

**ACCLIMATION OF APPLE PEEL TO LIGHT AND TEMPERATURE AND THE
EFFECT THEREOF ON RED COLOUR DEVELOPMENT AND TOLERANCE TO
SUNBURN**

By

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DECLARATION

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To Family,

In recognition of your patience, love, support and constant reminder that “we may grow apart in different directions like branches on a tree yet our roots remain the same”.

SUMMARY

Sunburn is the biggest abiotic quality defect affecting apple orchards in South Africa. In blushed cultivars, inadequate red colour development at harvest is of similar importance as sunburn as quality defect. The presence of these two quality defects negatively affects profitability of South African apple orchards. This study was undertaken to assess the response of apple peels from trees on different rootstocks of differing vigour to photothermal stress. Rootstocks are purported to produce different microclimatic conditions to developing fruit depending on their vigour. The objective was to identify whether previous acclimation to light and temperature affected the sensitivity of ‘Rosy Glow’ (RG) and ‘Golden Delicious’ (GD) apple peel from a range of rootstocks, to damage (photosystem and visible peel damage) under induced natural photothermal stress and whether such acclimation affected the ability of ‘Rosy Glow’ apples to colour under different temperature conditions.

We found that damage to peel photosystems occurred at all exposure periods in both cultivars, with peels under one hour exposure showing general indications of progressive recovery over the five-day period. Duration of exposure to the stress condition, the recovery period, and canopy position were identified as the dominant influences on damage and recovery of RG photosystems with duration of exposure and recovery period being the dominant influences on GD photosystems. Likewise, duration of exposure, the length of the evaluation period together with canopy position were the dominant influences on visible peel damage observed on both RG and GD apples. However, rootstock plays a role in the visible peel damage observed on RG apples in 2016. Fruit from the different canopy positions acclimated differently which showed in their response to the photothermal stress. Slightly lower peel sensitivity occurred in fruit from trees on the rootstock G3007 and a higher sensitivity in M793.

To ascertain the effect of rootstock on the colouring potential of RG apples, fruit peel discs were subjected to six temperature treatments. The effect on red colour development of RG apples under lab conditions is rootstock related and not related to vigour. Although fruit colour development varied between different rootstocks under different temperatures, results indicate different optimum temperature ranges for different rootstocks in the red colour development of RG apples. Geneva rootstocks G222 and G3007 rootstocks showed the highest potential for good colour development following a cold front under warm late-season conditions on par with the current

industry standard M793. In our final experiment, the effect of fruit cooling (as a means of modifying fruit microclimate) on the red colour response of ‘Cripps’ Pink’ (CP) apples at harvest was evaluated. The cooling treatments applied showed different responses on change in hue of CP apples at harvest, but all cooling treatments were beneficial to red colour development. Late cooling treatment from mid-February to mid-March was more effective in decreasing hue of CP apples at the end of the trial.

OPSOMMING

Sonbrand is die grootste abiotiese kwaliteitsdefek wat appelboorde in Suid-Afrika raak. In blos kultivars is onvoldoende rooi kleurontwikkeling by oes van soortgelyke belang as sonbrand as kwaliteitsdefek. Die teenwoordigheid van hierdie twee kwaliteitsdefekte beïnvloed winsgewendheid van Suid-Afrikaanse appelboorde negatief. Hierdie studie is onderneem om die reaksie van appelskil van bome op verskillende onderstamme van verskillende groeikragtigheid na foto-termiese stres te assesser. Onderstamme lei tot verskillende mikroklimatiese toestande vir ontwikkelende vrugte, afhangende van hul groeikragtigheid. Die doel was om te identifiseer of vorige akklimasie tot lig en temperatuur die sensitiwiteit van 'Rosy Glow' (RG) en 'Golden Delicious' (GD) appelskil van 'n reeks onderstamme vir skade (fotosisteme en sigbare skilskade) beïnvloed, onder geïnduseerde natuurlike foto-termiese stres, en of sodanige akklimasie die kleurontwikkelingsvermoë van 'Rosy Glow' appels beïnvloed het onder verskillende temperatuurtoestande.

Ons het bevind dat skade aan skil fotosisteme by alle blootstellingstydperke in albei kultivars plaasgevind het, maar skille onder een uur se blootstelling het algemene aanduidings van progressiewe herstel oor die vyf dae periode getoon. Die duur van stresblootstelling, die herstelperiode, en die blaardakposisie is geïdentifiseer as die oorheersende invloede op skade en herstel van RG fotosisteme, terwyl die duur van blootstelling en herstelperiode die dominante invloede op GD fotosisteme was. Net so was die duur van stresblootstelling, die herstelperiode, en die blaardakposisie die oorheersende invloede op sigbare skilskade wat op beide RG- en GD-appels waargeneem is. Onderstamme speel egter 'n rol in die sigbare skil skade wat in 2016 op RG appels waargeneem is. Vrugte uit die verskillende blaardakposisies het verskillend geakklimatiseer, wat in hul reaksie op die foto-termiese stres getoon is. Effens laer skilsensitiwiteit het plaasgevind in vrugte van bome op die onderstam G3007 en 'n hoër sensitiwiteit in M793.

