ten spyte van 'n relatiewe klein effek, het chlorofiel fluoressensie gedui op fotochemiese veranderinge in die skil vanweë waterstres. Waterstres het askorbiensuurvlakke in die skil geaffekteer, wat dui op die voorkoms van oksidatiewe stres. Netto koolstofassimilasie is verminder vanweë laer huidmondjiegeleiding en moontlik ook foto-inhibisie.

Pulsbesproeiing het sonbrand in 'Golden Delicious' en 'Granny Smith' verminder in vergelyking met 'n onderbesproeide kontrole. Die vermindering in sonbrand kan moontlik toegeskryf word aan enige van of beide verbeterde plant waterstatus wat stres verlaag het vegeleke met die onderbesproeide kontrole, of the relatief verhoogde vegetatiewe groei en gevolglike oorskaduwing van vrugte. Pulsbesproeiing het die temperatuur van die blaredak verlaag asook die plantwaterstatus verbeter.

Blaartoediening van S-ABA (aktiewe vorm van absissiensuur) oor drie groeiseisoene het geen effek op die voorkoms en graad van sonbrand onder Suid-Afrikaanse toestande gehad nie. Die hoof produksie-areas in Suid-Afrika (33-34°S breedtegraad) ondervind hoë straling terwyl temperature dikwels 40 °C oorskry. S-ABA toediening het huidmondjiegeleiding verlaag met 'n gevolglike verlaging in gaswisseling en vruggrootte. Boomwaterstatus, in terme van stamwaterpotensiaal, is verbeter vanweë die verlaging in transpirasie.

Vrugtranspirasie en waterverlies was hoër in onvolwasse vergeleke met volwasse vrugte. Dit stem oënskynlik ooreen met die toenemende vatbaarheid van vrugte vir sonbrand soos wat hul groei. Huidmondjies is aanwesig in onvolwasse vrugte en is onder regulatoriese beheer. Dit maak hul meer effektief vir evaporatiewe verkoeling. Gedeeltelik oop lentiselle was die mees algemene lentisel tipe in volwasse vrugte. Hierdie lentiselle is oneffektief vir transpirasie. Die digtheid van lentiselle asook hul morfologie het heelwat tussen en binne kultivars, tussen

vrugposisies in die blaredak en tussen produksie-area verskil. Alhoewel vrugtranspirasie en waterverlies gedurende die groeiseisoen afgeneem het, het dit nie altyd dieselfde patroon as vrugskilmorfologie gevolg nie. Bowendien is die bydrae van transpirasie en waterverlies tot die vrug hittebalans weglaatbaar klein. Gevolglik mag verskille in oppervlakmorfologie waarskynlik nie verskille in kultivar en vrug sonbrand sensitiwiteit verklaar nie.

In gevolgtrekking; hierdie studie verskaf insig in ekofisiologiese en biochemiese veranderinge wat tydens waterstres ervaar word en wat moontlik 'n strekking mag hê op die ontwikkeling van sonbrand in appels. Die studie verskaf verder ook bewyse van die skakel tussen plantwaterstatus en die ontwikkeling van sonbrand en dui daarop dat goeie bestuur van die plantwaterstatus deur besproeiing instrumentaal is tot die beheer van sonbrand in warm produksiestreke.

Publications and conference presentations from this thesis

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1. Mupambi G., Schmeisser M., Lötze E., Malan C., Dzikiti S. and Steyn, W.J. 2015. Effect of supplementary irrigation at high ambient temperatures on sunburn, plant physiology, soil and canopy environment of 'Granny Smith' apple. *Acta Hort.* 1150:239-244. <https://doi.org/10.17660/ActaHortic.2017.1150.33>
2. Mupambi G., Reynolds J.S. and Steyn, W.J. 2014. Foliar S-ABA application does not reduce sunburn in 'Granny Smith' apple. *Acta Hort.* 1042:303-309. <http://dx.doi.org/10.17660/ActaHortic.2014.1042.36>

Conference presentations

1. Mupambi G., Schmeisser M., Lotze E., Malan C., Dzikiti S., and Steyn W.J. 2015. Effect of supplementary irrigation at high ambient temperatures on sunburn, plant physiology, soil and canopy environment of 'Granny Smith' apple. VIII International Symposium on Irrigation of Horticultural Crops, Lleida, Spain, 8-11 June 2015. (Oral).
2. Mupambi G., Schmeisser M., Lotze E., Malan C., Dzikiti S., and Steyn W.J. 2015. Additional irrigation at high air temperatures reduces sunburn in 'Golden Delicious' apple. Combined Congress, Nelson Mandela Metropolitan University, George, 19-22, January 2015. (Oral)
3. Mupambi G., Reynolds J.S. and Steyn, W.J. 2014. Foliar S-ABA application does not reduce sunburn in 'Granny Smith' apple. Combined Congress, Rhodes University, Grahamstown 20-23, January 2014. (Oral)
4. Mupambi G., Reynolds J.S. and Steyn, W.J. 2013. Foliar S-ABA application does not reduce sunburn in 'Granny Smith' apple. XII International Symposium on Plant Bioregulators in Fruit Production, Orlando, Florida, United States, 28 July-1 August. (Oral).

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Dedications

This dissertation is dedicated to my grandmother Violet and my siblings Welly, Grace and Petros. Sorry it took me so long for me to finish this.

Also dedicated to the memory of my late parents Gavin and Vaidah, and my late brother Sebastian.

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This thesis was written according to the language and style required by the journals of the *American Society for Horticultural Science*. Each chapter represents an individual paper and some repetition between the chapters may occur.

General introduction

Due to global warming, the average temperatures of the Western Cape, which is the main apple production region of South Africa, is expected to increase by 2-3 °C over the next 30-40 years (Midgley et al., 2005). Although apples are produced under irrigation in South Africa, trees experience substantial water stress under hot conditions when tree water uptake does not match atmospheric evaporative demand (Croft, 1995; Ebel et al., 2001). In addition, water deficits due to drought are likely to increase, aggravating water stress (Lisar et al., 2012, Restrepo-Díaz et al., 2010).

Tree water stress has been linked to higher incidence of sunburn in apple fruit under South African conditions (Makredza et al., 2013). Sunburn is a physiological disorder associated with high irradiance and extreme fruit surface temperatures (Chen et al., 2008; Racsko and Schrader, 2012; Wünsche et al., 2004). The three types of sunburn that have been identified, viz. sunburn necrosis, sunburn browning and photooxidative sunburn, cause the discolouration of the fruit surface and results in the downgrading of fruit (Felicetti and Schrader, 2008; Schrader et al., 2001). This study mostly focused on sunburn browning and necrosis. Sunburnt fruit are not suitable for export, resulting in loss of earnings for the growers. Under South African growing conditions, up to 50% of the total yield may be unmarketable due to sunburn (Wand et al., 2006). Research on sunburn has focused mainly on the physiology underlying sunburn symptomology (Racsko and Schrader, 2012), determining thresholds for the environmental factors that induce sunburn (Racsko and Schrader, 2012), and technology to decrease sunburn such as pruning strategies, evaporative cooling, the use of shade nets and the application of reflective particle films (Gindaba and Wand, 2005).

Although water stress has been linked to sunburn development in apple fruit (Kader, 2002; Makredza et al., 2013; Piskolczi et al., 2004; Schrader et al., 2003; Woolf and Ferguson, 2000; Yuri et al., 2004), the relationship between water stress and sunburn is poorly understood. There is a gap in knowledge and lack of definitive data on the relationship between plant water status and sunburn occurrence in apple fruit. Makredza et al (2013) concluded that water stress aggravates sunburn development under conditions conducive for its development by increasing

fruit surface temperature, but did not elucidate the actual mechanism. The other references cited above just make casual mention of the relationship between plant water status and sunburn with no quantitative data to back up these claims. In addition, there is limited information available about the relationship between transient water stress and sunburn development in apple from a biochemical and ecophysiological perspective. In South Africa, growers believe that more water results in reduced sunburn incidence and use supplemental pulsing irrigation during heat wave conditions when water stress is likely to occur to mitigate the risk of sunburn. Pulsing irrigation is carried out by applying short pulses of irrigation during a heat wave using the existing microsprinkler irrigation system to wet the orchard floor. A typical pulsing cycle would involve application of water for 5 min and then 20 min off when the temperature exceeds 30 °C. However, no scientific evidence exists to support the claims that irrigation pulsing has any effect on sunburn.

Absciscic acid (ABA) is a plant growth regulator that is involved in the signalling and regulation of plant responses to water stress conditions, thereby regulating plant water status (Kim and van Iersel, 2011). Recent research in Japan found that foliar application of S-ABA (biologically active form of absciscic acid) reduced sunburn incidence in 'Tsugaru', 'Sensyu', 'Yataka' and 'Fuji' apples by up to 30% (Iamsub et al., 2008; Iamsub et al., 2009). The S-ABA application was associated with increased antioxidant levels, which, according to the authors, alleviated and prevented oxidative damage caused by high ambient temperatures and high irradiance (Iamsub et al., 2009). Racsco and Schrader (2012) in their review on sunburn in apple indicated that research is needed to assess the effectiveness of S-ABA to reduce sunburn in regions like South Africa with a harsher summer climate.

Very little information is available on how the macro and microclimate affects fruit surface anatomy and, hence, the evaporative cooling potential of fruit. Fruit surface temperature is normally much higher than ambient temperatures due to poor heat dissipating mechanisms and limited cooling capacity through transpiration (Cheng and Ma, 2004, Ferguson et al., 1998; Wünsche et al., 2004). Transpiration from the fruit surface results in the loss of heat and evaporative cooling of the fruit (Woolf and Ferguson, 2000). During periods of low transpiration, the evaporative cooling capacity of fruit is reduced and might lead to fruit temperature increases (Colaizzi et al., 2012). High fruit surface temperatures (46 to 49 °C, depending on cultivar) are

associated with the development of sunburn in apples (Chen et al., 2008; Wünsche et al., 2004). Sunburn necrosis occurs when fruit surface temperature reaches 52 °C for a minimum period of 10 min (Schrader et al., 2001). Sunburn browning, the most prevalent type of sunburn in apples, is induced by irradiance at threshold temperatures of 46 to 49 °C depending on the cultivar (Chen et al., 2008; Schrader et al., 2001; Wünsche et al., 2004).

Since the overall objective of the study was to establish how tree water status affects the occurrence of sunburn in apple fruit, a literature study was conducted on the ecophysiological, biophysical and biochemical responses of apple trees to water stress as well as fruit quality and sunburn development in relation to tree water stress. To orientate readers unfamiliar with sunburn in apple, a brief background was provided on the incidence, causal factors and underlying physiology of sunburn. The comprehensive review on sunburn in apple, published in 2012 (Racsko and Schrader, 2012), provided a great reference base and summation of knowledge in this field. We therefore rather focused our literature search more specifically on the changes that occur in apple trees in response to low plant water status and how these may relate to sunburn.

The aim of **Paper 1** was to establish the influence of changes in plant water status on apple tree ecophysiology and fruit biochemistry characteristics that influence the occurrence of sunburn. In order to achieve this, the effect of short term-induced water deficit on soil water status, canopy microclimate, leaf and fruit photochemistry and gas exchange, as well as fruit biochemistry was investigated in ‘Granny Smith’ and ‘Cripps’ Pink’ and related to the incidence and severity of sunburn.

Papers 2 and 3 examined the effect of cultural practices that effect tree water status on sunburn in apple. The aim of Paper 2 was to establish the effectiveness of irrigation pulsing in reducing sunburn in ‘Golden Delicious’ and ‘Granny Smith’, but during the course of the experiment the control was under irrigated. The trial therefore ended up assessing the effect of different levels of irrigation on sunburn incidence. The effect of irrigation treatments on soil water status, canopy microclimate, gas exchange, photochemistry, vegetative growth and fruit quality was assessed. Paper 3 evaluated the use of S-ABA application to decrease sunburn in ‘Granny Smith’ apples. The effect of S-ABA on fruit quality, gas exchange, plant water status, peel chemical

composition and fruit set under South African conditions were assessed. Different S-ABA treatments and application timings were evaluated with particular reference to their effect on sunburn incidence over three growing seasons.

The original objective of the study reported in **Paper 4** was to relate regional climate, canopy micro-climate and fruit surface anatomy to cooling capacity over the growing season in ‘Granny Smith’, ‘Golden Delicious’, ‘Starking’, ‘Fuji’ and ‘Royal Gala’ apples from the Ceres and Grabouw production areas in South Africa. Since the contribution of evaporative cooling from the apple surface to the fruit heat balance is negligible, the paper was refocused to assess the effect of climate (regional and micro-climatic) on the fruit surface anatomy.

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Literature review: Plant water status in relation to sunburn development in apple fruit.

1 Introduction

Water deficit is becoming the most common limiting factor in intensive cultivation all over the world due to population growth, deteriorating water quality and increased industrialization (Alizadeh et al., 2011; Behboudian and Mills, 1997). Although apples are generally produced under irrigation, trees experience transient water stress under hot and dry conditions when tree water uptake does not match the atmospheric evaporative demand (Ebel et al., 2001). Severe water stress affects hydraulic conductivity by inducing embolism in xylem vessels thereby reducing water supply to leaves (Tyree and Sperry, 1989). The primary stress experienced under water deficit is dehydration – as is also the case for various other abiotic stresses encountered by plants (Dinakar et al., 2012). The majority of plants have limited dehydration tolerance and will die at leaf water potentials between -5 and -10 MPa (Oliver et al., 2010). Water stress affects many biophysical, physiological, biochemical and molecular processes in plants, which influence growth and productivity (Osakabe et al., 2014; Šircelj et al., 2005). It also increases the sensitivity of plants to other biotic or abiotic stresses, thus further negatively affecting growth, productivity (Armaki et al., 2013; Chaves et al., 2002; Jiang and Zhang, 2002) and also fruit quality (Al-Yahyai, 2012). However, plant responses to water stress are complex and can be modified under field conditions by the superimposition of other environmental stresses (Chaves et al., 2002). The complex and interlinked nature of these processes makes it difficult to discern how they are related to each other and how they act to modify tree physiology, productivity and fruit quality (Morandi et al., 2014). Several processes are sensitive to water stress in deciduous trees, viz. flower bud differentiation and development, reproductive cell division, fruit drop and canopy growth (Naor, 2006). Observational data indicate that water stress may increase sunburn in apple (Racsko and Schrader, 2012) and Makedredza et al. (2012) increased sunburn in ‘Cripps’ Pink’ apple by withholding irrigation for two weeks.

Sunburn is a physiological disorder of apples that causes discoloration of the fruit surface thereby affecting fruit visual quality (Felicetti and Schrader, 2009). High ambient temperatures

and excessive levels of solar radiation cause overheating of the fruit surface leading to development of sunburn symptoms (Chen et al., 2008; Felicetti and Schrader, 2008a; Wünsche et al., 2004a). Overheating of the fruit surface occurs due to poor heat dissipation mechanisms and limited evaporative cooling capacity of the fruit peel (Cheng and Ma, 2004; Ferguson et al., 1998).

The recent comprehensive review on sunburn in apple (Racsko and Schrader, 2012) has explored the knowledge on the causal factors involved in sunburn development in detail. The objective of this literature review was therefore not to repeat the 2012 review, but to rather focus more specifically on the link between plant water status and sunburn. The reasons for this focus were because: 1) it was the focus of my research project, and 2) most information on the link between plant water status and sunburn seems to be observational, which includes the few studies mentioned in the 2012 review (these will be discussed in more detail).

2 Water stress terminology

Water deficit and water stress are terms commonly used when studying plant water relations. Water deficit occurs either when there is not enough available soil water for plant uptake or when the rate of transpiration exceeds water uptake (Bray, 1997; Dodd and Ryan, 2016; Lisar et al., 2012). Due to transpirational water loss, plants are seldom fully hydrated and therefore suffer from water deficits (Taiz and Zeiger, 2002; Shao et al., 2008). Dodd and Ryan (2016) defined stress in plants as any external factor that negatively influences plant growth, productivity, reproductive capacity or survival. Water stress is primarily caused by water deficit (Lisar et al., 2012, Restrepo-Díaz et al., 2010). There is no clear definition of water stress in plants. The terms water stress and water deficit are often used subjectively and with various meanings. There are two kinds of water stress, viz. transient water stress and long-term water stress. Transient diurnal water stress occurs during the day when evaporative demand exceeds water uptake with the plant recovering at night. Transient water stress is reversible and it is a result of the plants using internally stored water during the day causing stem or fruit diameter shrinkage and the refilling of the storage pools at night leading to expansion (Dzikiti et al., 2010; Steppe et al., 2006). Long-term water stress, on the other hand, occurs in response to diminishing soil water content. The plant is able to partially recover at night with gradual deterioration linked to decreasing soil

water availability. In apple production, long term or sustained water stress can cause a reduction in yield due to a reduction in fruit size (Lopez et al., 2014).

Plant water status refers to the actual amount of water in the plant. It is a useful measure for, amongst a variety of applications, water deficit research or research like the present study where different irrigation regimes are applied, breeding for drought tolerance, or irrigation systems management (Jones, 2007). Plant water status, as measured by a pressure chamber, can be expressed as leaf water potential, midday stem or xylem water potential and predawn leaf water potential. “Water potential” refers to the chemical potential of water divided by the volume of 1 mole of water and is expressed in units of pressure (Passioura, 2010). Leaf water potential, measured from an uncovered leaf fully exposed to solar radiation and assumed to be at steady state, is not a reliable measure of plant water status due to significant short-term variation in response to ambient environmental conditions (Jones, 2007). In fact, short term fluctuations in leaf water potential can exceed treatment differences (Jones, 2007). Predawn leaf water potential, which also uses an uncovered leaf to measure plant water status, provides an indication of the soil water availability and minimizes the variation obtained in daytime measurements. It is widely used in irrigation research as a measure of water stress (Jones, 2007 and references therein). Predawn leaf water potential gives an indication of the total amount of water available in the soil to the plant. Midday stem water or xylem potential is measured around solar noon (12:00 HR to 14:00 HR) and corresponds to the maximum water stress experienced by the plant, as this is assumed to be the period of maximum water demand. A healthy leaf is bagged for at least 1 hour in silver reflective bags to equalize the leaf and the tree’s xylem water potential before readings of midday stem water potential are obtained. According to Jones (2007), soil moisture measurements are preferred as indicator of drought stress for agronomic purposes, but midday stem water potential can also be a useful measurement and certainly preferably over the much more variable leaf water potential.

3 Ecophysiological responses to water stress

3.1 Stomatal conductance and gas exchange

Stomatal closure is one of the earliest responses in plants under water stress conditions (Naithani, et al., 2012). Stomatal aperture is controlled by the plant growth regulator abscisic acid (ABA),

which is produced in the roots and transported to the shoots via the xylem (Zhang and Davies, 1987). The importance of stomatal closure stems from the direct influence on transpiration and photosynthesis (Hasanuzzaman et al., 2014).

Some studies found stomata in apple leaves to be quite responsive to soil water deficit and to the vapor pressure deficit of the air. Stomatal conductance was reported to be reduced under water deficit in 'Braeburn', 'Royal Gala' and 'Imperial Gala' apple (Fernandez et al., 1997a; Mills et al., 1994; Yuan et al., 2013). Naor et al. (1995) also reported a reduction in stomatal conductance under reduced irrigation compared to normal irrigation in 'Golden Delicious' apple. The closure of stomata leads to decreased uptake of carbon dioxide (CO₂), which is required for photosynthesis (Pinheiro and Chaves, 2011). In young 'Imperial Gala' apple, Fernandez et al. (1997a) reported that the most consistent decreases due to water stress were in transpiration and net carbon assimilation. Maximum photosynthetic rates occur when the stomata are fully open (Yoder et al., 1994). 'Royal Gala' apple trees under stress showed a reduction in net photosynthetic rate (Yuan et al., 2013). Similar results were also observed in 'Braeburn' (Mills et al., 1996). Net photosynthesis and transpiration may be reduced by as much as 40% before the leaves show any wilting and over 90% at wilting, when stomata are less active or closed due to water deficit (Lakso et al., 1984). When water deficit is severe, stomata may not open at all to prevent water loss from the leaves, resulting in a decrease in internal CO₂ concentration (Maxwell and Johnson, 2000). In contrast, Yuan et al. (2013) reported that intercellular CO₂ concentration increased as water stress was prolonged in 'Royal Gala', signifying a non-stomatal limitation of leaf net photosynthetic rate. A slight water stress may also have desirable effects on productivity. For example, given that the CO₂ gradient across the stomatal pore is less steep than the water vapor gradient, a small degree of stomatal closure may in fact lead to increased leaf water use efficiency as transpiration is curtailed more than photosynthesis (Bacon, 2004). This is the principle behind deficit irrigation wherein controlled levels of soil water deficit are imposed to increase water use efficiency (Behboudian et al., 1998; Mills et al., 1996; Mpelasoka and Behboudian, 2002).

Chlorophyll fluorescence can be used to determine how photosynthesis is affected by water stress (Maxwell and Johnson, 2000). Changes in chlorophyll fluorescence parameters under water stress can indicate damage to the photosynthetic apparatus either on the donor or the

acceptor side of the photosystem II reaction centres (Yuan et al., 2013). Fernandez et al. (1997a) found that variable and maximal chlorophyll fluorescence and fluorescence quenching are not particularly sensitive to water stress and other fluorescence parameters showed little difference under water stress conditions in leaves of 'Imperial Gala' apple. Chlorophyll fluorescence is best used as an indicator of possible long term water stress in apple as it is not responsive in the short term (Brestic and Zivcak, 2013; Fernandez et al., 1997a). Makedredza et al. (2015) found measurement of the maximal photosynthetic efficiency of PSII (Fv/Fm) useful to indicate the presence of sunburn damage in apple fruit. These fruit were not showing any damage due to masking of the typical symptoms by anthocyanins.

3.2 Non stomatal limitations of photosynthesis

The inhibition of ribulose-1,5-bisphosphate carboxylase, which reduces photosynthesis has been reported to occur under water stress (Tezara et al., 1999). This is a result of lower ATP content resulting from the loss of ATP synthase (Escalona et al., 1999; Sharkey and Seemann, 1989; Tezara et al., 1999). Water stress inhibits chlorophyll synthesis; which is determined by the severity of water stress (Chernyad'ev, 2005). Water stress decreased carotenoid content in wheat seedlings, thereby decreasing photosynthesis, since carotenoids absorb additional light energy and transfer it to the reaction centers of photosystems I and II during photosynthesis (Chernyad'ev, 2005; Gamble and Burke, 1984). In contrast, Mohammadkhani and Heidari (2007) reported that carotenoid content increased under water stress in maize. The disruption of cyclic and non-cyclic electron transport occurs under water stress reducing photosynthesis (Heber and Walker, 1992). Water stress reduces chloroplast water content, whilst the duration and severity of water stress produces changes in chloroplast ultrastructure resulting in irreversible degradation (Chernyad'ev, 2005).

3.3 Plant strategies to cope with water deficit

Plant strategies to cope with water deficit normally involve a mixture of stress avoidance and tolerance 'strategies' (Chaves et al., 2002). The relationship between stomatal conductance and leaf water potential under water deficit gives rise to two contrasting water management strategies by plants (Klein, 2014; Tardieu and Simonneau, 1998). Isohydric plants aim to maintain constant leaf water potential by reducing stomatal conductance under water deficit conditions (Sade and

Moshelion, 2014). Anisohydric plants, on the other hand, keep their stomata open much longer under water deficit conditions and allow their leaf water potential to decrease to a specific threshold (Sade and Moshelion, 2014; Tardieu and Simonneau, 1998). Stomatal conductance is very sensitive to water status in isohydric species and less sensitive in anisohydric species (Jones, 2014). The molecular and cellular mechanisms underpinning these two types of behaviour are poorly understood. However, there is evidence pointing to the sensitivity of guard cells to a critical leaf water potential as playing some role (Sade et al., 2012). The agronomic importance of these behaviours is that for anisohydric crops, yield will increase under conditions of optimal to moderate water availability due to their higher stomatal conductance and resulting higher carbon assimilation (Alvarez et al., 2007; Sade et al., 2012; Sade et al., 2009). The classification of plants species as either isohydric or anisohydric should be done with caution as it has been shown that there is a continuum rather than dichotomy between isohydry and anisohydry (Klein 2014,).

The apple tree is generally considered to be isohydric (Jones, 2014). Under increasing vapor pressure deficits from 0.7 to 2.7 kPa, an isohydric response was observed among the apple progeny from 'Starkrimson' x 'Granny Smith', as they displayed water saving behaviour by regulation of stomatal closure (Regnard et al., 2009). Lauri et al. (2016) reported that 'Starkrimson', 'Granny Smith' and 19 hybrids selected from their progeny responded more or less strongly to drought, displaying more isohydric or anisohydric behavior depending on genotype. Similarly, Beikircher et al. (2013) found that 'Golden Delicious', 'Red Delicious' and 'Braeburn' displayed various degrees of iso-anisohydry. The rate of stomatal closure in plants can be modified by previous exposure to water deficit or the rate of desiccation, therefore making the classification of the species as either isohydric or anisohydric more complex (Jones, 2014). Grapevines have been shown to switch from anisohydric to isohydric-like behaviour in response to changing environmental conditions (Rogiers et al. 2012; Zhang et al. 2012).

The implication of these different strategies is that the use of plant water status as an indicator of water stress (Nadezhdina, 1999) should be done with caution since apple varieties can either be isohydric or anisohydric. Midday stem and leaf water potential may decrease under water stress as a result of soil water deficit (Nadezhdina, 1999; Naor et al., 2008). Midday stem water potential was found to be lower under reduced irrigation compared to normal irrigation in 'Golden Delicious' apple (Naor et al., 1995) and under deficit irrigation in 'Braeburn' (Mills et al., 1996).

Ebel et al. (1995a) reported that stem water potential values for severely stressed, moderately stressed and control 'Delicious' apple trees were -2.7, -2.0 and -0.8 MPa, respectively. For more isohydric apple varieties, no changes in leaf water potential may be observed, even if the tree is under water stress and has, as a result, closed its stomata. A low leaf water potential in such varieties may equate to considerable water stress. More anisohyric varieties may allow their leaf water potential to fluctuate in response to water availability and climatic conditions to a greater degree. Therefore, a low water potential in such varieties does not necessarily suggest that the plants are water stressed. Stomatal conductance for isohydric apple varieties could be sensitive to mild water stress, whilst anisohyric varieties may not show any changes in stomatal conductance until water stress is severe. Gas exchange in anisohyric varieties might be unaffected by water stress as these trees will generally keep the stomata open for longer under water stress conditions whereas in isohydric varieties carbon assimilation might be lower under the exact same water stress conditions. Considering the above, the term "water stress" should be used with caution. Unfortunately, much literature that relates to plant water status, water deficit and plant response, does not indicate or report the plant strategy to water deficit. In most cases, it is impossible to determine the position of the plants on the isohydry-anisohydry continuum used in different studies – hence, we are stuck with the somewhat "imprecise" terminology used in these papers.

4 Biochemical responses to water stress in apples trees

4.1 Changes in plant hormones

Abscisic acid is a plant growth regulator that is involved in the signaling and regulation of plant responses to water stress conditions (Kim and van Iersel, 2011). ABA is produced in the roots and transported to the shoots via the xylem where it regulates stomatal closure in leaves and young fruit whilst they still have stomata, which determines the rate of transpiration and thereby controls water loss from the plant (Zhang and Davies, 1987). ABA is synthesized from xanthophylls via violaxanthin, xanthoxin and ABA-aldehyde (C-40 pathway), with the conversion of violaxanthin to xanthoxin being the rate-limiting step in ABA biosynthesis (Reddy et al., 2004). The accumulated ABA in the xylem is transported to guard cells via passive diffusion in response to pH changes and by specific transporters (Osakabe et al., 2014). Water

stress was reported to induce increases in endogenous ABA content in two apple rootstocks, *Malus sieversii* and *M. hupehensis*, when measured 7 days after imposing drought stress (Zhang et al., 2014). Leaf ABA concentration was also increased by water stress in 'York Imperial' apple seedlings (Wang et al., 1987).

Exogenously applied ABA has been used to prime young 'Royal Gala' apple trees, so as to provide dehydration protection under conditions of water stress (Tworkoski et al., 2011). Zhang et al. (2014) reported that exogenous application of ABA was effective in enhancing stress tolerance of two apple rootstocks (*Malus sieversii* and *M. hupehensis*), by increasing activities of antioxidant enzymes and water relations under drought stress. ABA application has been found to increase total antioxidant capacity, total phenolics, chlorophyll concentration and ascorbic acid in apple fruit peel (Iamsub et al., 2009). ABA application can improve plant water status since it reduces stomatal conductance thereby limiting transpirational water loss. However, since ABA limits transpirational water loss, it might cause an increase in canopy temperature as convective cooling from the canopy is reduced. This might change the fruit heat balance as convective cooling of the fruit will decrease because the differences in fruit surface and surrounding air temperature will become negligible (W. Steyn, personal communication), which could potentially exacerbate sunburn development.

Jasmonic acid and its methyl esters (methyl jasmonate) are known to be involved in the response of plants to water stress by inducing the synthesis of various chemical compounds that act as osmoprotectants, such as polyols, amino acids and quaternary ammonium compounds (Gao et al., 2004). Kondo (2010) reported that water stress induced endogenous jasmonic acid synthesis in leaves of apple seedlings. YanPing et al. (2004) reported that a significant logarithmic correlation existed between leaf stomatal resistance and concentration of jasmonic acid in xylem, suggesting that jasmonic acid in xylem sap may be another signal responsible for communication between roots and shoots in apple trees under water stress.

4.2 Dehydrin production

Dehydrins are a distinct family of late embryogenesis abundant (LEA) proteins that are hydrophilic and glycine rich (Allagulova et al., 2003; Close, 1997; Wisniewski et al., 1996). They play an important role in protecting plants against water stress (Close, 1997; Liang et al.,

2012). Dehydrins can also accumulate under conditions that imitate water stress, for example, after application of ABA (Allagulova et al., 2003; Close 1996). They are the most noticeable soluble proteins induced by water stress (Close 1996). A total of 12 genes coding for the production of dehydrins were identified on various chromosomes in ‘Gala’ apple leaves (Liang et al., 2012). They provide stability to macromolecules such as proteins and nucleic acids by maintaining their structure during water stress, thereby preventing their denaturation (Liang et al., 2012; Wisniewski, 1996).

4.3 Production of free radicals and antioxidants compounds

Under water stress conditions, the production of reactive oxygen species such as hydroxyl radicals, singlet oxygen and hydrogen peroxide increases (Caverzan et al., 2012). In response to this, plants typically accumulate a wide range of antioxidants to quench the reactive oxidative species (Oh et al., 2010; Bolat et al., 2014). Increasing water stress resulted in higher peroxidase activities, phenol contents and catalase activity in ‘Visate Bella’ apple leaves (Bolat et al., 2014). Šircelj et al., (2005) reported that moderate water stress increased the concentrations of ascorbic acid, total glutathione and *b*-carotene, indicating an adaptation to oxidative stress in ‘Elstar’ and ‘Jonagold Wilmuta’ apple tree leaves. On the other hand, severe water stress caused a decline in ascorbic acid, total glutathione, together with the increases in oxidized glutathione concentration, indicating severe damage due to oxidative stress (Šircelj et al., 2005). Glutathione is a reliable drought stress indicator in apple trees, whereas ascorbic acid is only reliable under moderate drought stress (Šircelj et al., 2007).

4.4 Carbohydrate and sugar accumulation

To maintain turgor pressure under water stress, mature apple leaves can adjust osmotically by as much as 2 MPa as water stress develops (Lakso et al., 1984). The osmotic adjustment is due to increased concentration of soluble carbohydrates, such as sorbitol, glucose and fructose, which increases under water stress while sucrose and starch levels decrease (Wang and Stute, 1992). Wang and Stute (1992) observed that glucose and fructose concentrations increased, while sucrose and starch levels decreased significantly as water stress developed, strongly suggesting that sugar alcohols and monosaccharides are the most important for osmotic adjustment in ‘Jonathan’ apple.

4.5 Production of volatile organic compounds

The production and emission of volatile organic compounds occurs under water stress in apple trees (Naor, 2006). Volatile compounds are products of metabolism and their measurement increases the understanding of changes in apple trees under water stress (Ebel et al., 1995a). Water stress induced by late deficit irrigation from 102 days after full bloom to harvest increased the concentration of volatile compounds in 'Braeburn' fruit (Behboudian et al., 1998; Mpelasoka and Behboudian, 2002). Volatile organic compounds are considered to be important for apple aroma and can be classified into alcohols, aldehydes, ethyl esters and non-ethyl esters (Behboudian et al., 1998). Ebel et al. (1995a) reported that leaf emission rates of hexanal, (E)-2-hexenal, (E)-2-hexen-1-ol, 1-hexanol, hexyl acetate and (E)-2-hexenyl acetate were 5 to 310 times higher for severely stressed 'Delicious' apple trees, compared to those of the controls with the moderately stressed trees intermediate.

4.6 Changes in pigment concentrations

Red pigmentation in the apple peel is due to anthocyanins (Espley et al 2007; Saure, 1990). Anthocyanins have been reported to accumulate under water stress conditions (Ramakrishna and Ravishankar, 2011). Water stress enhanced red pigmentation in 'Braeburn' fruit (Kilili et al., 1996; Mills et al., 1994). The increase in anthocyanins under water stress is linked to accumulation of soluble sugars, which are important for anthocyanin production (Chalker-Scott, 1999; Mills et al., 1994). The exogenous application of ABA was reported to advance red color accumulation in 'MacIntosh' apples (Greene et al., 2011). The closing of stomata after exogenous ABA application will induce the plant into 'artificial' water stress mode increasing the accumulation of soluble sugars which are important for anthocyanin production (Chalker-Scott, 1999; Mills et al., 1994).

5 Effects of water stress on vegetative growth in apple

Water stress results in noticeable changes in growth parameters in apple trees (Ebel et al., 1995b). Leaf growth rate was reported to be reduced under water stress in 'Imperial Gala' (Fernandez et al., 1997b) and in 'Golden Delicious' (Robinson and Barritt, 1990). Other leaf related parameters that have been reported to be affected under water stress are leaf expansion

rate, leaf emergence rate and leaf folding (Robinson and Barritt, 1990; Steyn et al., 2002). However, Fernandez et al. (1997b) reported that leaf emergence had an inconsistent response to water stress. Early leaf senescence has been reported under water stress in ‘Autumn Rose Fuji’, possibly as a result of the production of ethylene (Fallahi et al., 2010). Ebel et al (2001) reported that as water stress increased in ‘Delicious’, leaves senesced acropetally starting with transition leaves near the bud-scale scar.

Shoot length and diameter has been reported to be reduced under water stress in MM.106, M.9, *Azayesh* and *Gami almasi* apple rootstocks (Alizadeh et al., 2011), in ‘Imperial Gala’ (Fernandez et al., 1997b) and in ‘Vista Bella’ apple (Bolat et al., 2014). Shoot growth is very sensitive to water stress and can be inhibited even before changes in plant water status occur (Sharp and LeNoble, 2002). Plant cell enlargement is driven by the water potential difference between the apoplast and symplast; under water stress, the driving force for water uptake into cells decreases and cell enlargement is inhibited resulting in reduced shoot growth (Molz and Boyer, 1978; Nonami et al., 1997). Only small water potential differences are required to cause cell enlargement when water flux enters a cell (Molz and Boyer, 1978). Rates of cell division were reported to be reduced under water stress in maize and could also potentially lead to a reduction in shoot growth (Sacks et al., 1997).

According to Huguet et al. (1992), the first indicator of water stress for many plants is the decrease and cessation of daily growth in stem diameter. Continuous recordings of trunk diameter fluctuations, using linear variable differential transducer gauges, have been used to determine the physical response of ‘Golden Delicious’ apple trees to water stress (Bonany et al., 2000; Huguet et al., 1992). Maximum daily shrinkage under severe water stress was smaller compared to a well-watered control in ‘Golden Delicious’ (Huguet et al., 1992). The maximum daily shrinkage of plant tissues appears to be linked to environmental factors affecting water stress, namely the water availability in the soil and potential evapotranspiration (Huguet et al., 1992). Measurements of trunk diameter fluctuations to determine water stress should be done with caution, however, as factors such as cold periods with low solar radiation or leaf damage by pests can affect readings (Huguet et al., 1992). The usefulness of trunk diameter measurements as indicators of water stress in olive trees was reported to decrease with tree age and crop load (Fernández et al., 2013). If trunk diameter measurements are to be used for measuring water

stress and scheduling irrigation, the independent verification of trunk diameter fluctuations with plant evapotranspiration or soil sensors is desirable (Goldhamer and Fereres, 2001).

Whole tree growth is reduced by water stress, with different rootstocks having an effect on the final tree growth (Fernandez et al., 1997b). Large, established trees with deep roots and trees on standard-sized or semi-dwarfing rootstocks, such as M.26 and MM.111, tolerate short periods of water stress, whereas young or newly planted trees and trees on dwarf rootstocks with shallow root zones, such as M.27 or M.9, are more sensitive to drought stress (Racsko, 2011). The deep roots from large trees are able to extract water more water from the soil compared to shallow root zone of small trees. However, Fernandez et al. (1997a) reported that trees on M.9 EMLA were least affected by water stress, MM.111 was intermediate, and Mark was the most sensitive. Dry matter accumulation was reduced under water stress in apple rootstocks (Alizadeh et al., 2011) and ‘Pink Lady’ apple (Lo Bianco et al., 2012). Fernandez et al. (1997b) reported a 16%, 27%, and 34% reduction in total plant dry weight for drought-stressed trees on M.9 EMLA, MM.111, and Mark, respectively, compared to corresponding controls in ‘Imperial Gala’. The increased production of fruiting spurs under moderate water stress has also been reported (Fallahi et al., 2010). Large crop loads significantly increase transpirational water loss in dwarfing rootstocks, as they bear more fruit per leaf area than standard-sized or semi-dwarfing rootstocks, leading to the increased sensitivity to water stress (Racsko, 2011).

6 Effects of water stress on yield and fruit quality in apple

Midday stem water potential is highly correlated to total yield and large fruit size in deciduous fruit, lower midday stem water potential result in yield reduction (Naor et al., 1999). Water stress can have an impact on yield in the current season or during subsequent years (Lopez et al., 2014). Water stress, in the form of continuous deficit irrigation during the whole growing season, decreased yield in ‘Braeburn’ (Kucukyumuk et al., 2013). Water stress that develops during mid-summer after canopy development and vegetative growth will have less effect on yield (Naor et al., 1995). Yield reductions under water stress are due to a reduction in fruit size in the current season, and reductions in flower initiation and return bloom in the subsequent years (Lopez et al., 2014). On the other hand, the increased production of fruiting spurs in ‘Autumn Rose Fuji’ under water stress resulted in a yield increase in the following season, although this could not be

sustained in the subsequent years (Fallahi et al., 2010). Leib et al. (2006) reported that deficit irrigation had no effect in the current year, but reduced yield in the second year in 'Fuji'.

Moderate water stress, by withholding irrigation late in the season through regulated deficit irrigation and partial root zone drying, can be used as a technique to improve fruit quality (Kilili et al., 1996; Leib et al., 2006) by improving attributes such as firmness, colour and concentrations of soluble solids, as well as enhance fruit maturity, storability and reduce weight loss in storage. (Lopez et al., 2014). However, severe water stress and stress during sensitive phenological stages can have negative effects on fruit quality, such as size reduction and increase in sunburn (Makredza et al., 2013; Naor et al., 2008). The effect of water stress on sunburn will be discussed in more detail in the next section.

Fruit size was reduced under water stress in 'Golden Delicious' (Naor et al., 2008) and in 'Braeburn' (Mpelasoka et al., 2001a). Water stress during the rapid fruit growth phase has a greater negative effect on fruit size (Mpelasoka et al., 2001a). The reduction in fruit size can be linked to a negative effect on the fruit growth rate, as found in 'Redspur Delicious' under water stress (Ebel et al., 1995b). Water stress has, however, been reported to increase fruit firmness in 'Fuji' (Leib et al., 2006) and 'Braeburn' (Kilili et al., 1996; Mpelasoka et al., 2001a). The increase in fruit firmness may be the direct result of smaller fruit, with smaller fruit being reported to have higher firmness (Mpelasoka et al., 2001a).

Water stress increased the concentration of total soluble solids, but had an inconsistent effect on titratable acidity in 'Fuji' (Leib et al., 2006). Mpelasoka et al. (2001b) also observed that water stress under deficit irrigation increased total soluble solids, but had little or no effect on total acidity in 'Braeburn'. Starch degradation was delayed under water stress in 'Braeburn' (Mpelasoka et al., 2001b) and in 'Delicious' (Ebel et al., 1993). This contrasts with earlier sugar accumulation in 'Braeburn' under water stress (Mills et al., 1994).

Water stress improved fruit color by enhancing red pigmentation in 'Braeburn' (Kilili et al., 1996; Mills et al., 1994) and in 'Autumn Rose Fuji' (Fallahi et al., 2010). However, in 'Red Delicious', fruit color was not affected by regulated deficit irrigation (Ebel et al., 1993). Increased bitterpit and a blotchy appearance were observed in 'Jonagold' apples that were exposed to a combination of water stress and high temperatures (Schrader et al., 2003). Calcium

deficiency is reported to cause bitterpit and under water stress, when calcium uptake is not optimal (Schrader et al., 2003). However, Mills et al. (1994) reported no incidence of any fruit disorders from water stressed trees that received no irrigation throughout the season either at harvest or after 12 weeks of 0 °C storage in 'Braeburn'.

7 Water stress and sunburn development in apple fruit

7.1 Sunburn types and main causal factors.

Three types of sunburn have been identified, namely sunburn necrosis, sunburn browning (Schrader et al., 2001) and photo oxidative sunburn (Felicetti and Schrader, 2008b; Schrader et al., 2003). Both sunburn browning and photo oxidative sunburn can eventually deteriorate into sunburn necrosis (Racsko and Schrader, 2012). Sunburn necrosis occurs in the orchard when fruit surface temperature reaches 52°C for a minimum period of 10 minutes (Schrader et al., 2001) and can be induced in sunlight or experimentally in the dark at the same fruit surface temperature (Schrader et al., 2001). It manifests as a dark brown or black necrotic lesion due to thermal death of tissue (Felicetti and Schrader, 2008a). Membrane integrity is destroyed and protein denaturation occurs at such high temperature resulting in cell death (Racsko and Schrader, 2010). Sunburn browning is the most prevalent sunburn type in apples characterized by yellowing or browning discoloration on the fruit surface (Felicetti and Schrader, 2008a). It was shown to be induced by ultraviolet-B (UVB) radiation at threshold temperatures of 46°C to 49°C depending on the cultivar (Schrader et al., 2001), although Hengari (2015) found that UVB only affected the photosystems of shaded apple peel. The discoloration associated with sunburn browning is due to degradation of chlorophylls and anthocyanins, xanthophyll cycle pool upregulation, carotenoid synthesis and phenolic accumulation (Felicetti and Schrader, 2008b; Hengari, 2015; Racsko and Schrader, 2012; Zhang et al., 2015). Photo oxidative sunburn occurs when previously shaded fruit are suddenly exposed to solar radiation and occurs even at fruit surface temperature below 31 °C (Felicetti and Schrader, 2008b; Schrader et al., 2003). Sudden exposure to solar radiation can occur after hand thinning, selective picking, shifting of branches as fruit load increases and on apples left uncovered in bins after harvest (Racsko and Schrader, 2012). The fruit surface is bleached, eventually turning whitish (Felicetti and Schrader, 2008b). Photo oxidative sunburn is induced by solar radiation in the visible range resulting in the

production of reactive oxygen species and singlet oxygen. UV-A and UV-B does not play a role in the formation of photo-oxidative symptoms (Felicetti and Schrader 2008b; Racsko and Schrader, 2012).

In terms of cultivars ‘Granny Smith’ and ‘Jonagold’ are considered more sensitive to sunburn compared to ‘Braeburn’, ‘Fuji’, ‘Golden Delicious’, ‘Boskoop’ and ‘Delicious’ whilst ‘Cripps’ Pink’, ‘Idared’ and ‘Topaz’ are least sensitive (Racsko and Schrader, 2012 and references therein). However, although some cultivars incur high sunburn losses, the incidence of sunburn and actual sunburn sensitivity does not necessarily correlate (Hengari, 2015). This is because factors such as tree architecture and cultivar bearing habit, harvest period, and masking by anthocyanin (in red cultivars) can all affect the incidence of sunburn.

7.2 Evidence for link between water stress and sunburn in apple.

Water stress has been reported to result in increased sunburn development in apple fruit (Kader, 2002; Makedredza et al., 2013; Piskolczi et al., 2004; Schrader et al. 2003; Woolf and Ferguson, 2000; Yuri et al., 2004). All these citations, apart from the study of Makedredza et al. (2013), were captured in the apple sunburn review of Racsko and Schrader (2012). Unfortunately, upon scrutiny, it is clear that none of these reported studies provided any empirical proof for their finding. Makedredza et al. (2013) concluded that that water stress aggravates sunburn development under conditions conducive for its development by increasing fruit surface temperature. However, these authors did not study the actual mechanism by which water deficit caused the increase in sunburn. Both partial root zone drying and deficit irrigation were reported to have no effect on sunburn in ‘Pink Lady’ (O’Connell and Goodwin, 1995). Schrader et al. (2003), however, reported that fruit on trees lacking vigor and on trees under water deficit are more likely to experience sunburn.

7.3 Possible ways in which plant water status may affect sunburn

7.3.1 Effect on the fruit heat balance

The fruit surface heat balance determines the extent of sunburn development in apples (Evans, 2004; Li et al., 2014). Fruit surface temperature is largely determined by radiation flux density

and wind velocity and to a smaller extent by fruit size, albedo and transpiration (Piskolczi et al., 2004). Li et al (2014) described the heat energy balance of the fruit surface during growth by specifying the normal heat flux at any point on the fruit surface to be:

$$R_{abs} = \lambda_e + H + R_e + E_f$$

where R_{abs} is the total incoming radiation (W/m^2), R_e is the emitted thermal radiation by the fruit (W/m^2), λ_e is the loss of energy by evaporation (latent heat loss), H is the loss or gain of sensible energy by convection and E_f is the total heat transfer within the fruit, including that from the plant to the fruit, the fruit temperature gradient, and metabolic activity. Under clear skies during the day, there is always a portion of exposed fruit surface receiving direct radiation (Smart and Sinclair, 1976; Thorpe, 1974). The λ_e is driven by the water vapor pressure deficit VPD between the surface and the surrounding air, and by the surface conductance to water vapor diffusion (Monteith and Unsworth, 1990), whilst H is a function of temperature difference between the fruit surface and the surrounding air (Sadreau et al., 2007). The fruit heat balance model is represented schematically in Figure 1.

Apple fruit can lose water via transpiration from the fruit surface (Gibert et al., 2005; Maguire et al., 2000). Compared to leaves, fruit have a much smaller surface to volume ratio resulting in lower net gas exchange and transpiration (Aschan and Pfan, 2003; Blanke and Lenz, 1989). Stomata, lenticels and microcracks form part of the transpiration pathway and contribute to fruit surface conductance (Blanke, 1995; Gibert et al., 2005). Water loss from apple fruit is dependent mainly on stomata in young fruit and on open lenticels and microcracks in mature fruit (Gibert et al., 2005; Maguire et al., 1999a, Veraverbeke et al., 2003a, 2003b). Stomata are present on the outer epidermis of young fruit, but at a 10 to 100-fold lower frequency compared to the leaf epidermis of the same species (Blanke and Lenz, 1989). Water loss in apple fruit is minimized by the presence of a cuticle, otherwise they will desiccate quickly (Kornaska, 2012). This might lead to temperature build up and occurrence of physiological disorders such as sunburn since the cooling capacity of the fruit is limited (Colaizzi et al., 2012). Results from modelling experiments by Li et al (2014), however, found fruit transpiration to be negligible compared to other sources of heat flux from fruit surface like convection and emitted radiation. Apple fruit have been reported to have high resistance to water vapor diffusion ($5000\text{-}7000 \text{ s cm}^{-1}$),

compared to grapes ($120\text{-}170 \text{ s cm}^{-1}$) and peach (33 s cm^{-1}) (Nobel, 1975), which might explain the negligible transpiration.

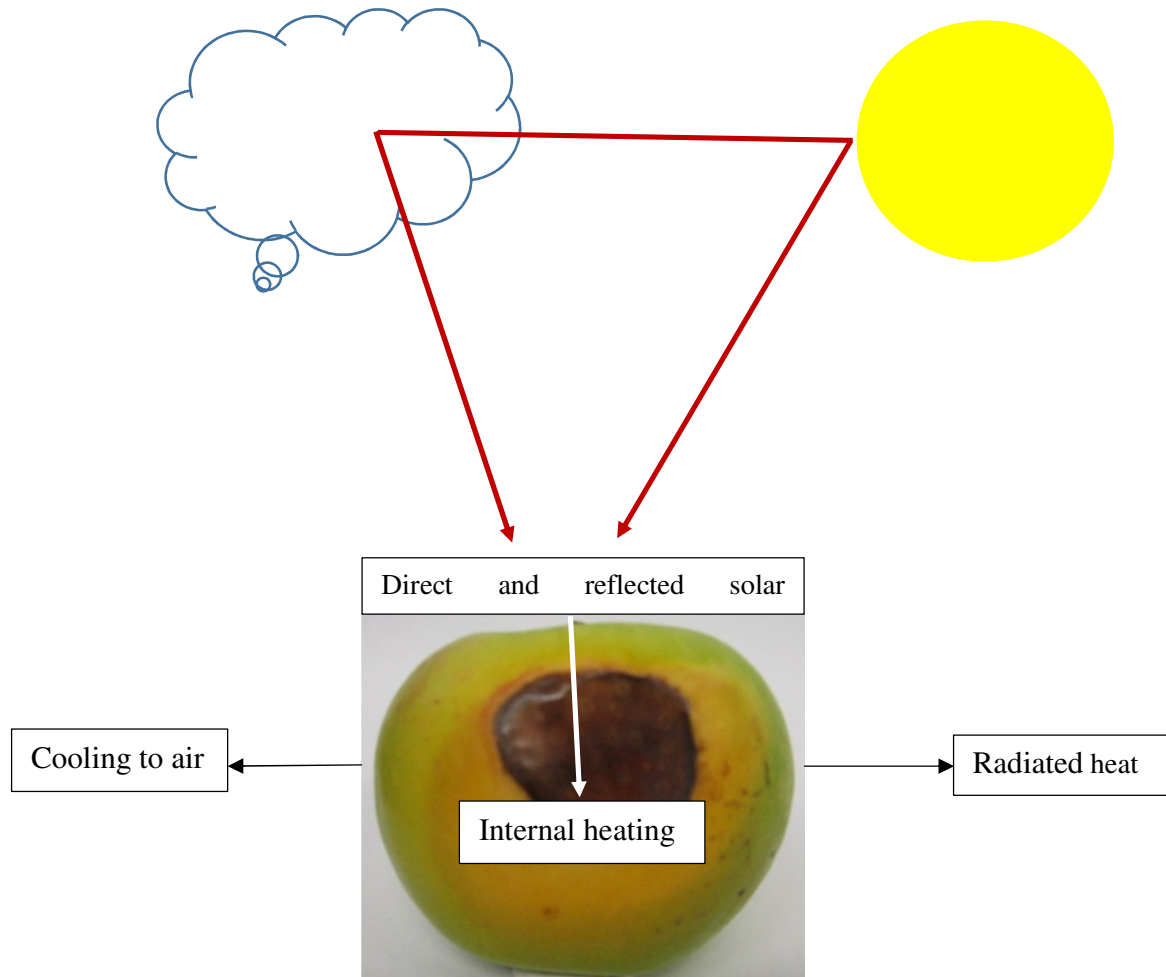


Figure 1. Energy balance model of apple fruit. Adapted from (http://www.tfrec.wsu.edu/pages/webdev/Apple_sunburn)

Water stress may indirectly increase the fruit surface temperature through reducing leaf transpiration. Closure of leaf stomata decreases evaporative cooling of the leaf surfaces resulting in an increase in canopy temperature (Colaizzi et al., 2012). This may decrease the ability of the fruit surface to radiate or lose heat to the surrounding air.

7.3.2 Excess radiation, plant water status and sunburn

Photoinhibition is the reduction in photosynthetic capacity of leaves due to excess radiation. The extent of damage caused by photoinhibition depends mainly on the proportion of solar radiation being absorbed in excess of the capacity of the photosynthetic organism to use that energy in photochemistry (Pastenes et al., 2005). High irradiance during periods of water stress can lead to photoinhibitory damage of photosystem II (PSII) (Jones 2014). Pastenes et al. (2005) reported that photoinhibition in bean is exacerbated under water stress due to the closure of stomata, limiting CO₂ availability at the carboxylation sites in the chloroplasts and, therefore, resulting in excessive excitation of the photosynthetic apparatus, particularly photosystem II (PSII). Photoinhibition damage of PSII of *Lycopersicon esculentum* Mill., *Solanum tuberosum* L. and *S. nigrum* L. was more pronounced in water-stressed leaves than in well-watered controls under intense solar radiation (Havaux, 1992). Water stress causes the rapid dephosphorylation of PSII proteins in barley. Since phosphorylation plays a major role in PSII repair, PSII damage is more pronounced under water stress combined with excessive solar radiation levels (Liu et al., 2009).

7.3.3 Physical responses to water stress

Plants have evolved mechanisms to protect against photoinhibition such as chloroplast movement, changes in chlorophyll concentration, increased antioxidant capacity, xanthophyll cycle-dependent energy dissipation, paraheliotropism and leaf rolling (Kadioglu et al., 2012; Pastenes et al., 2005). Paraheliotropism and leaf rolling are leaf movements that occur as an adaptive response to water deficit in plants (Kadioglu et al., 2012). Paraheliotropism entails the reorientation of the leaf blade to a more vertical position to avoid high irradiance thereby reducing photoinhibition, as has been observed under water deficit in beans (Pastenes et al. 2004; Yu and Berg, 1994). Paraheliotropic leaf movement have also been associated with long term water deficit in the presence of high irradiance (Yu and Berg 1994). Leaf rolling is a dehydration avoidance mechanism that protects leaves from the effects of photodamage under water deficit by reducing the leaf surface area exposed to solar radiation (Kadioglu and Terzi 2007). The reduction in the amount of excess solar radiation reaching the leaf surface helps protect the photosynthetic apparatus from damage during water stress (Kadioglu et al., 2012). Although there is no published literature on paraheliotropic leaf movement or leaf rolling in apple, it could

potentially lead to increased sunburn incidence in water stressed apples due to exposure of previously shaded fruit. Leaf rolling was observed in ‘Rosy Glow’ apples on M9 rootstock in the Witzenberg Valley in South Africa, while a section of the same orchard under 20% white shade net did not show any leaf rolling (W Steyn, personal observation, 2015).