Om die effek van onderstam op die kleurpotensiaal van RG appels te bepaal, is vrugskilskywe aan ses temperatuurbehandelings onderworpe. Die effek op rooi kleurontwikkeling van RG-appels onder laboratoriumtoestande was verwant aan die onderstam maar nie aan groeikragtigheid nie. Alhoewel vrugkleurontwikkeling verskil het tussen verskillende onderstamme onder verskillende temperature, dui resultate op verskillende optimum temperatuurreekse vir verskillende

onderstamme in kleurontwikkeling van RG-appels. Geneva onderstamme G222 en G3007 het die hoogste potensiaal vir goeie kleurontwikkeling na 'n laatseisoen koue front getoon, soortgelyk aan die potensiaal van die huidige bedryfstandaard M793. In ons finale proef is die effek van vrugverkoeling (as 'n metode om vrugmikroklimaat te wysig) op rooi kleurontwikkeling van 'Cripps' Pink' (CP) appels by oes geëvalueer. Die verkoelingsbehandelings wat toegedien is het verskillende reaksies gewys op kleurverandering van CP appels by oes, maar al die behandelings was voordelig vir rooi kleurontwikkeling. Die laat verkoelingsbehandeling vanaf middel Februarie tot middel Maart was die effektiëfste vir kleurontwikkeling van CP appels aan die einde van die proef.

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1. GENERAL INTRODUCTION

1.1 Background

To increase the profitability of their business, apple growers need to maximise yield while producing fruit crops of high quality. It has therefore become imperative that farmers utilise every available resource and technology to help achieve this target. Even where yield is maximised, downgrading of apple fruit due to defects such as sunburn (Racskó and Schrader, 2012) and insufficient colour of blushed and bi-colour cultivars (Steyn et al., 2005) limits the profitability of apple production in South Africa (Gindaba and Wand, 2005; Wand et al., 2005). Intense or excess solar irradiation may induce sunburn directly (Schrader et al., 2001), or indirectly through raised fruit surface temperatures, which in combination lead to photothermal stress to the peel photosystems. On the other hand, poor red colour of blushed and bi-colour cultivars is attributed to insufficient anthocyanin accumulation during the ripening-associated peak in synthesis. This is attributed to several factors ranging from genetic to environmental (Vimolmangkang et al., 2014), with light and temperature being the most important environmental factors (Lancaster et al., 1994; Saure, 1990).

The South African apple-growing region is mostly in the Western Cape which has a Mediterranean-type climate. This region is characterised by clear skies, high incident solar radiation and high temperatures during summer, which result in high risk of sunburn on apples (Gindaba and Wand, 2005). The high temperatures also give rise to poor red colour development of blushed apples (Gouws and Steyn, 2014). Maturing fruit that are exposed to direct solar radiation may initiate biochemical and physiological adjustments (also termed acclimation) to the gradual seasonal increase in solar radiation and thus reduce susceptibility to photothermal stress and sunburn, compared to fruit developing in shaded or semi-shaded positions (Felicetti and Schrader, 2008). Temperature fluctuations during fruit development also affect the potential for red colour (anthocyanin) synthesis, with optimum temperatures shifting in accordance with the seasonal climatic conditions (Gouws and Steyn, 2014). These studies indicate that biochemical and physiological acclimation occurs in peel tissues dynamically according to climatic conditions experienced in the preceding weeks.

The exposure of developing apples to high solar radiation and temperature is highly dependent on tree size, which is strongly influenced by rootstock. Bigger trees on more vigorous rootstocks

develop larger canopies and more foliage (Racskó and Schrader, 2012) and provide more shade to a higher proportion of developing fruit. More dwarfing rootstocks give rise to trees with smaller canopies and less foliage, and a higher proportion of fruit are exposed to direct sunlight and higher peel temperatures. Such fruit are generally thought to suffer more from sunburn (Racskó and Schrader, 2012) although the literature presents differing opinions since these fruit may also be better acclimated.

More exposed canopies on dwarfing rootstocks may also influence the temperature optimum of red colour (anthocyanin) synthesis in apple peel, with this optimum likely adjusting to reflect the conditions experienced during the course of the season. It is also possible that cooling of fruit surfaces at different developmental times leads to the acclimation of anthocyanin biosynthetic pathways and differing ability to colour up during ripening. Thus, the use of a range of rootstocks to test the ability of apple peel to withstand photothermal stress and to develop red colour before harvest provides a useful model for research. In addition, this can provide valuable information to guide rootstock choices for the apple industry as a whole and for individual sites.