8 Conclusion

The ecophysiological, biophysical and biochemical responses of apple tree to water stress are complex, working in tandem to protect the tree under conditions of water stress. These responses are often interlinked making it difficult to deduce how they are related to each other. Ultimately, tree water stress affects yield and fruit quality in apple fruit. There is limited information about the relationship between tree water status and sunburn development in apple tree. The possible effects of water stress on sunburn development in apple fruit is poorly understood and not well researched. There is only one scientific publication that attempted to establish the link between tree water stress and sunburn development in apple. Other published data generally entails only casual mentions of a link between water deficit and sunburn development in apple – these papers do not provide any empirical proof that water deficit increases sunburn in apple, nor do they cast any light of the potential mechanism(s) by which water deficit may increase sunburn. It is not known how water deficit affects fruit peel biochemistry and the resulting potential for sunburn development. No information is available on the relationship between tree ecophysiological parameters and water stress in terms of influence on sunburn development. There is clearly a need to address these knowledge gaps on the relationship between sunburn development and water deficit.

9. References

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Paper 1: The relation between water deficit and sunburn development in ‘Granny Smith’ and ‘Cripps’ Pink’ apple fruit.

Abstract

Water stress has been reported to increase sunburn development in apple fruit, but with little empirical proof and no data on how plant water status may relate to sunburn development. The aim of this research was to verify the effect of water deficit on sunburn development in apple and to test the hypothesis that water deficit predisposes apple peel photosystems to light stress thereby facilitating sunburn development. Water deficit was imposed during the 2013–2014 growing season by manipulating irrigation from 10–24 March and 13–18 April in mature ‘Granny Smith’ and ‘Cripps’ Pink’ apple trees, respectively. ‘Granny Smith’ treatments consisted of: 1) normal irrigation with nozzle flow rate of 35 L h⁻¹ hereafter called the control; 2) reduced irrigation by changing the nozzle to give a flow rate of 25 L h⁻¹ hereafter called 70% irrigation and; 3) no water hereafter called no irrigation. In ‘Cripps’ Pink’, two treatments, viz. 1) normal irrigation with nozzle flow rate of 37 L h⁻¹ hereafter called the control, and; 2) no irrigation were applied. Soil water status was measured to quantify the treatments while canopy microclimate, leaf and peel photochemical efficiency, leaf gas exchange, stem water potential, peel antioxidants and peel pigmentation. Trees were subjected to different levels of water deficit. Gas exchange, photochemical efficiency and midday stem water potential measurements in ‘Granny Smith’ were taken on 10, 17, and 24 Mar 2014 (hereafter called day 0, 7 and 14, respectively). In ‘Cripps’ Pink’, these measurements were taken only on 18 Apr 2014 (day 5). Sunburn, peel antioxidants and peel pigmentation was assessed at harvest. Volumetric soil water content steadily declined over the course of the experiment and stem water potential was lower in the no irrigation treatment in both cultivars. ‘Cripps’ Pink’ had higher sunburn severity and ‘Granny Smith’ had higher sunburn necrosis and incidence in no irrigation treatments. Fruit surface temperature was increased in the no irrigation treatment in both cultivars, whilst leaf temperature was not affected. The increase in fruit surface temperature did not relate to changes in canopy microclimate and decreased fruit

surface evapotranspiration was also ruled out as a cause. Maximal photochemical efficiency (F_v/F_m) and actual photochemical efficiency (oPSII) were reduced under water deficit in ‘Granny Smith’ leaves, whilst in ‘Cripps’ Pink’ leaves only F_v/F_m was reduced under water deficit with no effect on the other chlorophyll fluorescence parameters. In the fruit peel, F_v/F_m and photochemical quenching (qP) were reduced under water deficit in ‘Granny Smith’, whilst in ‘Cripps’ Pink’ only F_v/F_m was reduced. These changes, although slight, together with increased levels of antioxidants in the apple peel, suggest that water deficit caused excess excitation of PS II resulting in oxidative stress that increased sunburn.

1. Introduction

South Africa was the sixteenth biggest apple producer in the world in 2013 at ca. 812 million tons (HORTGRO, 2016). About 44% of the crop is exported, 27% is sold on the local market and 30% is processed into juice. In 2014/15, the net value of a ton of export and processed apples was R10 689 and R1 142 respectively (HORTGRO, 2016). The downgrading of fruit for processing therefore constitutes a major loss in potential income. Sunburn, the discoloration or necrosis of the apple peel in response to high irradiance and high temperature (Racsko and Schrader, 2012) is the major reason for this downgrading of apple fruit to lower value classes. Wand et al. (2006) reported that sunburn on apples in the Western Cape can result in yield losses of export quality fruit of up to 50%. South African apples are produced at lower latitude compared to other major producers under the Mediterranean-type climate of the Western Cape Province. The high temperatures and high irradiance experienced during the growing season (Sept. to Apr.) are conducive to the development of sunburn.

The direct factors, i.e. light and temperature, involved in sunburn development, the biochemical changes giving rise to sunburn symptoms, the various defense mechanisms as well as cultural management practices that can be employed to decrease sunburn in apple has received considerable research attention (reviews in Racsko and Schrader, 2012). However, very little is known about how water deficit and plant water status affects sunburn in apple. Racsko and Schrader (2012) cite several authors who reported increased sunburn development in apple fruit in trees under water stress (Schrader et al. 2003a; Woolf and Ferguson, 2000; Yuri et al., 2004). However, none of these studies provided any empirical proof for their findings. Makedredza et al.

(2013), in a study where irrigated apple trees were compared with trees from which water was withheld for 14 days found evidence of increased sunburn in response to water deficit. They linked the increase in sunburn to increased fruit surface temperatures on trees that did not receive irrigation, but failed to study the actual mechanism by which water deficit caused the increase in sunburn. Both partial root zone drying and deficit irrigation were reported to have no effect on sunburn in 'Pink Lady' (O'Connell and Goodwin, 1995). Schrader et al. (2003a), however, reported that fruit on trees lacking vigor and on trees under water deficit are more likely to experience sunburn.

Water deficit and water stress are terms commonly used when studying plant water relations. Water deficit occurs either when there is not enough available soil water for plant uptake or when the rate of transpiration exceeds the rate of water uptake (Bray, 1997; Dodd and Ryan, 2016; Lisar et al., 2012). Due to transpirational water loss, plants are seldom fully hydrated and therefore suffer from water deficits (Shao et al., 2008). There is an absence of a clear definition of water stress in plants. Dodd and Ryan (2016) defined stress in plants as any external factor that negatively influences plant growth, productivity, reproductive capacity or survival. Hence, in the context of commercial fruit production, water deficit that impacts negatively on plant growth and productivity can be considered as water stress. Water stress affects many biophysical, physiological, biochemical and molecular processes in plants (Šircelj et al., 2005). However, the complexity and interlinked nature of these processes makes it difficult to deduce how they are related to each other and how they act to modify tree physiology, productivity and fruit quality (Morandi et al., 2014).

The aim of the research reported in this paper was to verify the results of Makedredza et al. (2013) regarding the effect of water deficit on sunburn development in 'Granny Smith' and 'Cripps' Pink' apple and then to expand on this previous research by testing the hypothesis that water deficit predisposes apple peel photosystems to light stress thereby facilitating sunburn development. We also assessed the possibility that water deficit could increase the peel temperature by its effect on the canopy microclimate. The effect of short term induced water deficit on soil water status, leaf and fruit photochemistry, gas exchange and fruit biochemistry was assessed.

2. Materials and methods

2.1 Study sites and plant material

2.1.1 ‘Granny Smith’

The trial was conducted during the 2013–2014 growing season in a mature ‘Granny Smith’ orchard at Oak Valley Estate in Grabouw, South Africa (Latitude: 34°15’S; Longitude: 19°07’E, Alt. 375 m). The orchard was planted in 1998 on MM109 rootstock at a spacing of 4 m x 1.5 m, a north - south row orientation and at a slope of about 4%. The trees were approximately 3.6 m tall, 2 m wide, and had a conical shape. Normal commercial cultural practices were followed over the course of the experiment. The orchard soil was classified as Pinedene form with a sandy loam texture and 35% stone (Soil Classification Working Group, 1991). Clean cultivation was practiced on the tree row and mulch was not applied. Although no statistical data exists, ‘Granny Smith’ is widely considered to be the apple cultivar that incurs most losses due to sunburn under South African conditions.

2.1.2 ‘Cripps’ Pink’

The trial was conducted during the 2013–2014 growing season in a mature ‘Cripps’ Pink’ orchard at Stellenbosch University’s Welgevallen Experimental Farm (Latitude: 33°56’S; Longitude: 18°52’E, Alt. 165 m). The orchard was planted in 1998 on M793 rootstock at a spacing of 4 m x 1.5 m, a north-east by south-west row orientation and a slope of about 4%. The trees were approximately 3.2 m tall and conical in shape, with a width of approximately 1.8 m at 1.5 m above the ground. Normal commercial cultural practices were followed over the course of the experiment. The orchard soil was classified as Oakleaf form, a cambisol with a high clay percentage and a high water-holding capacity (Soil Classification Working Group, 1991). Clean cultivation was practiced on the tree row and mulch was not applied. ‘Cripps’ Pink’ was used because it is a late season cultivar that allowed us to supplement data from the ‘Granny Smith’ trial. Also, it was the same cultivar (in the same orchard) that Makedredza et al. (2013) previously used to study the effect of water deficit on sunburn.

2.2 Experimental design and treatments

2.2.1 ‘Granny Smith’

The trial was carried out over two weeks from 10 to 24 Mar. 2014. Three treatments used were viz: 1) normal irrigation with nozzle flow rate of 35 L h^{-1} , hereafter called the control; 2) reduced irrigation by changing the nozzle to give a flow rate of 25 L h^{-1} , hereafter called 70% irrigation, and; 3) no water, hereafter called no irrigation. The no irrigation treatment was achieved by blocking micro-sprinkler emitters with stoppers. Three trees were used per plot with a buffer tree in between. The normal irrigation was applied according to the scheduling of the farm. Each treatment was replicated six times in a complete randomized block design.

The normal irrigation scheduling at Oak Valley Estate during the experiment was 35 L h^{-1} for one and half hours three times a week, applied using Ein-Tal micro-sprinklers (Rain-Tal Ltd., Or-Akiva, Israel) spaced 1.5 m along the irrigation line and placed midway between two trees. However, since the trial was conducted close to harvest, only one irrigation event was recorded and there was also rainfall events which resulted in adjustment in the irrigation schedule. Given this application rate, the control treatment was therefore unlikely to be over or under-irrigated since a well-watered fully grown apple tree in South Africa typically transpires between 20 and 30 liters of water per day during hot and dry weather (Dzikiti et al [in press]; Gush and Taylor 2014; Volschenk et al., 2003).

2.2.2 ‘Cripps’ Pink’

The trial was done over five days from 13 to 18 Apr. 2014. Two treatments used were viz: 1) normal irrigation with nozzle flow rate of 37 L h^{-1} , hereafter called the control, and; 2) no irrigation. Three trees were used per plot with a buffer tree in between. Each treatment was replicated eight times in a complete randomized block design. The normal irrigation scheduling at Welgevallen Experimental Farm during the experiment was 37 L h^{-1} for two and half hours three times a week, supplied by Dan micro-sprinklers (NaanDanJain Irrigation Lt, Kibbutz Naan, Israel) spaced 1.5 m along the irrigation line and placed midway between two trees. The no irrigation treatment was achieved by blocking the micro-sprinkler emitters with stoppers.

2.3 Soil water content

Volumetric soil water content was measured during each trial using two water content reflectometers per treatment (Model: CS616, Campbell Scientific®, Logan, Utah, USA). The sensors were inserted vertically into the soil sampling the upper 30 cm of soil profile where most of the roots were found. The probes were placed halfway between the tree trunk and the micro-sprinkler. The probes were connected to a data logger (Model: CR1000, Campbell Scientific®, Logan, Utah, USA) and the whole system was powered by a 12 V car battery. Programming of the data logger was done using the Short Cut module (Loggernet 4.1, 2010, Campbell Scientific®, Logan, Utah, USA). Data were collected with a scan interval of 30 seconds and averages stored every 2 min. Within the treatment replication, only the middle tree was used to measure soil water content, to minimize interference from adjacent treatments.

2.4 Weather data and canopy microclimatic conditions

Weather data (hourly temperature, rainfall and reference evapotranspiration) were obtained from automatic weather stations located on site at Oak Valley Estate ('Granny Smith') and at Welgevallen ('Cripps' Pink'). The weather stations were located outside the orchards. Canopy microclimates were measured using temperature and relative humidity probes connected to data loggers and housed in radiation shields attached to tree trunks within the tree canopy at 2.0 m from the ground (Tinytag Plus 2, Gemini Data Loggers, West Sussex, UK). One treatment replication per cultivar was used to monitor the canopy microclimate, located in the middle of the orchard in order to minimize edge effects. The middle tree in each plot was used to minimize interference from adjacent treatments.

2.5 Tree ecophysiological status

Ecophysiological measurements were taken on 10, 17, and 24 Mar 2014, hereafter called day 0, 7 and 14, respectively, in 'Granny Smith'. In 'Cripps' Pink', measurements were taken only on 18 Apr 2014, hereafter called day 5. The 'Cripps' Pink' experiment was done in order to supplement data from 'Granny Smith', since it had rained during the execution of the latter trial.

2.5.1 Gas exchange

Gas exchange measurements were taken using an infrared gas analyzer (Model: LI-6400 Li-COR, Lincoln, Nebraska, USA). Reference carbon dioxide concentration was set at 380 ppm, leaf temperature at 25 °C, and the quantum flux of the photosynthetic photon flux density (PPFD) to 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Measurements were done on sun-exposed leaves on the western side of the tree. Two healthy leaves of the same age and size were used per treatment replication from the middle tree. Leaves were selected at shoulder height and all spot-readings were taken between 08:00 and 11:30 (Local time = GMT + 2 h).

2.5.2 Plant water status

Plant water status, measured as midday stem water potential, was assessed using a pressure bomb (Model: 600, PMS Instrument Co, Albany, Oregon, USA). Two healthy and fully expanded leaves per treatment replication from inside the tree canopy of the same age, size and proximity to the stem. Leaves were bagged for at least one hour in silver reflective bags to equilibrate the leaf and the stem's xylem water potential before readings were taken. Leaves were sampled from shoulder height and all readings were taken at midday between 12:00 and 13:30.

2.6 Leaf and fruit surface temperature

Leaf and fruit surface temperature measurements were taken on day 0, 7 and 14 in 'Granny Smith'. In 'Cripps' Pink', measurements were taken only on day 5. Leaf and fruit surface temperature was measured using a hand-held infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA) between 14:00 and 14:30. Leaf temperature was measured on sun-exposed leaves of similar size, health and age, whilst fruit surface temperature was measured on the sun-exposed part of the fruit. Five leaves and fruit per treatment replication were tagged for measurements.

2.7 Chlorophyll fluorescence

Chlorophyll fluorescence measurements were done on fruit and leaves according to the method described by Steyn et al. (2009). Measurements were made using a pulse-modulated fluorimeter (Model: FMS2, Hansatech Instruments Ltd., Norfolk, England). To obtain F_v/F_m , maximum quantum yield of photosystem II (PSII), F_o , was measured using a weak-modulating light unable

to induce photosynthesis. A saturating light pulse ($10800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD at the end of the fiber optic cable for 0.7 s) was then used to attain the maximal yield of fluorescence, F_m . Variable fluorescence, F_v , was calculated as $F_m - F_o$. Actual efficiency (photon yield) of PSII photochemistry (ϕPSII) was measured as $(F'_m - F)/F'_m$ at stepwise increments of actinic radiation levels to a maximum of $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD. Transient interruption of actinic radiation followed by irradiation with far-red radiation for 5 s was used to obtain F'_o . Photochemical quenching (qP), was determined as $(F'_m - F)/(F'_m - F'_o)$ and non-photochemical quenching of fluorescence (qNP) as $1 - (F'_m - F'_o)/(F_m - F_o)$. Healthy exposed, non-damaged leaf and fruit samples were taken from the same height and position on the branch at 17:00. Samples were stored in brown paper bags and dark adapted at 20 °C overnight with readings taken from 08:00 the following day. Measurements on fruit were done on the shaded side to avoid variation caused by possible sun damage on the exposed side of the fruit. Three leaves and fruit per tree replicate were used for F_v/F_m measurement, whilst only two leaves and fruit were used for quenching analysis per tree replicate as it was time consuming. Measurements were taken on day 0, 7 and 14 in ‘Granny Smith’ and on day 5 in ‘Cripps’ Pink’. In ‘Granny Smith’, chlorophyll fluorescence measurements were done only in control and no irrigation treatment due to time constraints.

2.7 Peel chemical composition

Peel chemical composition was assessed on samples collected on day 0, 7 and 14 in ‘Granny Smith’ and day 5 in ‘Cripps’ Pink’. Fruit peel tissue was separated from the flesh for chemical analysis. The collected peel tissue samples were flash frozen in liquid nitrogen before being finely milled. Milled samples were stored in a freezer at -80 °C. Ascorbic acid, glutathione and total phenolic concentration were determined for both cultivars, whilst total chlorophyll and carotenoid concentration were determined in ‘Granny Smith’ and total anthocyanin concentration in ‘Cripps’ Pink’. ‘Granny Smith’ fruit only contains anthocyanins under exceptional circumstances.

2.7.1 Ascorbic acid and glutathione

High - Performance Liquid Chromatography (HPLC) was used for ascorbic acid and glutathione analysis. The analysis was done in three phases according to the method by Jooste (2012) with

some minor modifications. In the extraction phase, 10 ml of extraction buffer was added to 2 g of fresh frozen sample in a centrifuge tube. The extraction buffer was made up of 3% metaphosphoric acid (MPA), 1 mM ethylenediaminetetracetic acid (EDTA) and 2% insoluble polyvinylpyrrolidone (PVPP). The mixture was vortexed and left to stand for 15 min, after which 1.8 ml was pipetted into a 2 ml Eppendorf tube and then centrifuged at 20 000 x g for 15 min. The subsequent analysis phase was done in two steps. The first step involved direct analysis of clean supernatant to identify the reduced forms of ascorbic acid and glutathione. Second step involved reducing ascorbic acid and glutathione from oxidized forms using 20 μ l of 400 mM DL-dithiothreitol (DTT) to measure the total ascorbic acid and glutathione in the sample. Oxidized ascorbic acid and glutathione in the sample was determined by subtracting reduced values from the total. HPLC analysis was done on Agilent Series 1100 HPLC system (Agilent Technologies, Inc., Waldbronn, Germany) using a photodiode array detector and C₁₈ 5- μ m stationary phase column protected by a 4.6 mm x 12.5 mm guard cartridge (Zorbax SB-C18, Agilent, USA). A known concentration of ascorbic acid and glutathione was used to identify retention peaks. The final phase involved quantification of ascorbic acid and glutathione in the sample and this was done using Chemstation for LC 3D systems software (Rev. B.10.03 (204), Agilent Technologies, Inc., Waldbronn, Germany). Results were expressed as micrograms per gram fresh weight (μ g.g⁻¹ FW).

2.7.2 Total phenolics

Total phenolics were measured by the Folin Ciocalteu's (FC) phenol colorimetric method. The method involved the addition of 5 mL of 80% ethanol to 1 g fresh frozen sample in a 50 ml centrifuge tube. The mixture was then ground finely with Ultra Turrax. A magnetic rod was added to the mixture and the tubes were placed in a fridge at 4 °C where they were constantly stirred using a magnetic stirrer. In triplicates 10 μ l of the sample, 40 μ l of 80% ethanol and 450 μ l of 0.1M FC reagent were added to plastic cuvettes. After 5 min, 500 μ l of 5.6% Na₂CO₃ was added to the cuvette and the mixture vortexed after which it was left to stand for 90 min before readings were taken. The spectrophotometer was blanked with 80% ethanol and a standard curve of Gallic acid (mg L⁻¹) from 0 to 2.0 was constructed. Total phenolic concentration was expressed as Gallic acid equivalent (GAE) in milligrams per gram fresh weight (mg.g⁻¹ FW).

2.7.3 Total chlorophyll and carotenoids

Total chlorophyll and carotenoids were analyzed according to the method of Lichtenthaler (1987) with some modifications. The method involved the addition of 3 ml cold, 100% acetone to 0.5 g fresh frozen sample in a 50 ml centrifuge tube. A magnetic rod was added to the mixture and the tube was placed in a fridge at 4 °C, where it was constantly stirred using a magnetic stirrer for 24 hours. After extraction, the sample was then centrifuged for 15 min at 3500 rpm. The supernatant was decanted and 2 ml of cold, 100% acetone was added to the remaining pellet. The sample was then centrifuged again for 15 min at 3500 rpm and the clean extract decanted into the vial with the first supernatant. This final supernatant was filtered using a 0.45 µm filter (Millex-HV, Millipore, Corporation, Milford, MA, USA) into a glass cuvette. Absorbance was measured at 470, 645 and 670 nm on the spectrophotometer (Cary 50 Bio, Varian, Australia (PTY) Ltd, Melbourne, Australia). The spectrophotometer was blanked with 100% cold acetone beforehand. The extinction coefficients of Lichtenthaler (1987) were used to calculate the chlorophyll and carotenoid concentrations in micrograms per gram fresh weight ($\mu\text{g}\cdot\text{g}^{-1}$ FW). The analysis was done in dim light, since chlorophyll is degraded at high light intensity.

2.7.4 Total anthocyanin

Total anthocyanins were extracted using acidified methanol (95 ml methanol: 5 ml 3 mol/HCL) as an extraction solvent. The method involved the addition of 5 ml cold extraction solvent to 2.0 g fresh frozen sample in a 50 ml centrifuge tube. A magnetic rod was added to the mixture and the tube was placed in a fridge at 4 °C, where it was constantly stirred using a magnetic stirrer for one h. After extraction, the sample was then centrifuged for 10 min at 10000 rpm. The supernatant was decanted into a glass vial which was kept in a fridge and 2 ml of cold, 100% acetone was added to the remaining pellet. The sample was then centrifuged again for 10 min at 10000 rpm and the clean extract decanted into the vial with the first supernatant. This final supernatant was filtered using a 0.45 µm filter (Millex-HV, Millipore, Corporation, Milford, MA, USA) into a plastic cuvette. Absorbance (A) was measured at 530 and 653 nm on the spectrophotometer (Cary 50 Bio, Varian, Australia (PTY) Ltd, Melbourne, Australia). The spectrophotometer was blanked with extraction solvent beforehand. Results were expressed as $\Delta A_{(530-653)} \text{g}^{-1}$ fresh weight (ABS).

2.8 Sunburn assessment

Sunburn was assessed on days 0, 7 and 14 on ‘Granny Smith’ and days 0 and 5 on ‘Cripps’ Pink’. The experiments were done late in the season, when most of the fruit already showed sunburn symptoms. Therefore, previously unexposed fruit was exposed to the sun by pruning and used for sunburn assessment. Ten fruit per tree replicate were tagged for sunburn assessment. Sunburn incidence and severity were assessed using the Schrader and McPerson scale (Schrader et al., 2003b), where 0 represented no sunburn, 1 to 4 refer to different stages of sunburn browning and 5 signifies sunburn necrosis (Appendix 1).

2.9 Statistical analysis

Data were analyzed using the General Linear Models (GLM) procedure of SAS Enterprise Guide 3.0 (SAS Institute Inc., 2004, Cary, NC, USA). Where significant differences occurred ($p \leq 0.05$), means were separated by the Least Significant Difference (LSD). Linear and quadratic contrasts were fitted where applicable.

3. Results

3.1 ‘Granny Smith’

3.1.1 Weather conditions, soil moisture and canopy environment

Daily maximum temperatures ranged from 18.7 to 29.4 °C, while total daily reference evapotranspiration ranged from 1.81 to 3.89 mm during the trial (Figure 1). Rainfall was recorded twice with 1.6 mm on 12 Mar 2014 and 6.4 mm on 19 Mar 2014 (Figure 1). Volumetric soil water content was similar between treatments at the beginning of the trial but increased in the control and 70% irrigation treatment due to an irrigation event on 12 Mar 2014, whilst in the no irrigation treatment, it steadily declined over time (Figure 2). On 19 Mar 2014, all three treatments recorded an increase in volumetric soil water content due to precipitation in the morning and in the early evening (Figure 2). Only one irrigation event was recorded, since it rained during the experiment and also irrigation scheduling was adjusted since it was close to harvest. There were no discernible differences in the daily patterns of canopy air temperature,

relative humidity and the air's vapor pressure deficit over the duration of the trial (Figures 3, 4 and 5).

3.1.2 Net gas exchange, stem water potential, leaf temperature and fruit surface temperature

There were no significant differences between the treatments in net carbon assimilation, stomatal conductance, transpiration rate, stem water potential, leaf temperature and fruit surface temperature on day 0 (Table 1). Stomatal conductance and transpiration rate showed a quadratic trend on day 0, being lowest in the 70% irrigation treatment compared to the control and no irrigation treatments, but these cannot be explained since treatments had not started (Table 1). Net carbon assimilation rate and stem water potential were significantly lower in the no irrigation treatment compared to the control and 70% irrigation treatments on days 7 and 14 (Table 1). Fruit surface temperature was significantly higher in the no irrigation treatment compared to control and 70% irrigation on days 7 and 14 (Table 1). No significant differences were observed between treatments and control in stomatal conductance, transpiration and leaf temperature on days 7 and 14 (Table 1). Net carbon assimilation rate and stem water potential decreased linearly with reduction in irrigation on days 7 and 14 (Table 1). Leaf and fruit surface temperature increased linearly with a reduction in irrigation on day 14 (Table 1).

3.1.3 Leaf and fruit chlorophyll fluorescence

There were no significant differences in leaf F_v/F_m , Φ PSII efficiency, qP and qNP between the control and no irrigation treatments on days 0 and 7 (Table 2). F_v/F_m and Φ PSII efficiency were significantly lower in the no irrigation treatment compared to the control on day 14 (Table 2). No significant differences were observed in qP and qNP between the control and no irrigation treatments on day 14 (Table 2).

There were no significant differences in fruit peel F_v/F_m , Φ PSII efficiency, qP and qNP between the control and no irrigation treatments on days 0 and 7 (Table 3). F_v/F_m and qP were significantly lower in the no irrigation treatment compared to the control on day 14 (Table 3). No significant differences were observed in Φ PSII efficiency and qNP between the control and no irrigation treatments on day 14 (Table 3).

3.1.4 Peel chemical composition

There were no significant differences in total ascorbic acid, reduced ascorbic acid, oxidized ascorbic acid, total glutathione, reduced glutathione and oxidized glutathione concentration between the treatments on day 0 (Table 4). Reduced ascorbic acid increased linearly, whilst oxidized ascorbic acid decreased on day 0 with reduction in irrigation (Table 4). These apparent differences at the onset of the trial should be considered in the interpretation of results obtained after treatments were affected. Oxidized ascorbic acid was significantly higher in the no irrigation treatment compared to the control and 70% irrigation treatments on day 7 (Table 4). No significant differences were observed in total ascorbic acid, reduced ascorbic acid, total glutathione, reduced glutathione and oxidized glutathione concentration between the treatments on day 7 (Table 4). There was a linear increase in total ascorbic acid, oxidized ascorbic acid and total glutathione with decreasing amount of irrigation water on day 7 (Table 4). There were no significant differences in total ascorbic acid, reduced ascorbic acid, oxidized ascorbic acid, total glutathione, reduced glutathione and oxidized glutathione concentration between the control and treatments on day 14 (Table 4). Reduced ascorbic acid showed a quadratic trend on day 14, being highest in the 70% irrigation treatment compared to the control and no irrigation treatments (Table 4).

No significant differences were observed in total chlorophylls, total carotenoids and total phenolic concentrations between the treatments on day 0 (Table 5). Total chlorophyll concentration was significantly lower in the no irrigation treatment compared to the control and 70% irrigation treatments on day 7 (Table 5). Total carotenoid concentrations were significantly lower in the no irrigation and 70% irrigation treatments compared to the control on day 7 (Table 5). There was no significant difference in total phenolic concentration between the treatments on day 7 (Table 5). Total chlorophyll and total carotenoid concentrations decreased linearly with decreasing amounts of irrigation water on day 7 (Table 5). There were no significant differences in total chlorophyll, total carotenoid and total phenolic concentrations between the treatments on day 14 (Table 5).

3.1.5 Sunburn incidence

There were no significant differences in sunburn severity of all fruit, sunburn severity of sunburnt fruit, and percentage sunburn browning and sunburn incidence between the treatments on day 0 (Table 6). No sunburn necrosis was recorded on day 0. Sunburn severity of all fruit, sunburn severity of sunburnt fruit, sunburn browning and sunburn necrosis did not differ significantly between the treatments on day 7 (Table 6). Sunburn incidence was significantly higher in the 70% and no irrigation treatments compared to the control on day 7 (Table 6). Sunburn severity of all fruit, sunburn browning and sunburn incidence increased linearly with reduction in amount of irrigation on day 7 (Table 6). Sunburn severity of all fruit, sunburn severity of sunburnt fruit, sunburn browning and sunburn incidence did not differ significantly between the treatments on day 14 (Table 6). Sunburn necrosis was significantly higher in the no irrigation treatment compared to the control and 70% irrigation treatments on day 14 (Table 6). Sunburn severity of all fruit, sunburn severity of sunburnt fruit and sunburn necrosis increased linearly with reduction in amount of irrigation on day 14 (Table 6). There was a high percentage of photooxidative sunburn, since previously unexposed fruit were exposed by pruning and used for sunburn assessment (Figure 6). The photooxidative sunburn eventually deteriorated into sunburn necrosis.

3.2 ‘Cripps’ Pink’

3.2.1 Weather conditions, soil moisture and canopy environment

Daily maximum temperatures ranged from 21.9 to 35.1 °C while the reference evapotranspiration ranged from 2.76 to 3.86 mm during the trial (Figure 7). Initial volumetric soil water content was similar at the beginning of the trial but increased in the control due to two irrigation events on 14 Apr. and 16 Apr., whilst in the no irrigation treatment it steadily declined over time (Figure 8). There were no discernible differences in canopy air temperature, relative humidity and vapor pressure deficit for the duration of the trial (Figures 9, 10 and 11).

3.2.2 Stem water potential, net gas exchange, leaf temperature and fruit surface temperature

Stem water potential, net carbon assimilation rate and stomatal conductance were significantly lower in the no irrigation treatment compared to the control on day 5 (Table 7). Although not significant ($p=0.0516$) transpiration rate was lower in the no irrigation treatment compared to the control (Table 7). No difference in leaf temperature was observed between the no irrigation treatment and the control (Table 7). Fruit surface temperature was significantly higher in the no irrigation treatment compared to the control (Table 7).

3.2.3 Chlorophyll fluorescence

There were no significant differences in F_v/F_m , ϕ PSII efficiency, qP and qNP between the no irrigation treatment and the control when measured in the fruit peel on day 5 (Table 8). In the leaves, F_v/F_m was significantly lower in the no irrigation treatment compared to the control (Table 8). ϕ PSII efficiency, qP and qNP did not differ between the no irrigation treatment and the control in leaves (Table 8).

3.2.4 Peel chemical composition

Total ascorbic acid and reduced ascorbic acid concentrations were significantly increased in the no irrigation treatment compared to the control (Table 9). There were no significant differences in oxidized ascorbic acid, total glutathione, reduced glutathione and oxidized glutathione concentrations between the no irrigation treatment and control (Table 9). Total phenolic concentration was significantly higher in the no irrigation treatment compared to the control (Table 10). There was no difference between the no irrigation treatment and the control in total anthocyanin concentration (Table 10).

3.2.5 Sunburn incidence

No significant differences in sunburn severity of all fruit and of sunburnt fruit, and percentage sunburn browning, necrosis and incidence were observed on day 0 between the no irrigation treatment and the control (Table 11). Sunburn severity of all fruit and of sunburnt fruit was significantly higher in the no irrigation treatment compared to the control on day 5 (Table 11).

Although not significant ($p=0.0692$), percentage sunburn necrosis was higher in no irrigation treatment compared to control. There were no significant differences in percentage sunburn browning and incidence on day 5 between the treatments (Table 11). There was a high percentage of photooxidative sunburn, since previously unexposed fruit were exposed by pruning and used for the sunburn assessment (Figure 12). This resulted in 100% sunburn incidence in both the no irrigation treatment and the control (Table 11). The photooxidative sunburn eventually deteriorated into sunburn necrosis.

4. Discussion

In this study, plant water status measured as midday stem water potential was negatively affected in both cultivars in the no irrigation treatment. Stem water potential was observed to be consistently more negative under water stress in young apple trees (Fernandez et al., 1997b). Ebel et al. (1995) reported that stem water potential values for severely stressed, moderately stressed and control 'Delicious' apple trees were -2.7, -2.0 and -0.8 MPa, respectively. Based on this data, at lowest midday leaf water potential of -1.39 MPa, non-irrigated 'Granny Smith' in our trial did not appear to be under significant water stress while at -1.97 MPa, the non-irrigated 'Cripps' Pink' trees would be moderately stressed. The apple tree is generally considered to employ an isohydric strategy to water deficit (Jones, 2014). However, Beikircher et al. (2013) found that 'Golden Delicious', 'Red Delicious' and 'Braeburn' displayed various degrees of iso-anisohydry. Lauri et al. (2016) reported that 'Starkrimson', 'Granny Smith' and 19 hybrids selected from their progeny on M9 NAKB rootstock responded more or less strongly to drought, displaying more isohydric or anisohydric behavior depending on genotype. Hence, using plant water status to infer water stress should be done with caution since in more isohydric cultivars, leaf water status does not necessarily correlate with water stress, while in more anisohydric cultivars, leaf water potential may be adjusted while keeping stomata open, giving the impression that the tree is under water stress. Hence, we refrain from relating our results to measures of water stress, but rather discuss the effect of contrasting levels of water availability and water deficit in 'Granny Smith' and 'Cripps' Pink' apple (as inferred from soil moisture content) on the incidence and severity of sunburn.

In agreement with Makedredza et al. (2013), ‘Granny Smith’ showed higher sunburn incidence and increased sunburn necrosis under water deficit, whilst in ‘Cripps’ Pink’ only sunburn severity increased under water deficit. The sunburn parameters measured in the study tended to increase in severity with increasing water deficit in ‘Granny Smith’. Treatment differences would possibly have been greater if the sudden exposure of fruit due to the removal of overshadowing leaves at the onset of the experiment did not cause photooxidative sunburn in almost all fruit. This high level of photooxidative sunburn explains why all treatments, including the control, showed chlorophyll and carotenoid loss by the end of the experiment in ‘Granny Smith’. The leaves were removed to rule out the contribution of transient leaf movement to treatment differences in sunburn development. Water deficit may cause leaf movement or folding (Kadioglu and Terzi, 2007), which could potentially increase the exposure of fruit to light and thereby result in an increase in sunburn. Photooxidative sunburn occurs when previously shaded fruit are suddenly exposed to radiation even at relatively low temperatures (Felicetti and Schrader, 2008; Schrader et al., 2003b). The photooxidative sunburn eventually deteriorated into sunburn necrosis, as also previously shown by Racsko and Schrader (2012). We realized that exposing fruit to high irradiance would result in sunburn, but the severity of sunburn was greater in apples on trees under water deficit. It would have been best for this experiment to have used fully exposed fruit without sunburn, but such fruit were not available at this stage of fruit development in this particular season. Nevertheless, the link between water deficit and increased sunburn development is still quite evident. Another indirect way by which irrigation can affect sunburn development is by affecting shoot growth. A reduction in tree growth under water stress has been reported in young apple trees (Fernandez et al., 1997b). However, the short duration of our trials ruled out differential growth effects on sunburn development. Also, the trials were conducted late in the season after shoot growth cessation.

After ruling out the contribution of shading effects to the increase in sunburn under water deficit, our next consideration was how water deficit increased fruit surface temperature. Makedredza et al. (2013) also reported an increase in fruit surface temperature under water stress conditions so this seems to be a consistent effect of water deficit. Considering that high fruit peel temperature is a major causal factor in sunburn development (Schrader et al., 2003a), an increase in peel temperature in response to water deficit might explain the increase in sunburn severity under water deficit. Fruit surface temperature is largely determined by radiation flux density and wind

velocity and to a smaller extent by fruit size, albedo and transpiration (Piskolczi et al., 2004). Water deficit and resultant lower plant water status may therefore potentially increase the apple fruit surface temperature by either decreasing evaporative cooling of the fruit surface or by decreasing convective heat loss to the surrounding air – we have ruled out increased radiation load on the fruit surface by removing overshadowing leaves.

Apple fruit can lose water through stomata in young fruit, and open lenticels and microcracks in mature fruit (Blanke and Lenz, 1995; Gibert et al., 2005; Maguire et al., 1999, Veraverbeke et al., 2003a, 2003b). Our experiments were conducted close to harvest ruling out the contribution of stomata to transpiration from the fruit surface. Apple fruit have high resistance to water vapor diffusion ($5000-7000 \text{ s cm}^{-1}$) (Nobel, 1975) and the contribution of transpiration to the fruit heat balance is therefore negligible compared to convection and emitted radiation (Li et al., 2014; Paper 4). The limited cooling capacity of apple fruit (Colaizzi et al., 2012) was evident from fruit surface temperatures exceeding leaf temperature by c. 8 to 13 °C in our trials (Table 1, 7).. Closing of stomata generally reduces evaporation from the leaf surface and this should result in an increase in leaf and canopy air temperature (Jones, 1992), which should limit the ability of fruit to lose heat through convection. In Italy, Portugal and Spain, non-irrigated peach tree leaves were about 5 – 6 °C warmer than leaves of adjacent well-irrigated trees (Massai et al., 2000). On a whole tree level, well-watered olive trees with a higher water potential had lower canopy temperatures than trees under deficit irrigation with a lower water potential (Sepulcre-Canto et al, 2006). We did not observe an effect on canopy air or leaf temperature in response to water deficit treatments. Although stomatal conductance was lower in ‘Cripps’ Pink’, leaf transpiration was not affected in either cultivar ($p = 0.0516$ in ‘Cripps’ Pink’), which would explain why we did not observe an effect of irrigation level on leaf temperature. Transpiration is expected to be lower with reduced stomatal conductance but also responds to vapor pressure deficit. However, on the days measured in our trials, vapor pressure deficit was not higher for the no irrigation compared to the control treatment. In young apples trees, Fernandez et al. (1997a) reported that the most consistent decreases due to water stress were in transpiration and stomatal conductance. The closing of stomata did, however, lead to lower net carbon assimilation in both cultivars.

It would be remiss not to mention an alternative explanation for the increase in fruit surface but not leaf temperature in response to the deficit irrigation. Increased severity of sunburn browning and necrosis in non-irrigated trees would result in a decrease in the albedo of the fruit peel. The lower albedo would increase the radiant heating of the fruit peel as illustrated by Smart and Sinclair (1976) through the white and black painting of grape berries. The increase in fruit temperature would then not be the cause of the increase in sunburn severity, but rather an indirect effect thereof. In this case, an explanation for how water stress may possibly increase sunburn without necessarily increasing fruit surface temperature is needed.

Sunburn results from photooxidative damage to the cells of the fruit peel due to inhibition of PSII complexes by high temperature and high light (Chen et al., 2008). High irradiance, in excess of what can be used photochemically or quenched by non-photochemical means, may cause oxidative damage to the photosynthetic apparatus (Foyer et al. 1994). Combination with other stresses can result in even moderate irradiance becoming excessive (Powles 1984). High irradiance coinciding with water stress can lead to photoinhibitory damage of photosystem II (Jones 2014). In bean leaves, water stress increases photoinhibition and gives rise to excessive excitation of the photosystems due to stomatal limitation of photosynthesis (Pastenes et al., 2005). Our assessments of photochemistry via chlorophyll fluorescence showed that F_v/F_m and $^o\text{PSII}$ efficiency were reduced under water deficit in ‘Granny Smith’ leaves. In ‘Cripps’ Pink’ leaves only F_v/F_m was reduced under water deficit with no effect on the other chlorophyll fluorescence parameters. In the fruit peel, F_v/F_m and qP were reduced under water stress in ‘Granny Smith’ whilst none of the fluorescence parameters were affected in ‘Cripps’ Pink’. It has to be considered that our measurements were taken on previously shaded peel. If taken on the exposed sides, we would have expected a correlation between photodamage, sunburn and a reduction in F_v/F_m as reported by Makedredza et al. (2015) in ‘Fuji’ and ‘Cripps’ Pink’. However, we would then have been unable to assess any changes in the photochemistry of the peel that might predispose it to sunburn development. However, Steyn et al. (2009) showed that ‘Forelle’ pear peel was considerably more sensitive to light stress than ‘Forelle’ leaves, possibly due to the different composition and biochemistry of fruit peel photochemistry (Blanke and Lenz, 1989). Furthermore, apple peel temperatures were 8 to 13 °C higher than leaf temperatures (Table 1, 7). In water stressed *Macroptilium atropurpureum* Dc. cv. Siratro leaves, photoinhibition increased concurrently with increases in leaf temperature in the 31 to 41 °C range (Ludlow and Björkman,

1984). It follows that the high irradiance in combination with high temperature, can have a more severe effect on the photochemistry of apple peel compared to apple leaves. The significant changes in some of the measured chlorophyll fluorescence parameters, even though the changes were slight, suggest that changes in the photochemistry of the fruit peel took place in response to the water deficit. Although not directly proven, these changes may relate to the increase in the severity of sunburn that was observed. With regard to the rather small changes in fluorescence parameters that we observed, Fernandez et al. (1997a) found little responsiveness in qP to water stress in leaves of 'Imperial Gala' apple whilst F_v/F_m was measured but not reported.

Higher concentrations of antioxidants in the fruit peel in response to abscisic acid application have been linked to lower sunburn incidence in apples (Iams et al., 2009). Free radical generation seems to serve as trigger for plants to develop tolerance of various stresses (Jiang and Zhang, 2001). Under stressful environment conditions like water stress, the production of reactive oxygen species increases (Davey et al., 2000). Whereas oxidative potential seems to respond to stress level, upregulation of antioxidant defenses seems to be less responsive, resulting in the defenses being overwhelmed at high stress levels. This is illustrated by Jiang and Zhang (2001) who found that treatment of maize seedlings with low abscisic acid concentrations (10 to 100 μM) upregulated the antioxidative defense response, but excessive free radical generation at 1 mM led to oxidative damage. Chen et al. (2008) found increased levels of ascorbic acid and glutathione in apples with sunburn, while Ma and Cheng (2004) found an upregulation of antioxidant enzymes and ascorbic acid in apple peel suddenly exposed to sunlight. Felicetti and Schrader (2008) found increasing levels of some phenolics with increasing severity of sunburn browning. Total phenolics as groups acts as the major antioxidant in apple peel (Wolfe et al., 2003). While glutathione was not affected under water deficit in both 'Granny Smith' and 'Cripps' Pink', total ascorbic acid, reduced ascorbic acid and total phenolics were increased under water deficit in 'Cripps' Pink'. At 7 days after the onset of treatments, oxidized ascorbic levels were higher in 'Granny Smith' peel of the non-irrigated treatments while total ascorbic acid levels increased linearly with irrigation level, although treatment differences were not significant. Seen together, these effects of water deficit on some of the cellular antioxidants provide further evidence of water deficit giving rise to oxidative stress in apple peel. No changes in anthocyanin levels were observed which is consistent with anthocyanins not generally being upregulated in response to water stress (Steyn et al., 2002).

5. Conclusion

This study is the first that attempts to explain the relationship between sunburn in apple and plant water deficit from an ecophysiological and biochemical perspective. Water deficit increased the fruit surface temperature as well as sunburn severity in ‘Cripps’ Pink’ and sunburn incidence and necrosis in ‘Granny Smith’. The design of our study ruled out the contribution of shading effects to the differences in sunburn that were observed. Also, measurements of leaf and canopy air temperature suggest that apples under water deficit did not have an impaired ability to lose heat through convection. However, chlorophyll fluorescence measurements pointed to some photochemical changes within the fruit peel in ‘Granny Smith’ under water deficit. Considering that sunburn is thought to result from photooxidative damage to fruit peel due to photothermal inhibition of PSII complexes (Chen et al., 2008) and that high irradiance combined with water stress can lead to photoinhibitory damage of photosystem II (Jones 2014), water deficit may predispose fruit to sunburn by interfering with the ability of the peel to cope with excess irradiance. The increased oxidative stress experienced in apple peel under water deficit is evident from the upregulation of some antioxidants.

6. References

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Table 1. The effect of water deficit on net carbon assimilation rate (A), stomatal conductance (g_s), transpiration (E), stem water potential (SWP), leaf temperature (LT) and fruit surface temperature (FST) of ‘Granny Smith’ at Oak Valley, Grabouw, during the 2013-2014 growing season.

Treatment	A	g_s	E	SWP	LT	FST
Day 0						
1. Control	11.6 ns	0.40 ns	5.50 ns	-1.14 ns	30.2 ns	42.8 ns
2. 70% irrigation	12.4	0.35	5.06	-1.11	31.0	42.7
3.No irrigation	12.7	0.41	5.67	-1.10	29.8	43.6
Pr>F						
Irrigation	0.5629	0.0764	0.0782	0.3449	0.1523	0.1848
Irrigation linear	0.3225	0.4012	0.4008	0.1891	0.3923	0.1109
Irrigation quadratic	0.7208	0.0348	0.0358	0.5478	0.0809	0.3505
Day 7						
1. Control	16.2 a ^z	0.47 ns	5.83 ns	-1.18 a	29.1 ns	41.0 b
2. 70% irrigation	14.8 ab	0.40	5.30	-1.22 a	29.5	41.3 ab
3.No irrigation	12.4 b	0.39	5.14	-1.39 b	29.5	42.6 a
Pr>F						
Irrigation	0.0178	0.2174	0.2512	0.0029	0.6201	0.0416
Irrigation linear	0.0056	0.1183	0.1284	0.0010	0.4145	0.0159
Irrigation quadratic	0.8468	0.4392	0.5244	0.3290	0.6095	0.5005
Day 14						
1. Control	16.6 a	0.35 ns	5.45 ns	-1.09 a	28.8 ns	36.8 b
2. 70% irrigation	16.2 a	0.33	5.22	-1.13 ab	29.0	38.0 ab
3.No irrigation	14.2 b	0.31	5.04	-1.22 b	29.7	39.0 a
Pr>F						
Irrigation	0.0486	0.2873	0.3210	0.0287	0.1284	0.0180
Irrigation linear	0.0202	0.3443	0.4569	0.0010	0.0531	0.0059
Irrigation quadratic	0.4191	0.2039	0.1925	0.3290	0.6157	0.6596

^zMeans with a different letter differ significantly at the 5% level (LSD); ns-Not significant.

Net carbon assimilation rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); Stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$); Transpiration rate ($\text{mol m}^{-2} \text{s}^{-1}$); Stem water potential (MPa); Leaf temperature ($^{\circ}\text{C}$); Fruit surface temperature ($^{\circ}\text{C}$)

Table 2. The effect of water deficit on F_v/F_m , o PSII, qP and qNP at $1400 \mu \text{ mol m}^{-2} \text{ s}^{-1}$ PFD of 'Granny Smith' apple leaves at Oak Valley, Grabouw, during the 2013-2014 season.

Treatment	F_v/F_m	o PSII	qP	qNP
Day 0				
1. Control	0.79 ns	0.07 ns	0.20 ns	0.90 ns
2.No irrigation	0.79	0.05	0.17	0.92
Pr>F				
Irrigation	0.6909	0.6177	0.7495	0.1406
Day 7				
1. Control	0.80 ns	0.10 ns	0.24 ns	0.89 ns
2.No irrigation	0.79	0.07	0.20	0.87
Pr>F				
Irrigation	0.4457	0.1958	0.3822	0.3376
Day 14				
1. Control	0.79 a ^z	0.15 a	0.32 ns	0.85 ns
2.No irrigation	0.76 b	0.10 b	0.23	0.89
Pr>F				
Irrigation	0.0099	0.0253	0.0986	0.0563

^zMeans with a different letter differ significantly at the 5% level (LSD)

ns-Not significant.

Table 3. The effect of water deficit on F_v/F_m , oPSII , qP and qNP at $1400 \mu \text{ mol m}^{-2} \text{ s}^{-1}$ PFD of ‘Granny Smith’ apple peel at Oak Valley, Grabouw, during the 2013-2014 season.

Treatment	F_v/F_m	oPSII	qP	qNP
Day 0				
1. Control	0.74 ns	0.16 ns	0.37 ns	0.90 ns
2.No irrigation	0.77	0.16	0.36	0.90
Pr>F				
Irrigation	0.3250	0.7958	0.8251	0.7446
Day 7				
1. Control	0.79 ns	0.14 ns	0.34 ns	0.80 ns
2.No irrigation	0.73	0.13	0.29	0.88
Pr>F				
Irrigation	0.2139	0.5225	0.1189	0.1919
Day 14				
1. Control	0.82 a ^z	0.18 ns	0.40 a	0.88 ns
2.No irrigation	0.67 b	0.10	0.23 b	0.86
Pr>F				
Irrigation	0.0081	0.0614	0.0097	0.4592

^zMeans with a different letter differ significantly at the 5% level (LSD)

ns-Not significant.

Table 4. The effect of water deficit on total ascorbic acid (TAA), reduced ascorbic acid (RAA), oxidized ascorbic acid (OAA), total glutathione (TG), reduced glutathione (RG), and oxidized glutathione (OG) concentrations ($\mu\text{g g}^{-1}$ FW) in the peel of ‘Granny Smith’ apple at Oak Valley, Grabouw, during the 2013-2014 season.

Treatment	TAA	RAA	OAA	TG	RG	OG
Day 0						
1. Control	284.3 ns	182.7 ns	101.7 ns	34.4 ns	32.6 ns	1.75 ns
2. 70% irrigation	266.8	177.6	89.24	34.4	32.2	1.61
3.No irrigation	301.8	236.2	65.7	37.1	35.5	2.19
Pr>F						
Irrigation	0.6959	0.0656	0.0645	0.4384	0.3306	0.5366
Irrigation linear	0.5623	0.0420	0.0286	0.2626	0.2300	0.7597
Irrigation quadratic	0.5406	0.1936	0.3820	0.5406	0.3709	0.2933
Day 7						
1. Control	476.2 ns	325.5 ns	150.7 b ^z	29.1 ns	24.5 ns	4.53 ns
2. 70% irrigation	523.8	308.2	215.7 b	35.6	30.4	5.16
3.No irrigation	658.3	195.0	463.3 a	37.6	32.0	5.63
Pr>F						
Irrigation	0.0864	0.1194	0.0008	0.1028	0.1116	0.3468
Irrigation linear	0.0342	0.0542	0.0003	0.0443	0.0530	0.1595
Irrigation quadratic	0.6280	0.4883	0.1668	0.5222	0.4293	0.8951
Day 14						
1. Control	733.6 ns	441.4 ns	292.2 ns	43.4 ns	38.2 ns	5.25 ns
2. 70% irrigation	816.2	632.4	183.3	50.5	46.7	3.79
3.No irrigation	737.4	511.8	225.5	49.0	44.2	4.76
Pr>F						
Irrigation	0.2468	0.0565	0.0971	0.1038	0.0811	0.2342
Irrigation linear	0.7365	0.3682	0.2500	0.1259	0.1440	0.9271
Irrigation quadratic	0.1052	0.0227	0.0517	0.0925	0.0581	0.0974

^zMeans with a different letter differ significantly at the 5% level (LSD), ns-Not significant

Table 5. The effect of water deficit on total chlorophyll, total carotenoid and total phenolic concentrations in the peel of 'Granny Smith' apple at Oak Valley, Grabouw, during the 2013-2014 growing season.

Treatment	Total chlorophyll concentration ($\mu\text{g g}^{-1}$ FW)	Total carotenoid concentration ($\mu\text{g g}^{-1}$ FW)	Total phenolic concentration (mg g^{-1} FW)
Day 0			
1. Control	286.8 ns	61.8 ns	140.6 ns
2. 70% irrigation	280.8	60.2	135.2
3.No irrigation	296.0	64.6	155.6
	Pr>F		
Irrigation	0.7074	0.5088	0.1875
Irrigation linear	0.5850	0.4291	0.1580
Irrigation quadratic	0.5421	0.4014	0.2282
Day 7			
1. Control	284.5 a ^z	62.1 a	153.1 ns
2. 70% irrigation	266.7 ab	55.7 b	172.0
3.No irrigation	228.2 b	54.5 b	182.0
	Pr>F		
Irrigation	0.0293	0.0446	0.4896
Irrigation linear	0.0099	0.0247	0.2776
Irrigation quadratic	0.6925	0.2273	0.6842
Day 14			
1. Control	262.1 ns	63.1 ns	156.2 ns
2. 70% irrigation	248.7	64.8	182.4
3.No irrigation	249.7	65.1	175.6
	Pr>F		
Irrigation	0.2763	0.9532	0.2240
Irrigation linear	0.1914	0.7754	0.2189
Irrigation quadratic	0.2966	0.9040	0.1808

^zMeans with a different letter differ significantly at the 5% level (LSD),

ns-Not significant

Table 6. The effect of water deficit on sunburn severity of all fruit (SVA), sunburn severity of sunburnt fruit (SVB), and percentage of fruit showing browning (SB), necrosis (SN) and incidence (SI) of ‘Granny Smith’ at Oak Valley, Grabouw during the 2013-2014 growing season.

Treatment	SVA ^y	SVB ^x	SB (%) ^w	SN (%) ^v	SI (%)
Day 0					
1. Control	0.40 ns	1.06 ns	37.5 ns	-	37.5 ns
2. 70% irrigation	0.48	1.17	41.7	-	41.7
3.No irrigation	0.52	1.17	47.9	-	47.9
Pr>F					
Irrigation	0.7514	0.6077	0.8128	-	0.8128
Irrigation linear	0.4771	0.3685	0.5302	-	0.5302
Irrigation quadratic	0.8407	0.7113	0.9835	-	0.9835
Day 7					
1. Control	1.21 ns	1.45 ns	83.3 ns	0.00 ns	83.3 b
2. 70% irrigation	1.60	1.62	97.9	0.00	97.9 a
3.No irrigation	1.77	1.77	97.9	2.08	100.0 a
Pr>F					
Irrigation	0.0615	0.4225	0.0603	0.4019	0.0492
Irrigation linear	0.0259	0.2023	0.0476	0.2299	0.0296
Irrigation quadratic	0.4441	0.8951	0.1490	0.5593	0.2052
Day 14					
1. Control	2.29 ns	2.33 ns	98.8 ns	0.00 b ^z	95.8 ns
2. 70% irrigation	2.52	2.52	100.0	0.00 b	100.0
3.No irrigation	2.91	2.92	87.5	12.5 a	100.0
Pr>F					
Irrigation	0.0681	0.0835	0.1317	0.0313	0.1317
Irrigation linear	0.0239	0.0305	0.1412	0.0170	0.0959
Irrigation quadratic	0.8548	0.7657	0.1488	0.2066	0.2317

^zMeans with a different letter differ significantly at the 5% level (LSD); ns-Not significant; ^y0-5 score, with 0 having no sunburn and 5 the most severe; ^x1-5 score, with 0 having no sunburn and 5 the most severe; ^w1-4 score; ^v5 score, (Schrader and McFerson sunburn chart).

Table 7. The effect of water deficit five days after the start of treatments on stem water potential, leaf gas exchange, leaf temperature and fruit surface temperature of ‘Cripps’ Pink’ at Welgevallen Experimental Farm (Stellenbosch) during the 2013-2014 growing season.

Treatment	SWP	A	g_s	E	LT	FST
1. Control	-1.61 a ^z	11.7 a	0.21 a	3.36 ns	30.6 ns	38.9 b
2. No irrigation	-1.97 b	9.6 b	0.15 b	2.69	31.6	40.8 a
Pr>F						
Treatment	0.0032	0.0073	0.0338	0.0516	0.1152	0.0233

^zMeans with a different letter differ significantly at the 5% level (LSD).

ns-Not significant.