1.2 Research hypothesis, aim and objectives

For this research, we hypothesised that acclimation of apple peel to preceding fruit surface temperature conditions affects their sensitivity to high irradiance and temperature stress, as measured by peel damage and the ability to develop red colour.

The overall aim of this study was to better understand the role of acclimation of apple peel photosystems and red colour (anthocyanin) biosynthesis to irradiance and temperature, as influenced by rootstocks of differing vigour and evaporative cooling of fruit surfaces.

The specific objectives of the study were:

- To determine the influence of a range of dwarfing to vigorous rootstocks on acclimation of apple fruit peel photosystems to high irradiance and temperature and to quantify the resulting visible sunburn symptoms;
- To explore whether different rootstocks can influence the temperature optimum for red colour (anthocyanin) development in apple peels;

- To evaluate whether acclimation of apple peel to reduced peel temperatures during the mid to late developmental period impacts on their subsequent ability to develop red colour at harvest.

1.3 Thesis structure

Chapter 2 presents a review of literature on South African apple production, rootstocks used and under evaluation, and an overview of sunburn and red colour development in apples and the factors which can influence these. In Chapter 3 the sensitivity of ‘Rosy Glow’ and ‘Golden Delicious’ apple fruit peel photosystems to high irradiance as influenced by a range of dwarfing to vigorous rootstocks is presented. The change in maximum light use efficiency (F_v/F_m) and scored visible peel damage after exposure to high irradiance and temperature was analysed and used to interpret whether tree canopy structure (and thus previous exposure to light and heat) and/or inherent (direct) rootstock effects influence the development of photosystem damage and visible peel damage. In Chapter 4, the influence of rootstock on the temperature optimum or optimum range for red colour development in ‘Rosy Glow’ apple peels during the ripening associated peak is presented. Chapter 5 focuses on the use of evaporative cooling of fruit surfaces of ‘Cripps Pink’ to evaluate whether acclimation of the fruit peel to lowered temperatures at different stages of fruit development affected red colour development at harvest. Chapter 6 covers a general discussion and conclusion from the findings from the three trials.

1.4 References

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2. LITERATURE REVIEW

2.1 INTRODUCTION

2.1.1 Overview of the South African apple industry

The South African apple industry covers a total land area of about 23,625 hectares (HORTGRO, 2016) and employs about 24.6% of labour for deciduous fruit production in South Africa (GAIN, 2013). Apples constituted almost 34% (R3.4 billion) of the total gross income for deciduous fruit in the 2013/2014 growing season (DAFF, 2016). Produce prices are determined by the market forces of demand and supply which is determined by fruit quality in both the foreign and local market settings (DAFF, 2013).

The South African apple industry is export-oriented with approximately half of the apples produced being absorbed by the export market (HORTGRO, 2016). This is mostly due to favourable foreign exchange return rates coming from the importing countries. South African apples are available in many northern hemisphere countries during their winter and spring seasons when their own stocks are depleted. In recent times, the African continent has fast become a prime destination for South African apples, absorbing about 31% of exported apples in the 2015/2016 season (PPECB, 2016). Asia and the Far East are also big importers of South African apples.

In South Africa, apples are mostly grown in areas of the Western Cape region (HORTGRO, 2016). The region has a Mediterranean-type climate with mild winters and warm summers (Midgley et al., 2014), favourable for apple production. This region accounts for approximately 90% of total production and exportation of apples (GAIN, 2016). Ceres is the largest apple production area at about 6,960 hectares, with the EGVV region (Elgin, Grabouw, Villiersdorp and Vyeboom) being the second largest (HORTGRO, 2016).

‘Golden Delicious’ and ‘Granny Smith’ are the main apple cultivars produced in South Africa. Currently, ‘Golden Delicious’ accounts for about 25% of the total production area, ‘Granny Smith’ accounts for about 19%, whilst ‘Royal Gala’ and ‘Topred’ account for 16% and 13% of total apple cultivation area, respectively (HORTGRO, 2016). Between 2008 and 2013 there has been a steady increase in cultivation and output of ‘Cripps’ Pink’ and ‘Fuji’ apples. However, they still constitute only about 10% and 8% of total production area, respectively.

2.1.2. Abiotic production constraints

Cultivation of apples in South Africa can be very challenging. The Western Cape has complex soil patterns and varied landscapes due to the diversity of its geology and topography of this region (Midgley et al., 2014). Nutrient-poor, shallow and rocky mountain soils are the predominant medium for cultivation (Costa and Stassen, 2011). The apple growing regions of South Africa are located around latitude 33-34°S as compared to other producing areas such as Washington State (48°N), France (46°N) and Poland (52°N). This region experiences high incident solar irradiation during summer. Summers are dry with a monthly average maximum air temperature of about 29°C and 26°C in Ceres and EGVV, respectively. Warm winter temperatures in some production regions (especially EGVV) result in insufficient chilling required for even budbreak and good fruit set (Costa and Stassen, 2011). As temperatures continue to increase due to climate change, these factors will become more difficult to manage (Midgley et al., 2014). The review of Midgley et al. (2014) on climate change and agriculture in the Western Cape also predicts a likely reduction in the surface water availability and some potential shifts in the seasonality of rainfall, as well as warming, due to climate change. Another challenge is the prevalence of pests and diseases, such as woolly apple aphid, which thrive in this region. These conditions limit the choice of cultivars and rootstocks to those that perform well in poor soil and under warm conditions (Costa and Stassen, 2008). Pome crop export estimates for the 2015-2016 season decreased from 47.8 million cartons to 44.7 million cartons (PPECB, 2016) due to heat waves and drought conditions experienced in the early part of 2016 that affected fruit size, yield and quality. Such events are expected to become more problematic as a result of climate change (Midgley et al., 2016).

2.2. APPLE ROOTSTOCKS

2.2.1. Current rootstocks and rootstock requirements

There is no other above-ground crop where as much attention has been dedicated to rootstocks than for apples. The South African climate and soil conditions are a major limiting factor in the selection of suitable rootstocks for apple production. Poor sandy, gravelly and shallow soils coupled with marginal winter chilling, high temperatures and high irradiance during the growing season are major factors affecting the selection of rootstocks for the apple industry in South Africa (Costa and Stassen, 2008; 2011). In order to produce superior quality fruit, producers must adopt

new and improved technologies to maximise production under these challenging conditions. The introduction of new and improved rootstocks is one focus area within the industry.

The South African apple industry relied heavily on Northern Spy rootstock in the early part of the twentieth century for its resistance to woolly apple aphid (WAA) (De Wet, 1953). The widespread predatory wasp *Aphalinus mali*, however, reduced the impact of WAAs. The industry, therefore, reverted to the use of apple seedlings from various commercial cultivars (Costa and Stassen, 2008). In addition, the Northern Spy rootstock was difficult to propagate and resulted in poor fruit quality. The use of seedling rootstocks also came with poor uniformity of trees and trees with poor root development (Costa and Stassen, 2008). After many trials and evaluation in South Africa, the M793 rootstock was widely planted after its release to growers in 1957 (Van Zyl et al., 1974).

Over the years, efforts to select and provide rootstocks best suited to the South African apple producing regions led to the implementation of rootstock trials in various locations. Bergh et al. (1978) reported on trials involving 20 different rootstocks grafted to 'Golden Delicious' and 'Granny Smith', which were planted from 1967 to 1972, in the Grabouw and Langkloof regions. Some of the rootstocks tested included M26, M7, MM106, Northern Spy, M.2, MM105, MM106, MM101, MM104, MM109, MM113 and MM114. Costa (1998) reported on encouraging performances of M7 and MM106 in trials established in Ceres, Langkloof and Grabouw in 1994, which included M9, M7, MM106, MM109, MM111, M25, M793 and the Israeli Hassabi selections, using 'Royal Gala' as the scion.

Currently, the industry still relies heavily on certified, virus-free M793 for a wide range of soil potentials and climates (Costa and Stassen, 2011). The vigorous MM109 rootstock is also used on low potential soils in warmer areas, in replant situations and for weaker scion cultivars, whilst growers also use the semi-dwarfing M7 rootstock on high potential soils. Other rootstocks that are used include M25 and MM106 (but both are susceptible to *Phytophthora*) and the strongly dwarfing M26 and M9 (less than 1%) on high potential soils in colder climates (Frederik Voigt, personal communication, 2016).

When most apple industries across the globe were moving towards the use of more dwarfing rootstocks under intensive systems in the latter part of the 20th century, South African growers could not immediately follow suit. The new generation of rootstocks first needed to be tested for the climatic and soil conditions in the country, including considerations of susceptibility to heat

stress (and resulting sunburn and poor red colour), WAA, and various root (e.g. *Phytophthora*) and replant diseases (Costa and Stassen, 2011).

Trees in South African orchards are usually spaced at 4×1.5 to 2 m, trellised and trained to single leaders. This is because most apple rootstocks commercially available are semi-vigorous. The resulting trees are relatively large and therefore labour intensive, requiring large inputs to manage. The demand for new and improved apple rootstocks is therefore based on the requirement for high yield efficiency, high precocity, tolerance to replant conditions, resistance or tolerance to WAA, resistance or tolerance to *Phytophthora*, and adaptability to the local South African climate and soils (Costa and Stassen 2008). Importantly, there is a need for reduced vigour so that more intensive production systems (higher tree densities) can be implemented with resultant increased yield efficiency and improved fruit quality (Costa, 2011). Therefore, the use of smaller and more efficient trees will result in higher and earlier production, more efficiency in pest and disease management thereby reducing cost of production greatly whilst improving fruit quality. The primary purpose currently (amongst others) is to introduce rootstocks which increase yield and provide resistance to WAA under South African conditions (Frederik Voigt, personal communication, 2016). Rootstocks that meet the above requirements can be considered for wider propagation and distribution to the South African industry to extend the current range of options (Voigt and Stassen, 2014). The industry is also working on testing and introducing rootstocks which might possess characteristics to improve bud break of apples under South African climatic conditions (Frederik Voigt, personal communication, 2016).