SWP-Stem water potential (MPa).

A-Net carbon assimilation rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

g_s -Stomatal conductance ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

E-Transpiration rate ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

LT-Leaf temperature ($^{\circ}\text{C}$).

FST-Fruit surface temperature ($^{\circ}\text{C}$).

Table 8. The effect of water deficit on F_v/F_m , PSII efficiency (ϕ PSII), photochemical (qP) and non-photochemical quenching (qNP) at $1400 \mu \text{ mol m}^{-2} \text{ s}^{-1}$ PFD of ‘Cripps’ Pink’ apple fruit peel and leaves at Welgevallen Experimental Farm (Stellenbosch) during the 2013-2014 growing season.

Treatment	F_v/F_m	ϕ PSII	qP	qNP
Peel				
1. Control	0.86 ns	0.21 ns	0.36 ns	0.87 ns
2. No irrigation	0.86	0.20	0.33	0.85
Pr>F				
	0.9721	0.5466	0.3010	0.3529
Leaves				
1. Control	0.84 a ^z	0.07 ns	0.16 ns	0.90 ns
2. No irrigation	0.82 b	0.06	0.16	0.91
Pr>F				
	0.0077	0.2209	0.8507	0.1152

^zMeans with a different letter differ significantly at the 5% level (LSD).

ns-Not significant.

Table 9. The effect of water deficit on total ascorbic acid, total glutathione, oxidized ascorbic acid, oxidized glutathione, reduced ascorbic acid and reduced glutathione in the peel of 'Cripps' Pink' apples five days after the start of treatments at Welgevallen, Stellenbosch, during the 2013-2014 season.

Treatment	Total ascorbic acid ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Reduced ascorbic acid ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Oxidized ascorbic acid ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Total glutathione ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Reduced glutathione ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Oxidized glutathione ($\mu\text{g}\cdot\text{g}^{-1}$ FW)
1. Control	544.1 b ^z	515.3 b	28.9 ns	29.6 ns	28.1 ns	1.47 ns
2. No irrigation	686.1 a	636.7 a	49.4	32.1	29.3	2.69
			Pr>F			
Treatment	0.0010	0.0001	0.2162	0.0998	0.2907	0.0632

ns-Not significant.

Table 10. The effect of water deficit on total phenolics total anthocyanins in the peel of ‘Cripps’ Pink’ apples five days after the start of treatments at Welgevallen Experimental Farm, Stellenbosch, during the 2013-2014 growing season.

Treatment	Total phenolics (GAE) (mg.g ⁻¹ FW)	Total anthocyanins (ABS)
1. Control	134.0 b	1.80 ns
2. No irrigation	162.4 a	1.55
	Pr>f	
Treatment	0.0068	0.3346

²Means with a different letter differ significantly at the 5% level (LSD).

ns-Not significant.

Table 11. The effect of water deficit on sunburn severity of all fruit (SVA), sunburn severity of sunburnt fruit (SVB), and percentage of fruit showing browning (SB), necrosis (SN) and incidence (SI) of ‘Cripps’ Pink’ at Welgevallen Experimental Farm, Stellenbosch, during the 2013-2014 growing season.

Treatment	SVA ^y	SVB ^x	SB (%) ^w	SN (%) ^v	SI (%)
Day 0					
1. Control	0.13 ns	1	13.8 ns	0	13.8 ns
2. No irrigation	0.23	1	18.8	0	18.8
Pr>F					
Treatment	0.1663	-	0.3506	-	0.3506
Day 5					
1. Control	3.71 b ^z	3.71 b	55.0 ns	45.0 ns	100.0 ns
2. No irrigation	4.40 a	4.40 a	32.4	67.6	100.0
Pr>F					
	0.0175	0.0175	0.062	0.0692	-

^zMeans with a different letter differ significantly at the 5% level (LSD).

^y0-5 score, with 0 having no sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^x1-5 score, with 0 having no sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^w1-4 score, (Schrader and McFerson sunburn chart).

^v5 score, (Schrader and McFerson sunburn chart).

ns-Not significant.

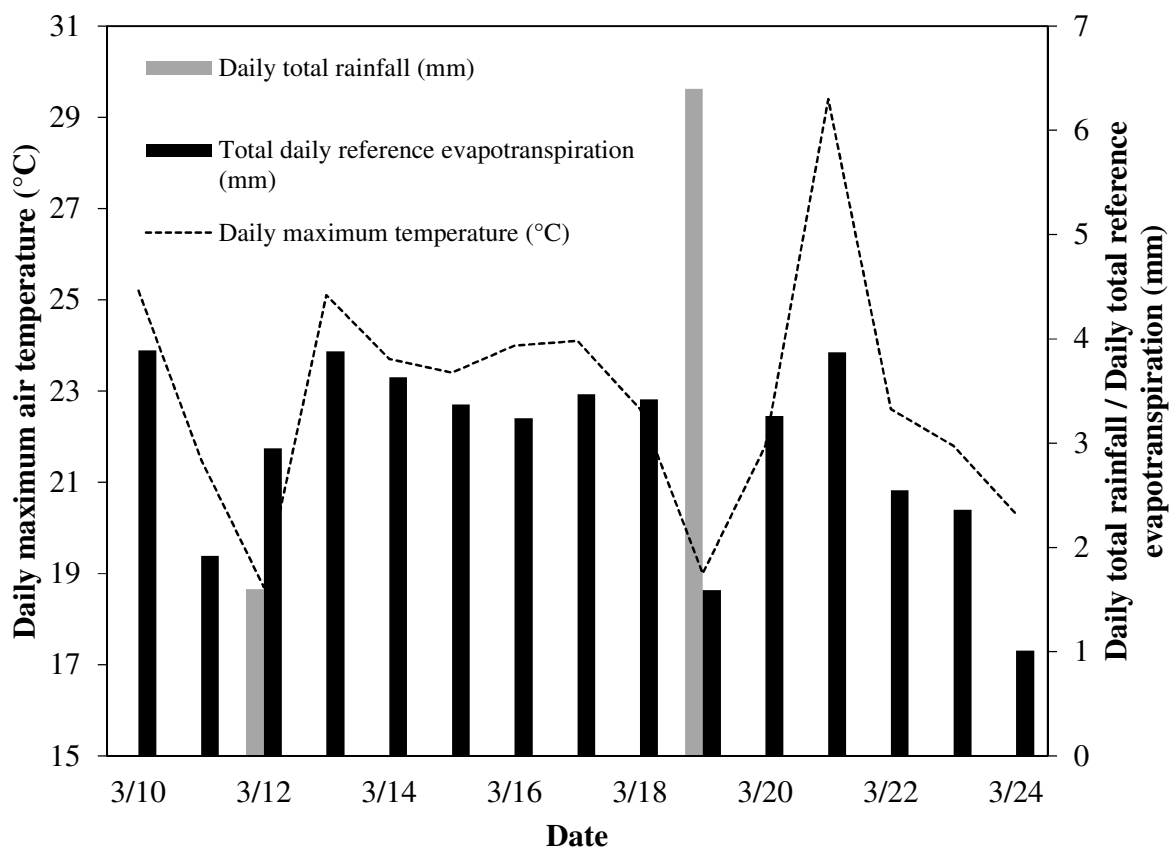


Figure 1. Daily maximum air temperature, total rainfall and reference evapotranspiration for the duration of the water deficit trial from 10 to 24 Mar 2014 at Oak Valley, Grabouw, as measured by the automatic weather station.

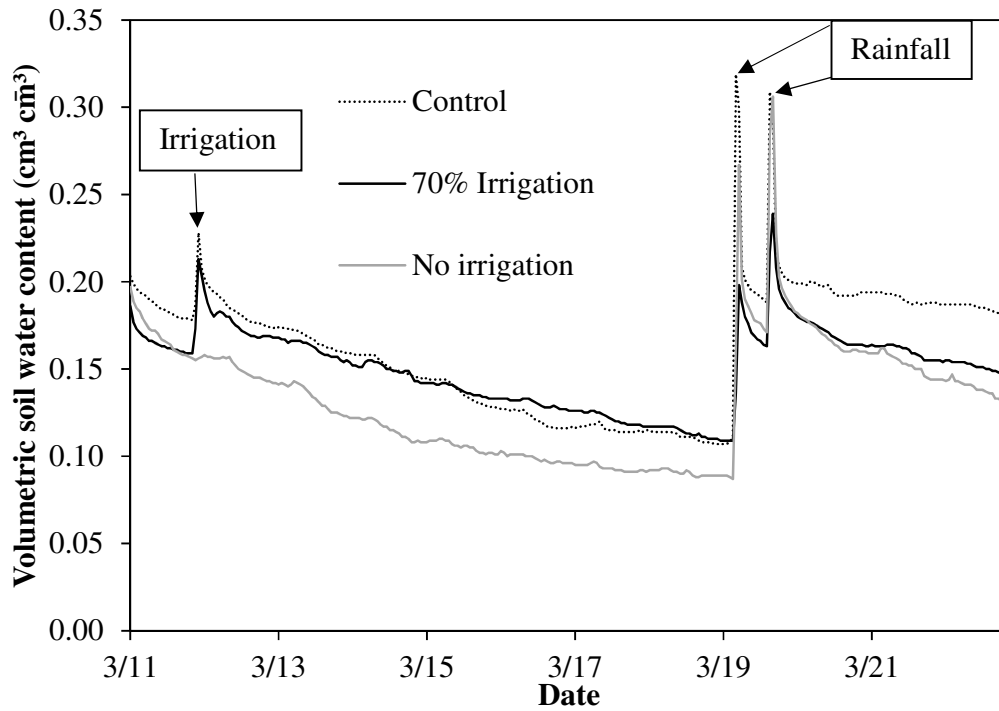


Figure 2. Volumetric soil water content in the top 30 cm root zone of 'Granny Smith' during the water deficit trial at Oak Valley, Grabouw, during the 2013-2014 growing season.

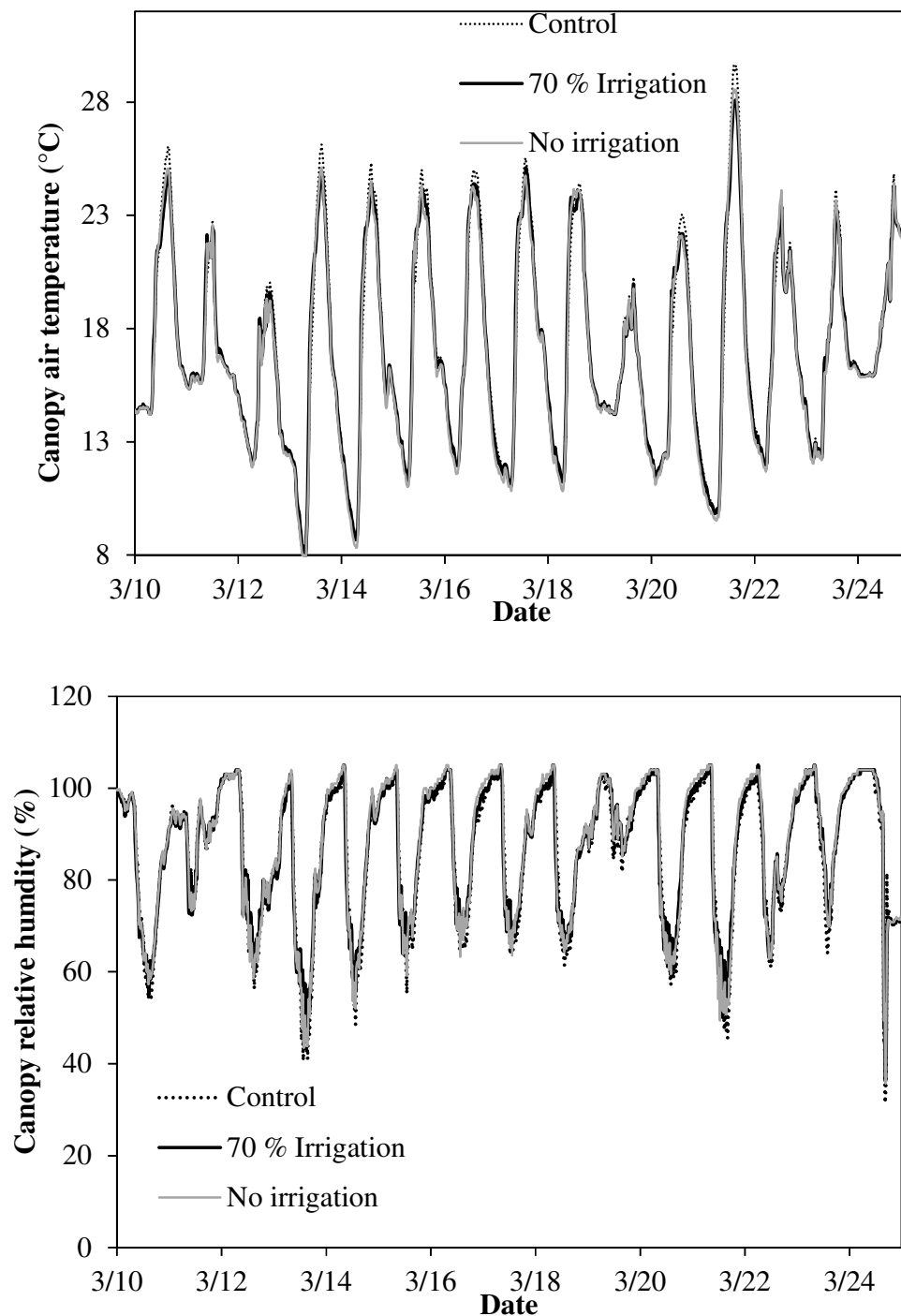


Figure 3. (Top) Canopy air temperature and (bottom) canopy relative humidity of 'Granny Smith' during the duration of the water deficit trial at Oak Valley, Grabouw, during the 2013-2014 growing season.

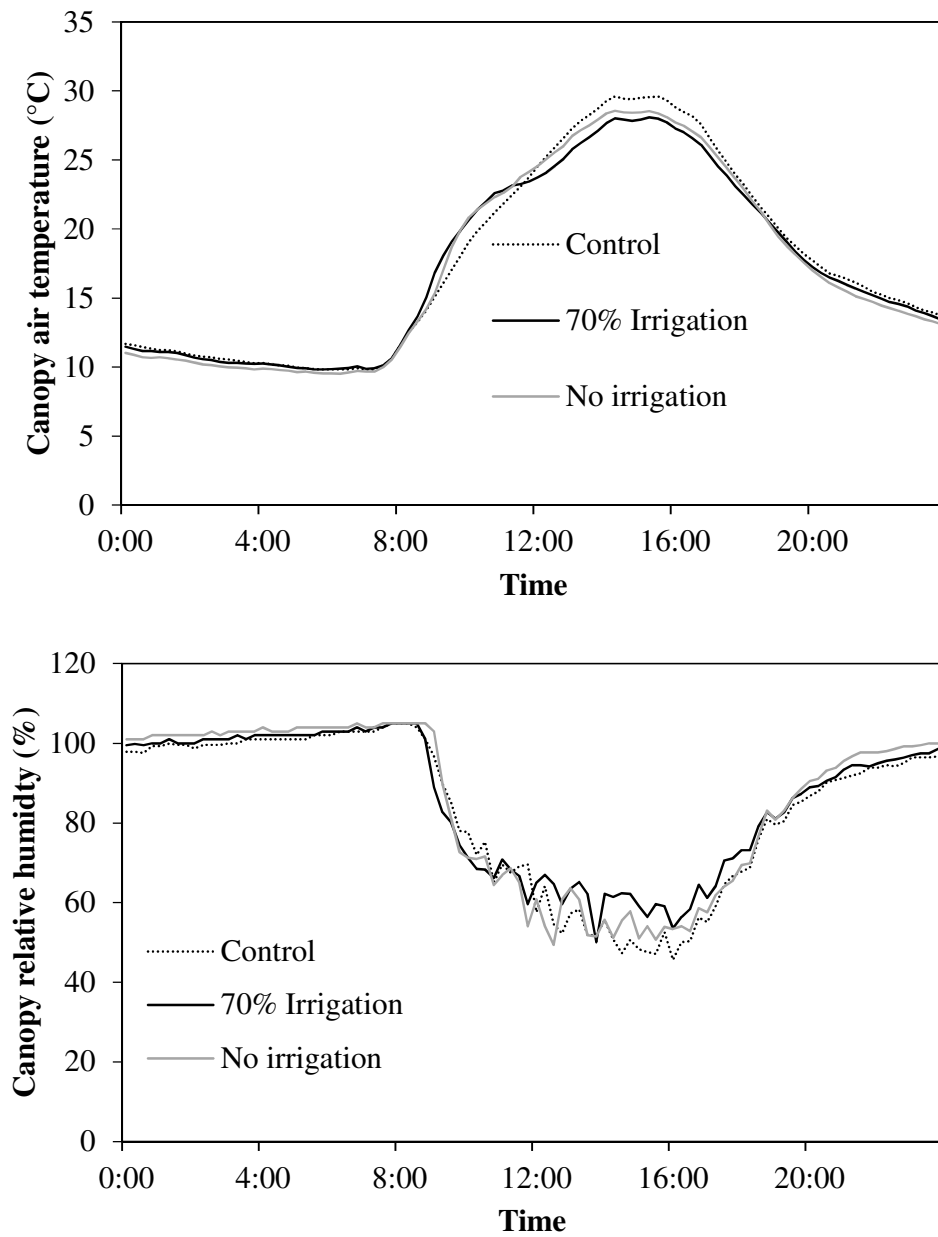


Figure 4. (Top) Daily canopy air temperature and (bottom) relative humidity on 21 Mar. 2014 of 'Granny Smith' during the water deficit trial at Oak Valley, Grabouw, during the 2013-2014 growing season.

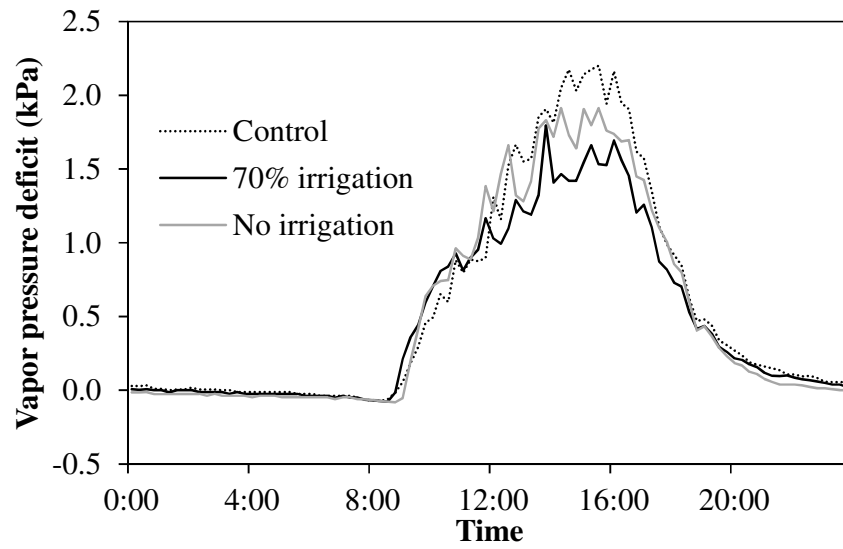


Figure 5. Canopy vapor pressure deficit of ‘Granny Smith’ on 21 Mar. 2014 during the water deficit trial at Oak Valley, Grabouw during the 2013-2014 growing season.



Figure 6. Photooxidative sunburn occurrence in 'Granny Smith' due to previously unexposed fruit being exposed by pruning, eventually turning to sunburn necrosis.

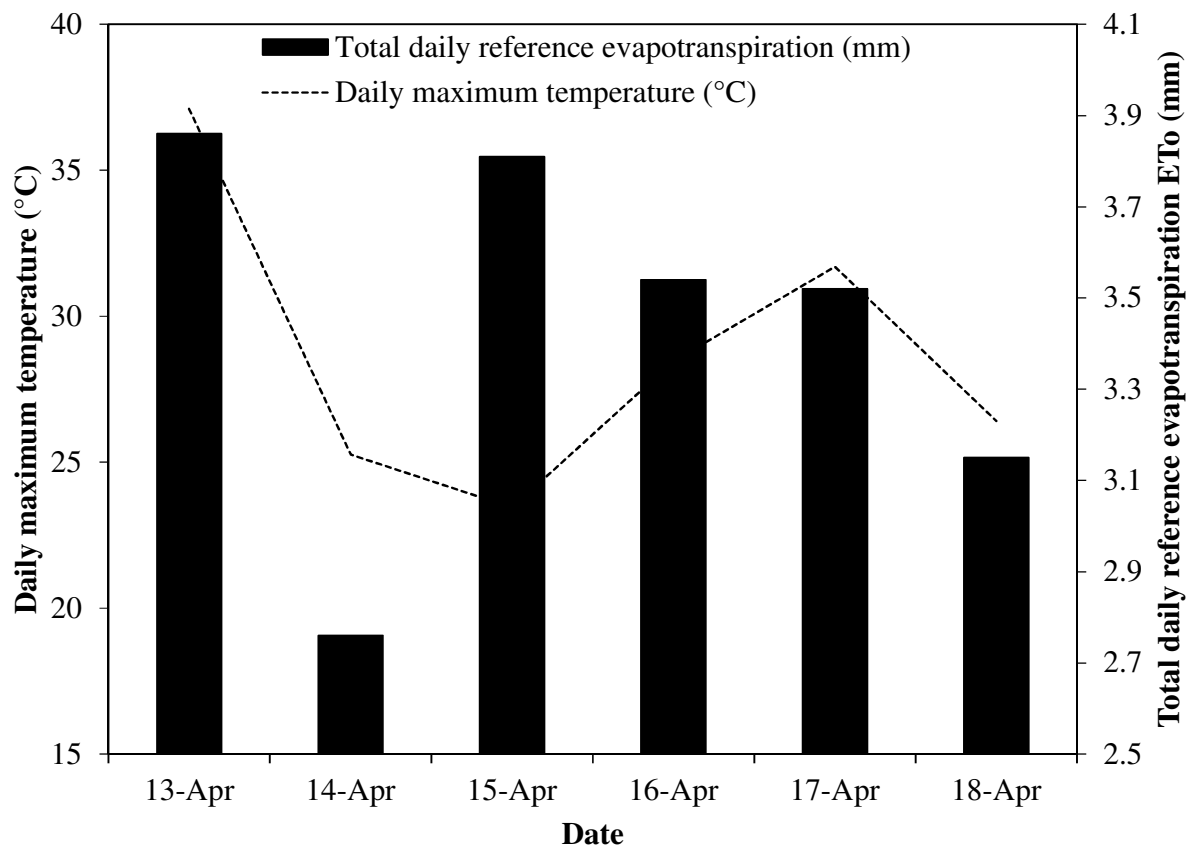


Figure 7. Daily maximum air temperature and reference evapotranspiration from 13 to 18 Apr 2014 at Welgevallen Experimental Farm, Stellenbosch as measured by the automatic weather station.

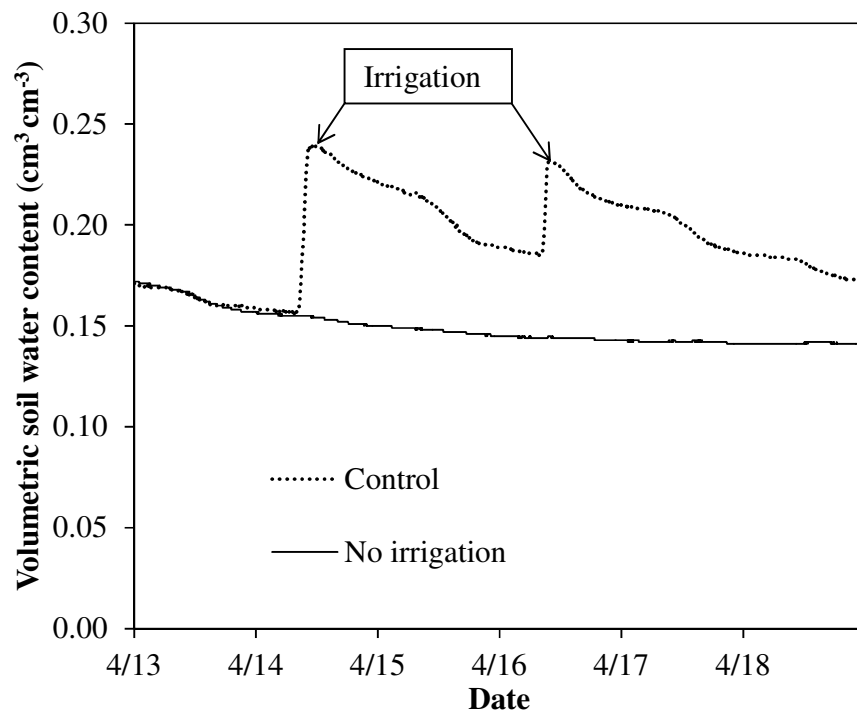


Figure 8. Volumetric soil water content of 'Cripps' Pink' during the water deficit trial at Welgevallen Experimental Farm, Stellenbosch 2013-2014 growing season. No rainfall was recorded during the trial.

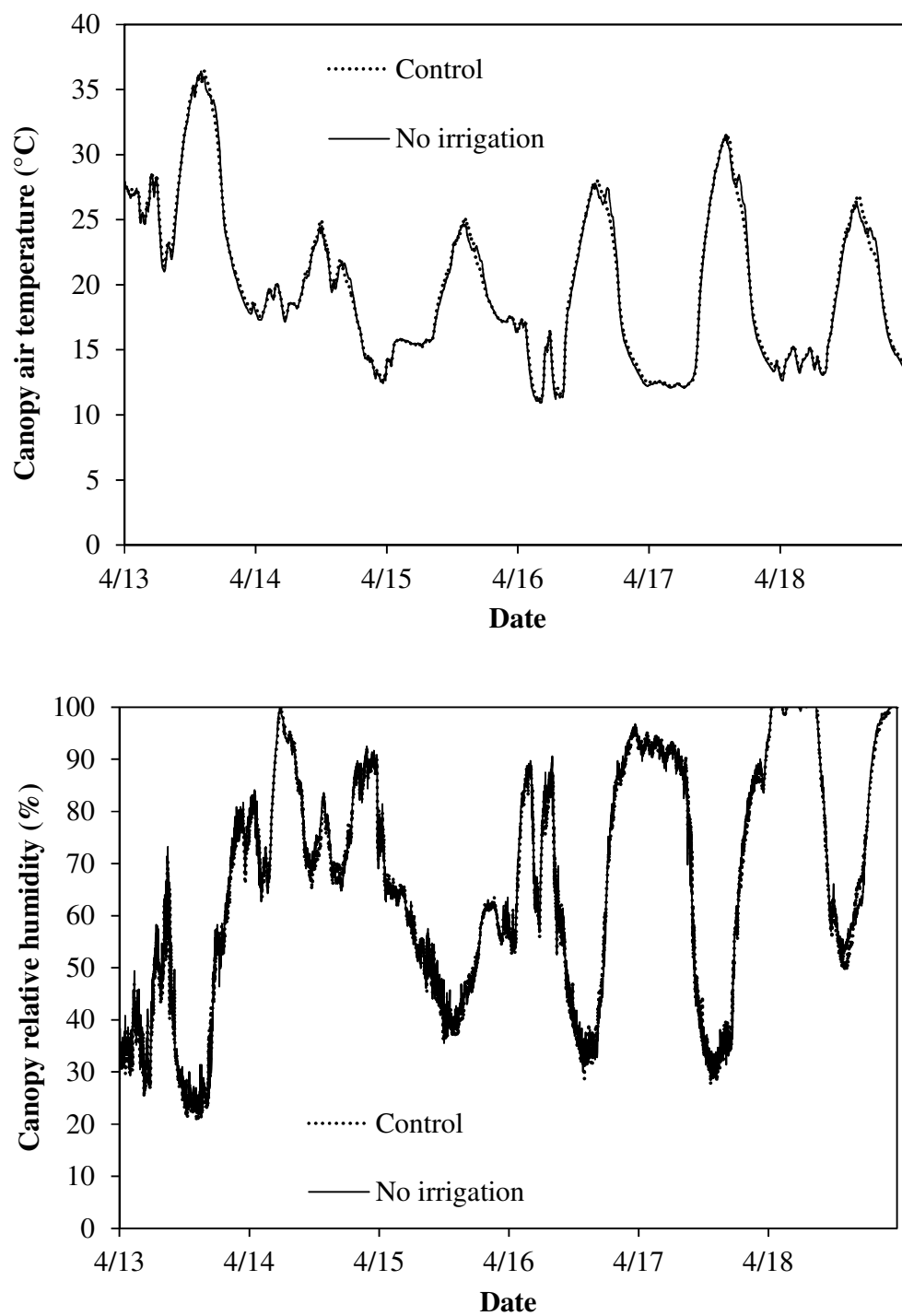


Figure 9. (Top) canopy air temperature and (bottom) canopy relative humidity of ‘Cripps’ Pink’ for the duration of the water deficit trial at Welgevallen Experimental Farm, Stellenbosch, during the 2013-2014 growing season.

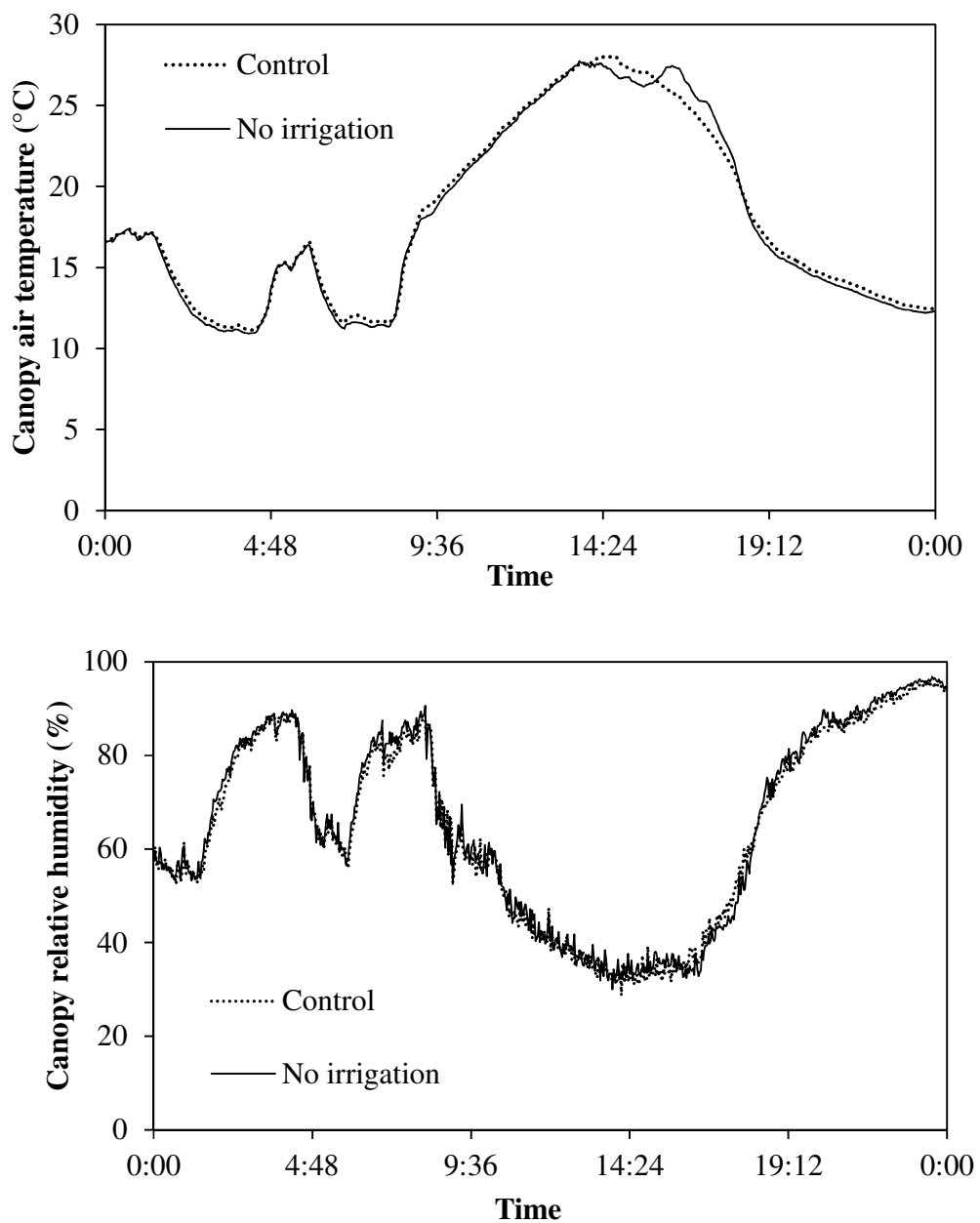


Figure 10. (Top) Daily canopy air temperature and (bottom) relative humidity of ‘Cripps’ Pink’ on 16 Apr. 2014 during the water deficit trial at Welgevallen Experimental Farm, Stellenbosch, during the 2013-2014 growing season.

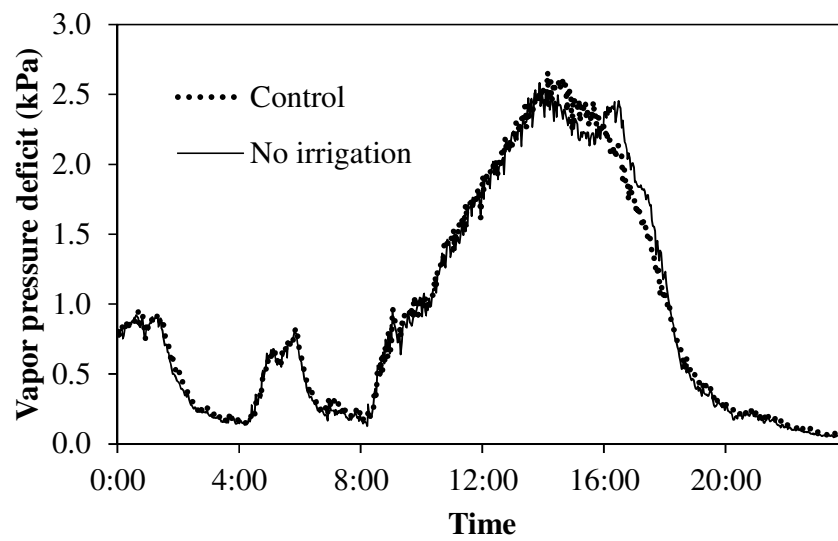


Figure 11 Vapor pressure deficit of the air in the canopy of the 'Cripps' Pink' on 16 Apr. 2014 during the water deficit trial at Welgevallen Experimental Farm, Stellenbosch, during the 2013-2014 growing season.



Figure 12. Photooxidative sunburn occurrence in 'Cripps' Pink' due previously unexposed fruit being exposed by pruning, eventually turning to sunburn necrosis.

Paper 2: Pulsing irrigation at high air temperatures reduces sunburn in under irrigated ‘Golden Delicious’ and ‘Granny Smith’ apple orchards.

Abstract

Pulsing irrigation is a supplemental irrigation strategy adopted by some South African growers to reduce sunburn in pome fruit. It involves the application of extra water to the orchard floor during a heat wave, with the intention to reduce tree stress and to modify tree canopy microclimate. The initial aim of this research was to examine the effect of pulsing irrigation on soil water status, canopy environment, tree ecophysiological status, vegetative growth and fruit quality in ‘Golden Delicious’ and ‘Granny Smith’ apple (*Malus ×domestica*). However, since the control was under irrigated, the focus shifted to studying the effect of pulsing irrigation on sunburn incidence in an orchard with suboptimal irrigation. Experiments were conducted during the 2013-2014 growing season at Meulrivier farm in Greyton, South Africa. Treatments consisted of a control scheduled per the farmer’s current practice using microsprinklers, and three treatments that received normal irrigation plus pulsing irrigation at 25, 37 and 75 L h⁻¹ per tree when ambient air temperature was ≥ 30 °C with a cycle of 5 min on and 20 min off. Soil water status and canopy environment were continuously monitored. Pulsing irrigation was started at 84 days after full bloom (DAFB) for ‘Golden Delicious’ and 92 DAFB for ‘Granny Smith’ and continued until commercial harvest at 135 DAFB for ‘Golden Delicious’ and 172 DAFB for ‘Granny Smith’. Tree ecophysiological assessments were done at 84, 102 and 116 DAFB in ‘Golden Delicious’, and 90, 114 and 158 DAFB in ‘Granny Smith’. At commercial harvest, a sample of at least 100 fruit per tree was collected for the assessment of sunburn and fruit color intensity. Fruit size, fruit firmness, and internal quality were measured. Vegetative growth was measured by pruning one-year-old shoots and weighing them. Pulsing irrigation increased volumetric soil water content compared to the under irrigated control. Volumetric soil water content in the control was below readily available water. Sunburn

incidence under pulsing irrigation was reduced by up to 11% in ‘Golden Delicious’ and 15% in ‘Granny Smith’ compared to the under irrigated control. Canopy maximum temperature was reduced by 2-3 °C on a hot day and relative humidity was increased by 10-12% under pulsing irrigation. Plant water status was improved under pulsing irrigation but there was no effect on leaf gas exchange. An improvement in fruit color was observed in ‘Granny Smith’. Total soluble solids were reduced under pulsing irrigation in ‘Golden Delicious’ whilst starch breakdown was increased by the highest pulsing treatment in ‘Granny Smith’. Vegetative growth was increased under pulsing irrigation. In conclusion, pulsing irrigation reduced the incidence of sunburn in ‘Golden Delicious’ and ‘Granny Smith’ compared to an under irrigated control. The reduction in sunburn might be attributed to either the improved plant water status, which reduced tree stress compared to the under irrigated control, or the comparatively more vegetative growth resulting in shading of fruit.

1. Introduction

So-called “pulsing irrigation” is a cultural practice adopted by some South African fruit growers to reduce sunburn in pome fruit. Sunburnt fruit are not suitable for export, resulting in loss of earnings for the growers. In some cases, up to 50% of the total yield may be unmarketable due to sunburn under South African growing conditions (Wand et al., 2006). It is a supplementary irrigation strategy whereby growers apply extra water to the orchard floor during a heat wave with the intention to reduce tree stress and thereby sunburn by modifying the canopy microclimate. However, no scientific evidence exists to support this claim and claims of positive results are entirely anecdotal. Pulsing irrigation is carried out by applying short pulses of irrigation during a heat wave. A typical pulsing cycle would involve the application of water for 5 min at temperatures exceeding 30 °C followed by 20 min off. There are widespread differences amongst the growers in terms of pulsing cycle methodologies and threshold temperatures used.

Pulsing irrigation is almost similar to evaporative cooling, the difference being that evaporative cooling involves an overhead irrigation system that cools the canopy temperature through evaporational heat exchange from the wetted fruit surface when ambient temperatures are high (Evans, 2004; Gindaba and Wand, 2005). Pulsing irrigation employs the standard irrigation

system and wets the orchard floor. Evaporative cooling has been shown to decrease fruit surface temperature in ‘Royal Gala’ by up to 5.8 °C (Gindaba and Wand, 2007), by 6.8 °C in ‘Mondial Gala’ (Iglesias et al., 2005), and decrease ambient air temperature by up to 9 and 10 °C respectively (Iglesias et al., 2002; Iglesias et al., 2005). Pulsing irrigation could potentially reduce the fruit surface temperature by increasing convectional heat loss from the fruit canopy. Colaizzi et al. (2012) reported that as plants transpire, the evaporation of water from liquid to vapor state consumes heat energy, which lowers the leaf temperature; this and the movement of water vapor away from the canopy removes heat and results in a cooling effect.

Other cultural practices that have been used for sunburn control in apples are the application of kaolin-based reflective particle films (Glenn et al., 2002; Gindaba and Wand, 2005; Wünsche et al., 2004) and installation of shade nets (Gindaba and Wand, 2005; Smit, 2007). Kaolin-based particle film sprays reflect UV-B wavelengths thereby reducing fruit surface temperature and reducing the incidence and severity of sunburn (Glenn et al., 2002). Shade nets decrease the irradiance and radiant heat load on fruit thereby significantly decreasing maximum fruit surface temperatures and sunburn incidence (Iglesias and Alegre, 2006). However, these cultural practices have limitations. Evaporative cooling requires large amounts of water (Evans 2004; Iamsb et al., 2009), which is undesirable in areas such as the Western Cape Province of South Africa with limited water supply. Poor quality water may also cause or make russetting on apples more conspicuous (Kotze´ et al., 1988.). Particle film sprays are not easy to wash off fruit and may require more labor prior to fruit packing (Lolicato, 2011). Shade nets are costly to establish over large areas and may require adaptation of farming practices to prevent excessive vegetative growth and yield alternation (Dussi et al., 2005; Gindaba and Wand, 2008).

The green apple cultivars ‘Golden Delicious’ and ‘Granny Smith’ are important to the export-orientated South African apple fruit industry, making up 24.6% and 18.4% of the total area planted (Hortgro, 2014). However, these cultivars are highly susceptible to sunburn (Fouché, 2009). High irradiance and extreme fruit surface temperatures (46 to 49 °C, depending on cultivar) are associated with the occurrence of sunburn in apple fruit (Chen et al., 2008). The three types of sunburn, viz. sunburn necrosis, sunburn browning and photo oxidative bleaching cause the discoloration of the fruit surface and result in the downgrading of fruit (Felicetti and Schrader, 2008; Schrader et al., 2001).

Tree water stress in apple production can be caused by below optimal irrigation or when tree water uptake does not meet evaporative demand. It has been linked to the development of sunburn in apple fruit (Makedredza et al., 2013; Schrader et al. 2003; Woolf and Ferguson, 2000; Yuri et al., 2004). All these citations, apart from the study of Makedredza et al. (2013), were captured in the apple sunburn review of Racsko and Schrader (2012). Unfortunately, upon scrutiny, none of these reported studies provided any empirical proof for their finding. Makedredza et al. (2013) concluded that that water stress aggravates sunburn development under conditions conducive for its development by increasing fruit surface temperature. The initial objective of this study was to examine the effect of pulsing irrigation when used as a sunburn control measure on soil water status, canopy environment, tree ecophysiological status, vegetative growth and fruit quality in ‘Golden Delicious’ and ‘Granny Smith’ under South African conditions. However, as the trial progressed, it became apparent that the control which was irrigated according the scheduling on the farm was under irrigated. Therefore, the focus of the trial shifted to examine the effect of under irrigation on sunburn development and tree ecophysiology.

2. Materials and methods

The trial was conducted during the 2013-2014 growing season in a ‘Golden Delicious’ and ‘Granny Smith’ orchard at Meulrivier farm in Greyton, South Africa (Latitude: 34°06’S; Longitude: 19°47’E, Alt 219 m) with a 0.2% slope. The region has a Mediterranean-type climate with precipitation primarily in winter (May to August) and generally little or no rainfall in the summer months. The region is characterized by high daily maximum air temperature and irradiance during the growing season (September to April) as shown by weather data obtained at Sunnyside Farm in Greyton, South Africa (Latitude: 34°05’S; Longitude: 19°40’E, Alt 243 m) (Figure 1). The orchard was planted in 1998 on M793 rootstock at a spacing of 4 x 1.5 m. The trees were 3.6 m tall and 3.0 m wide with a conical shape. Row orientation was north-south with two rows each of ‘Golden Delicious’ and ‘Granny Smith’ alternating in a 1 ha block. The orchard soil was classified as Pinedene with a sandy loam texture characterized by an orthic A horizon overlying a yellow-brown apedal B above a gleycutanic B horizon (Soil Classification Working Group, 1991). The irrigation on the farm was gravitational. Normal commercial

cultural practices of irrigation, pest management and fertilization were followed over the course of the trial. The yield was 79 and 71 tons per hectare for ‘Golden Delicious’ and ‘Granny Smith’, respectively.

2.2 Experimental design and treatments

Experimental design and treatments were similar for both cultivars. Trials were laid out according to a randomized complete block design with six replicates per treatment. Each replicate consisted of five trees with a guard tree in between. Two rows were used for each cultivar, with three full replications of each treatment in each row. There was a guard row in between each treatment row, with treatment rows 8m apart. The middle tree in each replicate was used for all the ecophysiological and canopy environment measurements. Treatments consisted of a control that received “normal” irrigation according to the scheduling of the farm and three treatments that received normal irrigation plus pulsing at 25, 37 and 75 L h⁻¹ for each tree. Pulsing was done at 37 L h⁻¹ to replicate the same rate as the grower. The lower pulsing rate (25 L h⁻¹), was to assess the effect of reduced water pulsing and the higher treatment (75 L h⁻¹) was to see what happens if you pulse at approximately twice the amount of normal irrigation. The normal irrigation scheduling at Meulrivier during the trial was 37 L h⁻¹ for 1.5 h three times per week (Mon, Wed and Fri), emitted by microsprinklers (Modular Group Naandanjain, Jain Irrigation Systems Ltd, Jalgaon, India), spaced 1.5 m apart and placed midway between the two trees with a wetting diameter of 1.7 m. The emitter rate for the control was provided by the grower and pressure was specified at 1.5 bar. The microsprinklers consisted of a bridge that holds the swivel and a nozzle connected by a 4/7 tube joined to the main line by a 4/7 barb. The bridge was mounted on a spike that was inserted into the ground. Pulsing irrigation was initiated when ambient air temperatures reached 30 °C with a cycle of 5 min on and 20 min off. An initial threshold of 28 °C was used but this was changed after two days as it resulted in excess runoff and water wastage. The system switched off when ambient temperatures fell below 30 °C and was automatically controlled using a thermal controller with built-in precision temperature and humidity sensors (MistGuard, Netafim Ltd, Tel Aviv, Israel) connected to a DC solenoid. A separate irrigation system was installed to effect the different treatments by having different nozzle sizes to obtain different delivery rates for each pulsing treatment. The new irrigation system was connected to the automatic controller and operated independently from the orchard

irrigation system. This trial was done on a farm with gravitational irrigation. The pulsing irrigation was started on 13 Jan 2014 which corresponded to 84 DAFB for ‘Golden Delicious’ and 92 DAFB for ‘Granny Smith’ and continued until commercial harvest at 135 DAFB for ‘Golden Delicious’ and 172 DAFB for ‘Granny Smith’.

2.3 Estimation of the volume of water used for pulsing irrigation

An estimation of the amount of water that was used for pulsing irrigation per treatment for each cultivar was done by adding the number of hours above 30 °C, from the beginning of the trial until harvest. From this, the number of pulsing events was calculated and converted to total number of hours under pulsing irrigation. This was used to calculate the extra amount of water applied per tree and per hectare. The amount of water applied for normal irrigation during the duration of the trial was calculated from the irrigation scheduling of the farm. Pulsing treatments also received irrigation whenever the control was irrigated.

2.4 Soil texture analysis and soil moisture measurement

Samples for soil analysis were collected at 0-300 mm and 300-600 depths. Two samples were collected from each of the four treatment rows, at the north end of the row. Soil samples were sent to Bemblab (Pty) Ltd. (Somerset West, South Africa) for soil texture analysis. Sand, silt and clay fractions were determined from analysis. Field capacity was determined at -10kPa and readily available water at -100kPa. A mathematical model was then used to calculate water holding capacity by from volumetric soil water content at field capacity and readily available water. Volumetric soil water content in the upper 300 mm of soil was measured using water content reflectometer probes (Model CS616: Campbell Scientific®, Logan, Utah, USA) installed halfway between the tree and microsprinkler vertically into the soil. The probe was connected to a data logger (Model: CR1000, Campbell Scientific®, Logan, Utah, USA) and the whole system was powered by a 12V car battery. Programming of the data logger was done using the ShortCut program (Loggernet 4.1, 2010, Campbell Scientific®, Logan, Utah, USA). Data loggers were downloaded twice to avoid filling up the logger, from 15 Jan 2014 to 06 Feb 2014 and from 07 Feb to 22 Feb 2014. During the first period, a reading was taken every 30 seconds and data were stored at hourly intervals as an average value. For the second period a reading was taken every 30 seconds and data were stored at 2 min intervals as an average. One treatment replication per cultivar with uniform soil and slope was used to monitor soil moisture content. However, only

results for ‘Golden Delicious’ are presented in this paper. Sensors malfunctioned in ‘Granny Smith’ and the data could therefore unfortunately not be used. Within the treatment replication, only the middle tree was used to measure soil moisture to minimize interference from adjacent treatments.

2.5 Canopy microclimatic conditions

Canopy microclimatic conditions were measured using air temperature and relative humidity sensors housed in radiation shields and connected to dataloggers (Tinytag Plus 2, Gemini Data Loggers, West Sussex, UK). The system was attached to tree trunks within the canopy at 2.5 m above the ground and the data were logged at 15 min intervals. One treatment replication per cultivar located in the middle of the orchard was used to monitor the canopy microclimate, in order to minimize fluctuations in canopy microclimatic conditions. The middle tree in each plot was used to minimize interference from adjacent treatments. Relative humidity was only obtained in the ‘Granny Smith’ treatments as the loggers in the ‘Golden Delicious’ trees had faulty sensors. Consequently, calculation of vapor pressure deficit was only done for ‘Granny Smith’.

2.6 Tree ecophysiological status

Baseline measurements of tree ecophysiological status were done before the beginning of the experiment at 84 DAFB for ‘Golden Delicious’ and 90 DAFB for ‘Granny Smith’ to establish initial conditions. Two further assessments of ecophysiological status were carried out for each cultivar at 102 and 116 DAFB for ‘Golden Delicious’ and 114 and 158 DAFB for ‘Granny Smith’. For ‘Golden Delicious’ at 102 DAFB, measurements were done one day after both normal irrigation and pulsing treatments took place, whilst at 116 DAFB, measurement were done one day after only normal irrigation took place. For ‘Granny Smith’, both measurements at 114 and 158 DAFB were done one day after both normal irrigation and pulsing treatments took place.

2.6.1 Gas exchange

Gas exchange measurements were done using an infrared gas analyzer, LI-6400 (Li-Cor, Lincoln, Nebraska, USA). Reference carbon dioxide concentration was set at 380 ppm, leaf

temperature at 25 °C and photosynthetic photon flux density (PPFD) to 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Measurements were done on sun-exposed leaves on the western side of the tree. Two leaves of the same age, size, and health were used per treatment replication. Leaves were sampled from shoulder height and all readings were done between 0800 HR and 1130 HR.

2.6.2 Plant water status

Plant water status, measured as midday stem water potential, was assessed using a Scholander pressure chamber (Model 600, PMS Instrument Co, USA). Two healthy leaves were chosen per treatment replicate from inside the tree canopy of the same age, size and proximity to the stem were bagged for at least 1 hour in silver reflective bags to equalize the leaf and the tree's xylem water potential before readings were taken. Leaves were sampled from shoulder height and all readings were done between 1200 HR and 1330 HR.

2.6.3 Leaf and fruit surface temperature

Leaf and fruit surface temperature was measured using a handheld infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA) between 1400 HR and 1430 HR. Leaf temperature was measured on sun-exposed leaves of similar size, health and age, whilst fruit surface temperature was measured on the sun-exposed part of the fruit. Five leaves and fruit per treatment replication were tagged for measurements. Leaf and fruit surface temperature measurements were performed at the same dates as tree ecophysiological measurements.

2.7 Sunburn and fruit color assessment

An initial sunburn assessment was done at the beginning of the experiment (84 DAFB 'Golden Delicious' and 92 DAFB 'Granny Smith'). At commercial harvest (135 DAFB 'Golden Delicious' and 172 DAFB 'Granny Smith'), a representative scaffold branch on both sides of the data collection tree in each replicate was strip picked resulting in a pooled sample of at least 100 fruit per treatment replicate. Sunburn incidence and severity were assessed using the Schrader and McPerson scale (Schrader et al., 2003), where 0 represented no sunburn, 1 to 4 refer to different stages of sunburn browning and 5 signifies sunburn necrosis. Samples were assessed for green color intensity on the greenest side of fruit using color charts, Set A 28 for 'Golden Delicious' and Set A 38 for 'Granny Smith' (Deciduous Fruit Board, South Africa).

2.8 Fruit quality

A sub-sample of 20 fruit was randomly selected and used to determine fruit quality parameters. Fruit diameter was measured using an electronic caliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan). Fruit firmness was measured twice for each fruit using a fruit texture analyzer at the equatorial region on opposite sides of the fruit (Güs, GS 20, Strand, South Africa). Percentage starch conversion was measured using the starch conversion chart (Unifruco Research Services, Bellville, South Africa). Sub-sample fruit were pooled together for juice extraction. A handheld refractometer (Model N1, Atago, Tokyo, Japan) was used to measure total soluble solids concentration (TSS). Titratable acidity (TA) was determined by titrating 5 g of juice with 0.1M NaOH with an automated titrator (Model 719 S, Metrohm AG, Hersiau, Switzerland) and expressed as percentage of malic acid.

2.9 Vegetative growth

Vegetative growth was measured by pruning all the one-year-old water shoots. One tree per treatment replicate was pruned on 15 Aug 2014 for ‘Golden Delicious’ and 4 Sept 2014 for ‘Granny Smith’ and the cuttings were placed in a plastic crate before being weighed on an electric field scale (W22 Series, UWE Co, Hsin Tien, Taiwan).

2.10 Statistical analysis

Data were analyzed using the General Linear Models (GLM) procedure of SAS Enterprise Guide 3.0 (SAS Institute Inc., 2004, Cary, NC, USA). Where significant differences occurred ($p \leq 0.05$), means were separated by the Least Significant Difference (LSD). Linear and quadratic contrasts were fitted where applicable including the control as pulsing level zero.

3. Results

3.1 Amount of water used for pulsing

The total number of days under pulsing irrigation was 51 for ‘Golden Delicious’ and 80 for ‘Granny Smith’ with 148 and 176 pulsing events per cultivar, respectively (Table 1). The distribution of the number of pulsing hours is shown in Figure 2. The amount of water per tree used for pulsing irrigation by the 25, 37 and 75 L h⁻¹ treatments in ‘Golden Delicious’ was 308,

456, and 924 L and for ‘Granny Smith’ 367, 542 and 1100 L (Table 1).’ This translated into 514, 760 and 1541 m³ per treatment per hectare in ‘Golden Delicious’ and for ‘Granny Smith’ 611, 904 and 1832 m³ ha⁻¹, respectively (Table 1). The total amount of water applied for normal irrigation during the duration of the trial was approximately 809 L per tree in ‘Golden Delicious’ and 1269 L per tree in ‘Granny Smith’ (Table 1). This translated into 1347 m³ per hectare in ‘Golden Delicious’ and 2113 m³ per hectare in ‘Granny Smith’ (Table 1).

3.2 Soil texture analysis and moisture status

Soil texture analysis showed average clay fraction was 9% in ‘Granny Smith’ and 8% in ‘Golden Delicious’. Silt fraction was 11% in ‘Granny Smith’ and 6.5% in ‘Golden Delicious’ (Appendix 1). Sand content was 80% in ‘Granny Smith’ and 85.5 % in ‘Golden Delicious’. Field capacity, readily available water and water holding capacity were an average 22.91%, 12.27 % and 106.36 mm/m respectively in ‘Granny Smith’ whilst in ‘Golden Delicious’, they were an average 20.21%, 10.20% and 101.79 (Appendix 2). Volumetric soil water content was increased by pulsing irrigation in the long term in the ‘Golden Delicious’ treatments (Figure 3). In the period 15 January to 06 February 2014, the average water content of the control treatment fluctuated around 0.10 cm³ cm⁻³. Pulsing irrigation increased the volumetric water content to peaks of 0.17, 0.18 and 0.22 cm³ cm⁻³ for normal + 25 L h⁻¹, normal + 37 L h⁻¹ and normal + 75 L h⁻¹ treatments, respectively. Normal irrigation had been slightly reduced during the second period of the campaign (06-22 February) culminating in lower soil water contents that fluctuated around 0.085 cm³ cm⁻³. Before the experiment began, from the 5th until the 10th of January, the trial site received 185 mm of rainfall (Figure 1). Average annual rainfall for this region calculated over a 22 year period is 450 mm, whilst for the month of January, it is 22 mm (NASA, 2017). The large amount of rainfall could possibly explain the higher volumetric soil water content at the beginning of the trial. Maximum soil water contents were not different between the normal + 25 L h⁻¹ and normal + 37 L h⁻¹ treatments (~ 0.192 cm³ cm⁻³) while the normal + 75 L h⁻¹ recorded the highest soil water content (0.21 cm³ cm⁻³). In addition, the changes in volumetric soil water content were also noticeable during pulsing irrigation on a hot day (Figure 4).

3.3 Canopy microclimatic conditions

Pulsing irrigation during the warmest part of the day reduced daily maximum canopy air temperature in both cultivars (Table 2, Figures 5 and 6). Canopy maximum air temperature was reduced by 2-3 °C on a hot day with pulsing (Figures 5 and 6). The reduction in canopy air temperature on hot days when pulsing irrigation was done was independent of the level of pulsing irrigation (Figures 5 and 6). Pulsing irrigation increased canopy relative humidity in ‘Granny Smith’ during a hot day and there were no differences during a cooler day (Figure 7). The increase in canopy relative humidity was also independent of the level of pulsing irrigation and was about 10-12 % higher during the hottest part of the day (Figure 7). Vapor pressure deficit within the tree canopy, calculated for a typical, hot day, was reduced by pulsing treatments compared to the control (Figure 8).

3.4 Tree ecophysiological status

There were no significant differences between pulsing treatments and the control in stem water potential, net carbon assimilation rate, stomatal conductance, transpiration rate, leaf temperature and fruit surface temperature at the beginning of the trial in both cultivars (Tables 3 and 4). At the second assessment, stem water potential was significantly higher in all pulsing treatments compared to the control in both cultivars (Tables 5 and 6). There were no significant differences between treatments and control in net carbon assimilation rate, stomatal conductance, transpiration rate, leaf temperature and fruit surface temperature in both cultivars (Tables 5 and 6). However, significant contrasts were observed in both cultivars in net carbon assimilation rate with pulsing irrigation increasing net carbon assimilation on average compared to the control (Table 5 and 6). There was a linear reduction in stem water potential and a linear increase in transpiration rate with increasing levels of pulsing irrigation in both cultivars (Table 5 and 6). There was a linear increase in net carbon assimilation and stomatal conductance with increasing levels of pulsing irrigation in ‘Granny Smith’ (Table 6).

Stem water potential of pulsing treatments was significantly higher compared to the control in both cultivars at the third assessment (Tables 7 and 8). There were no significant differences between pulsing treatments and the control in net carbon assimilation rate, stomatal conductance, transpiration rate, leaf temperature and fruit surface temperature (Tables 7 and 8). The leaf

temperature under the pulsing treatments was significantly lower on average compared to the control in ‘Golden Delicious’ (Table 7). Transpiration rate and net carbon assimilation rate were on average significantly higher compared to the control in ‘Granny Smith’ (Table 8). A linear increase in stem water potential with increasing levels of pulsing irrigation was observed in ‘Golden Delicious’ (Table 7). A quadratic trend in stem water potential was observed in ‘Granny Smith’, stem water potential was higher in the 25 L h⁻¹ pulsing treatment compared to normal irrigation, 37 and 75 L h⁻¹ (Table 8). Leaf temperature showed a quadratic trend in ‘Granny Smith’ with increasing levels of pulsing irrigation, being lowest in the 37 L h⁻¹ pulsing treatment compared to normal irrigation, 25 and 75 L h⁻¹ (Table 8).

3.5 Sunburn incidence and fruit color

Sunburn incidence prior to the beginning of the trial was 8% in ‘Golden Delicious’ and 11% in ‘Granny Smith’ (Table 9). At harvest, the percentage of fruit with sunburn browning was significantly lower for the 37 and 75 L h⁻¹ pulsing treatments, whilst the 25 L h⁻¹ treatment was not different compared to the control in ‘Golden Delicious’ (Table 10). In ‘Granny Smith’, all the pulsing treatments had a significantly lower percentage of fruit with sunburn browning compared to the control (Table 11). Both cultivars showed a linear reduction in the percentage of fruit with sunburn browning with increasing levels of pulsing irrigation (Tables 10 and 11). There were no significant differences between the treatments in the percentage of fruit with sunburn necrosis (Tables 10 and 11). However, significant contrasts were observed in both cultivars in the percentage of fruit with sunburn necrosis (Tables 10 and 11). The pulsing treatments had on average a lower percentage of fruit with sunburn necrosis compared to the control. There was a linear reduction in the percentage of fruit with sunburn necrosis with increasing levels of pulsing irrigation in both cultivars (Tables 10 and 11). Sunburn incidence was significantly reduced by 37 and 75 L h⁻¹ pulsing treatments, whilst the 25 L h⁻¹ treatment was not different compared to control in ‘Golden Delicious’ (Table 10). All the pulsing treatments significantly reduced sunburn incidence in ‘Granny Smith’ (Table 11). Increasing levels of pulsing irrigation resulted in a linear reduction in sunburn incidence in both cultivars (Tables 10 and 11). Sunburn severity of all fruit was significantly reduced by the 37 and 75 L h⁻¹ pulsing irrigation treatments whilst the 25 L h⁻¹ treatment was not significantly different from the control in ‘Golden Delicious’ (Table 10). In ‘Granny Smith,’ sunburn severity of all fruit was

significantly reduced by all the pulsing treatments (Table 11). A quadratic trend in sunburn severity of all fruit was observed in 'Golden Delicious'; it was lowest in the 37 L h⁻¹ treatment compared to normal irrigation, 25 and 75 L h⁻¹. In 'Granny Smith'; there was a linear reduction in sunburn severity of all fruit with increasing level of pulsing irrigation (Tables 10 and 11). There were no significant differences between the pulsing treatments and the control in sunburn severity of sunburnt fruits for both cultivars (Tables 10 and 11).

Pulsing irrigation at 37 and 75 L h⁻¹ resulted in significantly less green/ more yellow fruit in 'Golden Delicious' compared to the control, whilst the 25 L h⁻¹ treatment was not different (Table 10). In 'Granny Smith', all the pulsing irrigation treatments had significantly greener fruit compared to the control (Table 11). Significant contrasts were observed in both cultivars in fruit color (Tables 10 and 11). The treatments had on average less green/ more yellow fruit in 'Golden Delicious', in contrast 'Granny Smith' treatments had greener fruit. There was a linear reduction in intensity of green color in 'Golden Delicious' with increasing levels of pulsing irrigation (Table 10). In 'Granny Smith' a quadratic trend was observed; green color intensity was lowest with 25 and 75 L h⁻¹ pulsing treatments compared to normal irrigation and 37 L h⁻¹ (Table 11).

3.6 Fruit quality

There were no significant differences between pulsing treatments and the control in fruit mass, fruit diameter and fruit firmness (Tables 12 and 13). However, significant contrasts between the pulsing treatments and control in 'Granny Smith' in fruit mass and fruit firmness were observed; on average, pulsing treatments had larger and less firm fruit compared to the control (Table 13). There were no significant differences in TA between treatments in both cultivars (Tables 12 and 13). TSS was significantly reduced in all the pulsing treatments compared to the control in 'Golden Delicious' (Table 12). A quadratic trend in TSS was observed in 'Golden Delicious', TSS was lowest in the 37 L h⁻¹ pulsing treatment compared to normal irrigation, 25 and 75 L h⁻¹ (Table 12). In 'Granny Smith,' no significant differences in TSS were observed between treatments (Table 13). The 25 and 37 L h⁻¹ pulsing irrigation treatments significantly reduced TSS:TA compared to the control in 'Golden Delicious,' whilst the 75 L h⁻¹ treatment was not different from the control (Table 12). There were no significant differences between pulsing treatments and the control in TSS:TA in 'Granny Smith' (Table 13). However, a significant

contrast in TSS:TA between pulsing treatments and the control was observed (Table 13). On average, the treatments had lower TSS:TA compared to the control. A significant quadratic relationship in TSS:TA in ‘Golden Delicious’ was observed; TSS:TA was lowest in 37 L h⁻¹ pulsing treatment compared to normal irrigation, 25 and 75 L h⁻¹ (Table 12). In ‘Granny Smith,’ a linear reduction in TSS:TA occurred with increasing levels of pulsing irrigation (Table 13). Starch conversion was not affected by pulsing irrigation in ‘Golden Delicious’ (Table 12). In ‘Granny Smith,’ the highest level of pulsing irrigation (75 L h⁻¹) significantly increased starch conversion compared to the control, whilst the 25 and 37 L h⁻¹ treatments did not (Table 13). There was a linear increase in starch conversion with increasing levels of pulsing irrigation (Table 13).

3.7 Vegetative growth

In ‘Golden Delicious,’ vegetative growth was significantly increased by all the pulsing treatments, whilst in ‘Granny Smith’ only the two highest levels of pulsing irrigation (37 and 75 L h⁻¹) significantly increased vegetative growth compared to the control (Table 14). There was a linear increase in vegetative growth with increasing level of pulsing irrigation in both cultivars (Table 14).

4. Discussion

Sunburn is a major problem in the apple growing regions of South Africa due to the combination of high temperatures and high irradiance that are prevalent during the growing season (Oct – Apr). Sunburn incidence was lower in ‘Golden Delicious’ and ‘Granny Smith’ apple trees that received supplemental (pulsing) irrigation on the orchard floor on hot days compared to under irrigated control trees. This provides further evidence (see Paper 1) that water deficit increases sunburn in apple. In the untreated control, sunburn incidence was 21.2% in ‘Golden Delicious’ and 30.3% in ‘Granny Smith’. This is agreement with Wand et al (2006) who reported that South African producers generally estimate sunburn losses at 10–20% but sometimes as high as 30–50%. The reduction in sunburn incidence under pulsing irrigation was 11% in ‘Golden Delicious’ and 15% in ‘Granny Smith’. Makedredza et al. (2013), in a study where irrigated apple trees were compared with trees from which water was withheld for 14 days found evidence of

increased sunburn in response to water deficit. Considering the results of Makedredza et al. (2013), our results in Paper 1, and the anecdotal evidence reviewed by Rascko and Schrader (2012), increased susceptibility of apple fruit to sunburn seems to be a general response to water deficit. Of course, the effect of water deficit might be modulated by many factors such as cultivar, rootstock, the level of water stress, climatic conditions during the growing season, etc.

Overhead evaporative cooling also reduces sunburn in apples (Parchomchuk and Meheriuk, 1996; Gindaba and Wand, 2005; Evans et al., 1995). For evaporative cooling, Gindaba and Wand (2005) reported a 4% reduction in sunburn in 'Cripps' Pink' (15% sunburn incidence in control) and 8% in 'Royal Gala' (17% sunburn incidence in control) under South African conditions; Parchomchuk and Meheriuk (1996) reported reductions of 11 to 15% in 'Jonagold' (39 and 36% sunburn incidence in control) under Canadian growing conditions and Evans et al (1995) reported a 8% reduction in sunburn in 'Red Delicious' (18% sunburn incidence in control) in Washington State, USA. The effectiveness of evaporative cooling depends on many factors, including cultivar, season, system design, etc. (Evans, 2004). The mechanism by which pulsing irrigation and overhead evaporative cooling reduces sunburn is likely to differ. In the case of overhead evaporative cooling, the wetted fruit surface is cooled through evaporation of water from the surface (Evans, 2004). The intention is to only wet the fruit surface and to minimize water that reaches the orchard floor (Evans, 2004). Overhead evaporative cooling is thus not supposed to be a supplemental irrigation; nevertheless, some water still reaches the orchard floor. In the case of pulsing irrigation, by using the on-floor orchard irrigation system, the fruit surface is not wetted and water is applied directly to the orchard floor. Since our control was under irrigated, the difference in vegetative growth observed in our trial might be due pulsing irrigation having the most optimal amount of irrigation. Naor (2006) also reported increased vegetative growth with more optimal water supply. The increased vegetative growth could have contributed to sunburn reduction by increased shading.

Soil water status was better under pulsing irrigation compared to the under irrigated control resulting in an enhanced plant water status. Volumetric soil water content in the control was below readily available water indicating that the control was underirrigated. Plant water status plays a significant role in sunburn development in apple fruit and water stress on hot days increases the risk of sunburn (Lolicato, 2011; Makedredza et al., 2013). Plant water status was

improved irrespective of whether the day preceding the measurements received only normal irrigation or received both normal irrigation and pulsing irrigation treatments. At the start of the trial, midday stem water potential in the control was -1.05 MPa in ‘Granny Smith’ and -1.77 MPa in ‘Golden Delicious’. Ebel et al. (1995a) reported that stem water potential values for severely stressed, moderately stressed and control ‘Delicious’ apple trees were -2.7, -2.0 and -0.8 MPa, respectively. Since both cultivars were in the same block under the same irrigation system, this difference in plant water status might be explained by the isohydric/anisohydric behavior under transient stress. The lower leaf water potential in ‘Golden Delicious’ might be attributed to that particular cultivar allowing leaf water potential to fluctuate in response to water availability and climatic conditions compared to ‘Granny Smith’. Considering the above, the use of plant water status to quantify water stress should be done with caution. Plant water status in ‘Golden Delicious’ was consistently more negative compared to ‘Granny Smith’. An improvement in soil water status has also been observed under evaporative cooling, which could also help reduce tree stress and reduce the occurrence of sunburn, besides the direct cooling effect of evaporative cooling (Durner, 2013). Normal irrigation with full microjet sprinklers has also been reported to reduce sunburn compared to partial root zone drying or deficit irrigation, which induces some degree of stress in the tree (Fallahi et al., 2010).

Pulsing irrigation on hot days changed the microclimatic conditions within the tree canopy compared with the under irrigated control, resulting in lower temperatures and higher relative humidity. Fallahi et al (2010) are of the opinion that sprinklers create a cooler environment in the apple orchard. This opinion is not supported by quantitative data. Model calculated soil moisture has shown to influence the next month’s near-surface air temperature, with an inverse correlation between soil moisture and surface air temperature (Huang et al., 1996). Mahmood et al (2013) using historical weather data reported that growing season mean maximum temperature at irrigated areas is predominantly cooler than non-irrigated areas by up to 1.01 °C. A reduction in canopy air temperature and an increase in relative humidity were also observed under evaporative cooling (Iglesias et al., 2005). Vapor pressure deficits within the tree canopy were affected by changes in canopy air temperature and relative humidity, being lower in the irrigation pulsing treatments compared to the control. However, no changes were observed in leaf temperature, fruit surface temperature, stomatal conductance and transpiration, which are some of the aspects that could have been affected by a change in vapor pressure deficit under pulsing

irrigation (Andrews et al., 1992). Therefore, the observed change in vapor pressure deficit was not strong enough to affect tree water loss. However, it must be noted that our measurements of stomatal conductance and transpiration were done under controlled conditions inside a cuvette and results could be possible different if done under ambient conditions.

Pulsing irrigation on average increased fruit diameter and decreased fruit firmness in ‘Granny Smith’. Frequent irrigation was also previously reported to improve fruit size and reduce fruit firmness in ‘Gala’ (Opara et al., 1997). In contrast, deficit irrigation has been reported to result in increased fruit firmness (Kucukyumuk et al., 2013). With deficit irrigation, the grower intentionally under irrigates the orchard. The increase in fruit firmness under deficit irrigation can be linked directly to fruit size, which affects firmness, with smaller fruit having higher firmness (Naor, 2006). Evaporative cooling had no effect on fruit mass, fruit diameter and fruit firmness in ‘Royal Gala’ and ‘Cripps’ Pink’ under South African conditions (Van den Dool, 2006). Nemeskéri (2007) reported that irrigation schedules have an influence on the size and firmness of apples, particularly during fruit development. A decrease in fruit size with a reduction in rate of irrigation was observed in ‘Golden Delicious’ (Naor et al., 2008). The late start of the pulsing irrigation trial in the growing season may explain why ‘Golden Delicious’, which matures earlier than ‘Granny Smith’, did not show any treatment differences in fruit size. Fruit growth slows towards maturity in apple. In addition, small differences in fruit growth induced early during fruit development may compound during the remainder of fruit growth to give rise to considerable differences at harvest. Starch conversion was increased by the highest pulsing treatment in ‘Granny Smith’. The increased starch conversion under pulsing cannot be explained. Starch conversion would have been expected to be increased in the under irrigated control as in the case with ‘Granny Smith’. Irrigation affects fruit maturity, resulting in changes in fruit firmness as reported previously for deficit irrigation and partial root zone drying in ‘Autumn Rose Fuji’, ‘Braeburn’ and ‘Royal Gala’ (Fallahi et al., 2008; Mpelasoka, 2001; Zegbe et al., 2016). The increased starch breakdown under water deficit maybe be explained by earlier sugar accumulation as reported by Mills et al. (1994) in nonirrigated ‘Braeburn’ trees. It is not clear why ‘Golden Delicious’ did not show maturity differences in response to the treatments.

Internally, TA was not affected by pulsing irrigation whilst TSS was generally reduced under pulsing irrigation. A reduction in TSS was also observed with frequent irrigation (Opara et al., 1997). The reduction in TSS may be explained by a shading effect from increased vegetative

growth. Exposed outer canopy fruit have been reported to have higher TSS than shaded fruit from inside the canopy (Hamadziripi et al., 2014). On the other hand, deficit irrigation has been reported to increase TSS (Kucukyumuk et al., 2013; Naor, 2001). TSS:TA was also reduced by pulsing irrigation. Van den Dool (2006) reported no changes in TA, TSS or TSS:TA in response to overhead evaporative cooling whereas Parchomchuk and Meheriuk, (1996) found reductions in TSS and increased TA. Hence, fruit biochemical changes in response to irrigation treatments seem to be variable.

Fruit color was negatively affected by pulsing irrigation in ‘Golden Delicious’, whereas in ‘Granny Smith’ an improvement in fruit color was noted. Shaded ‘Granny Smith’ fruit from within the canopy have been reported to be lighter green in color, whereas partially shaded fruit have been found to have better color (Fouché et al., 2010). The improvement in fruit color in ‘Granny Smith’ could have occurred from the partial shading of fruit due to increased vegetative growth observed under pulsing irrigation. A decrease in vegetative growth has been reported under deficit irrigation (Kucukyumuk et al., 2013). Cell enlargement, and therefore vegetative growth, is highly sensitive to water stress, even when transient (Naor, 2006). Considering that pulsing did not affect fruit maturity in ‘Golden Delicious’, we cannot explain the negative effect of pulsing irrigation on fruit color in this cultivar. The possibility of nitrogen leaching occurring under high volumes of water applied through pulsing irrigation might have resulted in the negative effect on fruit color observed in ‘Golden Delicious’. However, since no negative effect on fruit color was observed in ‘Granny Smith’ we can be discounted. Also, vegetative growth was increased under pulsing irrigation which discounts nitrogen leaching. Lower nitrogen closer to harvest results in improved fruit color (Cheng, 2010; Racsó et al., 2005).

At the beginning of our experiment, pulsing was done at a threshold temperature of 28 °C and this resulted in runoff and water wastage as the orchard is planted on raised beds. Soil type should therefore be taken into consideration before deciding on an irrigation pulsing cycle and threshold temperature. The excess runoff might also have been due to lower soil infiltration rates exacerbated by the wet soils due to the high amount of rainfall received before the start of the trial. Heavy textured soils with lots of clay/silt will require shorter on cycles and longer off cycles to prevent excessive runoff compared to sandy soils. The changes in canopy microclimatic environment were independent of the level of pulsing irrigation in both cultivars. If this trial is to be repeated with a well-irrigated control, lower pulsing rates can be used.

5. Conclusion

In conclusion, pulsing irrigation reduced the incidence of sunburn in ‘Golden Delicious’ and ‘Granny Smith’ in an under irrigated orchard. This supports results from Paper 1 that show increased sunburn incidence under water deficit. The reduction in sunburn in response to pulsing irrigation might be attributed to the better plant water status compared to the under irrigated control. Drought stress has also been previously reported to increase sunburn in apples under South African conditions (Makaredza et al., 2013). The trial should be repeated with a well-irrigated control to see if pulsing irrigation still has the same effect on sunburn incidence. The unusually high amount of rainfall received in the week before the start of the trial should be also taken into consideration which might have resulted in the grower adjusting the irrigation. Unless the normal irrigation of the various treatments in a trial like this can be scheduled separately, pulsing irrigation would increase the amount of water applied compared to the control treatment. Even when down scaling normal irrigation for the increased water supplied through pulsing, longer duration irrigation events will still be required to wet the entire root volume as pulsing irrigation, due to its short duration and depending on soil type and infiltration rate, may preferentially wet the upper soil. The linking of pulsing irrigation scheduling with normally scheduled irrigation is problematic from an irrigation logistics perspective. This trial was done on a farm with gravitational irrigation and could be more challenging on a farm where the water is pumped in terms of costs and logistics. The reduction in sunburn that apple producers observe in response to pulsing irrigation might be because water is more readily available to roots and trees are therefore under less stress in-between normal irrigation events – this though requires scientific validation. There should therefore be no need to irrigate on hot days if sufficient water is readily available in the root zone. Management of irrigation by growers is therefore important to avoid under irrigation as observed in this study, which might lead to the increase of sunburn incidence. Future research needs to establish whether pulsing irrigation reduces sunburn in an orchard that is optimally irrigated.

6. References

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Table 1. Estimation of the total amount of water used for normal and pulsing irrigation during the duration of the experiments.

	Cultivar	
	'Golden Delicious'	'Granny Smith'
Start trial	13-Jan-14	13-Jan-14
Number of days	51	80
Total number of hours above 30 °C	74	88
Number of pulsing incidents	148	176
Total number of hours pulsing	13	15
Normal irrigation		
Number of irrigation incidents	22	35
Total amount/tree (L)	809	1269
Total amount/hectare (m ³)	1347	2113
Pulsing water applied/treatment/tree (L)		
1. Normal irrigation	0	0
2. Normal + 25 L h ⁻¹ pulse	308	367
3. Normal + 37 L h ⁻¹ pulse	456	542
4. Normal + 75 L h ⁻¹ pulse	924	1100
Pulsing water applied/treatment/hectare (m ³)		
1. Normal irrigation	0	0
2. Normal + 25 L h ⁻¹ pulse	514	611
3. Normal + 37 L h ⁻¹ pulse	760.0	904
4. Normal + 75 L h ⁻¹ pulse	1541	1832

Table 2. Average daily maximum air temperature and minimum relative humidity in the tree canopy under pulsing irrigation in ‘Golden Delicious’ (16 Feb 14 to 05 Mar 14) and ‘Granny Smith’ (19 Jan 14 to 05 Feb 14) at Meulrivier, Greyton, during the 2013-2014 growing season.

Treatment	‘Golden Delicious’		‘Granny Smith’	
	Daily max temperature (°C)	Daily min relative humidity (%)	Daily max temperature (°C)	Daily min relative humidity (%)
1. Normal irrigation	29.4 ± 1.07	-	30.8 ± 0.59	47.1 ± 1.97
2. Normal + 25 L h ⁻¹ pulse	27.8 ± 0.88	-	28.8 ± 0.49	56.1 ± 2.45
3. Normal + 37 L h ⁻¹ pulse	28.1 ± 0.93	-	29.1 ± 0.50	55.1 ± 2.15
4. Normal + 75 L h ⁻¹ pulse	28.0 ± 0.90	-	28.8 ± 0.49	57.0 ± 2.00

Table 3. Midday stem water potential, leaf gas exchange, leaf and fruit surface temperature of ‘Golden Delicious’ at 84 DAFB (daily maximum air temperature 27.5 °C) before the start of the trial at Meulrivier, Greyton, during the 2013-2014 growing season.

Treatment	SWP	A	g_s	E	LT	FST
1. Normal irrigation	-1.77 ns	15.0 ns	0.46 ns	4.95 ns	28.6 ns	38.4 ns
2. Normal + 25 L h ⁻¹ pulse	-1.69	14.4	0.48	5.17	29.4	39.8
3. Normal + 37 L h ⁻¹ pulse	-1.67	13.8	0.47	5.03	29.0	39.8
4. Normal + 75 L h ⁻¹ pulse	-1.66	14.0	0.43	5.15	28.3	39.4
			Pr>F			
Treatment	0.7622	0.2988	0.6261	0.8311	0.6585	0.5936
Pulsing vs Control	0.3111	0.9418	0.9418	0.4592	0.6522	0.2034
Pulsing linear	0.3861	0.4068	0.4068	0.5388	0.6505	0.4901
Pulsing quadratic	0.5593	0.3498	0.3498	0.7804	0.3046	0.2596

ns-Not significant.

SWP-Midday Stem water potential (MPa).

A-Net carbon assimilation rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

g_s -Stomatal conductance($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

E-Transpiration rate ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

LT-Leaf temperature (°C), FST-Fruit surface temperature (°C).

Table 4. Midday stem water potential, leaf gas exchange, leaf and fruit surface temperature of ‘Granny Smith’ at 90 DAFB (daily maximum air temperature 24.6 °C) before the start of the trial at Meulrivier, Greyton, during the 2013-2014 growing season.

Treatment	SWP	A	g_s	E	LT	FST
1. Normal irrigation	-1.05 ns	14.9 ns	0.39 ns	5.15 ns	29.1 ns	39.7 ns
2. Normal + 25 L h ⁻¹ pulse	-1.13	16.5	0.48	5.29	27.6	40.0
3. Normal + 37 L h ⁻¹ pulse	-1.05	14.5	0.36	4.94	29.1	39.2
4. Normal + 75 L h ⁻¹ pulse	-1.17	14.7	0.44	5.21	27.5	40.8
			Pr>F			
Treatment	0.0850	0.3346	0.2007	0.7105	0.3495	0.3422
Pulsing vs Control	0.1198	0.7394	0.4384	0.9931	0.2974	0.7220
Pulsing linear	0.0197	0.9903	0.2804	0.6923	0.1402	0.1619
Pulsing quadratic	0.8887	0.3499	0.6183	0.8781	0.8512	0.4019

ns-Not significant.

SWP-Stem water potential (MPa).

A-Net carbon assimilation rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

g_s -Stomatal conductance($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

E-Transpiration rate ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

LT-Leaf temperature (°C), FST-Fruit surface temperature (°C).

Table 5. The effect of pulsing irrigation at 102 DAFB (daily maximum air temperature 28.0 °C) on stem water potential, leaf gas exchange, leaf and fruit surface temperature of ‘Golden Delicious’ at Meulrivier, Greyton, during the 2013-2014 growing season.

Treatment	SWP	A	g_s	E	LT	FST
1. Normal irrigation	-2.03 b ^z	12.5 ns	0.32 ns	4.32 ns	29.1 ns	38.6 ns
2. Normal + 25 L h ⁻¹ pulse	-1.76 a	13.8	0.37	4.56	28.1	38.7
3. Normal + 37 L h ⁻¹ pulse	-1.72 a	14.2	0.43	5.12	27.8	38.7
4. Normal + 75 L h ⁻¹ pulse	-1.63 a	14.0	0.41	5.13	28.8	39.5
			Pr>F			
Treatment	0.0091	0.1087	0.3347	0.1440	0.4485	0.9057
Pulsing vs Control	0.0016	0.0210	0.1187	0.0760	0.2504	0.7561
Pulsing linear	0.0021	0.0632	0.1707	0.0481	0.8550	0.5116
Pulsing quadratic	0.1139	0.0946	0.2882	0.4426	0.1209	0.7752

^zMeans with a different letter differ significantly at the 5% level (LSD). ns-Not significant.

SWP-Stem water potential (MPa).

A-Net carbon assimilation rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

g_s -Stomatal conductance($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

T-Transpiration rate ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

LT-Leaf temperature (°C), FST-Fruit surface temperature (°C).

Table 6. The effect of pulsing irrigation at 114 DAFB (daily maximum air temperature 30.4 °C) on stem water potential, leaf gas exchange, leaf and fruit surface temperature of ‘Granny Smith’ at Meulrivier, Greyton, during the 2013-2014 growing season

Treatment	SWP	A	g_s	E	LT	FST
1. Normal irrigation	-1.48 b ^z	12.1 ns	0.32 ns	4.36 ns	36.3 ns	44.9 ns
2. Normal + 25 L h ⁻¹ pulse	-1.24 a	13.9	0.39	5.06	35.4	43.7
3. Normal + 37 L h ⁻¹ pulse	-1.11 a	13.0	0.38	4.92	36.2	42.8
4. Normal + 75 L h ⁻¹ pulse	-1.21 a	15.0	0.45	5.55	34.5	42.3
Pr>F						
Treatment	0.0174	0.0587	0.1682	0.1490	0.7420	0.8729
Pulsing vs Control	0.0034	0.0333	0.0738	0.0566	0.5353	0.5737
Pulsing linear	0.0530	0.0095	0.0318	0.0281	0.3200	0.9432
Pulsing quadratic	0.0231	0.7327	0.8297	0.7002	0.9056	0.5102

^zMeans with a different letter differ significantly at the 5% level (LSD). ns-Not significant.

SWP-Stem water potential (MPa).

A-Net carbon assimilation rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

g_s -Stomatal conductance($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

E-Transpiration rate ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

LT-Leaf temperature (°C), FST-Fruit surface temperature (°C).

Table 7. The effect of pulsing irrigation at 116 DAFB (daily maximum air temperature 35.3 °C) on stem water potential, leaf gas exchange, leaf and fruit surface temperature of ‘Golden Delicious’ at Meulrivier, Greyton, during the 2013-2014 growing season

Treatment	SWP	A	g	T	LT	FST
1. Normal irrigation	-1.81 b ^z	15.7 ns	0.38 ns	4.84 ns	35.6 ns	48.6 ns
2. Normal + 25 L h ⁻¹ pulse	-1.55 a	15.7	0.41	5.00	33.6	47.9
3. Normal + 37 L h ⁻¹ pulse	-1.53 a	16.1	0.43	5.25	33.3	48.2
4. Normal + 75 L h ⁻¹ pulse	-1.48 a	16.4	0.44	5.03	33.6	48.2
			Pr>F			
Treatment	0.0137	0.7651	0.6506	0.6366	0.3936	0.9729
Pulsing vs Control	0.0019	0.5511	0.3110	0.3376	0.0097	0.6939
Pulsing linear	0.0051	0.3287	0.2488	0.5303	0.2431	0.8470
Pulsing quadratic	0.0620	0.9134	0.7346	0.3490	0.2139	0.7597

^zMeans with a different letter differ significantly at the 5% level (LSD). ns-Not significant.

SWP-Stem water potential (MPa).

A-Net carbon assimilation rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

g_s-Stomatal conductance($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

E-Transpiration rate ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

LT-Leaf temperature (°C), FST-Fruit surface temperature (°C).

Table 8. The effect of pulsing irrigation at 158 DAFB (daily maximum air temperature 26.8 °C) on stem water potential, leaf gas exchange, leaf and fruit surface temperature of ‘Granny Smith’ at Meulrivier, Greyton during the 2013-2014 growing season

Treatment	SWP	A	g_s	E	LT	FST
1. Normal irrigation	-1.51 b ^z	11.6 ns	0.23 ns	3.65 ns	27.7 ns	36.8 ns
2. Normal + 25 L h ⁻¹ pulse	-1.17 a	13.0	0.35	5.06	26.3	35.6
3. Normal + 37 L h ⁻¹ pulse	-1.29 a	13.6	0.33	4.91	25.6	35.4
4. Normal + 75 L h ⁻¹ pulse	-1.28 a	13.3	0.42	5.38	27.2	35.7
			Pr>F			
Treatment	0.0073	0.2114	0.4413	0.1947	0.0776	0.3442
Pulsing vs Control	0.0015	0.0479	0.2577	0.0443	0.0618	0.0853
Pulsing linear	0.0137	0.1559	0.3931	0.0789	0.9207	0.2673
Pulsing quadratic	0.0069	0.1910	0.2968	0.2070	0.0207	0.1924

^zMeans with a different letter differ significantly at the 5% level (LSD). ns-Not significant.

SWP-Stem water potential (MPa).

A-Net carbon assimilation rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

g_s -Stomatal conductance($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

E-Transpiration rate ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

LT-Leaf temperature (°C), FST-Fruit surface temperature (°C).

Table 9. Sunburn assessment of ‘Golden Delicious’ and ‘Granny Smith’ at beginning of pulsing irrigation trial.

Cultivar	Sunburn severity (all fruit) ^z	Sunburn browning (%) ^y	Sunburn necrosis (%) ^x	Sunburn incidence (%)
‘Golden Delicious’	0.11	8	0	8
‘Granny Smith’	0.18	11	0	11

^z0-5 score, with 0 having no sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^y1-4 score, with 0 having no sunburn and 4 the most severe (Schrader and McFerson sunburn chart).

^x5 score, (Schrader and McFerson sunburn chart).

Table 10. The effect of pulsing irrigation on sunburn incidence and fruit color of 'Golden Delicious' at Meulrivier, Greyton during the 2013-2014 growing season.

Treatment	Sunburn severity (all fruit) ^y	Sunburn severity (sunburnt fruit) ^y	Sunburn browning (%) ^w	Sunburn necrosis (%) ^v	Sunburn incidence (%)	Fruit color ^u
1. Normal irrigation	0.54 a ^z	2.47 ns	20.2 a	1.00 ns	21.2 a	2.29 b
2. Normal + 25 L h ⁻¹ pulse	0.49 a	2.14	22.5 a	0.67	23.2 a	2.38 b
3. Normal + 37 L h ⁻¹ pulse	0.23 b	2.06	9.00 b	0.33	9.50 b	2.59 a
4. Normal + 75 L h ⁻¹ pulse	0.29 b	2.03	14.0 b	0.17	14.2 b	2.60 a
Pr>F						
Treatment	0.0001	0.4495	0.0012	0.0934	0.0007	0.0016
Pulsing vs Control	0.0003	0.1240	0.0514	0.0367	0.0288	0.0019
Pulsing linear	0.0001	0.1808	0.0151	0.0193	0.0070	0.0005
Pulsing quadratic	0.0328	0.3728	0.2013	0.4776	0.1788	0.1786

^zMeans with a different letter differ significantly at the 5% level (LSD). ns-Not significant.

^y0-5 score, with 0 having no sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^x1-5 score, with 0 having no sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^w1-4 score, (Schrader and McFerson sunburn chart). ^v5 score, (Schrader and McFerson sunburn chart).

^u1-12 score, with 1 being the greenest fruit and 12 the least green (A28 chart).

Table 11. The effect of pulsing irrigation on sunburn occurrence and fruit color of 'Granny Smith' at Meulrivier, Greyton during the 2013-2014 growing season.

Treatment	Sunburn severity (all fruit) ^y	Sunburn severity (sunburnt fruit) ^x	Sunburn browning (%) ^w	Sunburn necrosis (%) ^v	Sunburn incidence (%)	Fruit color ^u
1. Normal irrigation	0.55 a ^z	1.77 ns	28.8 a	1.50 ns	30.3 a	3.16 a
2. Normal + 25 L h ⁻¹ pulse	0.27 b	1.48	17.5 b	0.33	17.8 b	2.89 b
3. Normal + 37 L h ⁻¹ pulse	0.36 b	1.71	20.0 b	0.50	20.5 b	2.97 b
4. Normal + 75 L h ⁻¹ pulse	0.25 b	1.54	15.7 b	0.17	15.8 b	2.91 b
Pr>F						
Treatment	0.0085	0.4967	0.0111	0.1145	0.0081	0.0134
Pulsing vs Control	0.0014	0.2778	0.0018	0.0209	0.0013	0.0021
Pulsing linear	0.0030	0.2585	0.0030	0.0376	0.0022	0.0077
Pulsing quadratic	0.0551	0.5307	0.0892	0.2012	0.0747	0.0342

^zMeans with a different letter differ significantly at the 5% level (LSD). ns-Not significant.

^y0-5 score, with 0 having no sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^x1-5 score, with 0 having no sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^w1-4 score, (Schrader and McFerson sunburn chart). ^v5 score, (Schrader and McFerson sunburn chart).

^u1-12 score, with 1 being the greenest fruit and 12 the least green (A38 chart).

Table 12. The effect of pulsing irrigation on fruit mass, diameter, firmness and internal quality of ‘Golden Delicious’ at Meulrivier, Greyton, during the 2013-2014 growing season.

Treatment	Fruit mass (g)	Fruit diameter (mm)	Fruit firmness (kg)	TA (%)	TSS (°Brix)	TSS:TA	Starch conversion (%)
1. Normal irrigation	101.2 ns	60.9 ns	7.98 ns	0.37 ns	11.2 a ^z	30.5 a	34.5 ns
2. Normal + 25 L h ⁻¹ pulse	100.2	61.6	7.72	0.38	10.5 b	28.0 bc	40.4
3. Normal + 37 L h ⁻¹ pulse	109.4	62.5	7.64	0.39	10.4 b	26.8 c	43.9
4. Normal + 75 L h ⁻¹ pulse	101.9	61.0	7.85	0.36	10.7 b	29.5 ab	41.9
Pr>F							
Treatment	0.4061	0.5570	0.1202	0.3835	0.0034	0.0170	0.3454
Pulsing vs Control	0.5841	0.4262	0.0552	0.5815	0.0006	0.0194	0.0902
Pulsing linear	0.7858	0.9634	0.4129	0.6157	0.0451	0.5861	0.1732
Pulsing quadratic	0.3570	0.1966	0.0298	0.1285	0.0010	0.0026	0.2402

^zMeans with a different letter differ significantly at the 5% level (LSD).

ns-Not significant.

Table 13. The effect of pulsing irrigation on fruit mass, diameter, firmness and internal quality of ‘Granny Smith’ at Meulrivier, Greyton, during the 2013-2014 growing season.

Treatment	Fruit mass (g)	Fruit diameter (mm)	Fruit firmness (kg)	TA (%)	TSS (°Brix)	TSS:TA	Starch conversion (%)
1. Normal irrigation	148.1 ns	69.1 ns	7.97 ns	0.69 ns	11.0 ns	16.0 ns	18.9 b
2. Normal + 25 L h ⁻¹ pulse	149.8	70.3	7.80	0.73	10.6	14.7	21.9 ab
3. Normal + 37 L h ⁻¹ pulse	150.8	70.8	7.78	0.71	10.6	14.9	18.4 b
4. Normal + 75 L h ⁻¹ pulse	153.9	70.5	7.83	0.72	10.5	14.6	25.2 a
Pr>F							
Treatment	0.6133	0.1056	0.0647	0.3572	0.1621	0.1487	0.0168
Pulsing vs Control	0.3541	0.0205	0.0105	0.1141	0.0295	0.0291	0.1016
Pulsing linear	0.2161	0.1073	0.1081	0.1376	0.0674	0.0499	0.0037
Pulsing quadratic	0.9218	0.1103	0.0339	0.3702	0.2063	0.222	0.4256

²Means with a different letter differ significantly at the 5% level (LSD).

ns-Not significant.

Table 14. The effect of pulsing irrigation on vegetative growth of ‘Golden Delicious’ and ‘Granny Smith’ at Meulrivier, Greyton, during the 2013-2014 growing season.

Treatment	Cultivar	
	‘Golden Delicious’ (kg tree ⁻¹)	‘Granny Smith’ (kg tree ⁻¹)
1. Normal irrigation	1.17 b ^z	1.83 c
2. Normal + 25 L h ⁻¹ pulse	2.06 a	2.54 bc
3. Normal + 37 L h ⁻¹ pulse	1.94 a	2.63 b
4. Normal + 75 L h ⁻¹ pulse	2.32 a	3.67 a
	Pr>F	
Treatment	0.0035	0.0015
Pulsing vs Control	0.0006	0.0020
Pulsing linear	0.0010	0.0002
Pulsing quadratic	0.0933	0.9429

^zMeans with a different letter differ significantly at the 5% level (LSD).

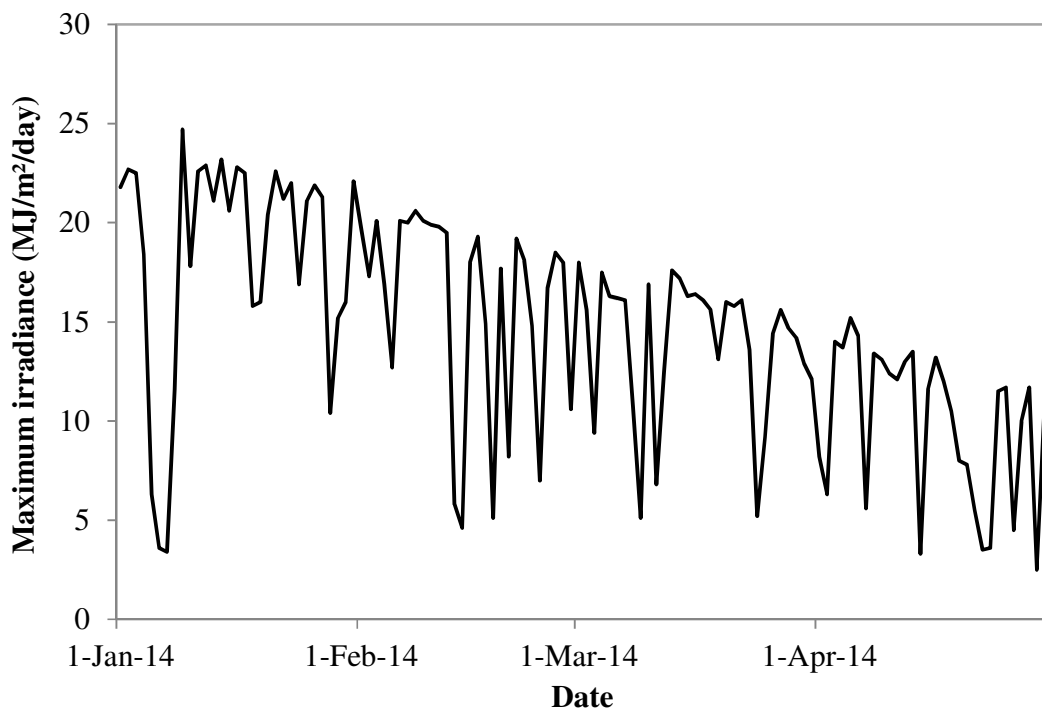
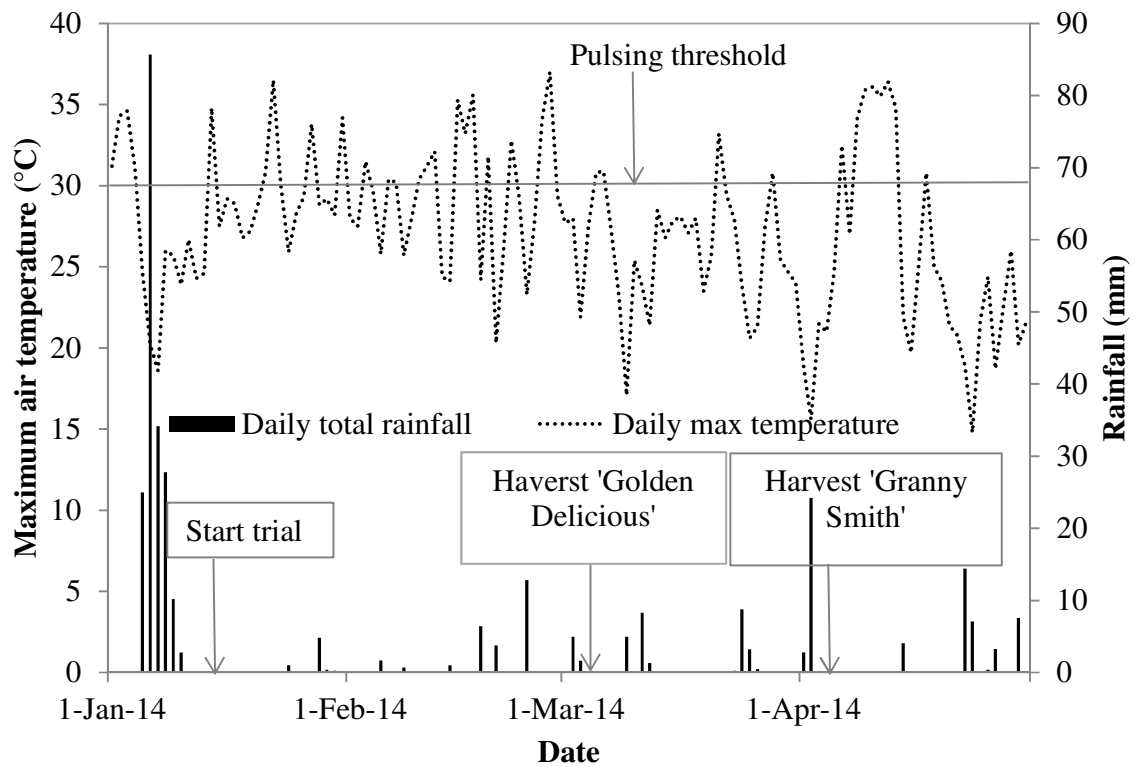


Figure 1. (Top) Daily maximum temperatures and total rainfall and (bottom) maximum daily irradiance during the pulsing trial.

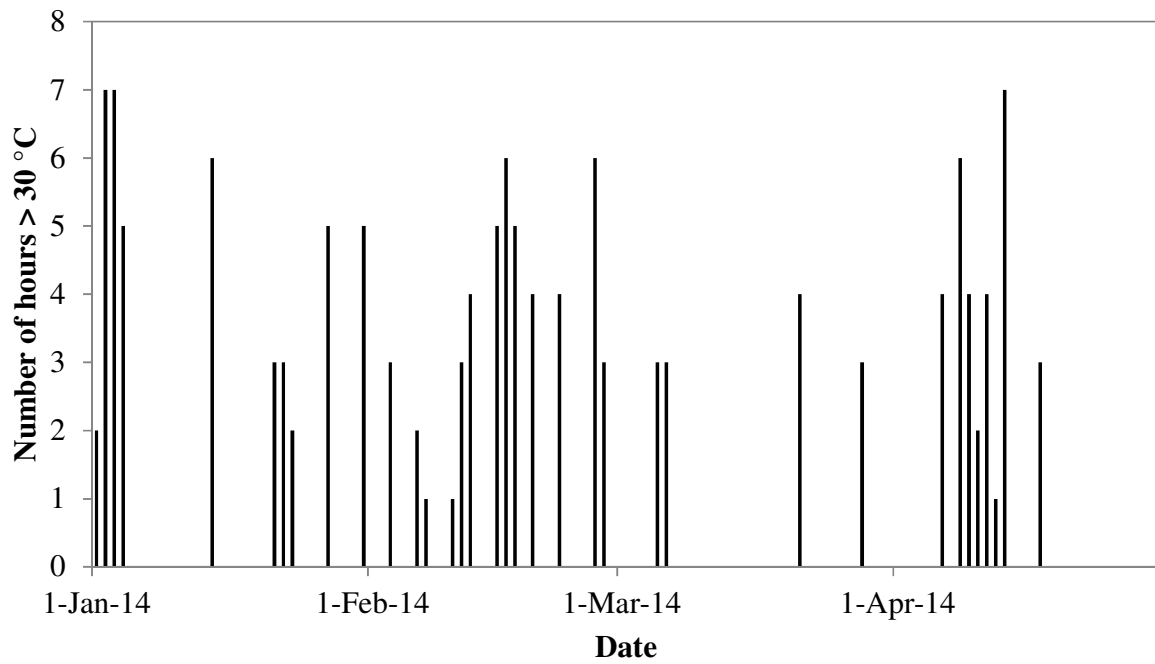


Figure 2. Distribution of number of hours above 30 °C during the pulsing trial

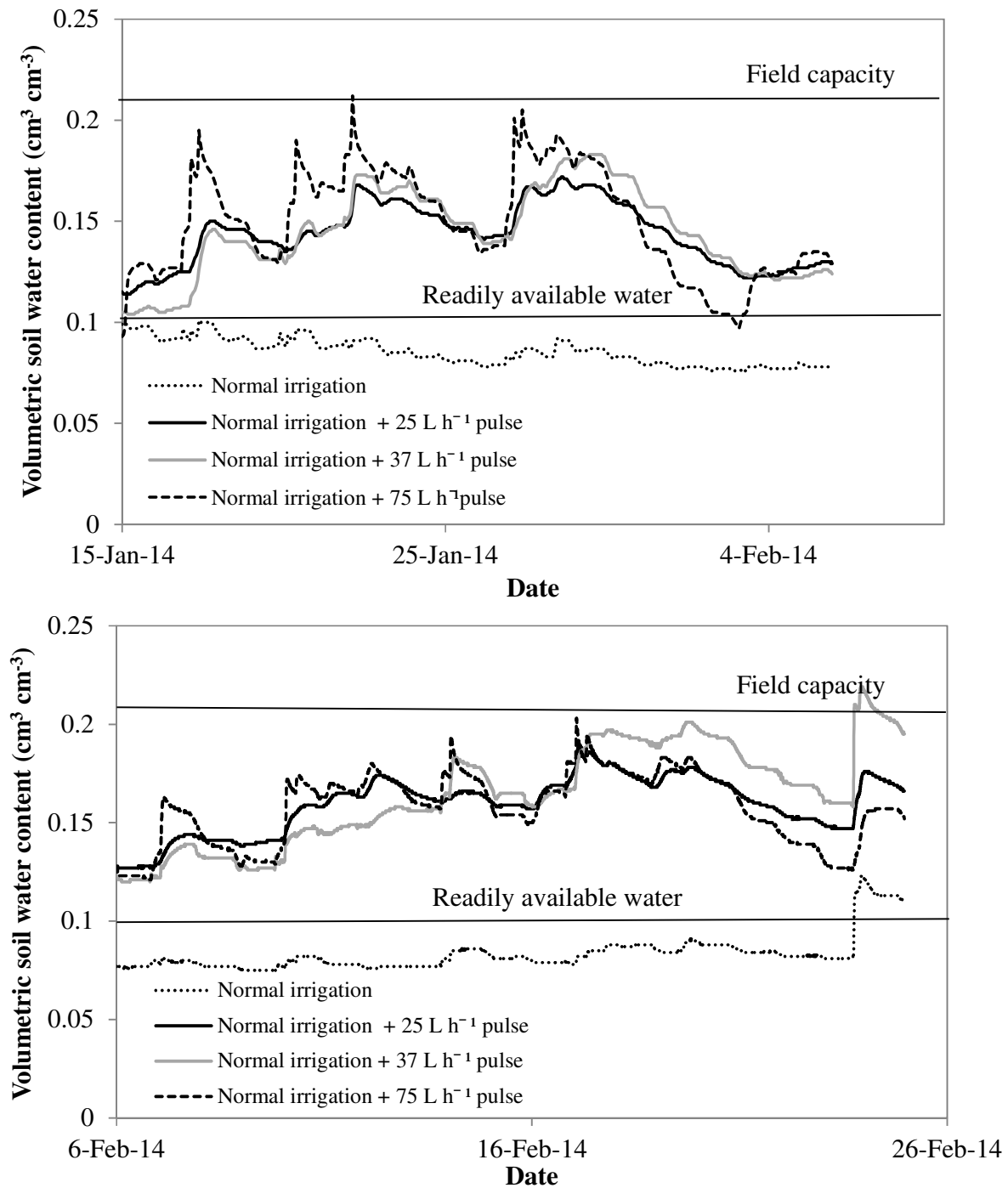


Figure 3. (Top) Long term changes in volumetric soil water content in 'Golden Delicious' under irrigation pulsing from beginning of the experiment from 15 Jan 2014 to 06 Feb 2014 and (bottom) 06 Feb to 22 Feb.

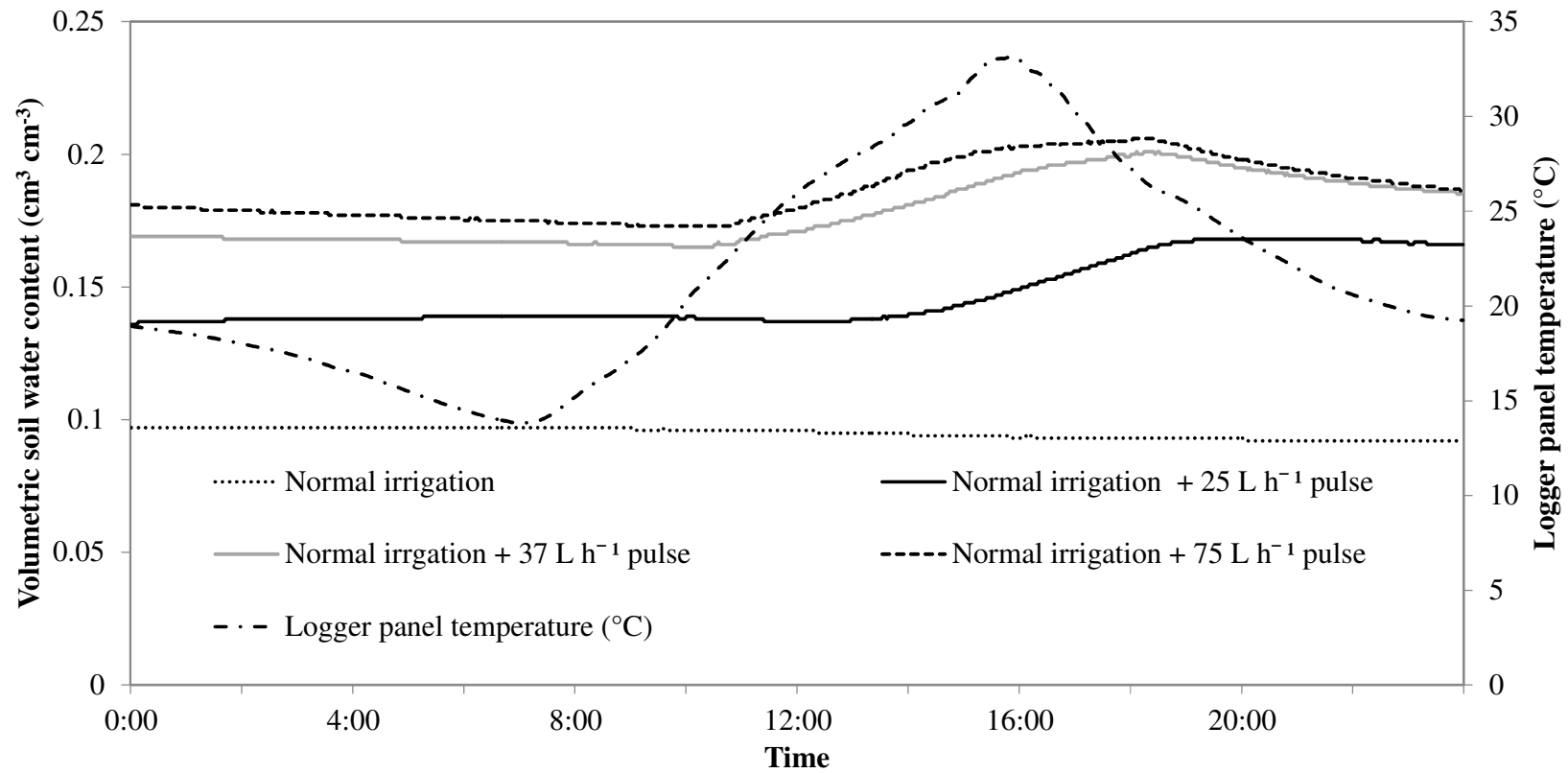


Figure 4. Daily changes in volumetric soil water content in ‘Golden Delicious’ under pulsing irrigation on a hot day (129 DAFB; 27 Feb 2014).