2.2.2. New and improved rootstocks

The South African apple industry is aware of the limitations of the rootstocks currently available and therefore has been taking measures to identify better options becoming available globally from rootstock development programmes such as the Geneva programme in the USA (Costa and Stassen, 2008). This led to the planting of the first trial site (Villiersdorp area) of some “second-generation” Geneva (G) rootstocks in 2000 (Costa, 2011). Due to the success of the Geneva rootstocks compared to the industry standards in this first trial, a randomised trial to evaluate various Geneva rootstocks was planted in 2010 (Costa and Stassen, 2011) in the Witzenberg Valley, using ‘Rosy Glow’ as the scion. Since then, three more rootstock trials have been established across the apple producing region. The objective of these trials is to evaluate the

various new rootstocks against the industry standards and to select rootstocks that can best maximise the limited resources of the South African apple industry. A few of the Geneva rootstocks such as G222 and G778 have been commercialised in the last three years, but most of the rootstock trials are still under evaluation (Voigt, 2014). The semi-commercialised Geneva rootstocks G222 and G202 possess less vigour than the industry standard M793, with G778 showing more vigour than M793. The semi-commercialised G228 is in the same vigour class as M793.

Table 1. Vigour of Geneva® rootstocks under evaluation in South Africa in relation to the Merton-Malling range (Voigt, 2016).

Merton-Malling rootstocks	Geneva® rootstocks		
	Semi-commercialised	Currently under evaluation	Imported for future evaluation
M9 size		G41	G214
M26 size	G202 G222	G757 G969 G890	G213, G210
M7 size			
M793 size	G228		
MM109 size	G778		

2.2.3. Rootstock-scion interactions and rootstock effects on fruit quality

Rootstocks can alter the characteristics of the scion. Jensen et al. (2003) found that the scions grafted to M9 T337 showed higher expression of a number of photosynthesis-related and cell division-related genes, while scions grafted to M7 EMLA showed increased stress-related gene expression. The differentially expressed genes in the two graft combinations affected tree stature, stress tolerance, photosynthetic activity, fire blight resistance and other differences conferred by the two rootstocks. Further research has shown that apple rootstocks have the innate ability to modify the basic physiology of the tree by modulating the expression of genes in the scion (Jensen et al., 2010). Studies on Geneva rootstocks have shown them to possess the innate ability to increase the number of lateral shoots in the nursery stage of the tree and flatten the branch angles of grafted scions (Fazio and Robinson, 2008). Fallahi et al. (2002) and Amiri et al. (2014) reported on rootstock influence on apple tree precocity, tree size, mineral uptake, and ability to withstand adverse environmental conditions. In their work, Fallahi et al. (2002), found that ‘Fuji’ trees on

the rootstock M9 possess the smallest trunk cross-sectional area, highest yield efficiency and were more precocious than ‘Fuji’ trees on rootstocks M26 and M7.

There are various indications that rootstocks are able to directly modify the quality attributes of deciduous fruit. Generally, rootstocks can influence ripening, colour and shape of fruit of the scion (Autio et al., 1996). The M9 rootstocks with smaller tree canopies were more yield efficient compared to other rootstocks (Meheriuk et al., 1994). In subsequent research, Fallahi et al. (2014) found that rootstock effects on the size and weight of ‘Pacific Gala’ fruit were very significant. In their studies, although rootstock impact on fruit quality was inconsistent, fruit on some rootstocks were slightly less red than fruit from others. Roberts et al. (2008) found that the dwarfing quince and OHxF rootstocks possessed the intrinsic ability to enhance red colour in ‘Forelle’ pears compared to other clonal pear rootstocks.

Rootstocks may also indirectly affect quality attributes of the scion fruit. Such effects are known to be related to the canopy size and architecture of the tree, which modifies the environment of the developing fruit. In apples, fruit ripening is correlated with tree vigour, with fruit from more dwarfing rootstocks ripening earliest. Fallahi et al. (2002) attributed the better fruit quality and yield of the rootstock M7 compared to M9 and M26 to the indirect influence of rootstock. Castle (1995) presented an argument that the quality of apple fruit was determined largely by factors related to crop load and canopy management, whereas the quality of citrus fruit was closely related to rootstock effects on plant water relations. This was based on evidence from field trial results, measurements of sucrose transport, and reciprocal grafting studies. Al-Hinai and Roper (2004) have shown that fruit quality of ‘Gala’ apples was not directly linked to rootstock, but that fruit firmness varies between rootstocks, indicating an indirect effect of rootstock on fruit quality. Over the years, rootstock breeding programmes have seen a huge leap in success in producing rootstocks with improvements on the old seedling rootstocks. Modern rootstock breeding programs aim at producing rootstocks with traits that are correlated with high fruit quality for future orchards (Fazio, 2014).