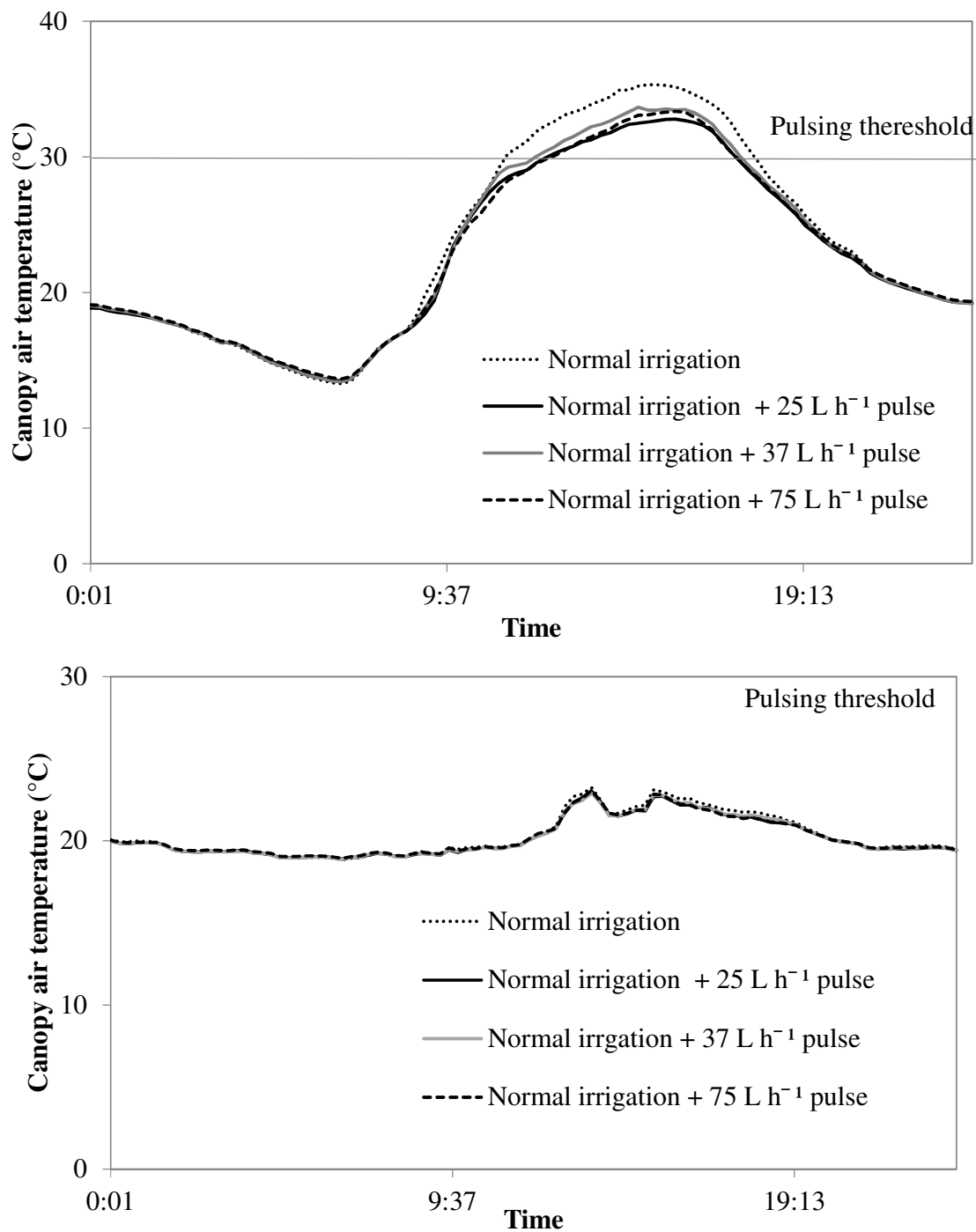


Figure 5. (Top) Effect of irrigation pulsing on canopy air temperature in 'Golden Delicious' on a hot day (129 DAFB, 27 Feb 2014, maximum air temperature 37 °C) compared to (bottom) a cooler day with no pulsing (126 DAFB, 24 Feb 2014, maximum air temperature 23.3 °C).

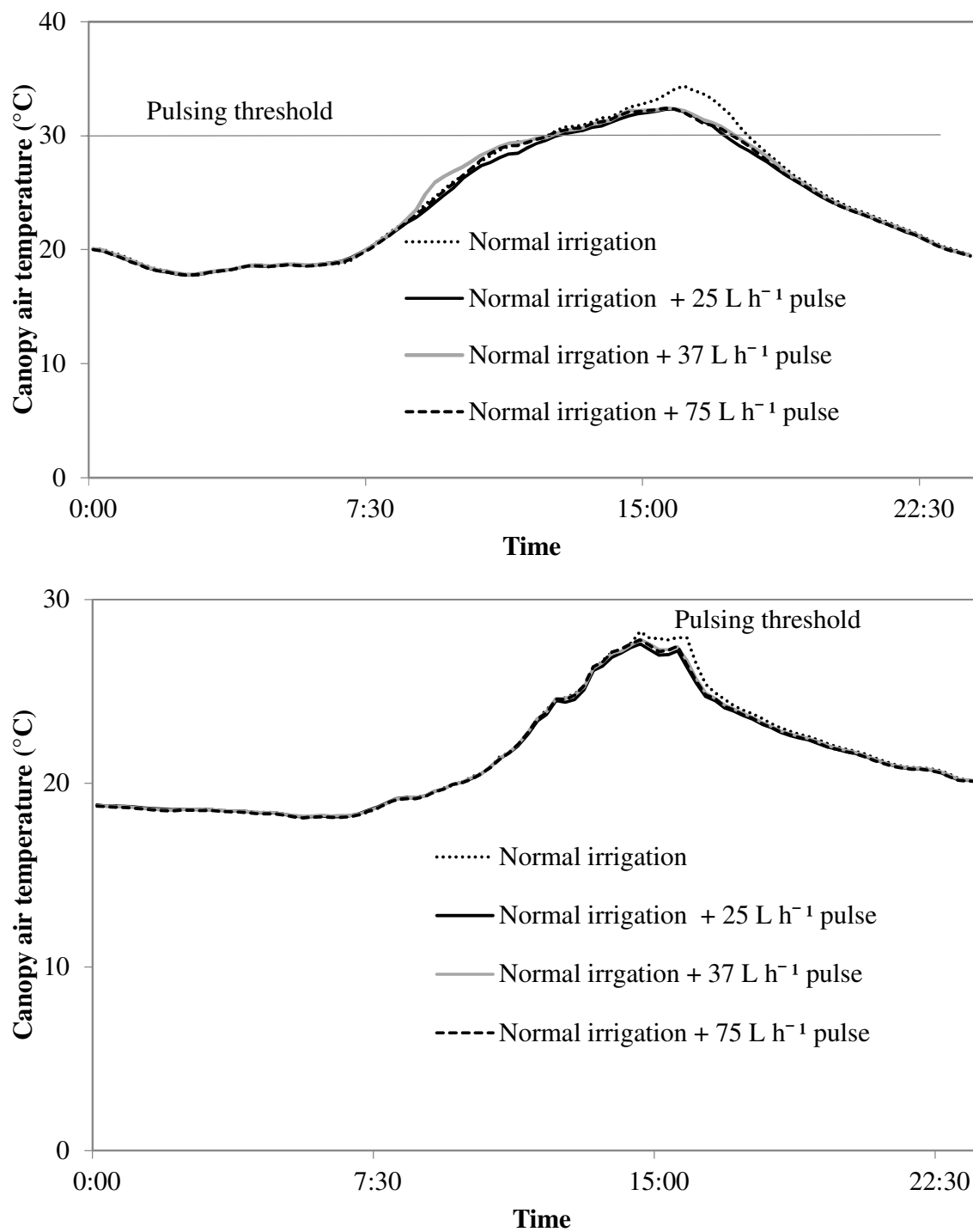


Figure 6. (Top) Effect of irrigation pulsing on canopy air temperature in 'Granny Smith' on a hot day (110 DAFB, 31 Jan 2014, maximum air temperature 34.2 °C) compared to (bottom) a cooler day with no pulsing (109 DAFB, 30 Jan 2014, maximum air temperature 28.2 °C).

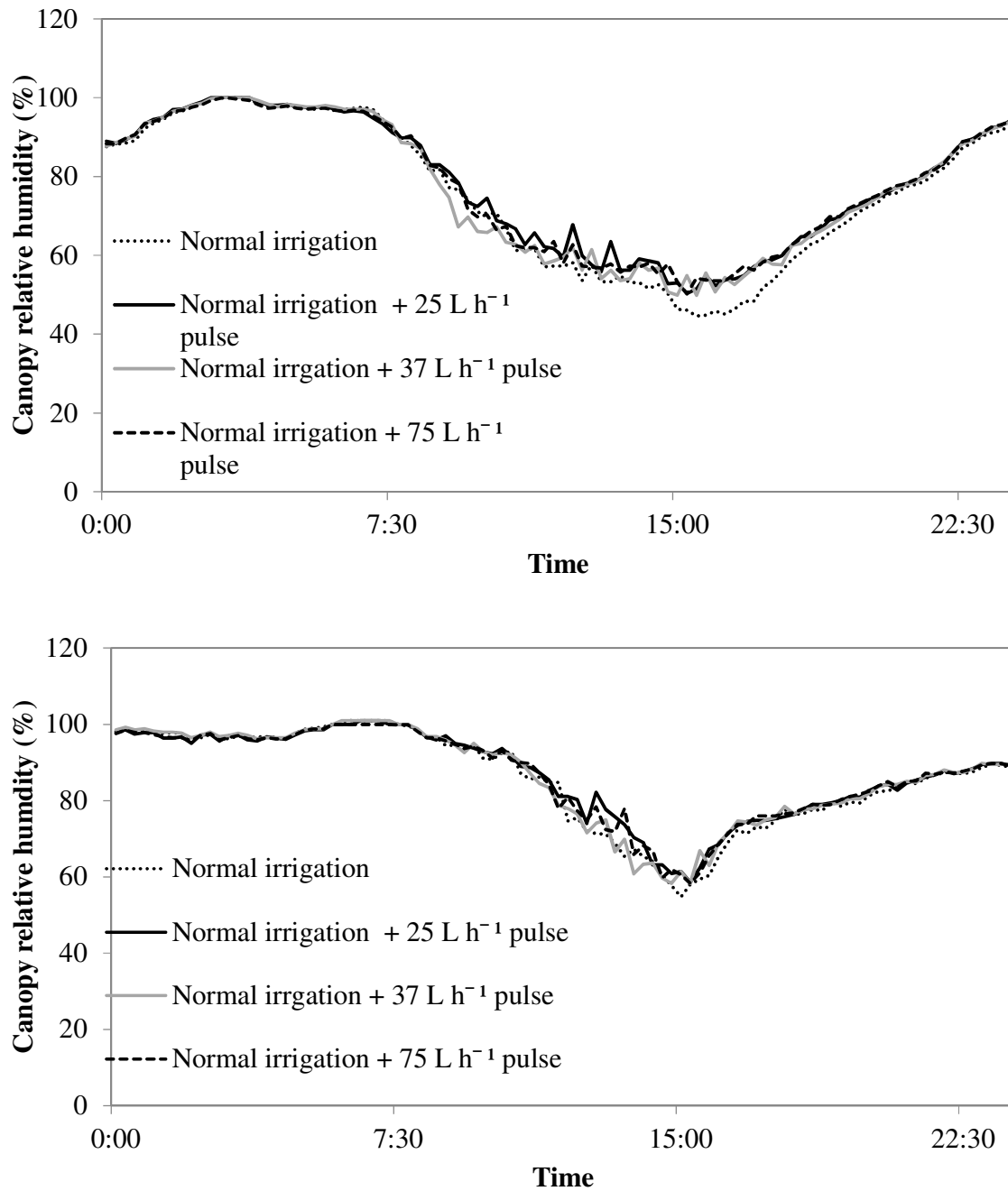


Figure 7. (Top) Effect of irrigation pulsing on canopy relative humidity in 'Granny Smith' on a hot day (110 DAFB, 31 Jan 2014, maximum air temperature 34.2 °C) compared to (bottom) a cooler day with no pulsing (109 DAFB, 30 Jan 2014, maximum air temperature 28.2 °C).

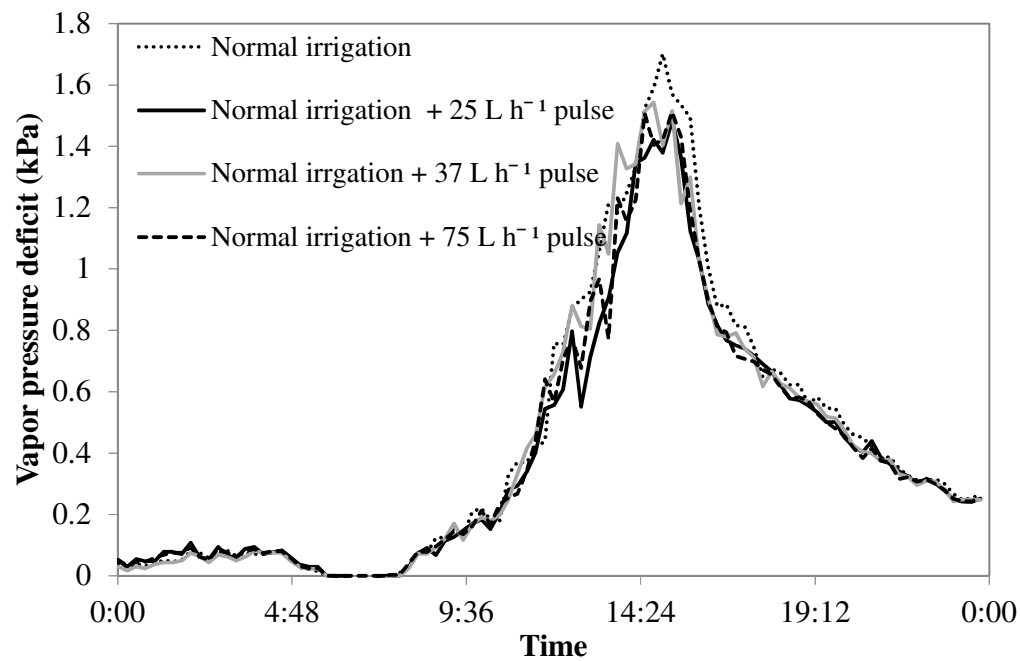
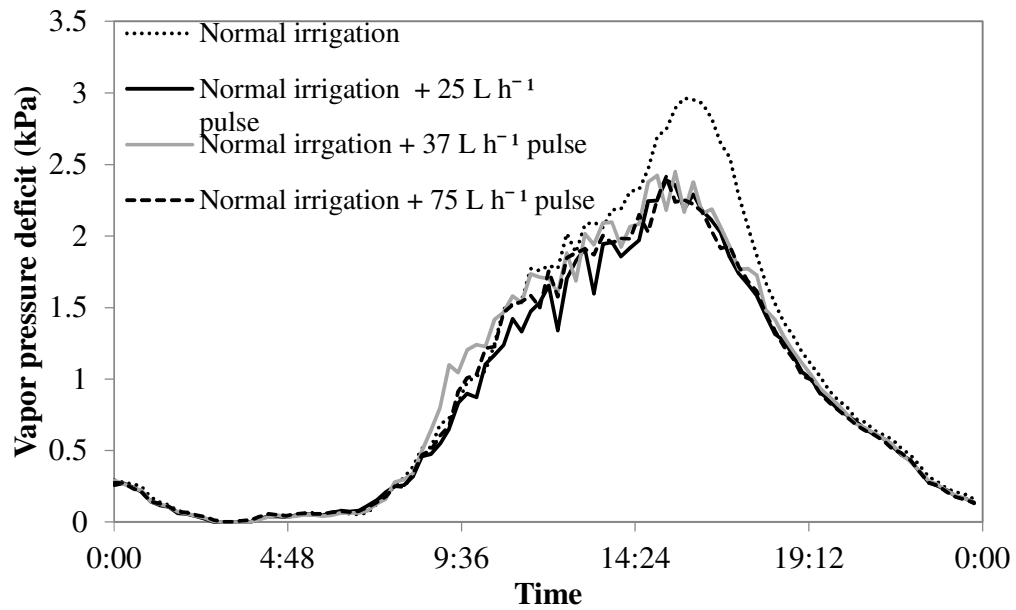


Figure 8. (Top) Canopy vapor pressure deficit in ‘Granny Smith’ on a hot day (110 DAFB, 31 Jan 2014, maximum air temperature 34.2 °C) compared to (bottom) a cooler day with no pulsing (109 DAFB, 30 Jan 2014, maximum air temperature 28.2 °C).

Appendix 1. Soil texture analysis at Meulrivier, Greyton, South Africa for 'Granny Smith' GS and Golden Delicious (GD).

Cultivar	Depth (mm)	Clay (%)	Silt (%)	Sand (%)	Fine Sand (%)	Medium Sand (%)	Coarse Sand (%)	Stone (%) (v/v)
GS Row 1	0-300	9	12	79	46.0	25.6	7.4	2.3
GS Row 2	0-300	9	12	79	44.3	21.7	13.0	3.8
GS Row 1	300-600	9	10	81	45.2	26.4	9.4	2.5
GS Row 2	300-600	9	10	81	40.7	29.6	10.7	8.1
GD Row 1	0-300	9	6	85	47.4	27.6	10.0	2.4
GD Row 2	0-300	7	8	85	46.3	28.0	10.7	4.0
GD Row 1	300-600	7	8	85	46.6	28.0	10.4	3.4
GD Row 2	300-600	9	4	87	41.1	35.6	10.3	4.7

Appendix 2. Field capacity (FC), readily available water (RAW) and water holding capacity (WHC) of soil at Meulrivier, Greyton, South Africa for ‘Granny Smith’ GS and Golden Delicious (GD).

Cultivar	Depth (mm)	FC (%)	RAW(%)	WHC mm/m
GS Row 1	0-300	23.93	12.74	111.90
GS Row 2	0-300	24.38	13.25	111.25
GS Row 1	300-600	22.88	12.06	108.24
GS Row 2	300-600	20.47	11.06	94.06
GD Row 1	0-300	21.37	10.68	106.91
GD Row 2	0-300	21.12	10.60	105.19
GD Row 1	300-600	21.27	10.66	106.18
GD Row 2	300-600	17.10	8.88	88.26

Paper 3: Effect of foliar S-ABA application on sunburn, fruit quality, peel chemical composition and the ecophysiological status of ‘Granny Smith’ apple trees under South African conditions

Abstract

Sunburn is a physiological disorder that affects the visual quality of apple fruit. In South Africa, producers estimate yield losses of up to 50% due to sunburn damage. Recent research in Japan has found that foliar application of abscisic acid (S-ABA) reduced sunburn in apples by up to 30%. The aim of this study was to examine the effect of S-ABA application on the occurrence of sunburn, other fruit quality parameters, gas exchange, plant water status, peel chemical composition and fruit set in ‘Granny Smith’ under South African conditions. Trials were conducted over three growing seasons from 2010–2011 to 2012–2013 in a ‘Granny Smith’ orchard in a prime apple producing area in the Western Cape Province. S-ABA was applied at concentrations between 250–1000 ppm and various timings during summer (from November until harvest in March/April). Fruit set was recorded in the 2011–2012 growing season when S-ABA was applied at full bloom. Tree ecophysiological measurements were taken in the 2010–2011 and 2012–2013 growing seasons. Peel chemical composition analysis was done in the 2012–2013 growing season. A representative scaffold branch on both sides of the tree was strip picked at commercial harvest resulting in samples of at least 100 fruit per tree for sunburn, fruit color, and red blush assessment. A sub-sample of 20 fruit was randomly selected and used to determine average fruit size, fruit firmness, and internal quality. The application of S-ABA had no effect on sunburn incidence and severity under South African growing conditions. In terms of peel composition, the concentration of total glutathione, oxidized glutathione, reduced glutathione, total chlorophylls and total carotenoids did not increase. In fact, S-ABA application had a negative effect by reducing the concentration of total antioxidants, total phenolics and reduced ascorbic acid whilst increasing oxidized ascorbic acid. Net carbon assimilation rate, stomatal conductance and transpiration rate were reduced by S-ABA application, while the stem water potential was increased. Concomitant with the decrease

in carbon assimilation, there was a significant reduction in fruit weight and diameter with repeated applications of S-ABA compared to the control treatment. Repeated applications of S-ABA also caused a significant reduction in titratable acidity and total soluble solids concentration. Fruit firmness and starch conversion were not affected by S-ABA application. A negative effect of S-ABA on green peel color was noted for some applications while red blush development was not affected by S-ABA application. Leaf necrosis was observed when S-ABA was applied during periods of high temperature. The application of S-ABA had no effect on fruit set. South Africa has a harsher climate than Japan and the application of S-ABA to reduce sunburn is ineffective and not recommended.

1. Introduction

Sunburn is a physiological disorder of apples that causes discoloration of the fruit surface thereby affecting fruit visual quality (Felicetti and Schrader, 2009). It is a serious problem in many apple-growing regions of the world. In South Africa producers estimate yield losses of up to 50% due to sunburn damage (Wand et al., 2006). High ambient temperatures and excessive solar radiation cause overheating of the fruit surface, leading to the development of sunburn (Chen et al., 2008; Wünsche et al., 2004a). Three types of sunburn have been identified namely, sunburn necrosis, sunburn browning (Schrader et al., 2001) and photo-oxidative sunburn (Felicetti and Schrader, 2008; Schrader et al., 2003).

Protection against sunburn includes natural defense mechanisms within the fruit peel and cultural practices adopted by growers. Fruit peel sunburn protection is offered by physiochemical properties of the peel such as homogeneity, thickness and composition of the epicuticular wax layer and the amount of hair on the skin surface, (Wünsche et al., 2004a), heat shock proteins (Ferguson et al., 1998; Ritenour et al., 1998), photoprotective pigments (Felicetti and Schrader, 2009) and antioxidant compounds (Yuri et al., 2010). Cultural practices that have been adopted to reduce sunburn include evaporative cooling (Evans, 2004; Gindaba and Wand, 2005), the application of kaolin-based reflective particle films (Glenn et al., 2002; Gindaba and Wand, 2005; Wünsche et al., 2004b) and installation of shade nets (Gindaba and Wand, 2005; Iglesias and Alegre, 2006; Smit, 2007). However, the complexity of the underlying physiological mechanisms and

environmental processes leading to sunburn has made it difficult to come up with a single solution to the problem (Wünsche et al., 2004a).

Absciscic acid (ABA) is a plant growth regulator that is involved in the signaling and regulation of plant responses to water stress (Kim and van Iersel, 2011). ABA is produced in the roots and transported to the shoots via the xylem where it regulates stomatal closure in leaves and fruit thereby controlling transpirational water loss from the plant (Zhang and Davies, 1987). Due to the high cost of production and chemical instability ABA has, until recently, had few applications in horticulture (Cao et al., 2013). However, recent advances in methods of production have made it economically feasible to use ABA in horticultural production (Peppi et al., 2006). ABA has been used in horticulture for the prevention of blossom end rot in tomato plants grown under low calcium conditions by improving calcium transport in xylem, resulting in higher tissue calcium concentrations (Tonetto de Freitas et al., 2011; Tonetto de Freitas et al., 2014). ABA has also been used as a physiological antitranspirant to improve shelf life and quality of pot plants by causing a delayed initiation of wilting (Kim and Van Iersel, 2011; Astacio and Van Iersel 2011; Waterland et al., 2010). Another application has been for the maintenance of postharvest quality and improving the color of red grapes by stimulating anthocyanin production (Cantin et al., 2007; Hiratsuka et al., 2001; Peppi et al., 2006), as a fruit thinner in apples and pears (Greene, 2012; Greene et al., 2011) and to prime young apple trees so as to provide dehydration protection under conditions of water stress (Tworkoski et al., 2011). Recent research in Japan has found that the foliar application of S-ABA (biologically active form of absciscic acid or [5-(1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)-3-methyl-2,4-pentadienoic acid] reduced sunburn incidence in 'Tsugaru', 'Sensyu', 'Yataka' and 'Fuji' apples by up to 30% (Iamsb et al., 2009; Iamsb et al., 2008). The S-ABA application was associated with increased antioxidant levels, thereby alleviating oxidative damage caused by high ambient temperatures and irradiance (Iamsb et al., 2009).

According to Racsko and Schrader (2012), further research is needed to determine whether S-ABA will also reduce sunburn in climatically harsher regions (such as South Africa) that experience higher summer temperatures and higher levels of irradiance. Therefore, the aim of this research was to examine the effect of S-ABA application on the incidence of sunburn in 'Granny Smith' apples under South African conditions. Considering the various effects of ABA

on plant physiology and ecophysiology, which may prove beneficial or detrimental in a commercial apple production setup, other fruit quality parameters, gas exchange, plant water status, peel chemical composition and fruit set were also assessed.

2. Materials and Methods

2.1 Study site and plant material

Trials were conducted over three growing seasons from 2010-2011 to 2012-2013 in a ‘Granny Smith’ orchard at Disseldraai farm in Grabouw, South Africa (Latitude: 34°16’S; Longitude: 19°03’E, Alt 266 m). The region has a Mediterranean-type climate with high daily maximum temperature and irradiance during the growing season as shown by weather data obtained at Oak Valley Estate in Grabouw, South Africa (Latitude: 34°15’S; Longitude: 19°07’E, Alt 375 m) (Figure 1). This is in contrast to Tsukuba, Japan where the original research was done, with a warm temperate climate with lower irradiance and maximum temperatures during the growing season (Figure 2 and Figure 3). The Disseldraai orchard was planted in 1993 on M793 rootstock at a spacing of 4.5 x 2 m in an east - west row orientation. Normal commercial cultural practices of irrigation, pest management and fertilization were followed. ‘Granny Smith’ is the second most cultivated apple cultivar in South Africa, making up 18.4 % of the total area planted (Hortgro, 2014) and it is highly susceptible to sunburn (Fouché et al., 2010). Therefore, considering the substantial economic losses due to sunburn, it is an important cultivar to conduct sunburn research on.

2.2 Treatments and experimental design

The treatments in the 2010-2011 growing season consisted of an untreated control, while 250 ppm S-ABA (Valent BioSciences, Libertyville, IL, USA), 500 ppm S-ABA and 1000 ppm S-ABA were applied monthly on 13 Jan. 2011 (92 days after full bloom - DAFB), 11 Feb. 2011 (121 DAFB) and 11 Mar. 2011 (149 DAFB). In the 2011-2012 growing season, treatments consisted of an untreated control, and 1000 ppm S-ABA applied at full bloom (FB, 17 Oct. 2011), monthly on 04 Dec. 2011 (48 DAFB), 05 Jan. 2012 (80 DAFB), 03 Feb. 2012 (109 DAFB) and 03 Mar. 2012 (140 DAFB) or whenever ambient temperature was forecasted to exceed 32 °C on 19 Jan. 2012 (94 DAFB), 13 Feb. 2012 (119 DAFB) and 09 March 2012 (144

DAFB) with a minimum gap of two weeks between applications. The arbitrary 32°C threshold for application was chosen considering the daily temperatures typically experienced during summer in this region and based on fruit peel temperature measurements done by WJ Steyn (pers. comm.) in previous seasons. The aim was to set a threshold that would balance the need for sunburn control with logistic and practical considerations of application and cost. Treatments in 2012-2013 consisted of an untreated control, and 400 ppm S-ABA applied 40 DAFB on 24 Nov. 2012 or 80 DAFB on 03 Jan. 2013. All treatments were applied using a motorized knapsack sprayer at a rate of 2 L tree⁻¹ (2 222 L ha⁻¹) as a full cover spray. In all three seasons, ten single-tree replicates per treatment were used with buffer trees between treatment plots and buffer rows between treatment rows in a randomized complete block design. Different trees were used in each season. Gas exchange measurements (methodology reported below) indicated that S-ABA application affected tree physiology and can thus be assumed to have been taken up effectively. The effect of S-ABA application on levels of active ABA within the plant falls outside the scope of this study.

The treatments during the first year (2010-2011) were adapted from Iamsub et al. (2008) and Iamsub et al. (2009), who originally reported the reduction of sunburn incidence after application of S-ABA. For the second season (2011-2012), an early application at full bloom was done with the aim to improve xylem connectivity, and therefore water supply to the fruit as reported by Tonetto de Freitas et al. (2014) in tomato. The treatment before the heat wave was done to increase the antioxidant capacity in the fruit peel just before photothermal stress – upregulation of antioxidant capacity was the mechanism reported for sunburn reduction by Iamsub et al. (2008) and Iamsub et al. (2009). For the 2012-2013 growing season treatments were imposed after full bloom and at lower concentration to avoid the negative effects observed in the 2011-2012 growing season on fruit size when sprayed at 1 000 ppm at FB.

2.3 Sunburn assessment

A representative scaffold branch on both the north and south facing sides of trees was strip picked at commercial harvest resulting in samples of at least 100 fruit per tree. Sunburn incidence and severity was assessed using the Schrader and McFerson scale (Schrader et al.,

2003) where 0 represented no sunburn, 1 to 4 refer to increasing levels of sunburn browning, and 5 signifies sunburn necrosis (Appendix 1).

2.4 Fruit quality

Samples were assessed for green color intensity on the greenest side of the fruit using a color chart (Set A 38, Deciduous Fruit Board, South Africa) and the occurrence of red blush using a color chart (Set A 32, Deciduous Fruit Board, South Africa). A sub-sample of 20 fruit was randomly selected and used to determine average fruit size. Fruit firmness was measured using a fruit texture analyzer at the equatorial region on opposite sides of the fruit (Güs, GS 20, Strand, South Africa). Percentage starch conversion was measured using the iodine test and starch conversion chart (Unifruco Research Services, Bellville, South Africa). Sub-sample fruit were pooled for juice extraction. A hand-held refractometer (Model N1, Atago, Tokyo, Japan) was used to measure total soluble solids concentration (TSS). Titratable acidity (TA) was determined by titrating 5 g of juice with 0.1 M NaOH with an automated titrator (Model 719 S, Metrohm AG, Hersiau, Switzerland) and expressed as percentage of malic acid.

2.5 Peel chemical composition

Peel chemical composition analysis was done in the 2012-2013 growing season on fruit collected 10 and 20 days after spraying (DAS) following both application dates. Ten fruit representative of those on tagged branches were randomly selected per tree replicate and fruit peel tissue separated from the flesh. The collected peel tissue samples were flash frozen in liquid nitrogen before being finely milled. Milled samples from each tree replicate were pooled together and then stored in a freezer at -80 °C.

2.5.1 Ascorbic acid and glutathione

High-performance liquid chromatography (HPLC) was used for ascorbic acid and glutathione analysis. The analysis was done in three phases according to the method by Jooste (2012) with some minor modifications. Firstly, in the extraction phase, 10 ml of extraction buffer was added to 2.0 g of fresh frozen sample in a centrifuge tube. The extraction buffer was made up of 3% metaphosphoric acid (MPA), 1.0 mM ethylenediaminetetracetic acid (EDTA) and 2% insoluble polyvinylpyrrolidone (PVPP). The mixture was vortexed and left to stand for 15 min, after

which 1.8 ml was pipetted into a 2.0 ml eppendorf tube and then centrifuged at 20 000 x g for 15 min. Secondly, the analysis phase was done in two steps. The first step involved direct analysis of clean supernatant to identify the reduced forms of ascorbic acid and glutathione. The second step involved reducing ascorbic acid and glutathione from oxidized forms using 20 μ l of 400 mM DL-dithiothreitol (DTT) to measure the total ascorbic acid and glutathione in each sample. Oxidized ascorbic acid and glutathione were determined by subtracting reduced values from the total. HPLC analysis was done on Agilent Series 1100 HPLC system (Agilent Technologies, Inc., Waldbronn, Germany) using a photodiode array detector and C₁₈ 5- μ m stationary phase column protected by a 4.6 mm x 12.5 mm guard cartridge (Zorbax SB-C18, Agilent, USA). A known concentration of ascorbic acid and glutathione was used to identify retention peaks. The final phase involved quantification of ascorbic acid and glutathione and this was done using Chemstation for LC 3D systems software (Rev. B.10.03 (204), Agilent Technologies, Inc., Waldbronn, Germany). Results were expressed as micrograms per gram fresh weight (μ g.g⁻¹ FW).

2.5.2 Total antioxidant capacity

Total antioxidant analysis was done using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay according to the method by Karioti et al. (2004) with some modifications. The method involved the addition of 40 mL distilled water to 0.5 g fresh frozen sample. The mixture was vortexed at 10 000 rpm for 10 min. In triplicates, 15 μ l of clean sample was added into eppendorf tubes together with 735 μ l of methanol and 750 μ l of 0.1 mM DPPH. The tubes were covered in aluminum foil and incubated at room temperature for 30 min. Absorbance was measured at 517 nm on the spectrophotometer (Cary 50 Bio, Varian, Australia (PTY) Ltd, Melbourne, Australia). The spectrophotometer was blanked with 100% methanol and a standard curve of ascorbic acid from 0 to 2.0 mM was constructed. Total antioxidant activity was expressed as milligrams per gram fresh weight (mg.g⁻¹ FW).

2.5.3 Total phenolics

Total phenolics were measured by the Folin Ciocalteu's (FC) phenol colourimetric method. The method involved the addition of 5 mL of 80% ethanol to 1.0 g fresh frozen sample in a 50 ml centrifuge tube. The mixture was then ground finely with Ultra Turrax. A magnetic rod was

added to the mixture and the tubes were placed in a fridge at 4 °C where they were constantly stirred using a magnetic stirrer. In triplicates, 10 µl of the sample, 40 µl of 80% ethanol and 450 µl of 0.1M FC reagent were added to plastic cuvettes. After 5 min 500 µl of 5.6% Na₂CO₃ was added to the cuvette and the mixture vortexed after which it was left to stand for 90 min before readings were taken. The spectrophotometer was blanked with 80% ethanol and a standard curve of Gallic acid (mg/L) from 0 to 2.0 was constructed. Total phenolic concentration was expressed as Gallic acid equivalent (GAE) in milligrams per gram fresh weight (mg.g⁻¹ FW).

2.5.4 Total chlorophyll and carotenoids

Total chlorophyll and carotenoids were analyzed according to the method of Lichtenthaler (1987) with some modifications. The method involved the addition of 3.0 ml cold, 100% acetone to 0.5 g fresh frozen sample in a 50 ml centrifuge tube. A magnetic rod was added to the mixture and the tube was placed in a fridge at 4 °C where it was constantly stirred using a magnetic stirrer. After extraction, the sample was then centrifuged for 15 min at 3500 rpm. The supernatant was decanted and 2.0 ml of cold 100% acetone was added to the remaining pellet. The sample was then centrifuged again for 15 min at 3500 rpm and the clean extract decanted into the vial with the first supernatant. This final supernatant was filtered using a 0.45 µm filter (Millex-HV, Millipore, Corporation, Milford, MA, USA) into a glass cuvette. Absorbance was measured at 470, 645 and 670 nm on the spectrophotometer (Cary 50 Bio, Varian, Australia (PTY) Ltd, Melbourne, Australia). The spectrophotometer was blanked with 100% cold acetone beforehand. The extinction coefficients of Lichtenthaler (1987) were used to calculate the chlorophyll and carotenoid concentrations in µg.g⁻¹FW (fresh weight).

2.6 Tree ecophysiological status

Tree ecophysiological measurements were done 7 DAS at 123 DAFB for the 2010-2011 growing season to determine whether the S-ABA application had an effect on tree physiology and could therefore be expected to have been taken up by the plant. Since these measurements indicated that S-ABA had physiological effects, no measurements were done in the 2011-2012 growing season. In the 2012-2013 growing season, it was decided to do measurements at 10 and 20 DAS at 40 and 80 DAFB to determine the duration of the S-ABA effects.

2.6.1 Gas exchange

Gas exchange measurements (net CO₂ assimilation rate, stomatal conductance, transpiration rate) were taken using an infrared gas analyzer, LI-6400 (Li-Cor, Lincoln, Nebraska, USA). Flow rate was set at 500 $\mu\text{mol s}^{-1}$, reference carbon dioxide concentration at 380 ppm, leaf temperature at 25 °C and photosynthetic photon flux density at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Measurements were taken on sun-exposed leaves on the northern side of the tree. Two leaves of the same age, size and health were used per treatment replication. Leaves were sampled from shoulder height and all readings were taken between 08:00 and 11:30.

2.6.2 Plant water status

The effect of S-ABA on plant water status was assessed by measuring midday stem water potential using a pressure bomb (Model 600, PMS Instrument Co, USA). For each tree replicate, two healthy leaves of the same age, size and proximity to the stem, from inside the canopy were bagged for at least one hour in silver reflective bags to equilibrate the leaf and the stem water potential before readings were taken. Leaves were sampled from shoulder height and all readings were taken between 12:00 and 13:30.

2.6.3 Fruit surface temperature

Fruit surface temperature was measured using a hand-held infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA) between 14:00 and 14:30 on the sun-exposed part of the fruit. Five fruit per treatment replication were tagged for measurements.

2.7 Fruit set measurements

As indicated for the description of the treatments, S-ABA application during early tomato fruit development was reported to improve xylem connectivity (Tonetto de Freitas et al., 2014). Hence, an early application was included in 2011-2012 to assess its effect on sunburn. More detailed anatomical assessment would have been made if the treatment showed any potential. Since S-ABA applied during early fruit development can induce fruit abscission (according to Phillagro SA, our source of S-ABA), fruit set was recorded in the 2011-2012 season by tagging

five flower clusters per tree at full bloom and counting the number of fruit set after natural fruit drop approximately 40 DAFB.

2.8 Statistical analysis

Data were analyzed using the General Linear Models (GLM) procedure of SAS Enterprise Guide 3.0 (SAS Institute Inc., 2004, Cary, NC, USA). Where significant differences occurred ($p \leq 0.05$), means were separated by the Least Significant Difference (LSD). Linear and quadratic contrasts were fitted where applicable.

3. Results

3.1 Sunburn incidence

The application of S-ABA at different concentrations and timings did not decrease the incidence and severity of the different sunburn types in ‘Granny Smith’ under South African growing conditions in any of the three growing seasons (Table 1, 2 and 3). In fact, for the 2011-2012 growing season, the application of 1000 ppm S-ABA at FB and preceding ambient temperature exceeding 32 °C increased sunburn necrosis compared to the control (Table 2). Sunburn incidence was also increased by the application of 1000 ppm S-ABA at FB (Table 2). Only in the 2011-2012 season was there a significant contrast between S-ABA applications and control, with S-ABA application resulting in higher sunburn necrosis compared to the control (Table 2).

3.2 Fruit quality

There were no significant differences in fruit weight and diameter between the treatments in the 2010-2011 growing season (Table 4). However, fruit diameter and weight, decreased linearly with increasing S-ABA concentration (Table 4). Repeated applications of S-ABA caused a reduction in fruit size compared to the control in the 2011-2012 growing season (Table 5). Fruit size was not affected by S-ABA applications in the 2012-2013 growing season (Table 6).

The application of 1 000 ppm S-ABA in the 2010-2011 growing season resulted in significantly greener fruit (Table 4). Fruit green color increased linearly with increasing S-ABA concentration in the 2010-2011 growing season (Table 4). Monthly applications of 1 000 ppm S-ABA and at FB application resulted in less green fruit during the 2011-2012 growing season (Table 5). There

was no significant difference in green color between treatments in the 2012-2013 season when S-ABA was applied at 40 DAFB and 80 DAFB (Table 6). S-ABA application did not affect the incidence of red blush in any of the seasons (Tables 4, 5 and 6).

Fruit firmness and starch conversion were not affected by S-ABA application in any of the seasons (Tables 4-9). Repeated applications of S-ABA reduced TSS in the 2010-2011 and 2011-2012 (Tables 7 and 8). TSS decreased linearly with increasing S-ABA concentration in the 2010-2011 growing season (Table 7). Single applications of S-ABA had no effect on TSS in the 2011-2012 and 2012-2013 growing seasons (Tables 8 and 9). A similar trend was observed for TA with repeated applications of S-ABA at high concentration (1 000 ppm) causing a significant reduction in TA in the 2010-2011 and 2011-2012 seasons (Tables 7 and 8). However, repeated applications at lower concentrations (250 and 500 ppm) and single applications of S-ABA had no effect on TA in any of the seasons (Tables 7, 8 and 9). There was a linear decrease in TA with increasing S-ABA concentration (Table 7). Except for the monthly 1 000 ppm S-ABA treatment (2011-2012 season) that had a higher TSS:TA ratio than the control (Table 8), there were no significant differences between the treatments and control in terms of the TSS:TA ratio in any of the seasons (Tables 7, 8, 9).

3.3 Peel chemical composition

The application of S-ABA at 40 DAFB did not affect oxidized and total ascorbic acid or oxidized, reduced and total glutathione concentrations in the fruit peel at both 10 and 20 DAS (Table 10). Reduced ascorbic acid was significantly lower in the treated peel compared to the control at 10 DAS, but there was no difference in reduced ascorbic acid concentration between the treatments and control at 20 DAS (Table 10). At 80 DAFB, total ascorbic acid and oxidized ascorbic acid concentrations were significantly higher in the treated peel compared to the control at 10 DAS, but there was no difference at 20 DAS (Table 11). There were no significant differences in oxidized, reduced and total glutathione concentrations, and reduced ascorbic acid concentration between the S-ABA and control in the fruit peel at both 10 and 20 DAS (Table 11). When sprayed at 40 DAFB, S-ABA significantly reduced the concentration of total antioxidants and total phenolics when measured 10 DAS, but not at 20 DAS (Table 12). Total chlorophyll and carotenoid concentrations were not significantly different at both 10 and 20 DAS (Table 12). There were no significant differences in concentration of total antioxidants,

phenolics, chlorophylls and carotenoids when S-ABA was applied at 80 DAFB, measured at both 10 and 20 DAS (Table 13).

3.4 Tree ecophysiological status

Net carbon assimilation rate, stomatal conductance and leaf transpiration rate were significantly reduced by S-ABA application compared to the control in the 2010-2011 growing season (Table 14). The application of S-ABA at 40 DAFB in 2012-2013 had no effect on net carbon assimilation rate, stomatal conductance or leaf transpiration rate when measured at 10 and 20 DAS (Table 15). Net carbon assimilation rate, stomatal conductance and leaf transpiration rate were significantly reduced compared to the control by S-ABA application at 80 DAFB when measured at 10 DAS, but not at 20 DAS (Table 16). There were no significant differences in fruit surface temperature between the treatments in any of the seasons (Tables 14, 15 and 16). Stem water potential was significantly higher for the 500 ppm S-ABA treatment when measured 7 DAS during the 2010-2011 growing season (Table 14). During the 2012-2013 growing season, stem water potential was significantly higher at 10 DAS for both 40 and 80 DAFB sprays, but not different compared to the control when measured at 20 DAS (Tables 15 and 16). Leaf necrosis occurred when S-ABA was applied at 1 000 ppm preceding a heat wave (Figure 4).

3.5 Fruit set

S-ABA application at FB had no effect on fruit set (Table 17).

4. Discussion

The application of S-ABA did not reduce sunburn incidence and severity in 'Granny Smith' under South African conditions. Sunburn incidence in the control was 45.2%, 30.7% and 39 % in the 2010-2011, 2011-2012 and 2012-2013 growing seasons, respectively, measured on the Schrader and McFerson scale (Schrader et al., 2003). Previously, exogenous applications of S-ABA have been reported to decrease sunburn in 'Tsugaru', 'Sensyu', 'Yataka' and 'Fuji' apples by up to 30% at rates of 100 ppm to 800 ppm in Japan (Iamsu et al., 2008; Iamsu et al., 2009). In both studies, sunburn incidence was around 70% but there is no information on how sunburn was graded. S-ABA application consistently increased total antioxidant capacity, total

phenolics, chlorophyll concentration and ascorbic acid in fruit peel (Iamsub et al., 2009). Under South African growing conditions, the peel concentration of total glutathione, oxidized glutathione, reduced glutathione, total chlorophylls and total carotenoids did not increase. In fact, S-ABA application had a negative effect by reducing concentration of total antioxidants, total phenolics, and reduced ascorbic acid whilst increasing oxidized ascorbic acid. This was observed 10 DAS at 40 DAS and 10 DAS at 80 DAFB. The main apple growing regions of South Africa have a harsher climate with higher temperatures often exceeding 32 °C and high radiation levels during the growing season. The application of S-ABA under such conditions can cause the production of large amounts free radicals (Jiang and Zhang, 2001). If these free radicals cannot be scavenged, sunburn is then likely to occur. This can be further corroborated by results from our research where the application of S-ABA on a day preceding a heat wave increased the incidence of sunburn at harvest. Under less stressful conditions, such as those found in Japan, the beneficial effects of increased concentration of antioxidant-related compounds after application of S-ABA may have led to the reduction in sunburn as reported. The increase in sunburn when applied at full bloom may be due to increased sensitivity of young fruit to S-ABA sprays. Although leaf damage occurred, no visible defoliation could be seen which might have affected sunburn incidence from increased photooxidative sunburn. Also, at this point in the growing season, ambient temperatures are still low and fruit surface temperature rarely reaches the threshold for sunburn development. The sensitivity of 'Rosemarie' pear to photo thermal stress increased with fruit development (Steyn et al., 2009), which may explain the increased sensitivity of pome fruit to sunburn as the fruit matures.

Significant effects of S-ABA application on carbon assimilation, plant water status and fruit size rule out that the lack of effect on sunburn could be due to inadequate absorption of S-ABA. Although these leaf parameters do not imply that S-ABA was taken up or exported to the fruit, the effects observed on fruit color (Tables 4, 5) as well as antioxidants in the apple peel (Tables 10-12) do suggest that fruit did take up or received S-ABA through the vascular system. Net carbon assimilation rate, stomatal conductance, and leaf transpiration were significantly reduced by S-ABA application when measured 7 and 10 DAS. Similar results have been reported in vivo with potato leaves (Baricevic and Stopar, 1994). The reduction in net carbon assimilation rate, stomatal conductance and leaf transpiration rate can be explained by the stomatal closure induced by S-ABA application. The response did not extend beyond 10 DAS and no significant

differences remained at 20 DAS. Baricevic and Stopar (1994) similarly reported that net gas exchange returned to normal 8-16 days after applications. Plant water status represented by the stem water potential was significantly improved immediately after application and the effect faded over time. Plant water status also returned to normal at 20 DAS, suggesting that the effect on plant water status was temporary. Improved plant water status with S-ABA application has also been reported in young apple trees (Tworkoski et al., 2011) and in artichokes (Shinohara and Leskovar, 2014).

Leaf necrosis was observed when 1 000 ppm S-ABA was applied during periods of high temperatures. Under stress conditions caused by high temperatures, high concentrations of S-ABA can cause phytotoxicity leading to leaf damage (Ibrahim and Jaafar, 2013; Waterland et al., 2010). Also, S-ABA application has been shown to result in increased leaf temperatures due to the reduction in transpiration which may lead to leaf necrosis (O'Donoghue et al., 2011).

Repeated applications of S-ABA caused a reduction in fruit size. Fruit size also decreased with increasing concentration of S-ABA applied. The reduction in fruit size in our work can be explained by the fact that the application of S-ABA causes stomatal closure resulting in a reduction of CO₂ assimilation rate (Waterland et al., 2010, Greene 2012). A reduction in stomatal conductance was observed 7 DAS in the 2010-2011 growing season. During the 2012-2013 growing season, no effect on stomatal closure was observed at 40 DAFB. At 80 DAFB, the application of S-ABA caused stomatal closure until 10 DAS, but not observed at 20 DAS. Therefore, the effect on stomatal conductance was not lasting and inconsistent in our study. Thus fruit in S-ABA treated trees are likely to be smaller due to a lack of assimilates arising from reduced photosynthesis. Higher S-ABA concentrations will have a more adverse effect on stomatal closure and photosynthesis, leading to smaller fruit with increasing S-ABA applications. In contrast, an increase in fruit size has been reported from single applications of S-ABA when used as a fruit thinner at petal drop (Greene, 2012). This increase in fruit size is due to the thinning effect of S-ABA at petal drop which leaves fewer fruit to compete for photoassimilates.

There was no consistent effect of the application of S-ABA on the green color of 'Granny Smith' apples. Monthly applications of S-ABA improved fruit green color in the 2010-2011 growing season. In contrast, monthly applications in the 2011-2012 season had a negative effect on fruit

color. S-ABA is a product of the carotenoid biosynthetic pathway (Barickman et al., 2014). The carotenoid biosynthetic pathway is involved in photosynthesis and photo protection in plants (Shumskaya and Wurtzel, 2013). S-ABA has been shown to improve leaf green color in tomato plants by increasing the chlorophyll concentration (Barickman et al., 2014). In contrast, Wang et al. (2005) reported a decrease in the chlorophyll content in the pericarp of litchi fruit after S-ABA application. The occurrence of red blush was not affected by S-ABA application. This is in contrast to previous work that has reported an improvement in the color of red apple cultivars due to the promotion of anthocyanin synthesis (Greene et al., 2011).

The application of S-ABA did not affect fruit maturity judging from fruit firmness and starch conversion data. This is in agreement with previous research (Greene et al., 2011), although an improvement in firmness when S-ABA was applied at petal drop has been observed (Greene, 2012). While single applications of S-ABA had no effect on TSS and TA in this study and as reported by Greene et al., (2011), we found that repeated applications of S-ABA reduced TSS and TA. The S-ABA-induced closure of stomata may decrease photosynthesis and this may result in lower levels of photoassimilates in the fruit, hence lower TSS and TA (Waterland et al., 2010). In contrast, the application of S-ABA at petal drop, which had a thinning effect has been found to increase TSS (Greene, 2012). Fewer fruit were left on the tree after thinning, therefore more photoassimilates are available to the remaining fruit.

The application of S-ABA at FB had no effect on fruit set. High levels of S-ABA are associated with fruitlet abscission in apples (Eccher et al., 2013). The application of S-ABA at FB has been reported to reduce fruit set and have a thinning effect in apples (Greene et al., 2011). However, the results were inconsistent, with positive results in 3 out of 5 years, suggesting that climatic conditions and other factors may influence the efficacy of the S-ABA (Greene et al., 2011).

5. Conclusion

This research, done over three seasons, showed that foliar S-ABA application at different timings and application rates had no positive effect on sunburn incidence and severity in ‘Granny Smith’ apple under South African conditions. In fact, the application of high concentrations of S-ABA at FB and before a hot day increased sunburn necrosis and incidence. The positive effect on fruit peel biochemistry observed in Japan was not obtained in our study. In fact, the increased sunburn

incidence and necrosis observed in response to high S-ABA concentration and application before hot days may relate to apparent increased oxidation of antioxidants. The effects of S-ABA at high concentration on stomatal aperture caused a transient decrease in gas exchange that led to a reduction in fruit size and negative effects on internal fruit quality. However, there was a positive effect on tree water status in terms of stem water potential due to stomatal closure and the reduction in transpiration. Based on our results, the application of S-ABA to reduce sunburn is not recommended in regions similar to South Africa that experience high temperature and high irradiance.

6. References

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Table 1. The effect of S-ABA application on sunburn severity and incidence of ‘Granny Smith’ apples at Disseldraai, Grabouw, during the 2010-2011 season.

Treatment	Sunburn severity (all fruit) ^z	Sunburn severity (sunburn fruit) ^y	Sunburn browning (%) ^x	Sunburn necrosis (%) ^w	Sunburn incidence (%)
1. Control	1.35 ns	2.99 ns	28.5 ns	16.7 ns	45.2 ns
2. 250 ppm S-ABA	1.26	2.86	29.4	14.9	44.3
3. 500 ppm S-ABA	1.28	2.97	28.9	14.8	43.7
4. 1000 ppm S-ABA	1.09	2.96	23.4	12.5	35.9
Pr>F					
Treatment	0.4066	0.8852	0.2165	0.5020	0.1519
S-ABA vs Control	0.2970	0.6813	0.6316	0.2410	0.2888
S-ABA linear	0.2330	0.6345	0.0664	0.3865	0.0661
S-ABA quadratic	0.5378	0.6272	0.3635	0.6555	0.3543

^z0-5 score, with 0 having no sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^y1-5 score, with 1 having least sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^x1-4 score, (Schrader and McFerson sunburn chart).

^w5 score, (Schrader and McFerson sunburn chart).

ns - Not significant.

Table 2. The effect of S-ABA application on sunburn severity and incidence of ‘Granny Smith’ apples at Disseldraai, Grabouw, during the 2011-2012 season.

Treatment	Sunburn severity (all fruit) ^y	Sunburn severity (sunburn fruit) ^x	Sunburn browning (%) ^w	Sunburn necrosis (%) ^v	Sunburn incidence (%)
1. Control	0.58 ns	1.87 ns	29.4 ab ^z	1.31 b	30.7 b
2. 1000 ppm S-ABA at FB	0.74	1.88	35.4 a	3.11 a	38.4 a
3. 1000 ppm S-ABA monthly	0.68	2.00	27.3 b	2.60 ab	29.9 b
4. 1000 ppm S-ABA (>32 °C)	0.68	2.11	27.5 b	3.54 a	31.0 b
	Pr>f				
Treatment	0.2124	0.4701	0.0481	0.0373	0.0384
S-ABA vs Control	0.0631	0.3695	0.7850	0.0080	0.3466

^zMeans with a different letter differ significantly at the 5% level (LSD).

^y0-5 score, with 0 having no sunburn and 5 the s most severe (Schrader and McFerson sunburn chart).

^x1-5 score, with 1 having least sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^w1-4 score, (Schrader and McFerson sunburn chart).

^v5 score, (Schrader and McFerson sunburn chart).

ns - Not significant.

Table 3. The effect of S-ABA application on sunburn severity and sunburn incidence of ‘Granny Smith’ apples at Disseldraai, Grabouw, during the 2012-2013 season.

Treatment	Sunburn severity (all fruit) ^z	Sunburn severity (burnt fruit) ^y	Sunburn browning (%) ^x	Sunburn necrosis (%) ^w	Sunburn incidence (%)
1. Control	0.67 ns	1.68 ns	37.5 ns	1.50 ns	39.0 ns
2. 400 ppm S-ABA, 40 DAFB	0.76	1.70	42.2	1.51	43.7
3. 400 ppm S-ABA, 80 DAFB	0.68	1.71	37.0	2.55	39.5
Pr>f					
Treatment	0.4007	0.9581	0.1999	0.4548	0.2090
S-ABA vs Control	0.4075	0.7835	0.4423	0.5228	0.2930

^z0-5 score, with 0 having no sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^y1-5 score, with 1 having least sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

^x1-4 score, (Schrader and McFerson sunburn chart).

^w5 score, (Schrader and McFerson sunburn chart).

ns - Not significant.

Table 4. The effect of S-ABA application on fruit size, flesh firmness, green color and red blush of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2010-2011 season.

Treatment	Fruit weight (g)	Fruit diameter (mm)	Fruit firmness (kg)	Fruit color ^y	Red blush ^x
1. Control	137.8 ns	67.5 ns	8.31 ns	3.18 ab ^z	15.2 ns
2. 250 ppm S-ABA	138.8	68.0	8.29	3.23 a	17.6
3. 500 ppm S-ABA	135.4	67.2	8.28	3.00 bc	15.5
4. 1000 ppm S-ABA	129.0	66.0	8.27	2.93 c	13.6
	Pr>f				
Treatment	0.0857	0.1628	0.9770	0.0092	0.5360
S-ABA vs Control	0.3052	0.5523	0.6981	0.1082	0.8690
S-ABA linear	0.0189	0.0315	0.8372	0.0062	0.1592
S-ABA quadratic	0.9696	0.8636	0.9502	0.1259	0.7496

^zMeans with a different letter differ significantly at the 5% level (LSD).

^y1-12 score, with 1 being the greenest fruit and 12 the least green (A38 chart).

^x1-12 score, with 1 having no blush and 12 the most blush (A32 chart).

ns-Not significant.

Table 5 The effect of S-ABA application on fruit size, flesh firmness, green color and red blush of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2011-2012 season.

Treatment	Fruit weight (g)	Fruit diameter (mm)	Fruit firmness (kg)	Fruit color ^y	Red blush ^x
1. Control	164.8 a ^z	71.1 a	8.66 ns	1.13 c	0.93 ns
2. 1000 ppm S-ABA at FB	163.0 a	71.6 a	8.77	1.32 b	0.62
3. 1000 ppm S-ABA monthly	143.6 b	67.2 b	8.63	1.44 a	0.51
4. 1000 ppm S-ABA (>32 °C)	152.5 b	70.1 a	8.60	1.17 c	0.67
Pr>f					
Treatment	0.0008	0.0001	0.4930	0.0001	0.2583
S-ABA vs Control	0.0082	0.0299	0.8973	0.0001	0.0699

^zMeans with a different letter differ significantly at the 5% level (LSD).

^y1-12 score, with 1 being the greenest fruit and 12 the worst least green (A38 chart).

^x1-12 score, with 1 having no blush and 12 the most blush (A32 chart).

ns - Not significant.

Table 6. The effect of S-ABA application on fruit size, flesh firmness, green color and red blush of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2012-2013 season.

Treatment	Fruit weight (g)	Fruit diameter (mm)	Fruit firmness (kg)	Fruit color ^y	Red blush ^x
1. Control	139.8 ns	68.5 ns	8.01 ns	2.62 ns	0.88 ns
2. 400 ppm S-ABA \approx 40 DAFB	146.2	69.5	8.05	2.56	0.70
3. 400 ppm S-ABA \approx 80 DAFB	143.9	69.4	7.90	2.64	0.92
	Pr>f				
Treatment	0.7549	0.7237	0.4659	0.4714	0.2687
S-ABA vs Control	0.4885	0.4287	0.7517	0.7531	0.5896

^z1-12 score, with 1 being the greenest fruit and 12 the least green color (A38 chart).

^y1-12 score, with 1 having no blush and 12 the most blush (A32 chart).

ns - Not significant.

Table 7. The effect of S-ABA application on internal quality of ‘Granny Smith’ apples at Disseldraai, Grabouw, during the 2010-2011 season.

Treatment	Starch conversion (%)	TSS (°Brix)	TA (%)	TSS:TA
1. Control	22.8 ns	11.1 a ^z	0.66 ab	16.7 ns
2. 250 ppm S-ABA	19.9	11.0 b	0.67 a	16.4
3. 500 ppm S-ABA	22.5	10.7 bc	0.63 bc	17.0
4. 1000 ppm S-ABA	23.7	10.6 c	0.62 c	17.1
		Pr>f		
Treatment	0.3223	0.0060	0.0390	0.5845
S-ABA vs Control	0.6570	0.0085	0.1515	0.7634
S-ABA linear	0.0985	0.0150	0.0180	0.2265
S-ABA quadratic	0.4757	0.4014	0.3035	0.5588

^zMeans with a different letter differ significantly at the 5% level (LSD).

ns - Not significant.

Table 8. The effect of S-ABA application on internal quality of ‘Granny Smith’ apples at Disseldraai, Grabouw, during the 2011-2012 season.

Treatment	Starch conversion (%)	TSS (°Brix)	TA (%)	TSS:TA
1. Control	19.2 ns	11.8 a ^z	0.96 a	12.3 b
2. 1000 ppm S-ABA at FB	19.7	11.8 a	0.93 a	12.8 ab
3. 1000 ppm S-ABA monthly	20.1	10.8 b	0.80 b	13.6 a
4. 1000 ppm S-ABA (>32 °C)	21.6	11.0 b	0.84 b	13.2 ab
	Pr>f			
Treatment	0.2097	0.0001	0.0003	0.0439
S-ABA vs Control	0.1782	0.0002	0.0015	0.0210

^zMeans with a different letter differ significantly at the 5% level (LSD).

ns - Not significant.

Table 9. The effect of S-ABA application on internal quality of ‘Granny Smith’ apples at Disseldraai, Grabouw, during the 2012-2013 season.

Treatment	Starch conversion (%)	TSS (°Brix)	TA (%)	TSS:TA
1. Control	17.0 ns	11.8 ns	0.79ns	15.2 ns
2. 400 ppm S-ABA \approx 40 DAFB	19.1	11.7	0.84	14.3
3. 400 ppm S-ABA \approx 80 DAFB	18.6	11.6	0.85	13.9
	Pr>f			
Treatment	0.5252	0.4119	0.4065	0.3549
S-ABA vs Control	0.2746	0.2555	0.1891	0.1743

ns - Not significant

Table 10. The effect of S-ABA application at 40 DAFB on the concentration of total ascorbic acid, total glutathione, oxidized ascorbic acid, oxidized glutathione, reduced ascorbic acid and reduced glutathione in the peel of ‘Granny Smith’ apples at Disseldraai, Grabouw, during the 2012-2013 season.

Treatment	Total ascorbic acid ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Total glutathione ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Oxidized ascorbic acid ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Oxidized glutathione ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Reduced ascorbic acid ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Reduced glutathione ($\mu\text{g}\cdot\text{g}^{-1}$ FW)
10 DAS						
1. Control	1070.3 ns	65.3 ns	325.2 ns	15.9 ns	745.1 a ^z	49.4 ns
2. 400 ppm S-ABA, 40 DAFB	841.0	58.6	288.3	15.5	552.7 b	43.1
Pr>F						
Treatment	0.2044	0.1404	0.7814	0.7673	0.0095	0.0999
20 DAS						
1. Control	912.8 ns	75.7 ns	207.1 ns	12.4 ns	705.6 ns	63.3 ns
2. 400 ppm S-ABA, 40 DAFB	947.7	74.0	230.8	13.3	716.9	60.7
Pr>F						
Treatment	0.4716	0.9368	0.3618	0.2725	0.6120	0.8003

^zMeans with a different letter differ significantly at the 5% level (LSD).

ns - Not significant.

Table 11. The effect of S-ABA application at 80 DAFB on the concentration of total ascorbic acid, total glutathione, oxidized ascorbic acid, oxidized glutathione, reduced ascorbic acid and reduced glutathione in the peel of ‘Granny Smith’ apples at Disseldraai, Grabouw, during the 2012-2013 season.

Treatment	Total ascorbic acid ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Total glutathione ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Oxidized ascorbic acid ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Oxidized glutathione ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Reduced ascorbic acid ($\mu\text{g}\cdot\text{g}^{-1}$ FW)	Reduced glutathione ($\mu\text{g}\cdot\text{g}^{-1}$ FW)
10 DAS						
1. Control	750.9 b ^z	92.1 ns	189.3 b	17.6 ns	561.6 ns	74.5 ns
2. 400 ppm S-ABA, 80 DAFB	842.9 a	90.8	316.4 a	24.4	526.5	66.4
Pr>F						
Treatment	0.0491	0.8974	0.0499	0.2334	0.6001	0.3137
20 DAS						
1. Control	717.4 ns	83.2 ns	194.4 ns	15.7 ns	523.0 ns	67.5 ns
2. 400 ppm S-ABA, 80 DAFB	675.8	83.4	162.4	15.7	513.4	67.7
Pr>F						
Treatment	0.7006	0.7669	0.9631	0.5330	0.6996	0.8173

^zMeans with a different letter differ significantly at the 5% level (LSD).

ns - Not significant.

Table 12. The effect of S-ABA application at 40 DAFB on the concentration of total antioxidants, total phenolics, total chlorophylls and total carotenoids in the peel of ‘Granny Smith’ apples at Disseldraai, Grabouw, during the 2012-2013 season.

Treatment	Total antioxidants (AAE) (mg.g ⁻¹ FW)	Total phenolics (GAE) (mg.g ⁻¹ FW)	Total chlorophylls (µg.g ⁻¹ FW)	Total carotenoids (µg.g ⁻¹ FW)
10 DAS				
1. Control	16.3 a ^z	385.8 a	421.2 ns	87.0 ns
2. 400 ppm S-ABA, 40 DAFB	13.6 b	342.5 b	416.0	82.7
Pr>F				
Treatment	0.0131	0.0032	0.8284	0.3844
20 DAS				
1. Control	15.5 ns	309.5 ns	442.6 ns	89.2 ns
2. 400 ppm S-ABA, 40 DAFB	11.8	312.4	448.5	89.6
Pr>F				
Treatment	0.0995	0.8629	0.7430	0.9008

^zMeans with a different letter differ significantly at the 5% level (LSD).

ns - Not significant.

Table 13. The effect of S-ABA application at 80 DAFB on the concentration of total antioxidants, total phenolics, total chlorophylls and total carotenoids in the peel of 'Granny Smith' apples at Disseldraai, Grabouw during the 2012-2013 season.

Treatment	Total antioxidants (AAE) (mg.g ⁻¹ FW)	Total phenolics (GAE) (mg.g ⁻¹ FW)	Total chlorophylls (µg.g ⁻¹ FW)	Total carotenoids (µg.g ⁻¹ FW)
10 DAS				
1. Control	19.0 ns	306.5 ns	465.8 ns	93.1 ns
2. 400 ppm S-ABA, 80 DAFB	16.0	313.1	463.3	96.1
Pr>F				
Treatment	0.1449	0.8066	0.9288	0.5574
20 DAS				
1. Control	14.6 ns	228.8 ns	431.2 ns	88.7 ns
2. 400 ppm S-ABA, 80 DAFB	12.8	172.2	440.7	91.9
Pr>F				
Treatment	0.4396	0.0846	0.8313	0.7196

ns - Not significant.

DAS-Days after spraying

Table 14. The effect of S-ABA application at 123 DAFB on net carbon assimilation rate, stomatal conductance, transpiration rate, fruit surface temperature and stem water potential of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2010-2011 season.

Treatment	A	g_s	E	FST (°C)	SWP
1. Control	16.7 a ^z	0.38 a	6.3 a	36.8 ns	-1.59 b
2. 250 ppm S-ABA	13.8 b	0.23 b	4.4 b	37.3	-1.61 b
3. 500 ppm S-ABA	12.4 b	0.19 b	3.7 b	35.6	-1.28 a
4. 1000 ppm S-ABA	13.7 b	0.19 b	4.2 b	37.2	-1.42 ab
Pr>F					
Treatment	0.0188	0.0001	0.0002	0.1127	0.0470
S-ABA vs Control	0.0033	0.0001	0.0001	0.1902	0.1752
S-ABA linear	0.8955	0.2813	0.7673	0.8554	0.1299
S-ABA quadratic	0.2496	0.6952	0.3248	0.0167	0.0397

^zMeans with a different letter differ significantly at the 5% level (LSD).

ns-Not significant.

A-Net carbon assimilation rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

g_s -Stomatal conductance ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

E-Transpiration rate($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

FST-Fruit surface temperature (°C).

SWP-Stem water potential (MPa).

Table 15. The effect of S-ABA application at 40 DAFB on net carbon assimilation rate, stomatal conductance, transpiration rate, fruit surface temperature and stem water potential of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2012-2013 season.

Treatment	A	g_s	E	FST (°C)	SWP
10 DAS					
1. Control	16.2 ns	0.14 ns	3.47 ns	31.8 ns	-1.74 b ^z
2. 400 ppm S-ABA ≈40	12.9	0.14	3.96	31.4	-1.28 a
DAFB					
Pr>F					
Treatment	0.1624	0.8346	0.3607	0.4704	0.0182
20 DAS					
1. Control	14.5 ns	0.38 ns	4.77 ns	35.3 ns	-0.78 ns
2. 400 ppm S-ABA ≈40	11.1	0.20	3.59	35.0	-0.77
DAFB					
Pr>F					
Treatment	0.1666	0.0940	0.0948	0.9579	0.7804

^zMeans with a different letter differ significantly at the 5% level (LSD).

DAS-Days after spraying

ns-Not significant.

A-Net carbon assimilation rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

g_s -Stomatal conductance($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

E-Transpiration rate ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

FST-Fruit surface temperature (°C).

SWP-Stem water potential (MPa).

Table 16. The effect of S-ABA application at 80 DAFB on net carbon assimilation rate, stomatal conductance, transpiration rate, fruit surface temperature and stem water potential of ‘Granny Smith’ apples at Disseldraai, Grabouw during the 2012-2013 season.

Treatment	A	g_s	E	FST (°C)	SWP
10 DAS					
1. Control	19.1 a ²	0.41 a	4.97 a	39.8 ns	-1.88 b
2. 400 ppm S-ABA ≈80	15.2 b	0.25 b	3.82 b	39.3	-1.50 a
DAFB					
Pr>F					
Treatment	0.002	0.0136	0.0069	0.7123	0.0001
20 DAS					
1. Control	16.8 ns	0.35 ns	4.81 ns	40.2 ns	-1.79 ns
2. 400 ppm S-ABA ≈80	14.6	0.24	3.90	39.6	-1.64
DAFB					
Pr>F					
Treatment	0.1440	0.0619	0.1793	0.4555	0.1068

²Means with a different letter differ significantly at the 5% level (LSD).

DAS-Days after spraying

ns-Not significant.

A-Net carbon assimilation rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

g_s -Stomatal conductance($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

E-Transpiration rate ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

FST-Fruit surface temperature (°C).

SWP-Stem water potential (MPa).

Table 17. The effect of S-ABA application on the fruit set of ‘Granny Smith’ at Disseldraai, Grabouw during the 2011-2012 season.

Treatment	Fruit set (%)
1. Control	17.7 ± 4.14
2. 1000 ppm S-ABA at flowering	15.7 ± 2.10

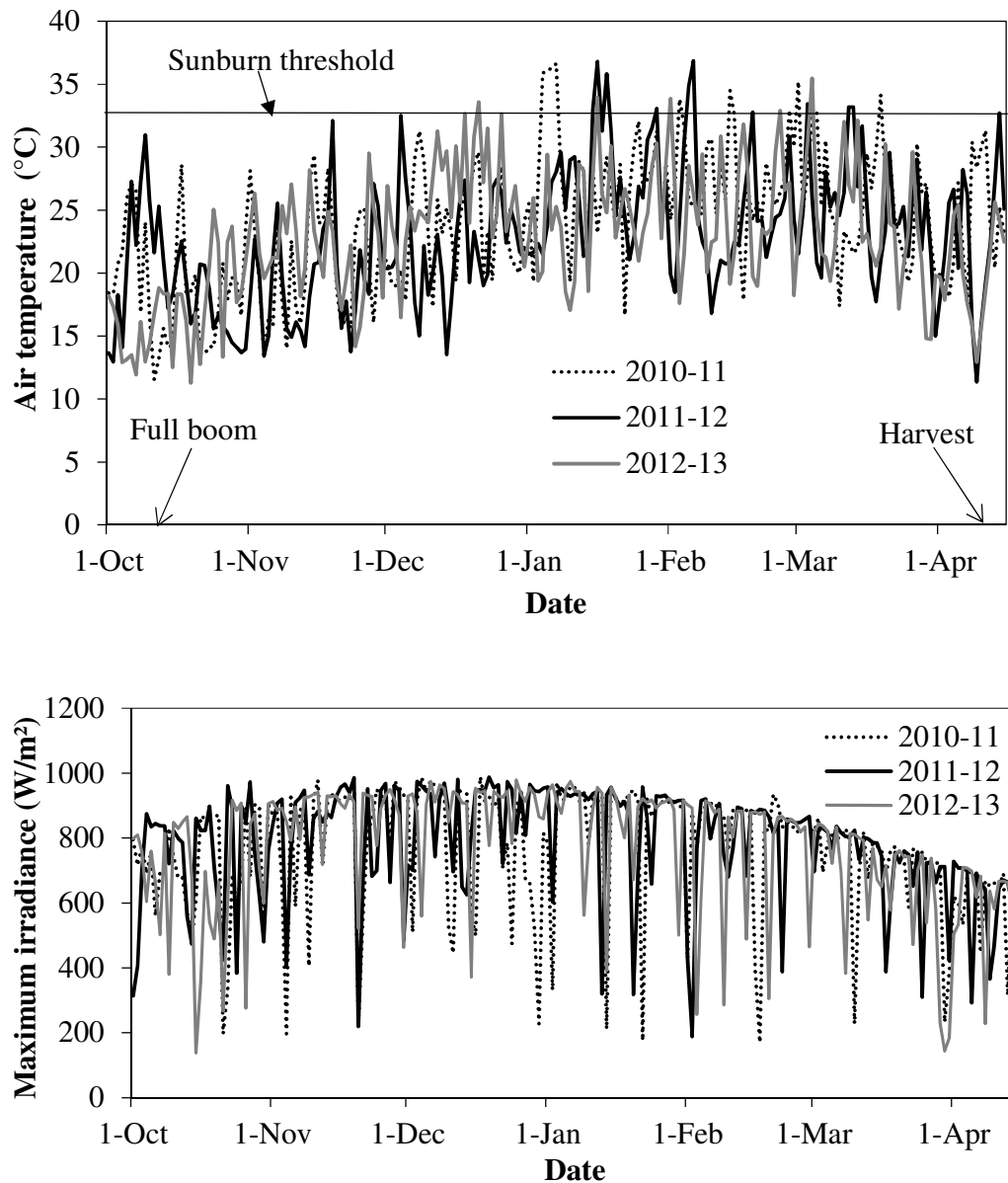


Figure 1. (Top) Daily maximum air temperatures and (bottom) daily maximum irradiance for Grabouw during the 2010-11, 2011-12 and 2012-13 growing seasons. The arbitrary 32°C sunburn threshold was chosen based on previous observations and in accordance with previous evaporative chilling trials at Stellenbosch University as threshold for the treatment in the 2011-12 season where S-ABA was applied prior to high, sunburn-inducing temperatures

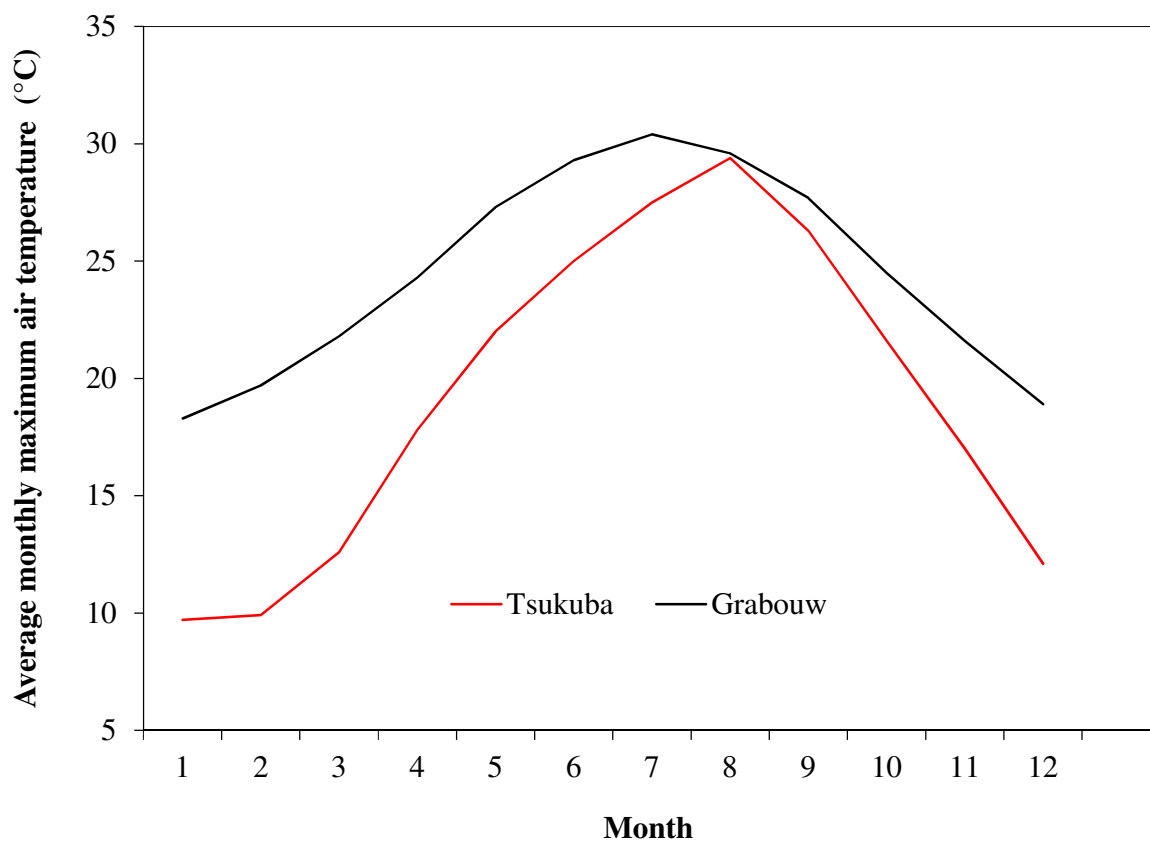


Figure 2. Historical average monthly maximum air temperature (Jan 1983-Dec 2004) for Tsukuba Japan, where Iamsub et al. (2009) conducted their study on the effect of S-ABA application on sunburn in apple at the University of Tsukuba ($36^{\circ}10' N$, $140^{\circ}10' E$) and Disseldraai, Grabouw, South Africa ($34^{\circ}15' S$; $19^{\circ}07' E$) used in the current study (<https://eosweb.larc.nasa.gov/cgi-bin/sse/grid.cgi?email=skip@larc.nasa.gov>). The data from Grabouw has been shifted by six months so that summer months for both hemispheres overlap.

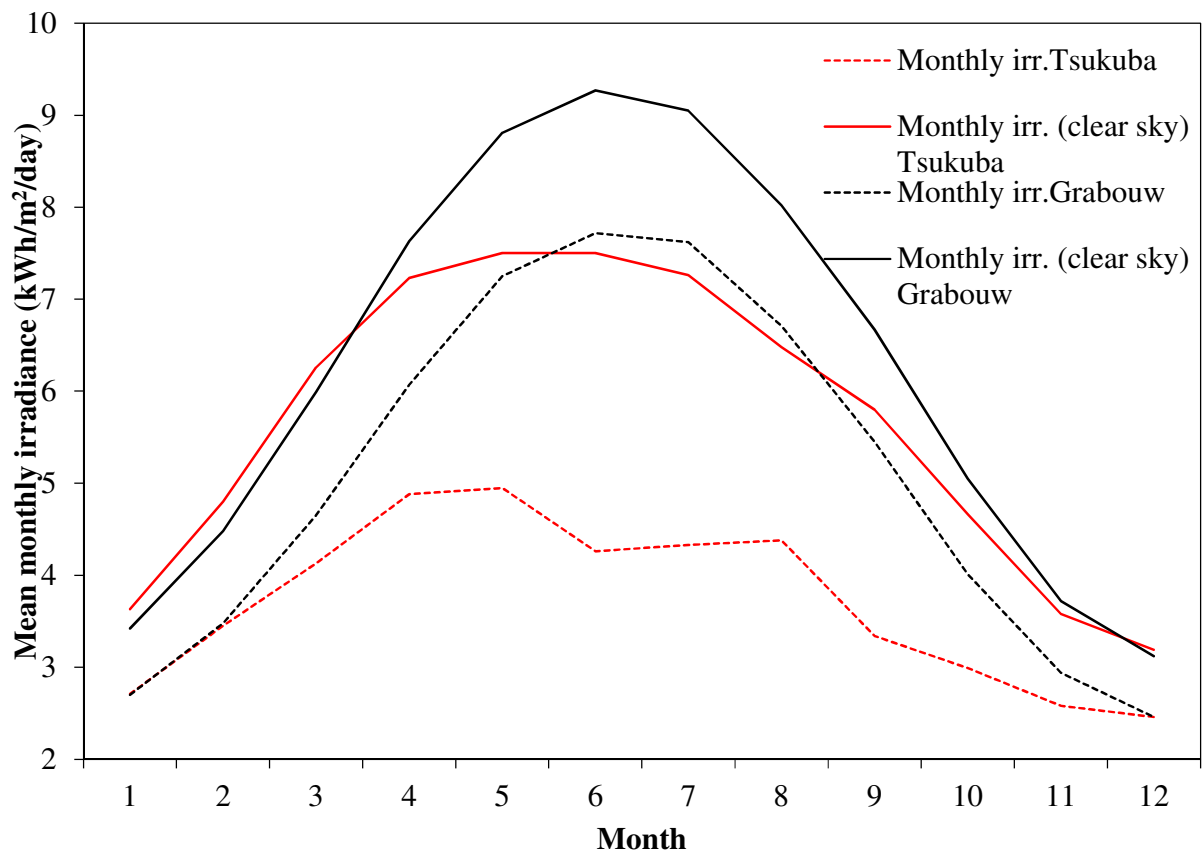


Figure 3. Historical monthly average amount of the total solar radiation (Jul 1983-Jun 2005) for all days and for clear days (<10% cloud cover) for Tsukuba Japan, where Iamsub et al. (2009) conducted their study on the effect of S-ABA application on sunburn in apple at the University of Tsukuba (36°10' N, 140°10' E) and Disseldraai, Grabouw, South Africa (34°15'S; 19°07'E) used in the current study (<https://eosweb.larc.nasa.gov/cgi-bin/sse/grid.cgi?email=skip@larc.nasa.gov>). The data from Grabouw has been shifted by six months so that summer months for both hemispheres overlap.



Figure 4. Although not of good quality, this picture does show the symptoms of leaf necrosis that were observed when S-ABA was applied at 1000 ppm, one day before it was forecasted to be $>32^{\circ}\text{C}$ the following day.

Paper 4: Effect of microclimate on the fruit surface anatomy of five apple cultivars grown in different production regions in South Africa

Abstract

Apple fruit can lose water via transpiration from the fruit surface which could potentially lead to evaporative cooling. Stomata and lenticels are involved in the transpiration pathway in apple fruit. Apple fruit transpiration is negligible at maturity, but might potentially contribute to cooling during early fruit development. This might explain why fruit susceptibility to sunburn increases during development and differs between cultivars. Hence, the objective of the study was to investigate the effect of regional microclimate and canopy micro-climate on fruit stomatal and lenticel density, and to relate this to the transpirational cooling capacity of different cultivars at various stages of fruit development. Experiments were conducted during the 2012–2013 growing season in ‘Granny Smith’, ‘Starking’, ‘Royal Gala’, ‘Golden Delicious’ and ‘Fuji’ orchards at Ceres (Latitude: 33°15’ S; Longitude: 19°15’ E) and Grabouw (Latitude: 34°12’ S; Longitude: 19°05’ E), South Africa. Fruit surface stomatal distribution and density were determined in young fruit at 40 days after full-bloom (DAFB) while lenticel type, density, distribution and size were determined in more mature fruit at 80 and 120 DAFB. Fruit transpiration rate and fruit water loss (during oven drying) was measured at 40, 80 and 120 DAFB for each cultivar. Latent heat flux was calculated from fruit transpiration rate and fruit water loss. The two production areas experienced different climatic conditions. Ceres had, on average, higher maximum temperatures, lower minimum temperatures, lower relative humidity, higher vapor pressure deficit and higher daily total solar radiation compared to Grabouw. Stomatal density did not differ between the two production areas. In addition, stomatal density on outer and inner canopy fruit showed no distinguishing patterns between the production areas. On outer canopy fruit, stomatal density tended to be higher on the shaded side of the fruit compared to the sun-exposed side. Stomatal density was generally highest at the calyx-end, followed by the equator of the fruit, and lowest on the stem-end. For both production areas, the predominant lenticel type was partially open. Closed and open lenticels were less usual. Average lenticel density from both production areas

generally decreased from 80 to 120 DAFB, although this was not always the case. The younger fruit (40 DAFB) which still had stomata, had a higher rate of transpiration and water loss compared to more mature fruit. Rate of fruit water loss decreased over the season in all cultivars as measured by both infrared gas analyzer and gradual oven drying at 40 °C. Latent heat flux from water loss also decreased in all cultivars. Rates of fruit transpiration and water loss were negligible and therefore had no effect on heat flux on the surface of apple fruit. In conclusion, there were large variations in fruit surface morphology in terms of lenticels within and between cultivars, between different canopy positions and between production regions. Heat loss from transpiration and water loss was negligible, and did not contribute to the evaporative cooling capacity of the fruit. Consequently, apple fruit transpiration had no influence on sunburn development.

1. Introduction

The Western Cape is a major production region for pome fruit in South Africa with its Mediterranean-type climate accompanied by hot, dry summers and mild, wet winters. High irradiance and extreme fruit surface temperatures are associated with the development of sunburn in apples (Chen et al., 2008; Wünsche et al., 2004). Sunburn necrosis occurs in the orchard when fruit surface temperature reaches 52 °C for a minimum period of 10 min (Schrader et al., 2001). Sunburn browning, the most prevalent type of sunburn in apples, is induced by light at threshold temperatures of 46 to 49 °C depending on the cultivar (Chen et al., 2008; Schrader et al., 2001; Wünsche et al., 2004). The majority of apple cultivars are susceptible to sunburn, although some are more sensitive than others (Racsko and Schrader, 2012). ‘Granny Smith’, for example, is more sensitive to sunburn compared to ‘Braeburn’, ‘Fuji’, ‘Golden Delicious’ and ‘Delicious’, whilst ‘Cripps’ Pink’ is least sensitive (Racsko and Schrader, 2012 and references therein). Fruit surface temperature can be 18 °C higher than ambient air temperature on the exposed side of the fruit, and 8-9 °C higher on the shaded side of the fruit (Piskolczi et al., 2004).

The fruit surface heat balance determines the extent of sunburn development in apples (Evans, 2004; Li et al., 2014). Fruit surface temperature is largely determined by radiation flux density and wind velocity and to a smaller extent by fruit size, surface albedo, and transpiration (Piskolczi et al., 2004). The differences in evaporative cooling potential between bulky fruit tissues and leafy tissues might explain why the apple fruit surface reaches such high

temperatures while leaves do not (Woolf and Ferguson, 2000). Compared to leaves, fruit have a much smaller surface to volume ratio resulting in lower net gas exchange and transpiration (Aschan and Pfanz, 2003; Blanke and Lenz, 1989). Makedredza et al. (2013) found higher fruit surface temperatures in trees under water deficit and argued that this might be explained by reduced fruit transpiration. Colaizzi et al. (2012) postulated that during periods of low transpiration under water stress, the evaporative cooling capacity of fruit is negatively affected and might lead to temperature build up (Colaizzi et al., 2012).

Stomata, lenticels and microcracks form part of the transpiration pathway and contribute to fruit surface conductance (Blanke, 1995; Gibert et al., 2005). Water loss from apple fruit is dependent mainly on stomata in young fruit and on open lenticels and microcracks in mature fruit (Gibert et al., 2005; Maguire et al., 1999a, Veraverbeke et al., 2003a, 2003b). Stomata are present on the outer epidermis of fruit, but at a 10 to 100-fold lower frequency compared to the leaf epidermis of the same species (Blanke and Lenz, 1989). The number of stomata present is set at anthesis and remains constant, resulting in a decrease in the stomatal density as the fruit surface expands (Blanke and Lenz, 1989). Stomata develop into lenticels after June drop (northern hemisphere) in apple as observed in ‘Golden Delicious’, ‘Cox Orange Pippin’ and ‘Boskoop’ (Schlegel and Schönherr, 2002). A lenticel is any opening in the cuticle that is shaped like stomata or sometimes resembles a crack (Turketti, et al., 2012). Microcracks are generated on the apple fruit surface during periods of high growth rates and increase fruit surface conductance to water loss (Gibert et al., 2005; Konarska, 2013). Differences in fruit water loss between cultivars can be attributed to differences in the number of lenticels and microcracks, as reported for ‘Szampion’ and ‘Jonagold’ (Konarska, 2013). ‘Szampion’ fruit had fewer lenticels, but a larger number of microcracks, which led to greater water loss compared to ‘Jonagold’.

Apple fruit are covered by a continuous extracellular cuticle that consists of a triglyceride matrix or cutin layer and an epicuticular wax layer (Konarska, 2012; Veraverbeke et al., 2001). The wax layer is deposited on the cutin matrix and forms a natural barrier against water loss (Gibert et al., 2005; Maguire et al., 1999a, Veraverbeke et al., 2001). The wax layer can change in composition during fruit development and in response to environmental conditions, in the process covering stomata, lenticels and microcracks further preventing water loss (Veraverbeke et al., 2001). The frequency and size of these openings are more important than cuticle thickness, which has been observed to have no effect on water vapor permeability (Maguire et al 1999b).

No preharvest studies have been done on apple fruit transpiration over the growing season. Postharvest studies have shown that there is a large variation in fruit water loss within a population of apple fruit and between growers due to differing cultural practices, with marginal differences between different growing regions as reported for 'Braeburn' in New Zealand (Maguire et al., 1999b; Maguire et al. (2000)). Maguire et al. (2000) reported harvest date to explain 50% of the total variation in fruit permeance, fruit to fruit differences accounted for 22% of the total variation, interaction between harvest dates explained 7% of the total variation and only 1% of the variation could be attributed to tree effects in 'Braeburn'. In another study of four cultivars, namely 'Braeburn', 'Pacific Rose', 'Granny Smith', and 'Cripps' Pink', 30% of the postharvest variation in fruit water vapor permeance was due to cultivar differences, over 20% was associated with harvest date, fruit to fruit variation accounted for 25% while inter tree variation accounted for 4% (Maguire et al., 2000). Apple fruit that were in contact with adjacent fruit within a cluster had on average 54% higher water vapour permeability than single fruit, whilst there was no difference in water loss between the sun and shaded sides of fruit (Maguire et al., 1999b). This might be caused by elevated permeance in fruit with multiple contact points, the direct contribution of contact areas themselves and different physiology of fruit that develop in clusters (Maguire et al., 1999b). In contrast, grape berries in large clusters were reported to heat more than those on loose clusters due to reduced transpiration (Smart and Sinclair, 1976). These various interlinked factors which influence fruit permeance give rise to large variations in fruit water loss between cultivars, between different canopy positions and between climatic regions.

Climate affects lenticel development due to its influence on fruit growth rate and cuticle development (Turketti et al., 2012). The effect of climate on stomatal development in apple has not been studied. Different microclimatic conditions exist within the canopy. Outer canopy fruit are exposed to much higher solar radiation and radiant heat load compared to inner canopy fruit (Fouché et al., 2010). In grapefruit, the sun exposed surface had larger wax platelets than the shaded side of the same fruit (McDonald et al., 1993). This was attributed to the climatic influence on wax morphology and composition (McDonald et al., 1993; Nordby and McDonald, 1995). Therefore, microclimate might also affect fruit surface anatomy in apple fruit in addition to canopy position and exposure to solar radiation. The extensive review on apple tree morphology and anatomy by Pratt (1990) stated that differences in leaf structure exist between

shaded and exposed leaves. Exposed leaves are thicker and larger compared to shaded leaves. On the other hand, Hunsche et al. (2010) reported that the microclimate under hail netting with a 10-12 % reduction in photosynthetically active radiation, had no impact on leaf micromorphology, amount of cuticular wax and leaf thickness in 'Fuji' and 'Pinova' apple cultivars. Apple fruit transpiration is negligible at maturity, but might potentially contribute to cooling during early fruit development. This might explain why susceptibility to sunburn increases during fruit development and differs between cultivars. Evaporative cooling potential through lenticels and stomata might possibly reduce sunburn development and incidence in different apple cultivars. The objective of the study was to investigate the effect of regional climate and canopy microclimate on fruit stomatal and lenticel density, and to relate this to the transpirational cooling capacity of 'Granny Smith', 'Golden Delicious', 'Starking', 'Fuji' and 'Royal Gala' at different stages of fruit growth.

2. Materials and Methods

2.1 Study sites and plant material

Trials were conducted during the 2012-2013 growing season in 'Granny Smith', 'Starking', 'Royal Gala', 'Golden Delicious' and 'Fuji' orchards at Vastrap Farm (Latitude: 33°15'S; Longitude: 19°15'E, Alt 894 m) in the Koue Bokkeveld, Ceres, and at Glenbrae Farm (Latitude: 34°12'S; Longitude: 19°05'E, Alt 321 m) in Grabouw, South Africa. The sites used in the study were representative of the two production regions which had different microclimates. Information on planting dates, full bloom dates, rootstocks, row orientation and direction is presented in Table 1 for each location and cultivar. Weather data was collected at De Keur (Latitude: 32°98'S; Longitude: 19°30'E, Alt 947 m) for the Ceres production region, and at Oak Valley (Latitude: 34°15'S; Longitude: 19°07'E, Alt 375 m) for the Grabouw production region. De Kleur was about 30 km away from Vastrap and Oak Valley was 7 km from Glenbrae. Normal commercial cultural practices of irrigation, pest management and fertilization were followed over the course of the trials.

2.2 Fruit surface anatomy

Fruit surface stomatal distribution and density were determined in young fruit at 40 DAFB; and lenticel type, density, distribution and size were determined in more mature fruit at 80 and 120 DAFB. Table 2 shows average fruit size per cultivar and production region during sampling for stomata and lenticels morphology experiments.

2.2.1 Stomatal density and distribution

Three exposed (outer canopy) fruit from each side of the row and three fully shaded, inner canopy fruit were collected on each sampling date. A thin, 1 cm² section was obtained from the stem-end, equator and calyx-end on the surface of the fruit using a razor blade. Samples were collected from the exposed and shaded side of outer canopy fruit, and from the opposite sides of inner canopy fruit. Samples were fixed in formalin-acetic acid (FAA) and stored until analysis. At analysis, the samples were transferred sequentially to the following solutions in order to dehydrate: 50% ethanol, 75% ethanol, 95% ethanol and 100% ethanol. Critical point drying was done to avoid deformation and collapse of structures that occurs when fresh samples are viewed under a scanning electron microscope, using a Balzers CPD020 critical point drier (Balzers Instruments, Balzers, Liechtenstein). Samples were loaded in a basket that was placed in a transfer boat and filled with 100% ethanol after which cold water was used to decrease temperature below 20 °C. The gas inlet valve was opened to 54 bar and samples were left for 1 hour to allow impregnation of tissue with liquid CO₂ gas. The inlet valve was then closed and a hot water supply at 35-40 °C turned on, which caused the gas to reach critical point and evaporate. After drying, samples were removed from the transfer boat and mounted on microscope studs before being vacuum-coated in a Bio Rad Polaron E5400 SEM Sputter Coating System (Bio-Rad, Hercules, California, USA). The sputter coating was performed using a gold palladium target and was approximately 10 angstroms thick. Samples were then mounted in a FEI Nova NanoSEM 230 (FEI, Hillsboro, Oregon, USA) scanning electron microscope for viewing. Magnification was 200 x and the voltage was 5.00 to 7.00 keV. Eighteen images were captured from each 1 cm² section and stomatal density was obtained by counting the number of stomata in each section. Figure 1 shows an example of an image obtained from the scanning electron microscope used to calculate stomatal density.

2.2.2 Lenticel type, density, distribution and size

Lenticel counts were done using a dye staining method. Five exposed fruit (outer canopy) from each side of the row and five fully shaded inner canopy fruit were collected on each sampling date. Whole fruit were immersed in methylene blue ($7.5 \text{ mg } 100 \text{ ml}^{-1}$) for up to 24 hours. Methylene blue only penetrates the open lenticels leaving halos of blue dye due to staining of the underlying cells (Harker and Ferguson, 1988). Lenticels were classified into open, partially open and closed depending on penetration of the dye (Figure 2). Open lenticels were deeply stained. Partially open lenticels were slightly stained and closed lenticels not stained by immersion in methylene blue. After immersion, a thin 1 cm^2 fruit surface section was obtained from the stem-end, equator and calyx region of the fruit, using a razor blade. The sections were then viewed under a stereo microscope (Leica M125, Leica Biosystems, Wetzlar, Germany) using Leica Application Suite software (Leica Biosystems, Wetzlar, Germany) and images captured for counting. Figure 2 shows an example of an image used to count and classify lenticel density and type. One open lenticel was chosen from each section to measure lenticel length and width (Figure 3). The same lenticel was then dissected to measure lenticel depth as shown in Figure 3.

2.3 Fruit transpiration and latent heat flux

Measurements were done on ‘Royal Gala’, ‘Granny Smith’, ‘Fuji’, ‘Starking’ and ‘Golden Delicious’ from Grabouw and Ceres at 40, 80 and 120 DAFB. Two well-exposed attached fruit on five replicate trees were enclosed in a conifer chamber attached to a LI-6400 infrared gas analyzer (Li-Cor, Lincoln, Nebraska, USA). Measurements were taken with reference CO_2 concentration set at 380 ppm, chamber VPD_{air} ranged from 1.0 to 2.5 kPa and block temperature was set at $25 \text{ }^\circ\text{C}$ in full sunlight measured using a LI-690A quantum sensor (Li-Cor, Lincoln, Nebraska, USA). All measurements were done between 08:00 and 11:30. After measurement, fruit were detached and fruit diameter measured. Fruit surface area was then calculated assuming fruit to be spherical and values expressed per unit surface area. Latent heat flux was calculated from fruit transpiration using the coefficient of latent heat of vaporization.

2.4 Fruit water loss at high temperature

Fruit samples were collected at 40, 80 and 120 DAFB from ‘Royal Gala’, ‘Granny Smith’, ‘Fuji’, ‘Starking’ and ‘Golden Delicious’ from Grabouw and Ceres. Five exposed (outer canopy)

fruit from each side of the row, as well as five inner canopy fruit were taken on each sampling date. Initial fruit weight and diameter was recorded for each fruit. Fruit diameter was used to calculate fruit surface area assuming the fruit to be spherical. The rate of water loss was determined in an oven set to 40 °C over 48 hours, with vents open at the top of the oven to avoid accumulation of water vapor, which would slow down the rate of water loss. Water loss was then calculated as fruit weight loss. Latent heat flux was calculated from fruit water loss using the coefficient of latent heat of vaporization.

2.5 Statistical analysis

Mean values and standard error of the mean were calculated for the data.

3. Results

3.1. Weather data

Monthly mean maximum temperatures were higher in Ceres compared to Grabouw in all months except for Dec 2012 and Apr 2013 (Figure 4). On the other hand, monthly mean minimum temperatures were always lower in Ceres compared to Grabouw (Figure 4). Monthly mean maximum relative humidity and mean minimum relative humidity were higher in Grabouw than in Ceres (Figure 5). Monthly mean vapor pressure deficit (Figure 6) and monthly mean of daily total solar radiation (Figure 7) were higher in Ceres than in Grabouw.

3.2 Stomatal density and distribution

There were no differences in average stomatal density between Ceres and Grabouw (Figure 8). For Ceres, 'Starking' and 'Granny Smith' had the highest average stomatal density followed by 'Fuji', whilst 'Golden Delicious' and 'Royal Gala' had the lowest (Figure 8). For Grabouw, 'Granny Smith' 'Starking' and 'Fuji' had the highest average stomatal density whilst 'Golden Delicious' and 'Royal Gala' had the lowest (Figure 8). There were no discernible differences in stomatal density from outer and inner canopy fruit (Figure 8). In outer canopy fruit, stomatal density was higher on the shaded side compared to the exposed side of 'Starking' and 'Fuji' in Ceres, and 'Granny Smith', 'Royal Gala' and 'Fuji' in Grabouw (Figure 8). The highest stomatal density was always found on the calyx-end of the fruit in both production areas, the only

exception being ‘Granny Smith’ at Ceres which had highest stomatal density at both the calyx-end and equator (Figure 9). The second highest stomatal density was found on the equator of the fruit, except for ‘Royal Gala’ in Ceres and ‘Fuji’ in Grabouw for which both the equator and stem-end were second highest (Figure 9). The stem-end of the fruit generally tended to have the lowest stomatal density (Figure 9).

3.3 Lenticel type, density, distribution and size

In Ceres, lenticel density was higher at 80 DAFB compared to 120 DAFB in ‘Granny Smith’, ‘Starking’ and ‘Royal Gala’, lower in ‘Golden Delicious’ and did not change in ‘Fuji’ (Figure 10). At 80 DAFB, lenticel density was highest in ‘Royal Gala’ and ‘Starking’, followed by ‘Fuji’ and then ‘Golden Delicious’, with ‘Granny Smith’ having the lowest lenticel density (Figure 10). At 120 DAFB, lenticel density was highest in ‘Fuji’ and ‘Golden Delicious’, followed by ‘Starking’ and ‘Royal Gala’ with ‘Granny Smith’ having the lowest lenticel density (Figure 10). Partially open lenticel was the most frequent type at both sampling dates in all cultivars, followed by closed lenticels, with open lenticels being the least frequent except for ‘Royal Gala’ which had more open than closed lenticels at both sampling dates, and ‘Golden Delicious’ which was not different at both sampling dates (Figure 10).

Although not always significant, lenticel density tended to be highest at the calyx-end, except in ‘Fuji’ the stem-end was highest although not significantly at both sampling dates (Figure 11). The number of lenticels at the stem-end and equator followed no distinct pattern at both sampling dates for all the cultivars (Figure 11).

In Grabouw, lenticel density was higher at 80 DAFB compared to 120 DAFB for all cultivars except for ‘Golden Delicious’ (Figure 12). At 80 DAFB, lenticel density was highest in ‘Starking’ and ‘Golden Delicious’, with ‘Granny Smith’, ‘Royal Gala’ and ‘Fuji’ having lower lenticel densities (Figure 12). At 120 DAFB, lenticel density was highest in ‘Golden Delicious’, followed by ‘Starking’ and ‘Fuji’, with ‘Granny Smith’ and ‘Royal Gala’ having the lowest lenticel density (Figure 12). Partially open lenticel was the most frequent type at both sampling dates in all cultivars (Figure 12). Closed lenticel was the second most frequent type in ‘Granny Smith’ and ‘Fuji’ at both sampling dates, and ‘Starking’ at 120 DAFB, whilst open lenticel was the second most frequent type in ‘Golden Delicious’ at 80 DAFB, and ‘Royal Gala’ at 120

DAFB (Figure 12). There were no differences between open and closed lenticel densities for ‘Golden Delicious’ at 120 DAFB, ‘Starking’ at 80 DAFB, and ‘Royal Gala’ at 80 DAFB.

Although not always significant, lenticel density tended to be highest at the calyx-end, except for ‘Granny Smith’ and ‘Royal Gala’ at 120 DAFB (Figure 13). There were no discernible patterns for lenticel density at the equator and stem-end (Figure 13).

There was no trend observed in lenticel density from shaded and exposed sides of outer canopy fruit and inside canopy fruit for both ‘Ceres’ and ‘Grabouw’ (Figures 14 and 15). Length and width of lenticels decreased between 80 and 120 DAFB in ‘Granny Smith’ and ‘Starking’, whilst it increased in ‘Golden Delicious’, ‘Royal Gala’ and ‘Fuji’ at Ceres (Figure 16). Depth of lenticels decreased between 80 and 120 DAFB at Ceres for ‘Granny Smith’ and ‘Starking’, remained the same for ‘Golden Delicious’ and ‘Fuji’, whilst it increased for ‘Royal Gala’ (Figure 16). Length, width and depth of lenticels increased for all cultivars between 80 and 120 DAFB at Grabouw (Figure 17). No discernible patterns in lenticel length, width and depth could be observed between the two regions (Figure 16 and 17).

3.4 Fruit transpiration and latent heat flux

Ceres

Fruit area-based transpiration rate as measured by infra-red gas analysis decreased in all cultivars from 40 to 120 DAFB, although not always significantly (Figure 18). At 40 DAFB, fruit transpiration rate was highest in ‘Golden Delicious’ followed by ‘Fuji’ and ‘Starking’, with ‘Granny Smith’ and ‘Royal Gala’ having the lowest fruit transpiration rate (Figure 18). Thereafter at 80 DAFB, fruit transpiration rate was highest in ‘Golden Delicious’ and ‘Starking’ followed by ‘Fuji’ with ‘Royal Gala’ and ‘Granny Smith’ having the lowest fruit transpiration (Figure 18). Finally, at 120 DAFB, fruit transpiration rate was highest in ‘Fuji’, followed by ‘Royal Gala’, ‘Golden Delicious’ and ‘Starking’, with ‘Granny Smith’ having the lowest fruit transpiration rate (Figure 18). Latent heat flux followed the same trend as fruit transpiration rate since values were directly calculated from the latter (Figure 18).

Grabouw

Fruit area-based transpiration rate decreased in all cultivars from 40 to 120 DAFB although not always significantly (Figure 19). Fruit transpiration rate at 40 DAFB was highest in ‘Starking’ and ‘Fuji’, followed by ‘Royal Gala’ and ‘Golden Delicious’, with ‘Granny Smith’ having the

lowest rate of transpiration (Figure 19). At 80 DAFB, fruit transpiration was highest in ‘Fuji’, followed by ‘Golden Delicious’ and ‘Starking’ and ‘Royal Gala’, with ‘Granny Smith’ having the lowest fruit transpiration rate (Figure 19). Finally, at 120 DAFB, fruit transpiration rate was highest in ‘Fuji’, ‘Golden Delicious’, ‘Starking’ and ‘Royal Gala’ with ‘Granny Smith’ having the lowest fruit transpiration rate (Figure 19). Latent heat flux followed similar trends as fruit transpiration since values were directly calculated from the latter (Figure 19).

Regional comparison

At 40 DAFB, fruit transpiration rate ranged from 1.25 to 2.78 $\text{mg m}^{-2} \text{s}^{-1}$ at Ceres whilst at Grabouw it ranged from 1.50 to 2.89 $\text{mg m}^{-2} \text{s}^{-1}$ (Figures 18 and 19). At 80 DAFB fruit transpiration ranged from 1.07 to 1.71 $\text{mg m}^{-2} \text{s}^{-1}$ at Ceres whilst at Grabouw it ranged from 1.04 to 2.06 $\text{mg m}^{-2} \text{s}^{-1}$ (Figures 18 and 19). Finally, at 120 DAFB fruit transpiration ranged from 0.55 to 1.28 $\text{mg m}^{-2} \text{s}^{-1}$ at Ceres whilst at Grabouw it ranged from 0.88 to 1.06 $\text{mg m}^{-2} \text{s}^{-1}$ (Figures 18 and 19). Latent heat flux followed similar trends since it was derived directly from transpiration rate (Figures 18 and 19).

3.5 Fruit water loss and latent heat flux

Ceres

Rate of fruit water loss at 40 °C decreased in all cultivars from 40 to 120 DAFB (Figure 20). At 40 DAFB, fruit water loss was highest in ‘Fuji’ and ‘Golden Delicious’ followed by ‘Starking’ and then ‘Royal Gala’, with ‘Granny Smith’ having the lowest rate of water loss (Figure 20). At 80 DAFB rate of fruit water loss was highest in ‘Fuji’, ‘Starking’ and ‘Royal Gala’, followed by ‘Golden Delicious’ with ‘Granny Smith’ having the lowest rate of water loss (Figure 20). At the last sampling date at 120 DAFB, rate of fruit water loss was highest in ‘Fuji’, ‘Golden Delicious’ and ‘Royal Gala’, followed by ‘Starking’, with ‘Granny Smith’ having the lowest rate of water loss (Figure 20). Latent heat flux followed similar trends as fruit water loss since values were directly calculated from the latter (Figure 20).

Grabouw

Rate of fruit water loss at 40 °C decreased in all cultivars from 40 to 120 DAFB (Figure 21). It was highest in ‘Starking’ at 40 DAFB, followed by ‘Fuji’, ‘Golden Delicious’ and ‘Royal Gala’

with ‘Granny Smith’ having the lowest rate of fruit water loss (Figure 21). At 80 DAFB, rate of fruit water loss was highest in ‘Golden Delicious’ and ‘Fuji’ with ‘Royal Gala’, ‘Starking’ and ‘Granny Smith’ having the lowest rate of water loss (Figure 21). At the last sampling date at 120 DAFB, rate of water loss highest in ‘Golden Delicious’, ‘Royal Gala’, and ‘Fuji’, followed by ‘Starking’ with ‘Granny Smith’ having the lowest rate of water loss (Figure 21). Latent heat flux followed similar trends as fruit water loss since values were directly calculated from the later (Figure 21).

Comparison of two production areas with different microclimates

At 40 DAFB, rate of fruit water loss ranged from 2.15 to 4.06 $\text{mg m}^{-2} \text{s}^{-1}$ at Ceres whilst at Grabouw it was 1.64 to 2.80 $\text{mg m}^{-2} \text{s}^{-1}$ (Figures 20 and 21). At 80 DAFB fruit water loss ranged from 0.77 to 1.93 $\text{mg m}^{-2} \text{s}^{-1}$ at Ceres whilst at Grabouw it ranged from 1.43 to 1.81 $\text{mg m}^{-2} \text{s}^{-1}$ (Figures 20 and 21). Finally, at 120 DAFB, rate of fruit water loss ranged from 0.71 to 1.32 $\text{mg m}^{-2} \text{s}^{-1}$ at Ceres whilst at Grabouw it ranged from 0.78 to 1.65 $\text{mg m}^{-2} \text{s}^{-1}$ (Figures 20 and 21). Latent heat flux followed similar trends since it was derived directly from transpiration (Figures 12 and 21).

4. Discussion

The apple fruit surface is dynamic and constantly changing during fruit development (Curry, 2009). Stomata are present on the fruit surface in young fruit but develop into lenticels as the fruit matures (Schlegel and Schönherr, 2002). The average stomatal density did not differ between the sites in the two regions. Stomatal density on outer canopy fruit (averaged over the exposed and shaded sides of fruit) and inner canopy fruit showed no distinguishing trends between the production areas. For outer canopy fruit, stomatal density tended to be higher on the shaded side of the fruit compared to the exposed side. Stomatal density was generally highest at the calyx-end, followed by the equator of the fruit, and lowest on the stem-end.