2.3. SUNBURN OF APPLES

2.3.1. Symptoms and causes of sunburn in apples

Sunburn is damage to fruit surfaces caused by exposure to intense or excess solar irradiation at high peel temperature (Racskó and Schrader, 2012). It remains a serious disorder in many apple-growing areas around the world (Racskó and Schrader, 2012). Sunburn in apples has been classified into three distinct categories, namely sunburn browning, sunburn necrosis and photo-oxidative sunburn (Schrader et al., 2001; Felicetti and Schrader, 2008a). Sunburn browning, the most prevalent and costly (Felicetti and Schrader, 2009a), results in a yellow, bronze, or brown spot on the sun-exposed side of an apple and occurs when the fruit surface temperature reaches 46 to 49°C in the presence of sunlight. Sunburn necrosis results in a necrotic spot on the sun-exposed side of the fruit leading to thermal death of cells in the peel when temperatures reach $52 \pm 2^\circ\text{C}$ (Schrader et al., 2001). Photo-oxidative sunburn occurs when non-acclimated apple fruit previously under shade are suddenly exposed to full sunlight. The sudden exposure may cause the symptom (a white spot on the affected side) to appear within 24 hours (Felicetti and Schrader, 2008a). Visible radiation and the production of reactive oxygen species are the induction factors for the occurrence of photo-oxidative sunburn.

The green (chlorophyll pigments) and red colour (anthocyanin pigments) in the apple fruit peel decrease or disappear depending on the severity of sunburn (Felicetti and Schrader, 2009b; Makedredza et al., 2015). The appearance of symptoms and the extent of visible damage may be due in part to the physiochemical properties of the fruit peel (Wünsche and Lakso, 2000; Wünsche et al., 2004).

2.3.2. Occurrence and impacts of sunburn

On the tree, exposed fruit surfaces heat up significantly above air temperature, sometimes by as much as 12° to 14°C (Racskó and Schrader, 2012). The severity of the impact of sunburn depends on the irradiance level, temperature and the duration of the photothermal stress (Felicetti and Schrader, 2009a). Though temperature and high irradiance are primarily responsible for the occurrence of sunburn, other indirect factors such as relative humidity, air movement, and various cultural practices can facilitate its development (Racskó and Schrader, 2012). Sunburn is common to various fruits and vegetables but the specific causes and nature of its occurrence may vary from crop to crop. Racskó and Schrader in their review paper of 2012, found that sunburn in apples differs from the ultraviolet (UV) damage that occurs in peppers and squash at high altitudes. The

severity and appearance of sunburn symptoms in apples also vary from cultivar to cultivar under the same environmental conditions. ‘Granny Smith’ is more prone to sunburn than ‘Fuji’ and ‘Golden Delicious’ (Racskó et al., 2005). However, cultivar sensitivity also relates to the colour of fruit (i.e. presence or absence of anthocyanins) and time of maturity and ripening. Red cultivars are generally known to show less symptoms (Makedredza et al., 2015) whilst cultivars that ripen early can burn easily or not depending on whether the fruit surface temperature threshold required for sunburn is attained before harvest (Racskó and Schrader, 2012). Minimum threshold temperatures for sunburn induction differ between cultivars (Schrader et al., 2003). Sunburn is always worse on the north and north-west sides of the trees in the southern hemisphere and on the south and south-west sides in the northern hemisphere (van den Ende, 1999).

Sunburn remains the most important cull factor in orchards in many parts of the world (Brunner et al., 2003). In Washington State in the USA, sunburn accounts for up to 10% of losses in apples (Schrader et al., 2004). In apple production regions with warmer climates, such as Australia (Racskó and Schrader, 2012) and Spain (Carbó et al., 2005), losses in unprotected orchards can often be up to four times higher than those of Washington State. In South Africa, losses are generally 10-20% but can go as high as 30-50% in unprotected orchards (Gindaba and Wand, 2005; Wand et al., 2006). In the 2015/2016 season, sunburn was a major factor leading to a decrease in the expected export volume of South Africa apples (PPECB, 2016).

2.3.3. Physiological and biochemical mechanisms of sunburn development

Fruit are more prone to heat stress than leaves due to their very limited cooling capacity by transpiration (Woolf and Ferguson, 2000). High temperature combined with high light alters the balance between photo-oxidation and photo-protection in sun-exposed apple peel (Chen and Cheng, 2009). Once fruit surface temperatures reach a threshold, peel damage occurs because of the imbalance between the production of reactive oxygen species and the ability of the fruit peel to detoxify the reactive intermediates and repair the resulting damage (Schrader et al., 2001).

In sunburn necrosis, membrane integrity is mostly destroyed leading to denaturing of several proteins during the inception of thermal death (Schrader et al., 2001). Electrolyte leakage increases significantly in the peel of apples with sunburn necrosis. Damage may extend a few millimetres to several centimetres into the fruit flesh and can damage the apple epidermis so seriously that pathogens may gain entry through the affected area (Racskó et al., 2005). Sunburn browning,

however, is the predominant type of sunburn occurring in sun-exposed apples in the orchard (Racskó and Schrader, 2012) but does not cause as much serious structural damage in the cuticle or the epidermal and sub-epidermal tissues as in the case of sunburn necrosis. The initial expression of this physiological disorder is discolouration of the peel surface, which is associated with changes in its pigments (Yuri et al., 2010). The discolouration has been correlated to decreased concentrations of chlorophylls and anthocyanins and increased concentrations of carotenoids and quercetin glycosides in the peel (Felicetti and Schrader, 2008b; Felicetti and Schrader, 2009a; Felicetti and Schrader, 2009b). Besides the primary external damage caused by pre-harvest exposure to elevated solar irradiation, sunburn severely damages fruit finish and quality (Racskó and Schrader, 2012). Apples with sunburn also frequently develop other peel disorders such as lenticel marking, with some of these disorders appearing before or after harvest (Schrader et al., 2004).

In photo-oxidative sunburn, exposed fruit surfaces become photo-bleached and this eventually leads to necrotic symptoms. Tissues experience photo-oxidative stress due to the presence of excessive reactive oxygen species (ROS) (Felicetti and Schrader, 2008a; Yuri et al., 2010). Photo-bleaching may occur at fruit surface temperatures (FST) below 31°C (Felicetti and Schrader, 2008a). Literature suggests that the exposure to higher surface temperature under high light does not make sun-exposed peel more tolerant of heat stress than the shaded peel in apples (Chen et al., 2009). However, Ma and Cheng (2003) found that sun-exposed apple peel possesses a larger xanthophyll cycle pool size and a higher conversion state than shaded peel. They concluded that both the xanthophyll cycle and the ascorbate glutathione pathway in apple peel are acclimated to the prevailing light exposure within the tree canopy to meet the respective needs of dissipating excess light and detoxifying reactive oxygen species.

Although sunburn occurs on fruit exposed to full sunlight, not all fruit in such positions are sunburnt. Fruit size also affects the resistance to, and occurrence of sunburn (Barber and Sharpe, 1971). Smaller fruit have a higher resistance to both sunburn browning and sunburn necrosis than larger fruit because their surface area is not usually sufficient to absorb enough solar radiation to increase fruit surface temperature to the minimum threshold of these sunburn types (Barber and Sharpe, 1971). This indicates that individual fruit characteristics affect the incidence of sunburn (Racskó and Schrader, 2012). Internal quality of the side of the fruit with sunburn is different from

the side without sunburn (Wünsche et al., 2000). Portions of apples with sunburn generally tend to have lower concentrations of acids (titratable acidity) than areas without sunburn. However, there are higher soluble solid concentrations in severely sunburnt fruit due to the lower relative water concentration in the tissue of the sunburnt area (Schrader et al., 2009). Torres et al. (2013) found that water relations and osmoregulation greatly influence the external and internal quality of sun-injured fruit. Exposure to sunlight induced increased sorbitol and glucose accumulation in sun-exposed parts of the peel surface, apparently as a response to counter the effects of the abiotic stress.

2.3.4. Environmental factors influencing sunburn development

Racskó and Schrader (2012) agreed with Yuri et al. (2010) that, although other factors may influence sunburn occurrence, high temperature and exposure to intense sunlight remain the primary direct factors that determine whether sunburn damage occurs.

Many authors identified the interplay between ambient air temperature and fruit surface temperature as being directly related to the risk of sunburn in apple fruit (Racskó and Schrader, 2012; Schrader et al., 2001; van den Ende, 1999). The heat load on the apple fruit surface that is exposed to the sun is influenced by the direct radiant heat from the sun and advective heat from hot air surrounding the tree and circulating around the fruit (Evans, 2004). Although only the direct radiant heating on the fruit can increase fruit surface temperature to levels that can induce the occurrence of temperature dependent sunburn (sunburn browning and sunburn necrosis), the ambient temperature can facilitate or mitigate the rate at which fruit surface temperature increases (Racskó and Schrader, 2012). As postulated by Evans (2004): to cool the fruit surface, the sources of heat energy load that increase fruit surface temperature must be curtailed during the warmest part of the day using temperature ameliorating techniques such as evaporative cooling. Sunburn browning does not, however, occur in similar attached apples heated to the threshold fruit surface temperature for sunburn development when sunlight is excluded (Racskó and Schrader, 2012).