For both production areas, the predominant lenticel type was partially open. Closed and open lenticels were less usual. As in other tree fruit, the apple maintains a cuticle to protect the inner cells from desiccation, contamination, and excessive water absorption (Curry et al., 2008). Wax covers the lenticels, but there might still be a few cells exposed, explaining the high number of

partially open lenticels. Except for 'Golden Delicious' at Ceres, average lenticel density generally decreased from 80 to 120 DAFB. Lenticels are formed from stomata and their number is "fixed" - as the fruit surface area increases, lenticel density decreases (Schlegel and Schönherr, 2002). The increase in lenticel density for 'Golden Delicious' at Ceres from 80 to 120 DAFB cannot be explained. Lenticels are formed when the cuticle develops over vestigial stomata in early fruit development (Curry, 2003), their number is not supposed to increase. In general, more lenticels were found at the calyx-end, as this is also where most stomata are found in young fruit. The calyx-end of the fruit expands less compared to the equator (Tukey and Young, 1942). Unlike stomata, however, no discernable trends could be found in terms of lenticel density for shaded and exposed sides of outer and inner canopy fruit.

Area based stomatal density per cm^2 at 40 DAFB was lowest in 'Royal Gala' (10.6) and highest in 'Starking' (45.9) at Ceres, whilst at Grabouw it was lowest in 'Royal Gala' (11.1) and highest in 'Granny Smith' (30.1). Stomatal density per cm^2 of apple rootstock leaf surface area was reported to be 350 for M.9, 440 for M.26, 505 for MM106 and 600 for M.25 (Beryl Beakbane and Majumder, 1975). Stomatal density is much higher in leaves than fruit, since the main function of leaves is gas exchange. Average lenticel density per cm^2 at 80 DAFB was lowest in 'Granny Smith' (3.35) and highest in 'Royal Gala' (9.16) at Ceres, whilst at Grabouw it was lowest in 'Granny Smith' (5.49) and highest in 'Starking' (8.53). At 120 DAFB average lenticel density per cm^2 was lowest in 'Granny Smith' (2.73) and highest in 'Fuji' (5.50) at Ceres, whilst at Grabouw it was lowest in 'Granny Smith' (2.39) and highest in 'Golden Delicious' (6.78). Turketti et al. (2012) reported lenticel densities per cm^2 of 7.42 to 16.0 (2009-2010 season) and 6.67 to 8.83 (2010-2011 season) at 70 DAFB and 7.08 to 11.3 (2009-2010 season) and 4.08 to 4.67 (2010-2011 season) for 'Royal Gala' at three different sites in Ceres comparing well to the values found in our study. The apparent incongruence between stomata and lenticel densities is because the number of stomata is set at flowering causing the stomatal density to decrease as the fruit surface expands (Blanke and Lenz, 1989). In addition, as the fruit matures, a wax layer is deposited on the cutin, covering some of the lenticels thereby reducing lenticel density (Gibert et al., 2005; Maguire et al., 1999a, Veraverbeke et al., 2001).

The apple cuticle is environmentally responsive (Curry, 2009). The two production areas experienced different climatic conditions. Ceres had, on average, higher maximum temperatures, lower minimum temperatures, lower relative humidity, higher vapor pressure deficit and higher

daily total solar radiation compared to Grabouw. Previous studies have found that the higher the relative humidity, the higher the number of lenticels of larger size (Turketti et al., 2012). The size of lenticels generally increased from 80 to 120 DAFB at Grabouw, whilst at Ceres, lenticel size showed no distinctive pattern as the season progressed. Grabouw experienced higher relative humidity, which might explain the increase in lenticel size. The increase in size of lenticels in the late stage of fruit development can also be explained by cracking and/or splitting of the cuticle around the lenticel (Guan et al., 2015; Harker and Ferguson, 1988). Microcracks, which affect fruit transpiration and surface conductance, are more frequent under conditions of high ambient temperature and low relative humidity (Curry et al., 2008).

Fruit transpiration was higher in young fruit compared to more mature fruits. This could be explained by stomatal density which was greater compared to lenticel density. In addition, the majority of lenticels were partially open resulting in a lower rate of water loss. Compared to stomata, lenticels seem to be under no regulatory control and therefore are less effective at controlling fruit water loss (Lendzian, 2006). Rate of fruit water loss decreased over the season in all cultivars as measured by both infrared gas analyzer and by drying at 40 °C in an oven. The decrease in rate of fruit water loss can be explained by a decrease in fruit surface conductance which occurs over the growing season (Gibert et al., 2005). In addition, apple fruit were reported to have high resistance to water vapor (5000-7000 s cm⁻¹), compared to grapes (120-170 s cm⁻¹) and peach (33 s cm⁻¹) (Nobel, 1975). Peach fruit have been reported to have high transpiration rates even at maturity, which can be explained by their low resistance to water vapor (Lescourret et al., 2001; Morandi et al., 2007). The higher fruit transpiration rate in peach and grape might possibly explain why these fruits are not prone to sunburn when compared to apple even when grown in close vicinity in the Western Cape (personal observations). Latent heat flux from water loss also decreased in all cultivars during fruit growth. In contrast, as fruit matured, cooling capacity was reported to increase in *Ficus* due to increased conductance to water vapor (Patino et al., 1994). The reason for the increase in conductance was not determined. Even though the Ceres and Grabouw regions differed climatically, there were no differences in the range of values obtained for the rate of fruit transpiration and fruit water loss. The values obtained for fruit transpiration and water loss were in the same range although they did not follow similar trends, maybe because different fruit samples were used or because of differences in

experimental conditions, i.e., dehydration of detached fruit at constant high temperature compared to gas exchange measurements using attached fruits.

Water loss in apple fruit is minimized by the presence of a cuticle, otherwise the fruit will desiccate quickly (Konarska, 2012). This might lead to temperature build up and occurrence of physiological disorders such as sunburn since the cooling capacity of the fruit is limited (Colaizzi et al., 2012). Results from modelling experiments in mature fruit by Li et al (2014) found fruit transpiration to be negligible compared to other sources of heat flux from the apple surface like convection and emitted radiation. They found that, at 12:00, total incoming radiation was 975 W m^{-2} , whilst outgoing heat fluxes were emitted radiation (550 W m^{-2}), convection (400 W m^{-2}) and transpiration was negligible (0 W m^{-2}) (Li et al., 2014). It is interesting though that ‘Granny Smith’, which is perceived to be the most sunburn sensitive cultivar, also had the lowest rate of transpiration, even though we can discount the contribution of transpiration to the fruit surface heat flux. Hence, the finding the ‘Granny Smith’ had the lowest rate of transpiration is possibly coincidental.

Although not accounted for in this study, microcracks contribute a significant number of natural openings in the cuticle of some cultivars of apple fruit, especially in more mature fruit (Guan et al., 2015). The formation of microcracks involves fracturing of the outer layer most resistant to water loss to reveal the cutin matrix, which has poorer barrier properties (Maguire et al., 1999a). Guan et al. (2015) reported numerous microcracks on surfaces of ‘Golden Delicious’ and ‘Golden Spur’ fruit, accounting for 90% of the natural openings, whilst no microcracks were observed in ‘Jiguan’, ‘Starkrimson’, ‘Jonathan’ and ‘Ralls Janet’, ‘Jonagold’, ‘Kuihua’, ‘Jinhong’ and ‘Red Fuji’. Fruit can naturally repair microcracks by covering them with wax (Curry et al., 2008). Under conditions of rapid fruit enlargement, repairing of the microcracks may lag fruit enlargement, which might cause large variance in fruit transpiration and fruit surface conductance (Curry et al., 2008; Maguire et al., 1999a). This may explain why the rate of transpiration and water loss generally did not correspond with the fruit anatomical parameters measured. Nobel (1975) reported temporal variations in fruit surface conductance and cuticular crack surface area, which may explain why it was difficult to relate transpiration and fruit water loss to the measured anatomical parameters.

5. Conclusion

No microclimatic response was easily discernable in terms of fruit surface morphology, despite there being clear climatic differences between the two production areas. Fruit canopy position did not influence stomatal density, although on outer canopy fruit, the calyx-end generally had higher stomatal density, followed by the equator of the fruit, with stem-end having the lowest stomatal density. The fruit surface morphology in terms of lenticels was variable between cultivars, between fruit of the same cultivar, between canopy position and between climatic regions. Rate of fruit transpiration and water loss decreased over the growing season. Heat loss from transpiration and water loss was negligible, making it difficult to relate cooling capacity to fruit surface morphology. Microcracks, which might form a significant number of openings in mature fruit, were not measured in this study and could have helped explain some of the trends in fruit transpiration and fruit water loss.

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Table 1. Study sites, plant material information and full bloom dates in 2012-2013.

Ceres				
Cultivar	Planting date	Rootstock	Tree spacing and row direction	Full bloom dates
'Granny Smith'	1950	Seedling	6.71 X 3.35 E/W	28 Sep. 2012
'Golden Delicious'	1950	Seedling	6.71 X 3.35 E/W	10. Oct. 2012
'Starking'	1950	Seedling	6.71 X 3.35 E/W	09 Oct. 2012
'Royal Gala'	1996	M793	4.0 X 1.25 E/W	06. Oct. 2012
'Fuji'	1996	M793	4.0 X 1.25 E/W	07 Oct. 2012
Grabouw				
'Granny Smith'	1967	M793	3.00 X 4.50 N/S	12 Oct. 2012
'Golden Delicious'	1967	M793	3.00 X 4.50 N/S	17 Oct. 2012
'Starking'	1975	Seedling	6.00 X 3.20 N/S	22 Oct. 2012
'Royal Gala'	1985	M793	4.00 X 2.00 E/W	14 Oct. 2012
'Fuji'	2002	M793	4.00 X 1.50 N/S	19 Oct. 2012

N/S, north by south

E/W, east by west

Table 2. Average fruit size (mm) per cultivar and production region at sampling for stomatal (40 DAFB) and lenticel (80 and 120 DAFB) morphology and distribution

Ceres					
	'Granny Smith'	'Golden Delicious'	'Starking'	'Royal Gala'	'Fuji'
40 DAFB	37.2	38.6	35.2	32.9	33.6
80 DAFB	53.9	49.8	53.5	54.2	51.4
120 DAFB	65.6	62.6	61.7	60.3	60.1
Grabouw					
	'Granny Smith'	'Golden Delicious'	'Starking'	'Royal Gala'	'Fuji'
40 DAFB	35.0	37.6	34.9	36.6	32.3
80 DAFB	49.1	55.4	48.8	52.5	48.9
120 DAFB	60.0	66.3	63.3	62.0	62.9

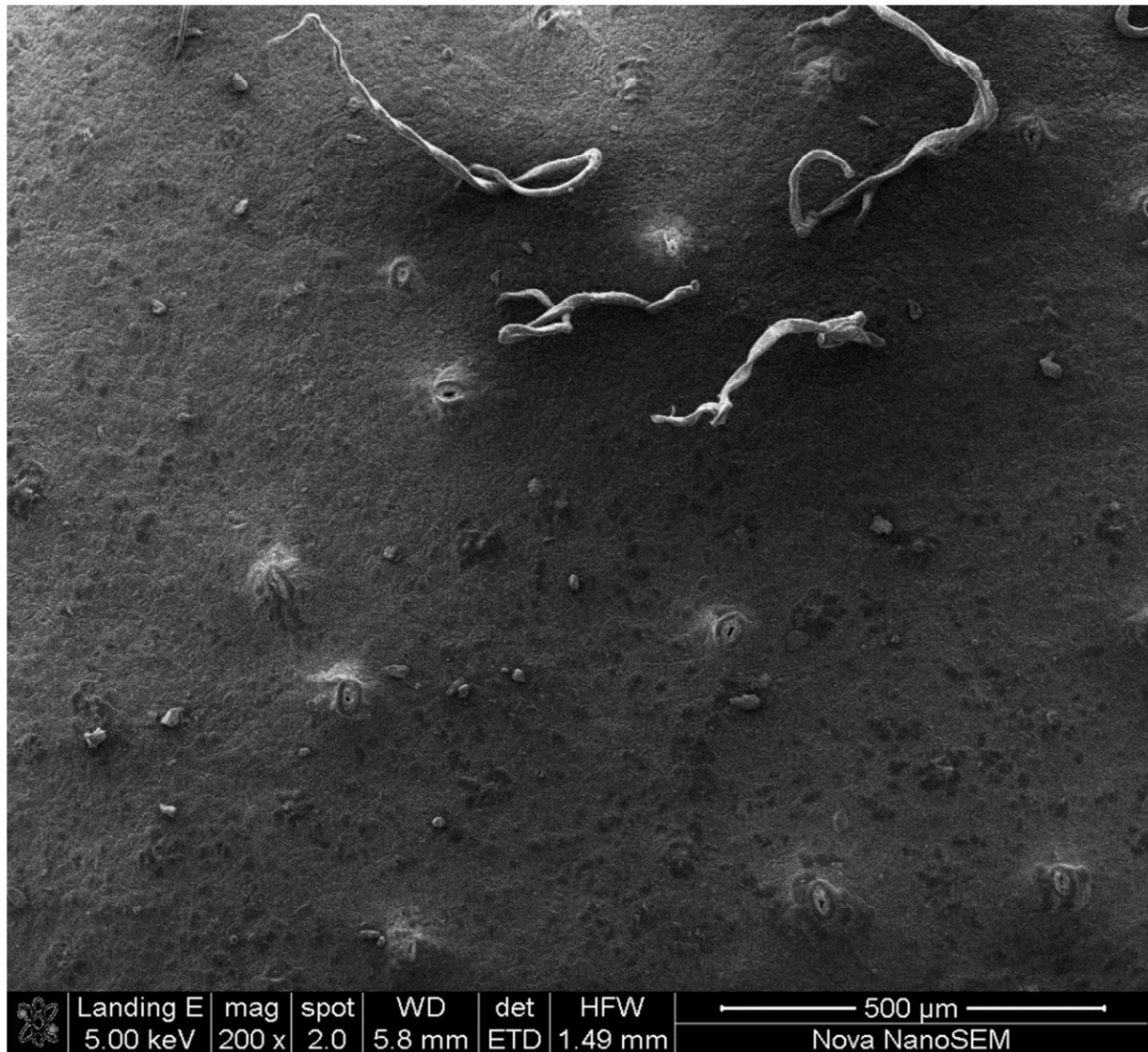


Figure 1. Image obtained from scanning electron microscope showing stomatal density at the calyx-end of 'Granny Smith' at 40 DAFB from Grabouw.

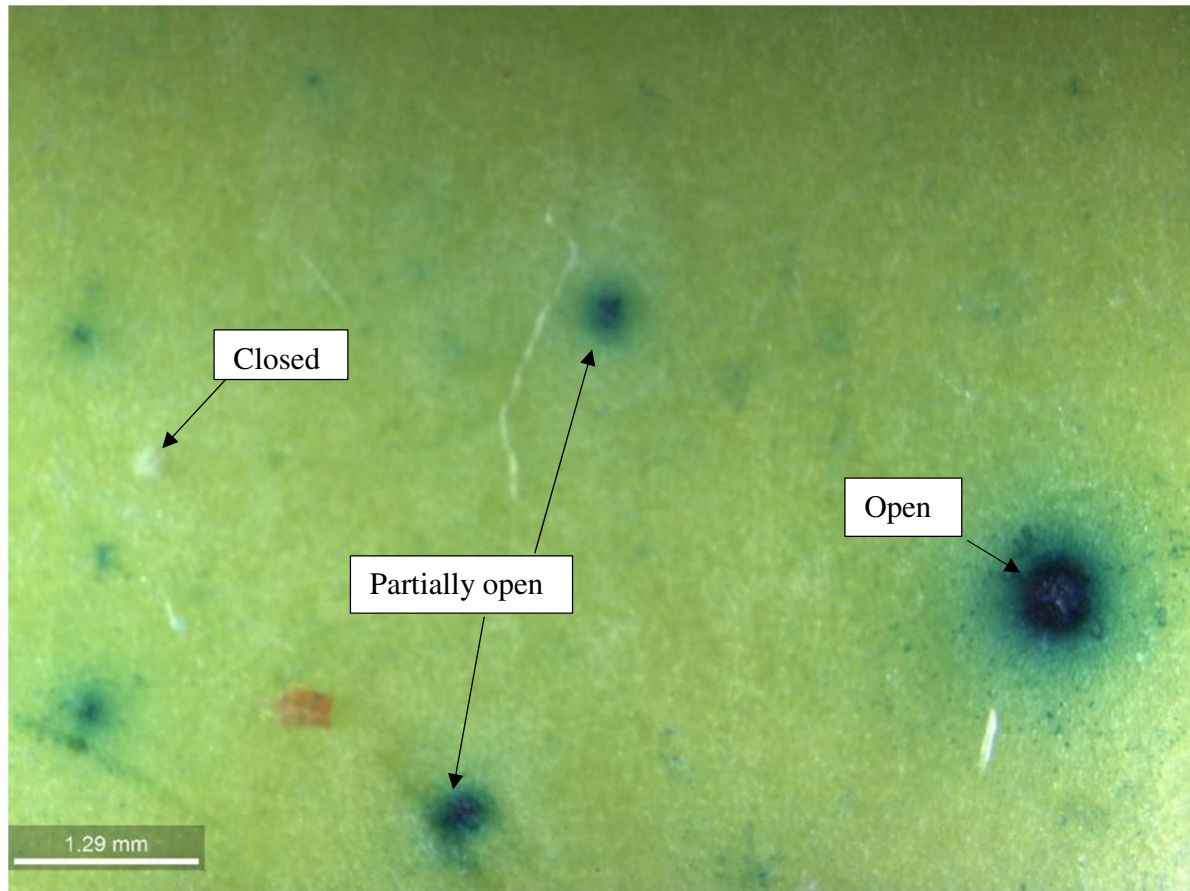


Figure 2. Image obtained from stereo microscope of 'Fuji' fruit surface from Ceres at 80 DAFB after immersion in methylene blue, showing the different lenticel types.

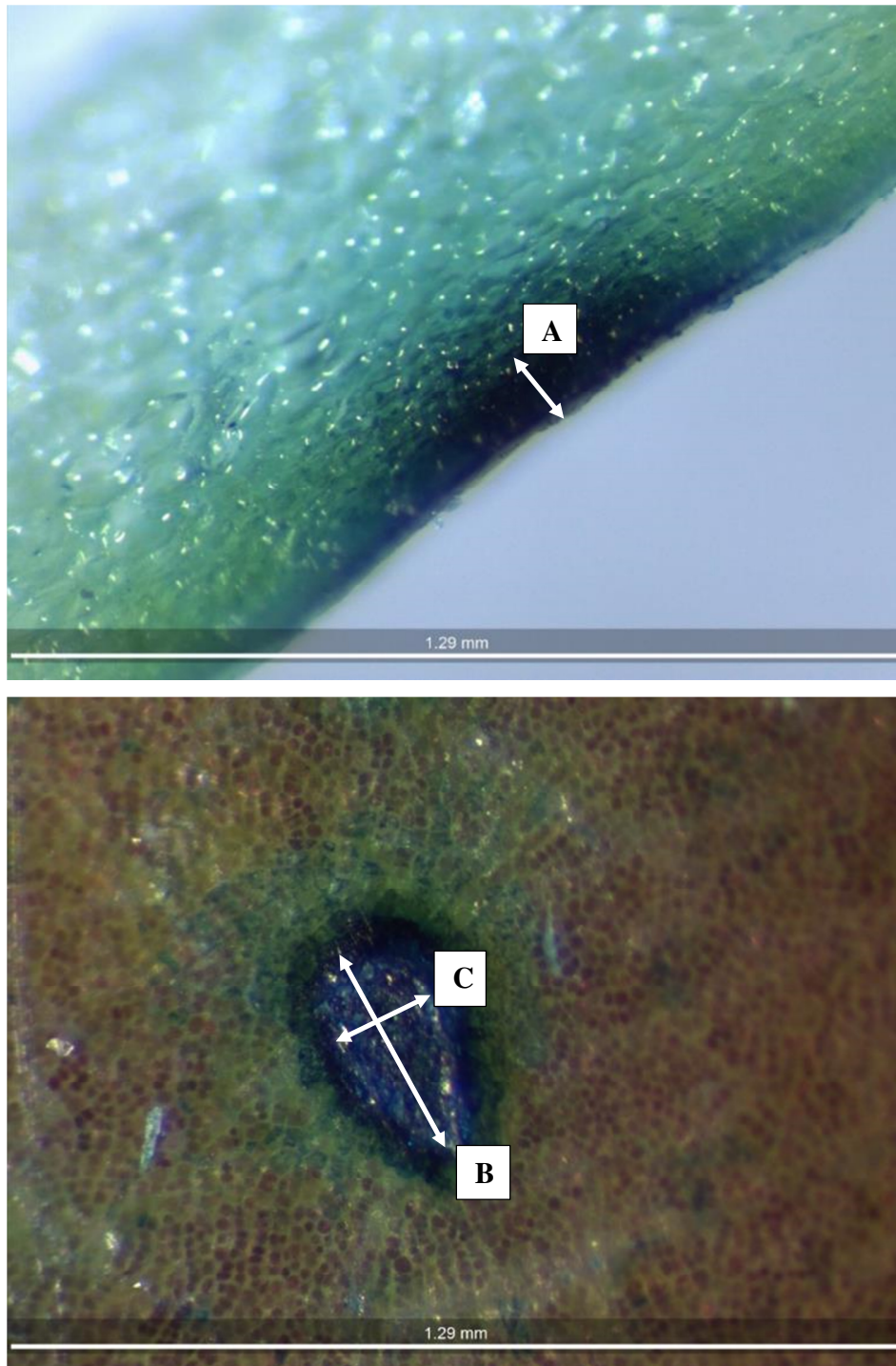
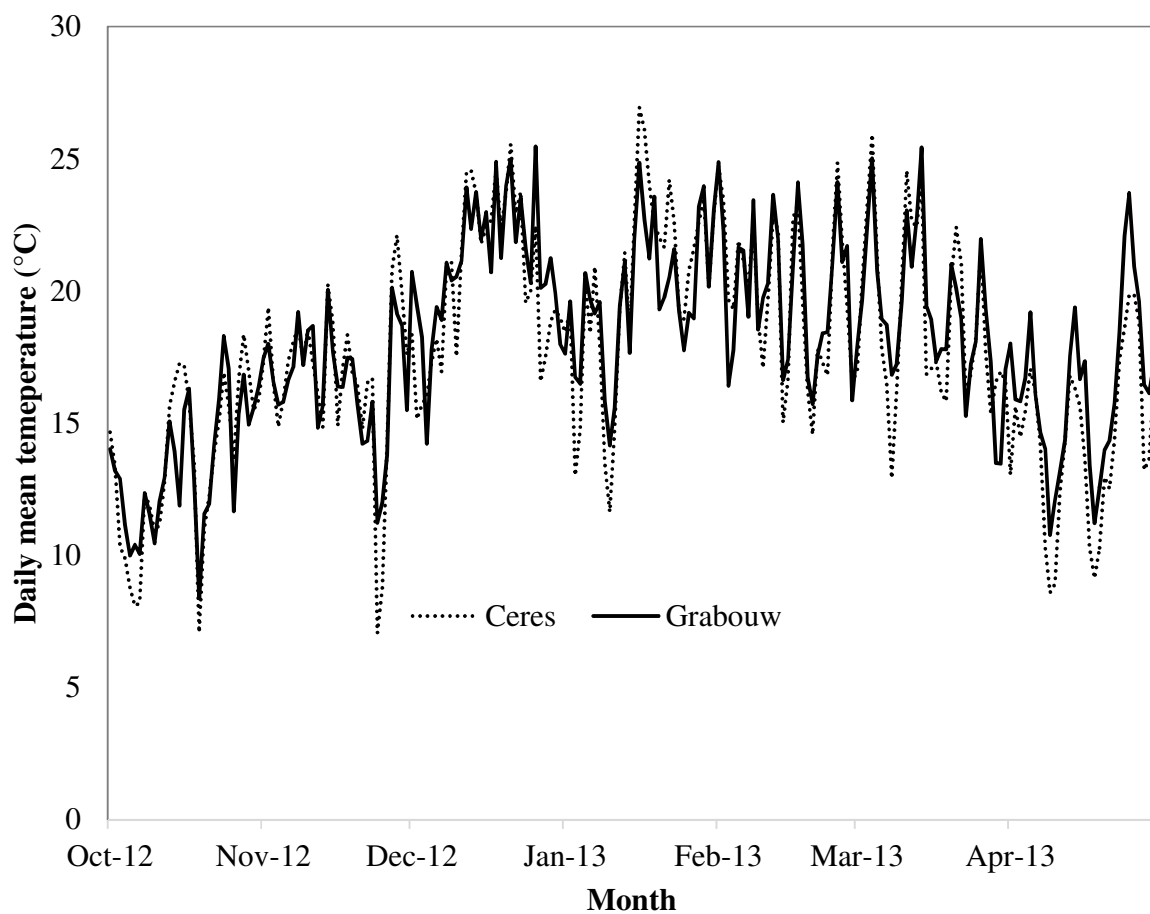
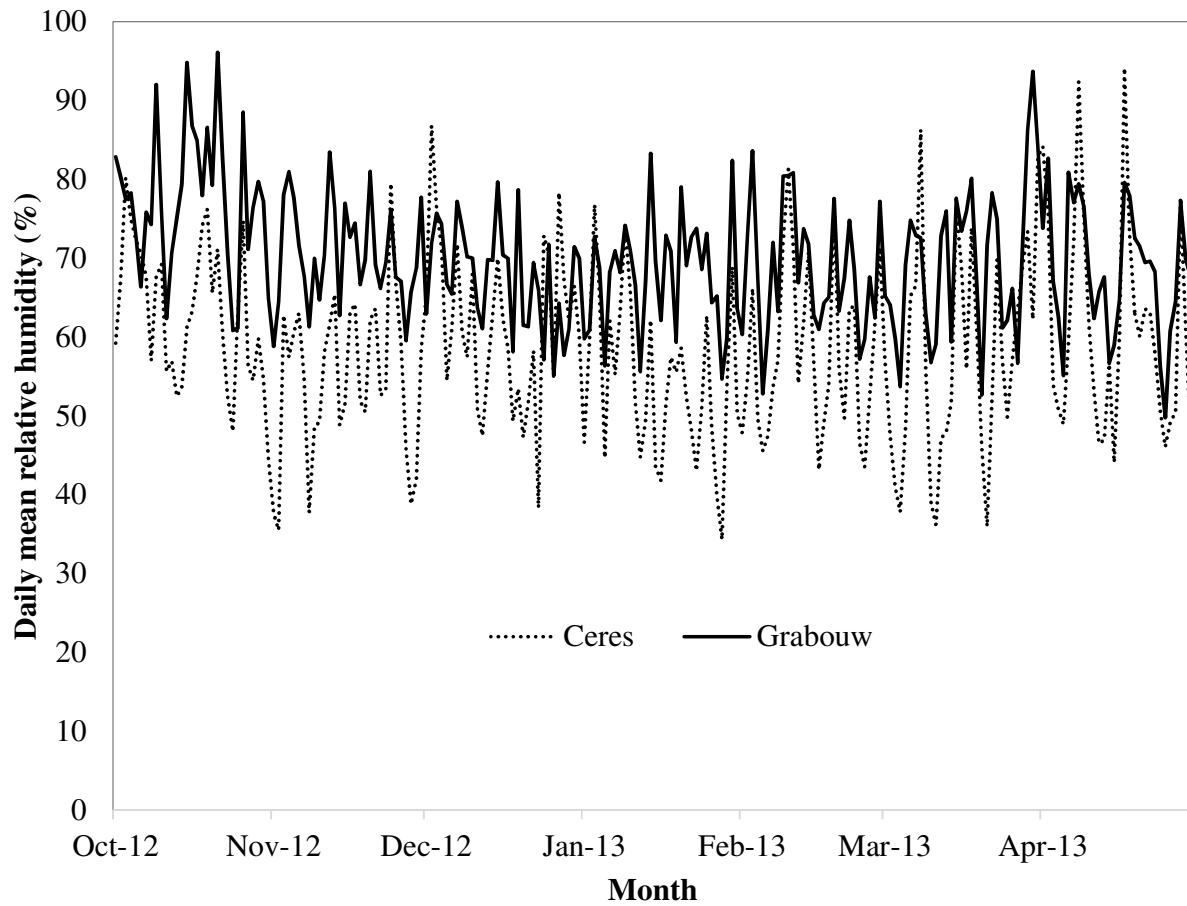


Figure 3 (Top) Cross-section of open lenticel used for anatomical measurements of depth (A) and (bottom) image of lenticel used for anatomical measurements of length (B) and width (C)



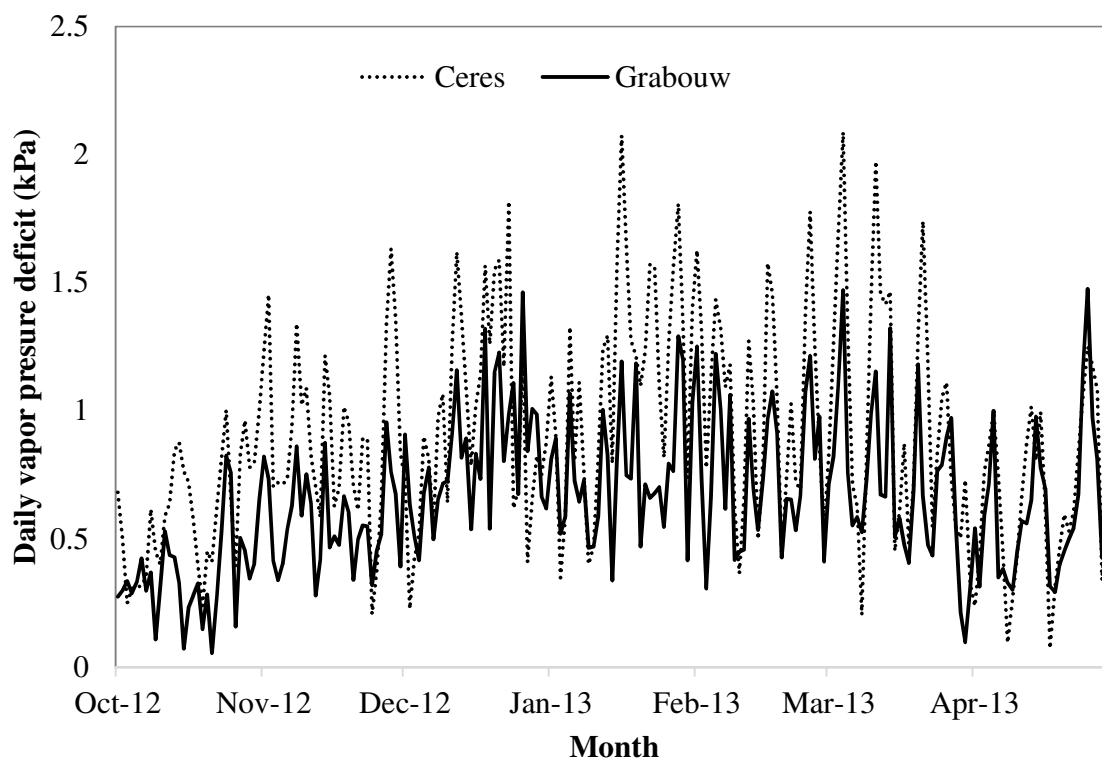
Month		Oct	Nov	Dec	Jan	Feb	Mar	April
Mean maximum temperature	Ceres	19.5	24.1	26.7	27.5	27.0	26.1	20.5
	Grabouw	17.0	22.6	27.1	25.6	25.9	24.6	21.4
Mean minimum temperature	Ceres	7.60	9.36	14.2	12.7	12.8	11.8	7.3
	Grabouw	9.17	10.4	15.1	14.0	14.2	13.7	10.3

Figure 4. Daily mean temperature at Ceres and Grabouw during the 2012-2013 growing season (figure) and monthly mean maximum and minimum temperature (table below).



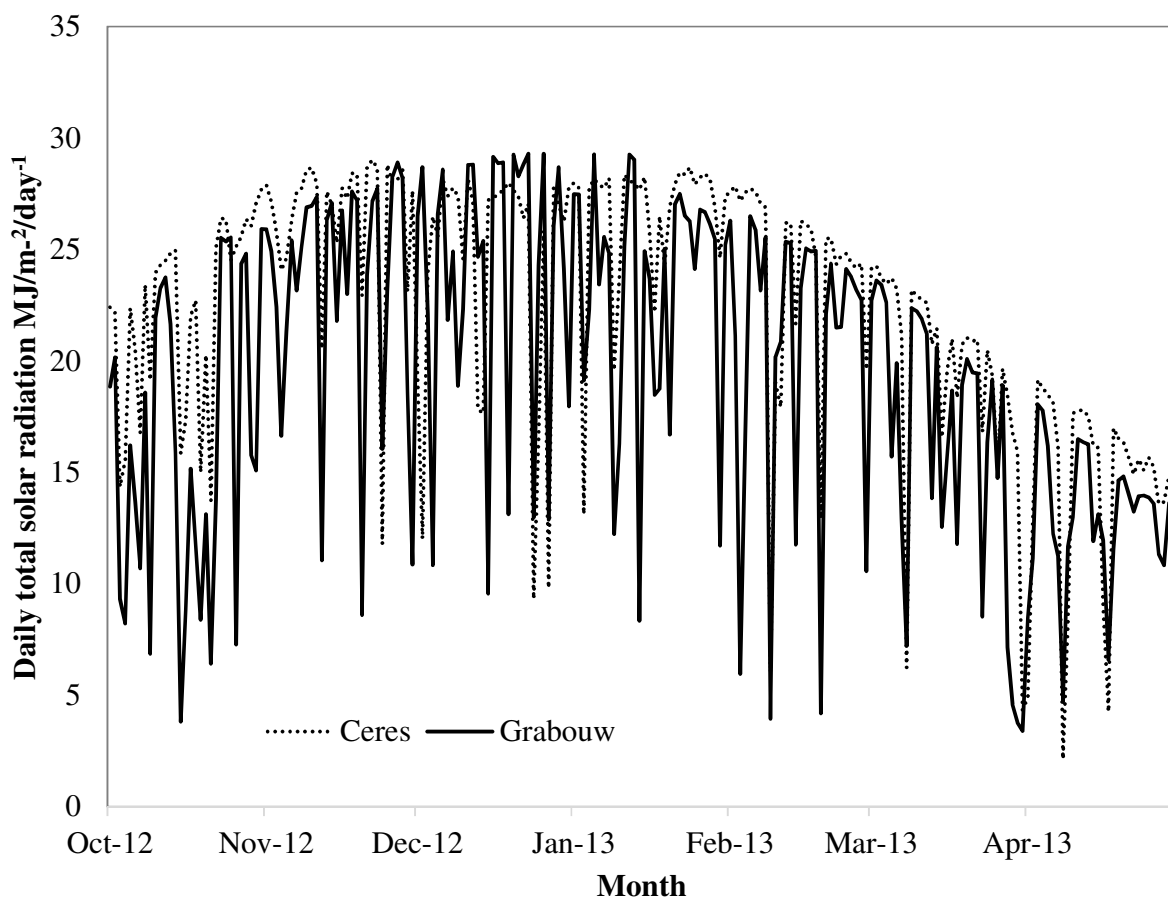
Month		Oct	Nov	Dec	Jan	Feb	Mar	April
Mean maximum RH	Ceres	85.1	81.4	84.3	79.3	83.9	81.83	85.7
	Grabouw	94.6	93.3	91.1	90.7	91.9	91.7	92.4
Mean minimum RH	Ceres	40.9	28.2	39.0	29.5	31.9	33.1	36.9
	Grabouw	61.5	47.8	44.1	44.8	45.0	47.1	45.3

Figure 5. Daily mean relative humidity at Ceres and Grabouw during the 2012-2013 growing season (figure) and monthly mean maximum and minimum relative humidity (table below)..



Month	Oct	Nov	Dec	Jan	Feb	Mar	April	
Mean vapor pressure deficit	Ceres	0.60	0.90	0.97	1.14	1.02	1.00	0.67
	Grabouw	0.36	0.57	0.83	0.76	0.77	0.71	0.62

Figure 6. Daily vapor pressure deficit at Ceres and Grabouw During the 2012-2013 growing season (figure) and monthly mean vapor pressure deficit (table below).



Month		Oct	Nov	Dec	Jan	Feb	Mar	April
Monthly mean solar radiation	Ceres	22.1	26.4	24.2	26.5	23.9	19.7	14.5
	Grabouw	16.1	23.2	23.9	23.5	20.5	16.2	13.0

Figure 7. Daily total solar radiation Ceres and Grabouw During the 2012-2013 growing season (figure) and monthly mean solar radiation (table below).

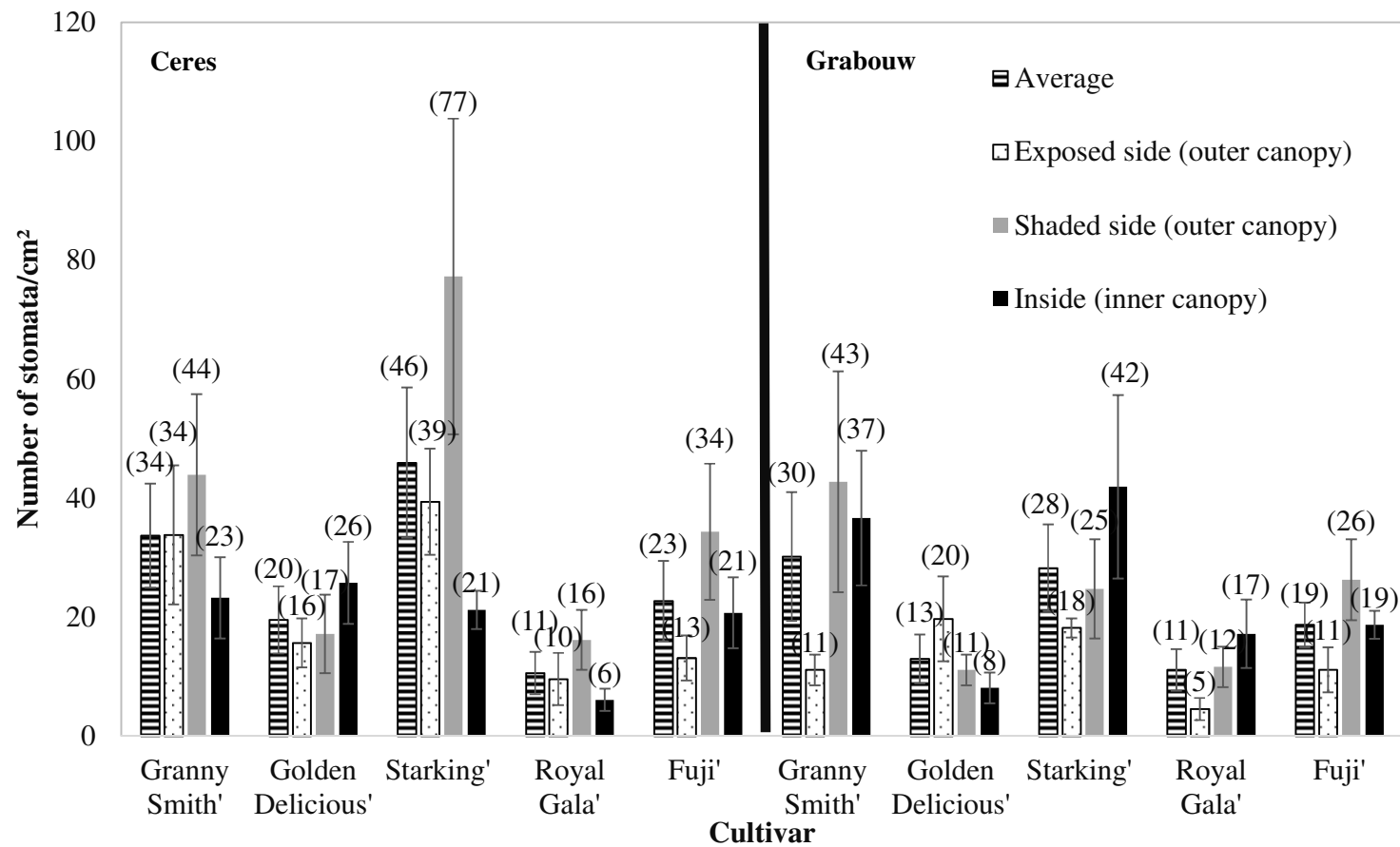


Figure 8. Stomatal density in fruit from various canopy positions in 'Fuji', 'Granny Smith', 'Golden Delicious', 'Starking' and 'Royal Gala' at 40 DAFB from two contrasting climatic regions.

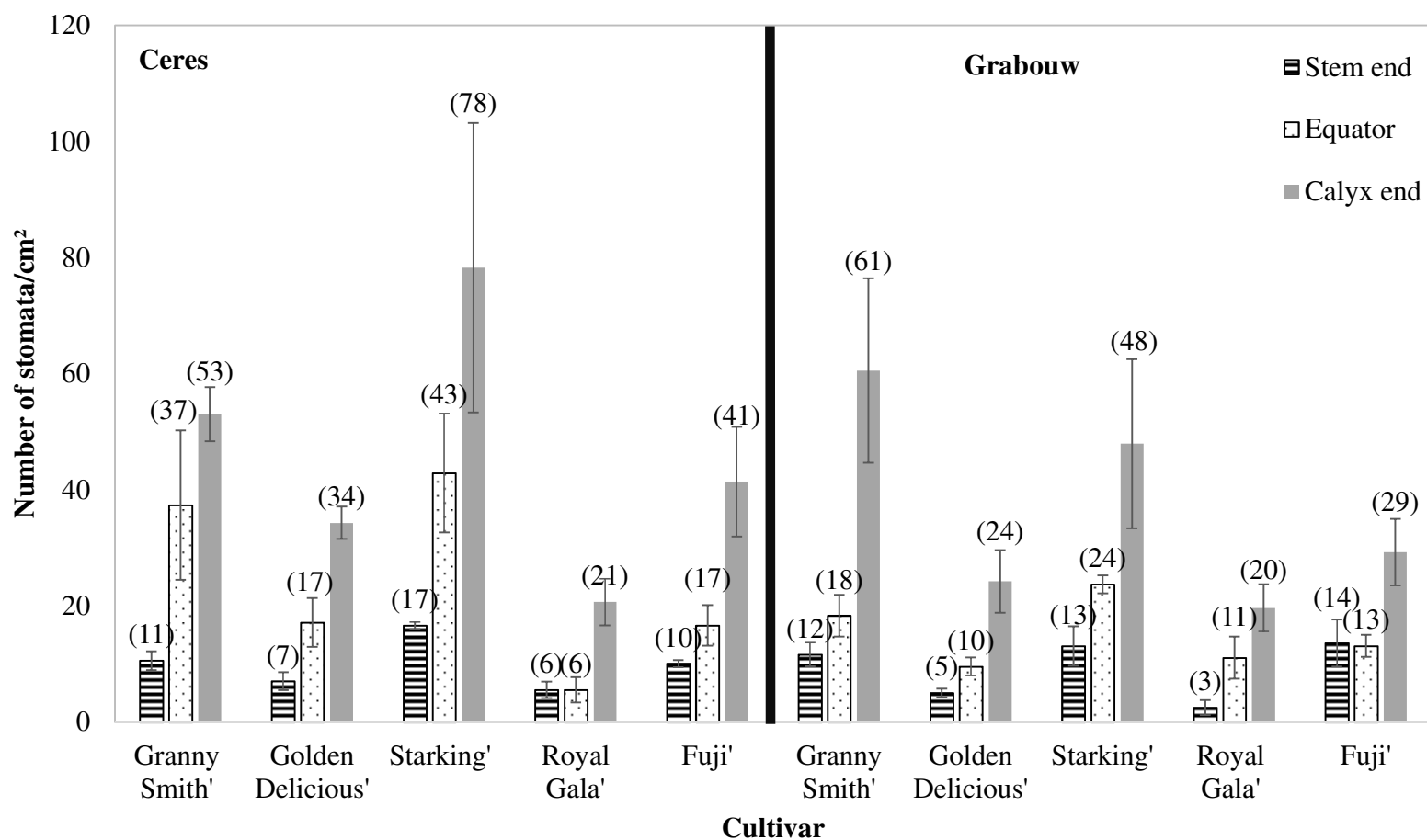


Figure 9. Stomatal density at various positions on the fruit in 'Fuji', 'Granny Smith', 'Golden Delicious', 'Starking' and 'Royal Gala' at 40 DAFB from two growing regions.

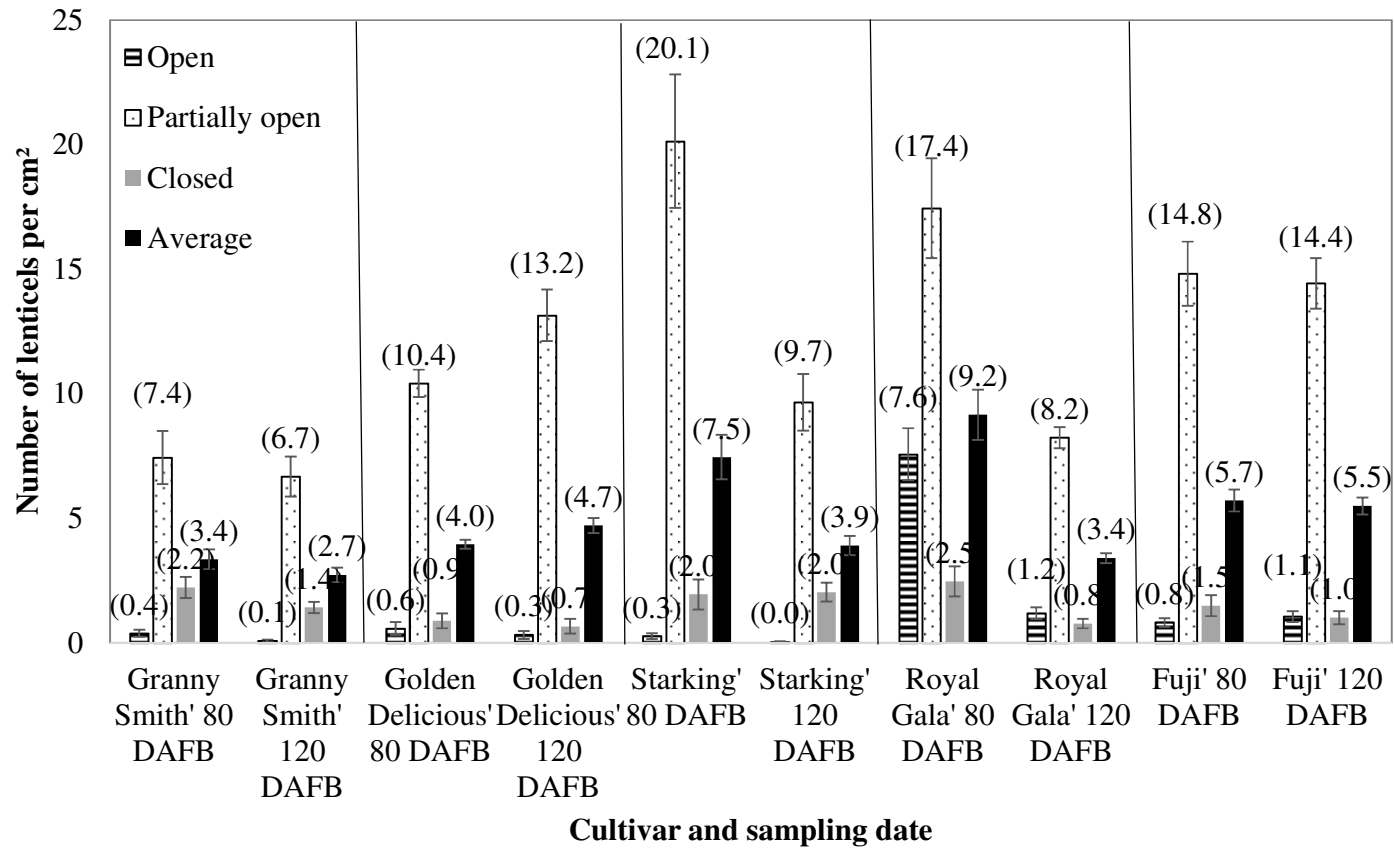


Figure 10. Density and type of lenticels in ‘Granny Smith’, ‘Golden Delicious’, ‘Starking’, ‘Royal Gala’ and ‘Fuji’ at Ceres at 80 and 120 DAFB.

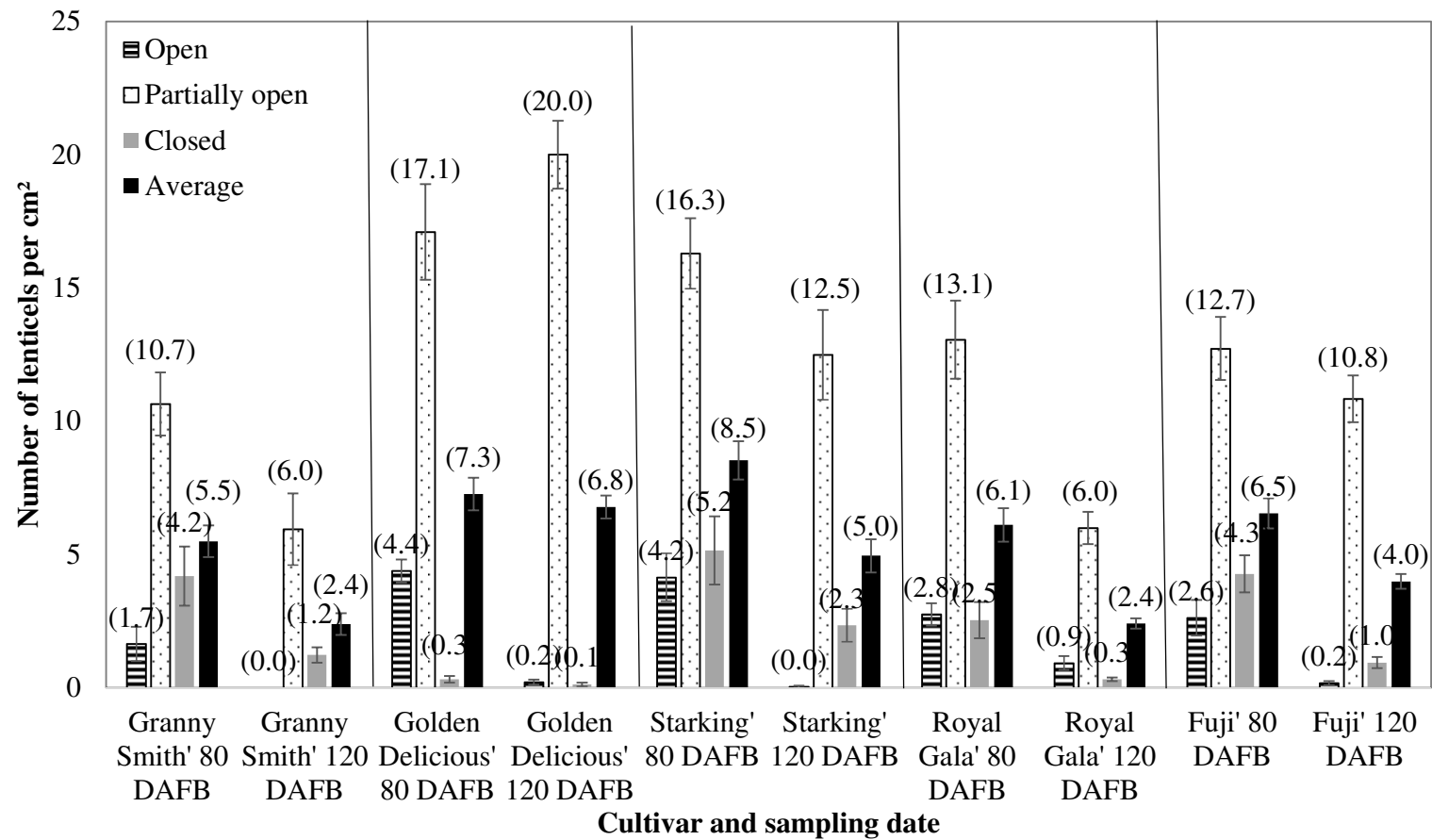


Figure 11. Density and type of lenticels in 'Granny Smith', 'Golden Delicious', 'Starking', 'Royal Gala' and 'Fuji' at Grabouw at 80 and 120 DAFB.

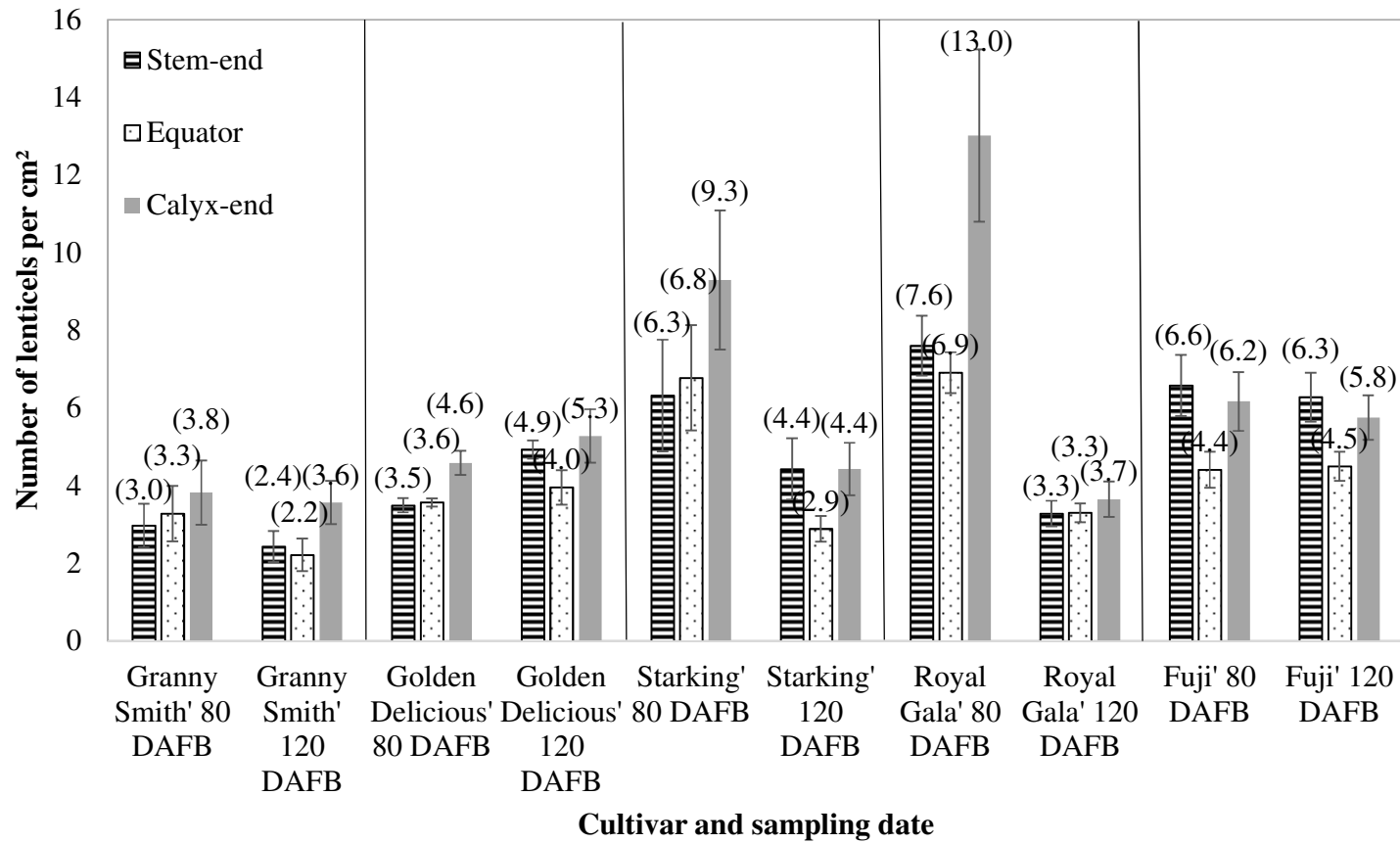


Figure 12. Density at various positions on the fruit of lenticels in 'Granny Smith', 'Golden Delicious', 'Starking', 'Royal Gala' and 'Fuji' at Ceres at 80 and 120 DAFB.

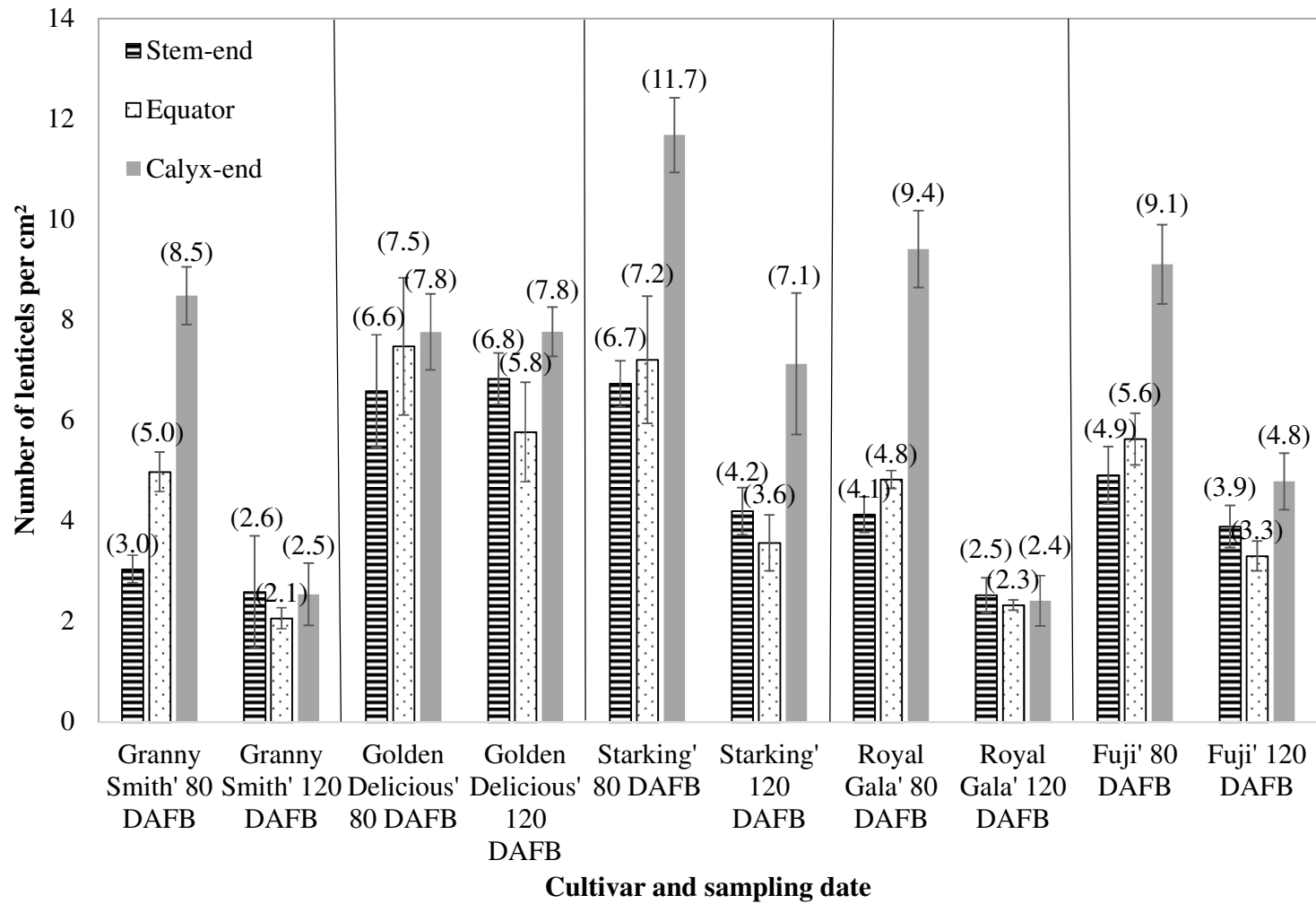


Figure 13 Density at various positions on the fruit of lenticels in ‘Granny Smith’, ‘Golden Delicious’, ‘Starking’, ‘Royal Gala’ and ‘Fuji’ at Grabouw at 80 and 120 DAFB.

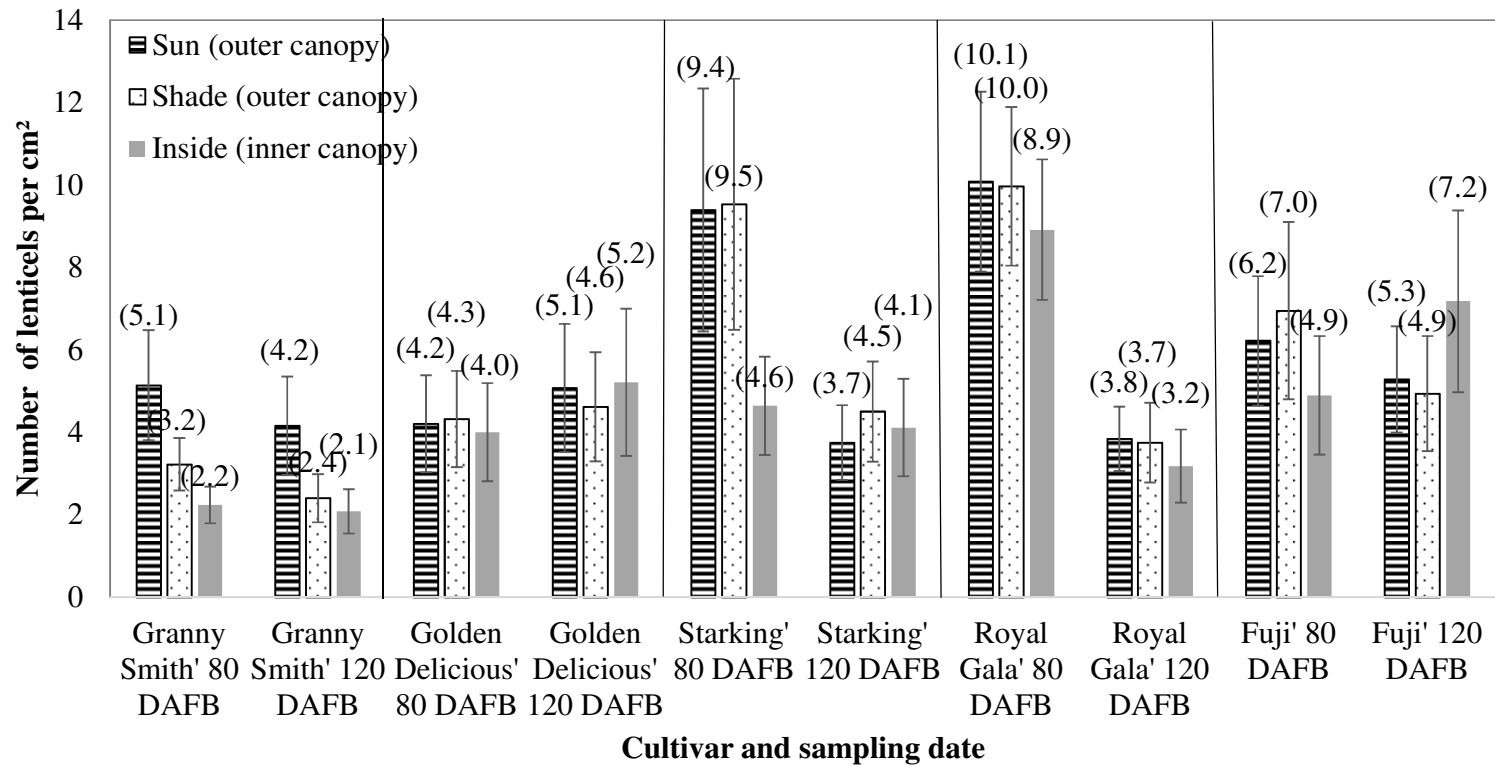


Figure 14. Density of lenticels on outer canopy (sun and shade side) and inside (inner canopy) fruit position in 'Granny Smith', 'Golden Delicious', 'Starking', 'Royal Gala' and 'Fuji' at Ceres at 80 and 120 DAFB.

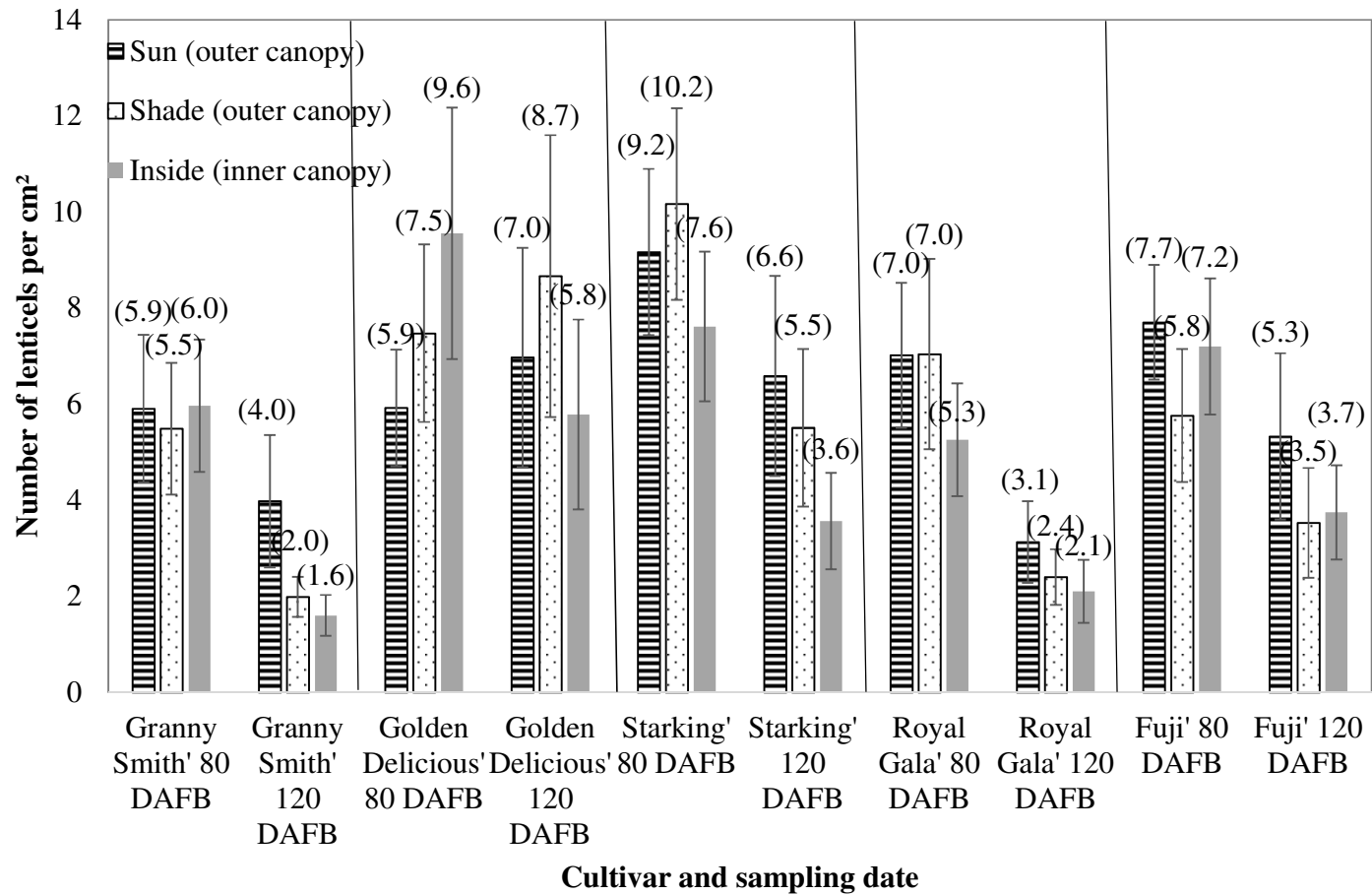


Figure 15. Density of lenticels on outer canopy (sun and shade side) and inside (inner canopy) fruit position in 'Granny Smith', 'Golden Delicious', 'Starking', 'Royal Gala' and 'Fuji' at Grabouw at 80 and 120 DAFB.

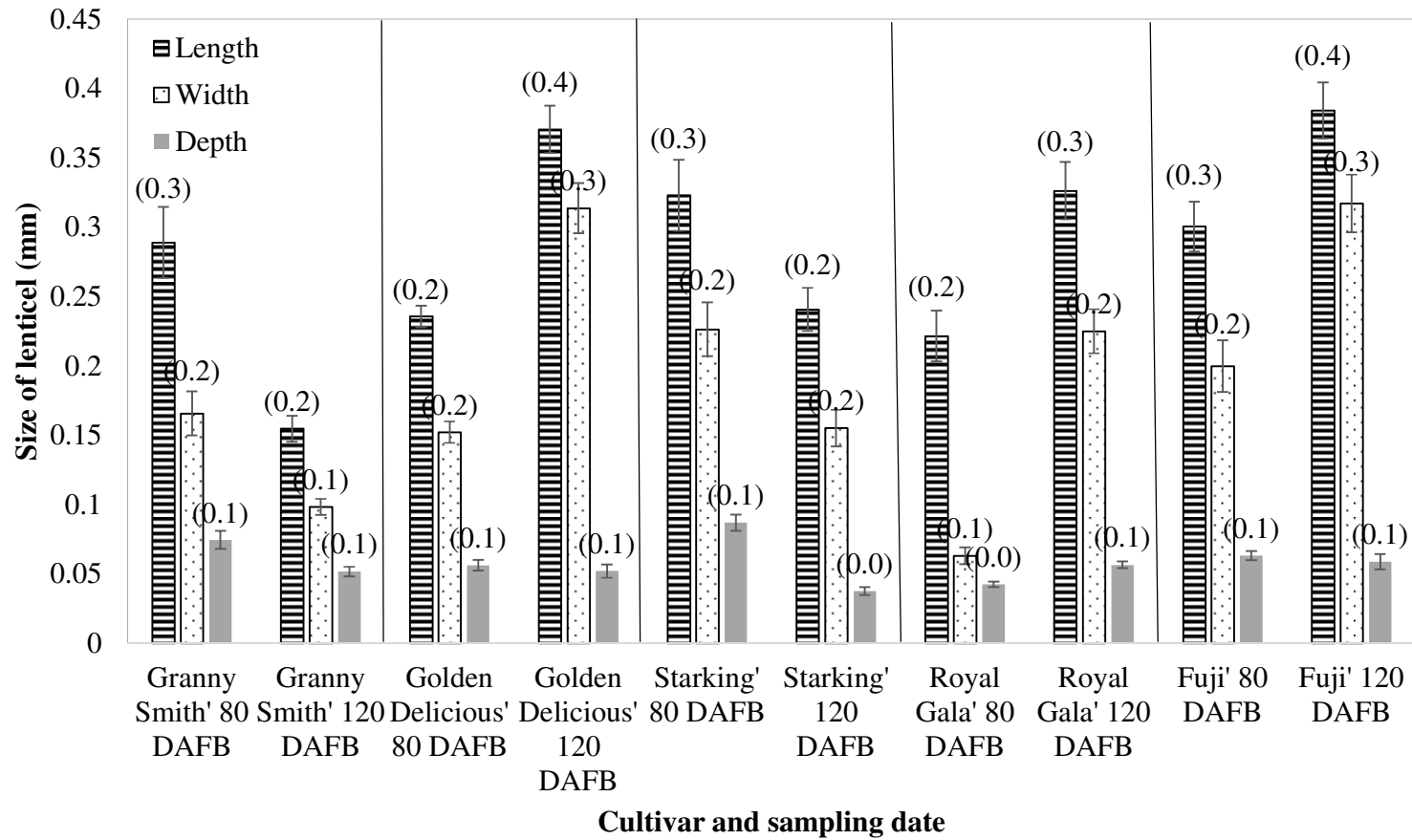


Figure 16. Length, width and depth of lenticels in ‘Granny Smith’, ‘Golden Delicious’, ‘Starking’, ‘Royal Gala’ and ‘Fuji’ at Ceres at 80 and 120 DAFB.

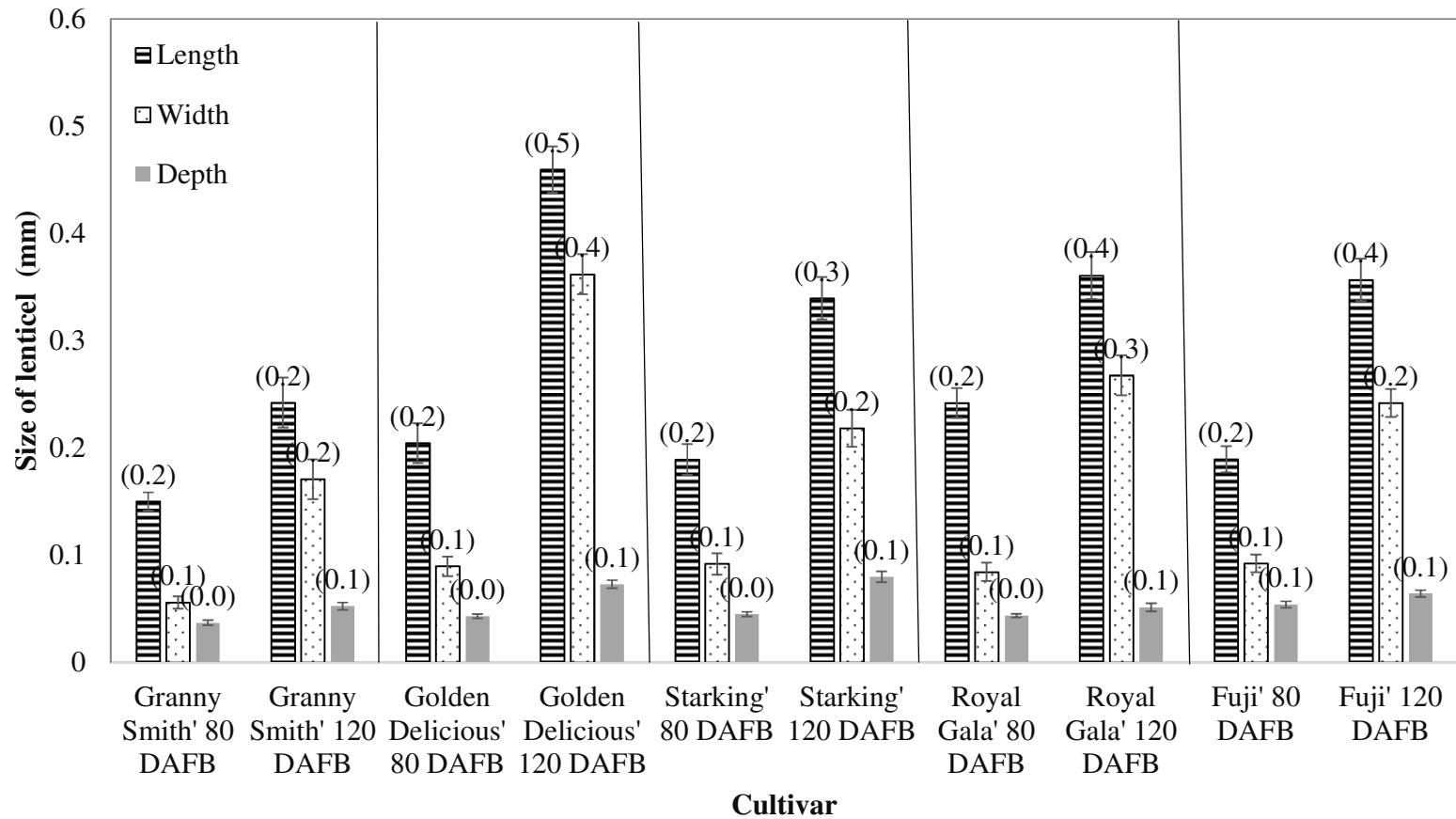


Figure 17. Length, width and depth of lenticels in ‘Granny Smith’, ‘Golden Delicious’, ‘Starking’, ‘Royal Gala’ and ‘Fuji’ at Grabouw at 80 and 120 DAFB.

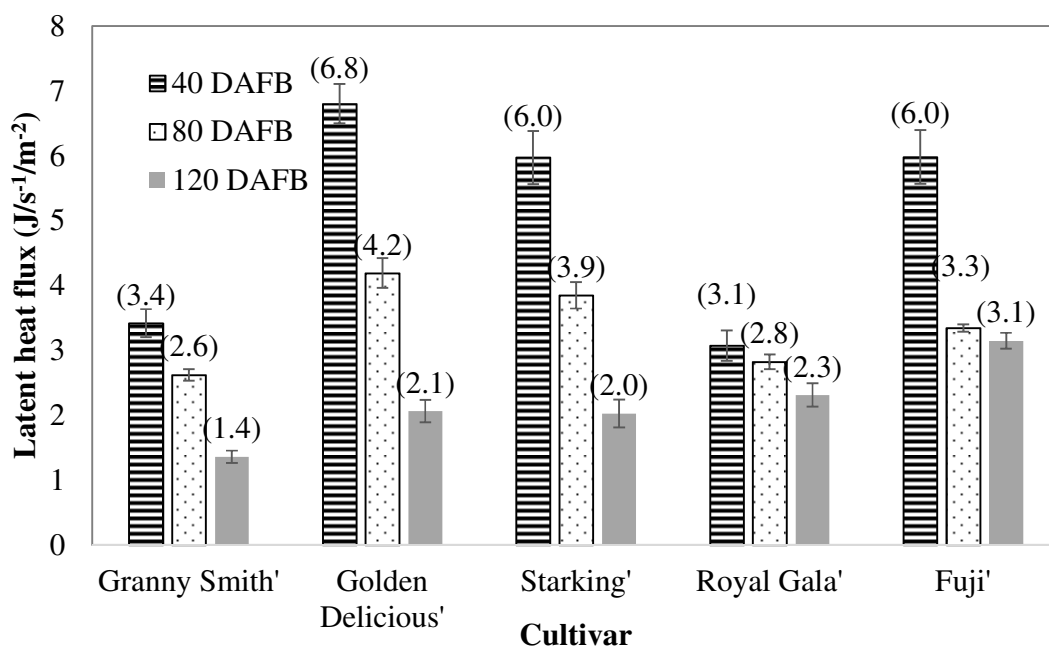
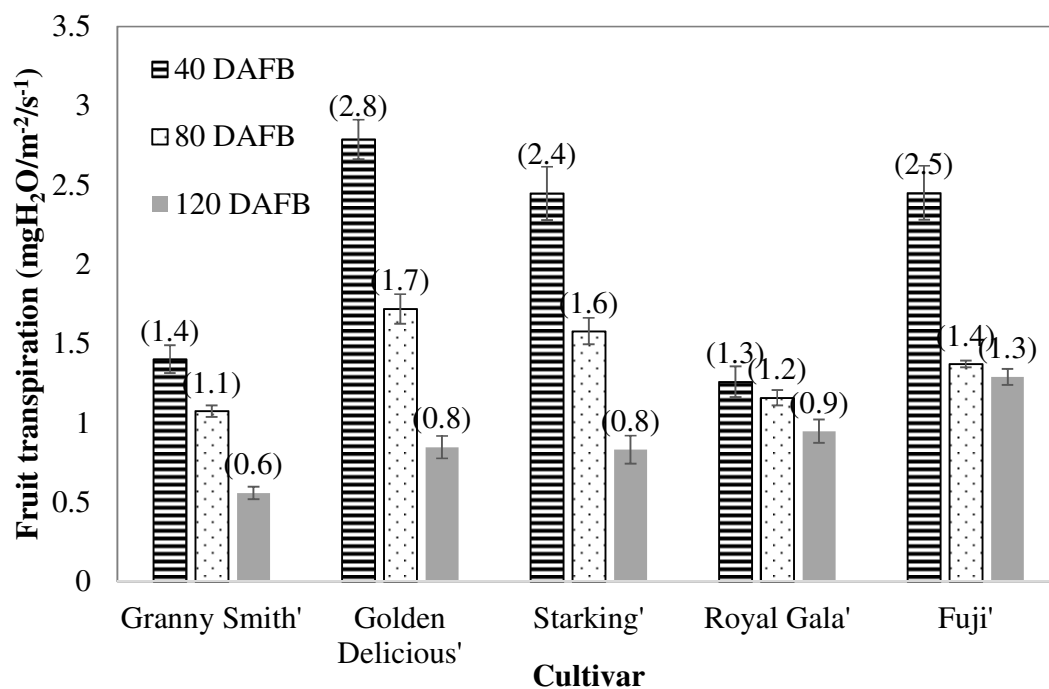


Figure 18. (Top) Fruit area-based transpiration rate and (bottom) latent heat flux in 'Fuji', 'Granny Smith', 'Royal Gala', 'Starking' and 'Golden Delicious' at Ceres measured using an IRGA.

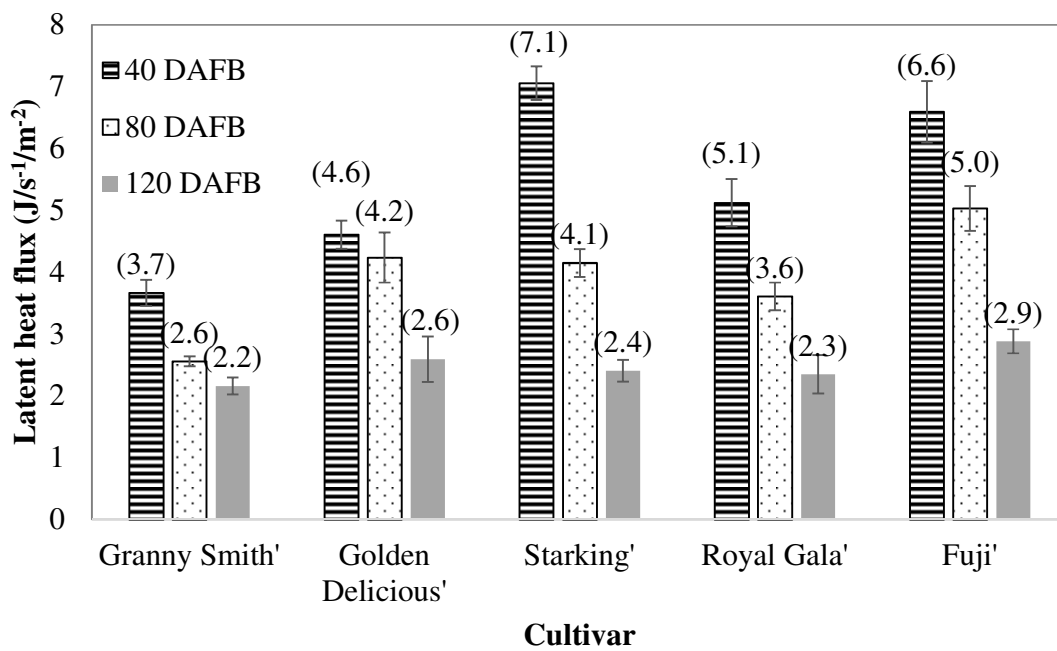
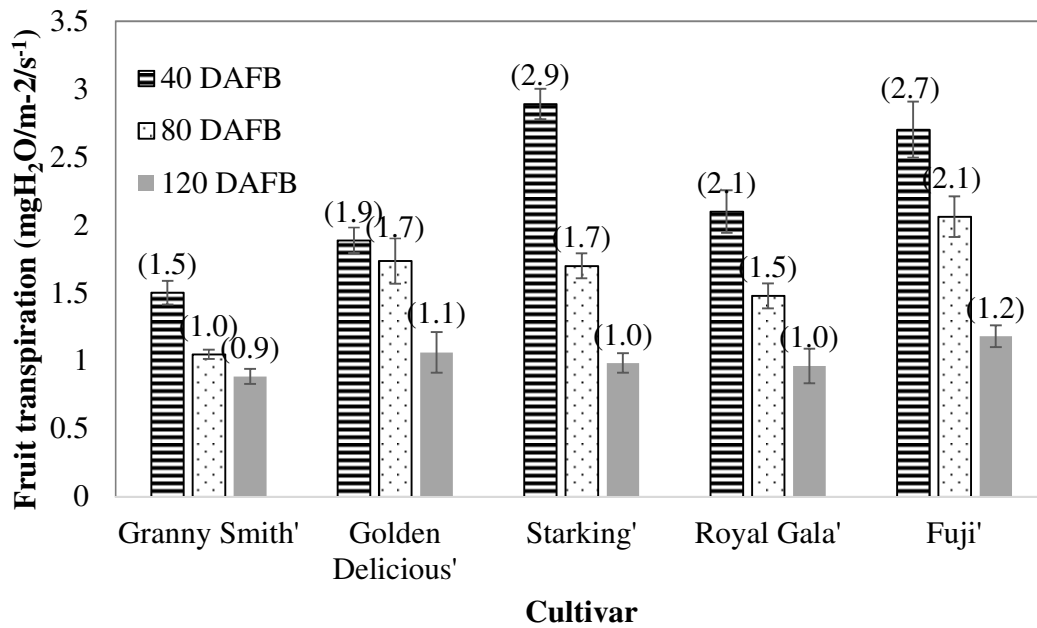


Figure 19. (Top) Fruit area-based transpiration rate and (bottom) latent heat flux in ‘Fuji’, ‘Granny Smith’, ‘Royal Gala’, ‘Starking’ and ‘Golden Delicious’ measured at Grabouw using an IRGA.

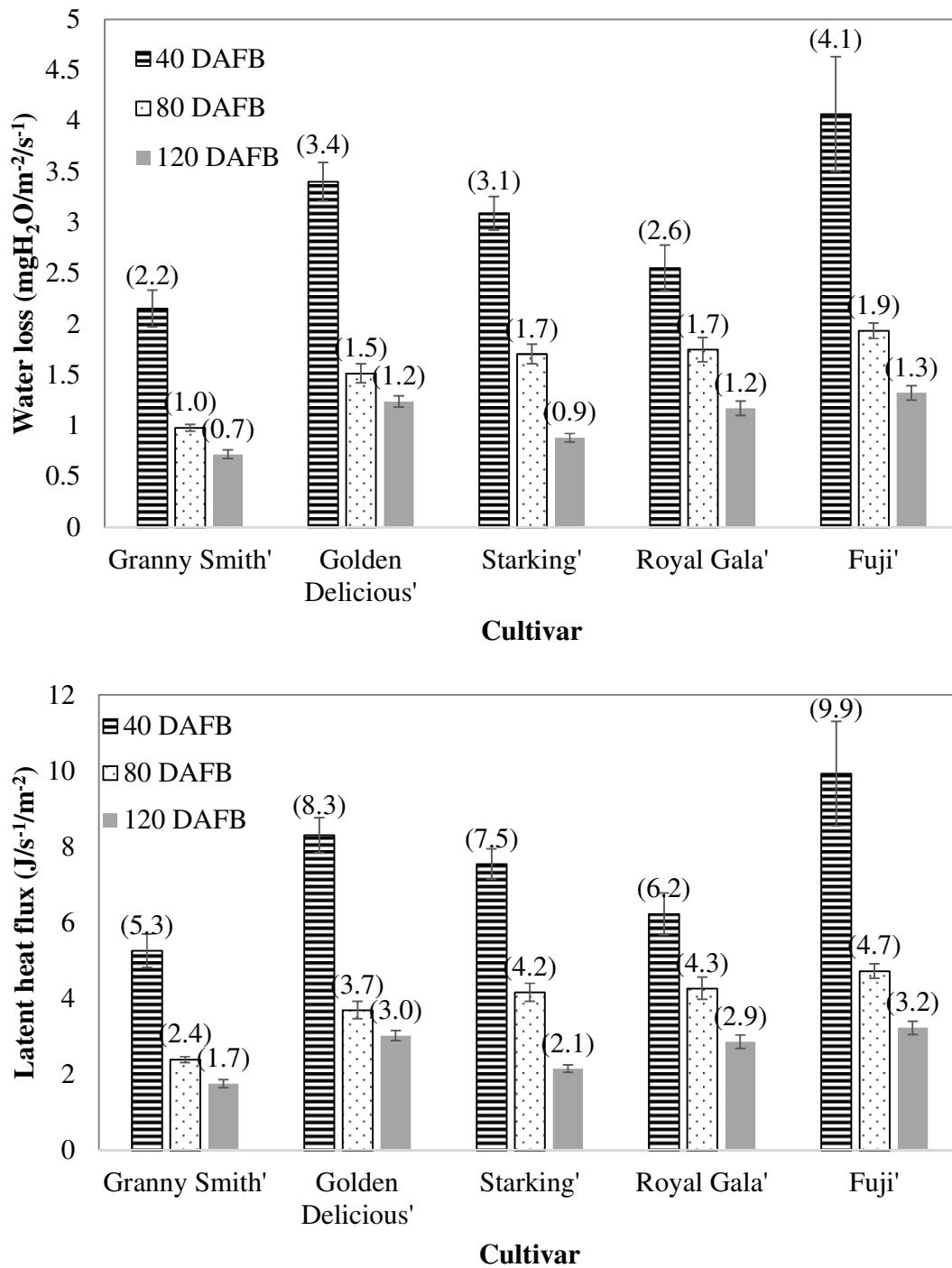


Figure 20. (Top) Rate of fruit water loss and (bottom) resulting latent heat flux in 'Fuji', 'Golden Delicious', 'Starking', 'Granny Smith' and 'Royal Gala' over 48 hours in an oven at 40 °C in fruit from Ceres.

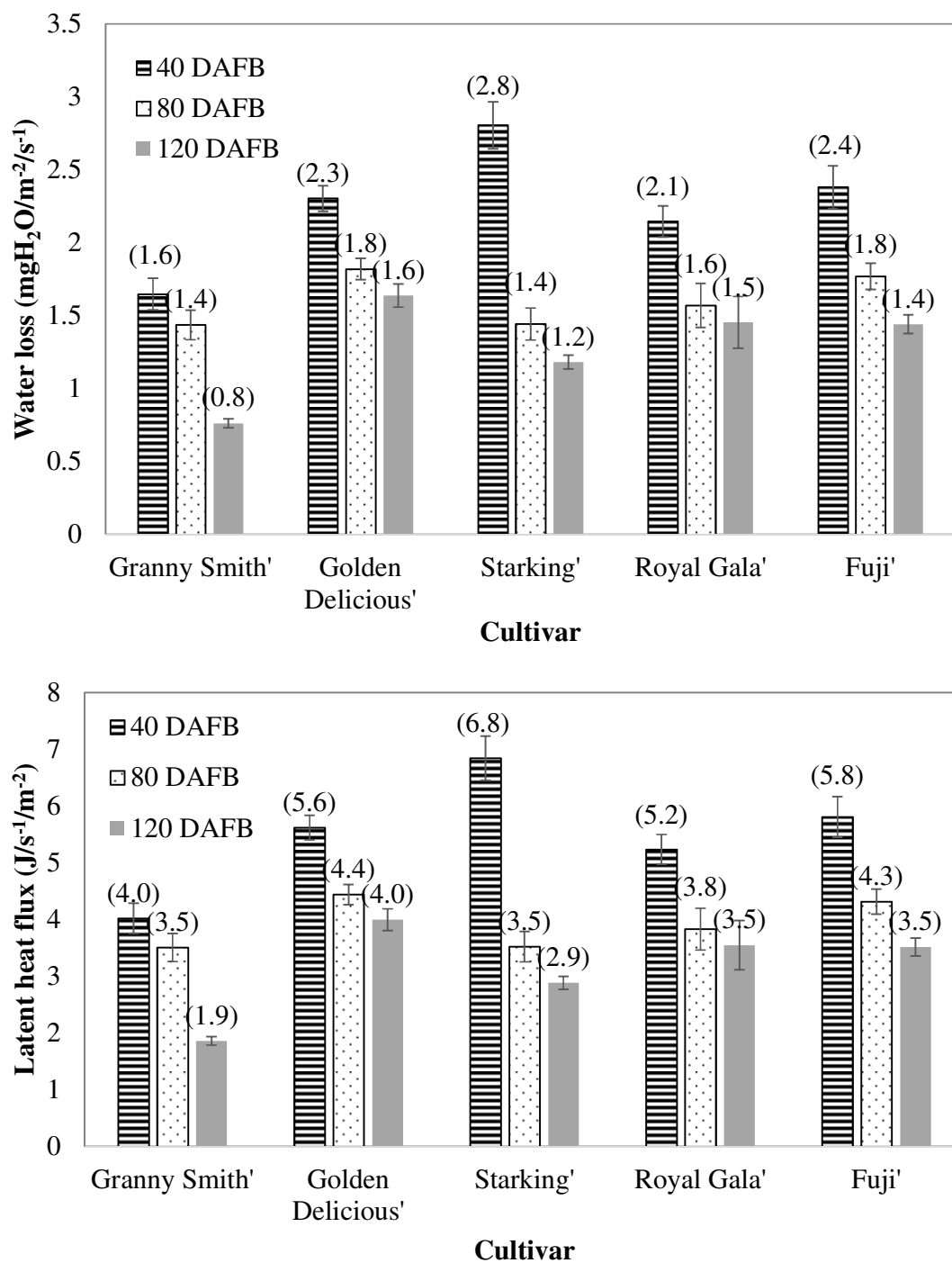


Figure 21. (Top) Rate of fruit water loss and (bottom) resulting latent heat flux in 'Fuji', 'Golden Delicious', 'Starking', 'Granny Smith' and 'Royal Gala' over 48 hours in an oven at 40 °C in fruit from Grabouw.

General discussion and conclusion

1. General background

The major apple production regions in South Africa at 33-34° S have a Mediterranean-type climate characterized by hot, dry summers and mild, wet winters. High temperatures and high irradiance during the growing season increase fruit surface temperatures and are associated with the occurrence of sunburn in apples (Chen et al., 2008). Sunburn is a serious problem for apple growers in the Western Cape and producers experience sunburn incidences of up to 50% of total yield in the worst years (Wand et al., 2006). Culling or downgrading of apples due to sunburn has a considerable impact on the profit margins of apple producers in South Africa. Tree water deficit has been linked to the occurrence of sunburn in apple fruit under South African growing conditions (Makaredza et al., 2013). However, the mechanisms by which tree water deficit is related to sunburn development in apple fruit are poorly understood. Scientific validation for the effect of water deficit on sunburn in apple is scant with the studies cited in the review of Racsko and Schrader (2012) on sunburn in apple providing only anecdotal or at best indirect evidence of any relationship.

This study was undertaken to verify and improve our understanding of how tree water deficit exacerbates sunburn. Such knowledge would contribute to the current knowledge on sunburn ontology and also assist growers in their efforts to reduce downgrading of fruit due to sunburn. In order to achieve this, the effect of short-term induced water deficit on sunburn development and plant water status was investigated. Radiant heating increases the temperature of apple fruit considerably above ambient air temperature. Apple fruit can cool by radiating heat to the environment and by convective heat loss to surrounding air. The temperature gradient between the canopy air and the heated apple fruit would determine the potential for convective cooling. We assessed the possibility that water deficit could increase the peel temperature by its effect on the canopy microclimate. We hypothesized that closure of stomata due to transient water deficit, would reduce evaporative cooling of leaves and result in an increase in canopy air temperature. This would then limit the ability of apple fruit to lose heat to the surrounding air, resulting in elevated fruit surface temperature and higher risk of sunburn. The effect of short term induced

water deficit on soil water status, leaf and fruit photochemistry, gas exchange and fruit biochemistry was assessed. An attempt was also made to evaluate the use of pulsing irrigation as practiced by South African apple growers to decrease sunburn and as an aid to reduce tree stress under heat stress conditions. The initial objective of this study was to examine the effect of pulsing irrigation when used as a sunburn control measure on soil water status, canopy environment, tree ecophysiological status, vegetative growth and fruit quality in ‘Golden Delicious’ and ‘Granny Smith’ under South African conditions. However, as the study progressed, it became apparent that the control which was irrigated according the scheduling on the farm was under irrigated. Therefore, the focus of the trial shifted to examine the effect of under irrigation on sunburn development and tree ecophysiology. The application of the plant growth regulator S-ABA, which has been shown to reduce sunburn development in Japan, supposedly by upregulating the antioxidant capacity of apple peel (Iams et al., 2009), was also assessed. We investigated the various effects of S-ABA on plant physiology and ecophysiology, which may prove beneficial or detrimental in a commercial apple production setup, other fruit quality parameters, gas exchange, plant water status, peel chemical composition and fruit set. Finally, we investigated the effect of regional climate and canopy micro-climate on fruit stomatal and lenticel density, and attempted to relate this to the transpirational cooling capacity of ‘Granny Smith’, ‘Golden Delicious’, ‘Starking’, ‘Fuji’ and ‘Royal Gala’ at different stages of fruit growth.

2. Water deficit and sunburn development

Water stress results in increased sunburn development in apple fruit, i.e., trees under water deficit are more likely to experience sunburn (Makredza et al., 2013; Schrader et al., 2003). We set out to study the mechanism by which water deficit increases sunburn. Plant water status was negatively affected by water deficit and was always the most responsive ecophysiological indicator of water deficit. Hence, our experimental setup did provide the necessary contrast between the control and water deficit treatments to study the effect of differences in plant water status on sunburn severity. Our results ruled out a shading effect on sunburn levels by exposing fruit to full sunlight. It would have been preferable to use fruit without sunburn that have been sun exposed during their entire development. However, late in the season when the experiment

was conducted, most fully exposed fruit had sunburn and could not be used in our experiment. Exposing fruit resulted in high sunburn incidence in both the control and the water deficit treatments. Sunburn necrosis and incidence increased under induced water deficit in ‘Granny Smith’, and in ‘Cripps’ Pink’ sunburn severity was increased relative to the control. Under water stress conditions the production of reactive oxygen species increases (Caverzan et al., 2012), which have the potential to cause damage to the fruit peel and predispose it to sunburn development. Fruit biochemistry in the peel was affected by water deficit as shown by changes in ascorbic acid levels in both cultivars and total phenolic concentration in ‘Cripps Pink’. Ascorbic acid and total phenolics act as antioxidants under water deficit to negate the effect of reactive oxygen species. Chlorophyll fluorescence, measured on the shaded, non-damaged sides of fruit, pointed to some photochemical changes within the fruit peel under water deficit, possibly by limiting quenching of absorbed light energy, potentially giving rise to free radical production and thereby predisposing fruit peel to sunburn. The results for chlorophyll fluorescence were not clear cut as chlorophyll fluorescence is not responsive to short term water stress (Fernandez et al., 1997). Our assessments of photochemistry via chlorophyll fluorescence showed that F_v/F_m and $^o\text{PSII}$ efficiency were reduced under water deficit in ‘Granny Smith’ leaves. In ‘Cripps’ Pink’ leaves only F_v/F_m was reduced under water deficit with no effect on the other chlorophyll fluorescence parameters. In the fruit peel, F_v/F_m and qP were reduced under water deficit in ‘Granny Smith’ whilst none of the fluorescence parameters were affected in ‘Cripps’ Pink’. The reduced qP in ‘Granny Smith’ at Oak valley showed possible damage to photosystem II and reduction in its efficiency. The differences in fluorescence parameters on the shaded sides of fruit, although slight, are of considerable significance since they possibly indicate that water deficit decreased the efficiency of the photo-apparatus. Considering the involvement of visible light in the development of sunburn (Felicetti and Schrader, 2008a), any decrease in the ability of the fruit peel photosystems to utilize light or cope with excessive light energy, may potentiate free radical-induced damage or the mobilization of protective mechanisms. The initiation of these protective mechanisms, i.e. xanthophyll cycle pool upregulation (Racsko and Schrader, 2012; Zhang et al., 2015), carotenoid synthesis (Felicetti and Schrader, 2008b), phenolic accumulation (Yuri et al, 2014) may manifest as visible sunburn symptoms (Hengari, 2015). Water deficit reduced net carbon assimilation due to a combination of reduced leaf stomatal conductance and possibly also photoinhibition. The reduction in leaf stomatal conductance under

water deficit might increase the canopy temperature, as transpirational cooling capacity is reduced leading to the formation of reactive oxygen species and increased sunburn occurrence.

The use of pulsing irrigation to avoid water stress during periods of high ambient temperatures and thereby decrease sunburn was investigated. Pulsing irrigation employs the standard micro-sprinkler irrigation system and wets the orchard floor. It is almost similar to overhead evaporative cooling, the difference being that evaporative cooling involves an overhead irrigation system that directly cools the canopy and the fruit surface through extraction of the latent heat energy needed for evaporation (Evans, 2004). Some apple growers in South Africa have been using pulsing irrigation to reduce water stress under heat stress conditions, although there is no empirical data showing the effectiveness of pulsing irrigation. There are widespread differences amongst the growers in terms of pulsing cycles and threshold temperatures, and claims of positive results appear to be anecdotal. Growers believe that pulsing irrigation decreased the air temperature in the orchard thereby cooling the fruit surface. However, Evans (2004) indicated that overhead irrigation systems that creates a misting effect and fail to effectively wet the fruit surface, do slightly cool the orchard air, but is not effective in controlling sunburn. Therefore, for overhead evaporative cooling to be effective as sunburn control strategy, wetting of the fruit surface is required. Bearing this in mind, we thought that pulsing irrigation through the regular irrigation system, if effective, might cool the tree canopy through maintenance of leaf transpiration on hot days, thereby facilitating convective heat loss from the fruit surface.

The initial objective of the study was to examine the effectiveness of pulsing irrigation as a sunburn control measure. However, as the trial progressed it became apparent that the control, which was irrigated according to the scheduling on the farm, was under irrigated. Therefore the focus of the trial shifted to examine the effect of pulsing on sunburn development in an under irrigated orchard. Results from our experiments showed that pulsing irrigation reduced the incidence of sunburn in ‘Golden Delicious’ and ‘Granny Smith’ in a under irrigated orchard. Soil moisture data showed that the control which was irrigated according to the schedule of the farm did not receive enough water. Therefore, the reduction in sunburn in response to pulsing irrigation might be attributed to better plant water status under pulsing irrigation compared to the under irrigated control. This supports results from Paper 1 which shows a relationship between

tree water deficit and sunburn occurrence in apple. Pulsing irrigation on hot days changed the microclimatic conditions within the tree canopy compared with the under irrigated control, resulting in lower temperatures and higher relative humidity. Optimal irrigation is therefore important to reduce sunburn development. In addition, irrigation practices like deficit irrigation and partial root zone drying should be avoided in cultivars that are more sensitive to sunburn, especially near harvest, as they might result in some degree of water stress. This trial was done on a farm with gravitational irrigation and in actual practice pulsing could be more challenging in terms costs and logistics on a farm where the water is pumped. Management of irrigation by growers is therefore important to avoid under irrigation as observed in this study which might lead to the increase of sunburn incidence. In terms of the effectiveness of pulsing irrigation in decreasing sunburn, considering our finding in Paper 1 that water deficit is associated with higher incidence of sunburn, further research is needed to establish whether pulsing irrigation reduces sunburn in orchards that are optimally irrigated. Our research suggests that in some instances where growers found decreased sunburn in response to pulsing irrigation, the orchards in question might otherwise not be optimally irrigated and pulsing irrigation may reduce sunburn simply by decreasing incidents of water deficit.

Abscisic acid (ABA) is a plant growth regulator that is involved in the signaling and regulation of plant responses to water stress (Kim and van Iersel, 2011). Recent research in Japan found that foliar application of S-ABA (biologically active form of abscisic acid) decreased sunburn incidence in 'Tsugaru', 'Sensyu', 'Yataka' and 'Fuji' apples by up to 30% (Iamsu et al., 2008; Iamsu et al., 2009). The application of S-ABA resulted in increased antioxidant levels, thereby alleviating or preventing oxidative damage caused by high ambient temperatures and high irradiance (Iamsu et al., 2009). Our research over three growing seasons showed that foliar S-ABA application had no effect on sunburn incidence and severity under South African conditions. The main apple growing regions of South Africa at 33-34° S latitude have a harsher climate with temperatures often exceeding 40 °C and higher radiation levels due to much less cloud cover during the growing season compared to Japan (Paper 3). Under harsher climatic conditions, the application of S-ABA can actually increase oxidative stress and cause the production of large amounts free radicals, instead of the beneficial antioxidant production observed in Japan (Jiang and Zhang, 2001). Under South African conditions, S-ABA application reduced the concentration of total antioxidants, total phenolics, and reduced ascorbic acid, whilst

increasing oxidized ascorbic acid - this indirectly suggests increased free radical production. The differences in response to S-ABA application in South Africa and Japan, may also relate to the enclosure of fruit in a bag in Japan – “fruit bagging” is a common practice used in Japan to etiolate the fruit peel resulting in bright red color development upon re-exposure of the fruit just prior to harvest.

S-ABA application resulted in lower stomatal conductance with the consequent decrease in gas exchange, reduction in fruit size and negative effects on internal fruit quality. There was a positive effect on tree water status in terms of stem water potential due to the reduction in transpiration. The potential use of the application of S-ABA to reduce water loss under severe water stress conditions when irrigation water is severely constrained or runs out under drought conditions should be researched. Under such conditions, growers may need to remove the tree canopy for the root system to survive. However, this use of S-ABA needs to be properly researched and carefully considered. Ultimately though, based on our results, the application of S-ABA to reduce sunburn is not recommended in regions that experience high sunburn incidences and where fruit bagging is not practiced.

3. Fruit morphology and evaporative cooling

The apple fruit surface is dynamic, constantly changing throughout the season (Curry, 2009). Water loss in apple fruit is minimized by the presence of a cuticle, otherwise they would desiccate quickly (Kornaska, 2012). This in turn tends to limit the evaporative cooling potential of apple fruit. Fruit transpiration and water loss was highest in young fruit compared to mature ones. Stomata are present in young fruit and are under regulatory control which makes them more effective for evaporative cooling. In mature fruit there are no functional stomata anymore and the predominant lenticel type was partially open and not effective for transpiration and water loss regulation. Stomatal density tended to be highest at the calyx-end in young fruit at 40 DAFB. There is a large variation in fruit surface morphology in terms of lenticels within cultivars, between cultivars, from different fruit position and climatic regions. Although fruit transpiration and water loss decreased over the growing season, it did not always follow the same pattern as fruit surface morphology. In addition, heat loss from transpiration and water loss is

negligible making it difficult to relate cooling capacity and fruit surface morphology. Emitted radiation and convection were reported to have a greater influence on apple fruit surface heat fluxes compared to transpiration in modeling experiments (Li et al., 2014; Paper 4). Evaporative cooling from the apple fruit surface therefore has no bearing on sunburn development.

Microcracks, which might form a significant number of openings in fruit, were not measured in this study and could have explained some of the trends in fruit transpiration and surface conductance. Since the fruit surface is not able to regulate temperature using evaporative cooling, cultural practices that help lower fruit surface temperatures should be considered to reduce sunburn development. The use of shade netting is a viable alternative to reduce fruit surface temperatures. Evaporative cooling can also be used, although it might not be an alternative in the future due to concerns over water quality and water scarcity from increasing frequency of droughts.

4. Conclusion and recommendations

The results of Paper 1 and, to an extent, also Paper 2 confirm the finding of Makedredza et al. (2013) that water deficit may increase sunburn development in apple fruit. As discussed in the review of Racsco and Schrader (2012), various studies have suggested that drought stress and water deficit might increase sunburn in apple, but none of these studies provided empirical proof or suggested the potential mechanism by which plant water status affect sunburn. Our research shows that water deficit possibly affects the ability of the photoapparatus in apple peel to utilize or quench absorbed light energy. It can be inferred that water deficit therefore increases free radical formation in sun exposed apple peel thereby predisposing the peel to sunburn development or resulting directly in damage that manifests as sunburn.

In terms of recommendations to growers; optimal irrigation is advised whereby growers should strive to prevent water deficits. The optimal irrigation of apple orchards to avoid tree water stress would be an important step toward reducing losses from sunburn losses. This can be done by having more proactive irrigation schedules supported by real-time soil and plant data instead of rigid schedules which might lead to water deficit as obtained in our studies. The exact plant

water potential at which sunburn risk might increase, might be difficult to determine, considering that sunburn development is influenced by multiple factors and also due to different apple genotypes to various levels employing iso- and anisohydric strategies in coping with water deficit. We did not in this study address the effect on sunburn of the transient water stress that might occur due to evaporation demand outstripping the ability of the tree to supply water through the xylem. Such conditions might quite commonly occur in South African apple growing regions characterized by low relative humidity, prevalent strong southeasterly winds and, therefore, high vapor pressure deficits during the growing season. Evaporative cooling may decrease the vapor pressure deficit, but is not a feasible option in the water scarce apple growing regions in South Africa. Shade netting may decrease transient water stress by decreasing wind speed and increasing humidity (Middleton and McWaters, 2002). However, the effectiveness of shade nets in decreasing sunburn relates more to decreased sunlight incident on fruit.

Further research should assess free radical formation in apple peel under conditions conducive to sunburn development in trees experiencing water deficit. Continuous logging of relevant ecophysiological parameters, more specific description of water availability to the plant and movement through the tree to the fruit would be recommended. Little is known about how the ability of the fruit peel to utilize and quench absorbed light energy may relate to sunburn development.

The pulsing experiment reported in Paper 2 did not provide conclusive results on the effectiveness of irrigation pulsing to decrease sunburn in apple. Since the control was under irrigated and pulsing irrigation did decrease sunburn, it would seem that in some instances where growers have attributed a decrease in sunburn to pulsing irrigation, the irrigation of the orchard might not have been optimal in seasons prior to commencing pulsing irrigation. In these instances, pulsing irrigation might decrease water deficit incidents, thereby decreasing sunburn. Further research is needed to study the effectiveness of pulsing irrigation in decreasing sunburn compared to a control that is optimally irrigated. The irrigation in both the pulsing treatments and the control would require careful management because pulsing will invariably alter the wetting profile and thereby may differentially affect root growth and function compared to the control. For this reason, more encompassing monitoring of soil water across treatment plots is required, while assessment of root growth is also recommended.

Results reported in Paper 3 indicate that foliar S-ABA does not decrease sunburn under conditions conducive to sunburn development in South Africa. It is unlikely that S-ABA will be effective in other regions where conditions are also conducive to sunburn development. When Racsco and Schrader (2012) in their review suggested that S-ABA is assessed in “harsher” regions like South Africa, they did not realize that apples in the Japanese study (Iams et al. 2008, 2009) were enclosed in bags after application. The decrease of sunburn in Japan in response to S-ABA application is likely to have more to do with the bagging of the fruit than with the less harsh Japanese climate. It would be relatively easy to test this hypothesis by conducting a factorial trial with bagging versus no bagging and S-ABA application versus no application as the factors.

Paper 4 showed that heat loss through transpiration is limited in apple fruit and has no bearing on sunburn development. Hence, factors that may affect convective heat loss, such as canopy temperature, may have a greater effect on sunburn development. However, this study did not establish the link between canopy temperature and fruit peel temperature. More research is therefore required on the factors that affect the fruit heat balance as influenced by plant water status.

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Appendix 1: Sunburn rating chart