In the field, direct sunlight falling on fruit surfaces contains high energy ultraviolet (UV) radiation. Within the UV spectrum penetrating the atmosphere, UV-B photons (280–320 nm) possess the highest energy levels and have the potential to damage macromolecules, generate reactive oxidative species (ROS) and impair cellular processes (Racskó and Schrader, 2012). In addition to a threshold fruit surface temperature, UV-B radiation is often considered a requirement for the

induction of sunburn browning (Schrader et al., 2001). Hengari et al. (2014) found that apples have a stronger light reflection response to UV-B radiation at the juvenile stage than when more mature. UV-B stress induced photoinhibition to previously shaded apples but sun-exposed fruit were acclimatised to the UV-B stress induced. Research suggests that apple fruit exposed to sunlight from early development are protected against UV-B and are unlikely to develop sunburn in response to UV-B exposure (Hengari et al., 2014). Since sunburn necrosis can be induced experimentally in the dark, sunlight is not directly necessary for the induction process; rather, high fruit surface temperature is what causes thermal death of fruit peel (Schrader et al., 2003).

Several indirect factors can contribute to the severity of sunburn. Low relative humidity is considered an important indirect factor in enhancing the severity of sunburn symptoms (Schrader et al., 2008). Another important indirect factor is wind velocity. Greater wind velocity lowers fruit surface temperature by disturbing and dissipating temperature of the boundary layer around the fruit. One major factor that cannot be overlooked is geographical location. Sunburn can occur in all apple growing regions in the world, but the severity and extent of damage varies from region to region. In tropical regions with predominantly clear weather conditions, sunburn incidence in heat tolerant low chill cultivars can often exceed 20% of harvested fruit, whereas in some tropical highlands such as Ethiopia no excessive sunlight or heat stress is experienced that may induce sunburn damage due to the continuous cloud cover (Racskó and Schrader, 2012). Areas like South Africa that are characterised by clear summer skies with high light and temperatures have higher levels of sunburn damage to apples (Racskó and Schrader, 2012).

2.3.5. Orchard management practices influencing sunburn occurrence and control

Cultural practices play a significant role in the incidence and severity of sunburn (Racskó and Schrader, 2012). Makedredza et al. (2013) found that water stress in apple trees aggravates the occurrence of sunburn symptoms. Transpiration is one mechanism that plants use to regulate temperatures, and plant water status affects leaf and fruit transpiration, thereby playing an important role in heat dissipation mechanisms that influence fruit surface temperature (Gonda et al., 2006). Makedredza et al. (2013) indicated that when soil moisture and stem water potential decreased, fruit surface temperature increased and so did sunburn incidence. The severity increased with a decrease in irrigation level, leading to an increase in the occurrence of sunburn

necrosis. Therefore, reducing water stress in orchards may ultimately reduce the incidence of sunburn on apples (van den Ende, 1999).

Cultural practices such as summer pruning, when done properly, should not overexpose the fruit through too much leaf removal, particularly on the northern/western sides of the row (van den Ende, 1999). Branches can be prevented from bending over and exposing fruit that were previously in shade to the sun. When there are many fruit and fewer leaves, more fruit are likely to be exposed to the sun. Thinning of fruit to one or two fruit per bearing position maximises colour development and can reduce sunburn in red and blushed apple cultivars; high crop load is thought to make fruit more vulnerable to sunburn (Bergh et al., 1980; van den Ende, 1999). However, there are contradictory reports on how crop load affects the incidence of sunburn. Wilton (1994) found low crop load to reduce the risk of sunburn damage. However, in South Africa, fruit thinning was found to have no impact on the incidence of sunburn of ‘Cripps’ Pink’ apples at harvest (Fouché et al., 2009).

Since high temperatures and high irradiation are known to cause sunburn, measures towards reducing fruit surface temperatures would help to alleviate the occurrence of sunburn. Many orchard practices and technologies have been tested for their ability to achieve this purpose, some with considerable success (Racskó and Schrader, 2012). Evaporative cooling (EC) is very efficient in reducing the incidence of sunburn in orchards (Evans, 2004; Gindaba and Wand, 2005; 2007). However, EC cannot prevent all the types of sunburn, it is costly to set up and maintain, and it is not suitable in water scarce areas (Evans, 2004).

The use of particle film treatment (Surround[®]) has proven to be an effective alternative to EC in reducing solar injury and improving apple quality in high-risk apple cultivars (Glenn, 2009; Wand et al., 2006; Gindaba and Wand, 2005; 2007; Glenn et al., 2001). According to Wünsche et al. (2004), the beneficial temperature reduction by the white particle cover makes it a promising treatment for alleviating excessive heat stress and sunburn in apple trees grown in warmer and dry locations. However, for particle film treatment and other protectants to be effective as sunburn controls, they need to fully cover fruit exposed to sunlight, and be able to be completely washed off after harvest, and this may prove difficult and costly (Racskó and Schrader, 2012). In Australia, sunscreens (Surround[®] and Parasol[®]) have been found to reduce the severity of sunburn in pomegranate fruit (Weerakkody et al., 2010). However, more work is still needed to ascertain the

effects of sunscreen treatments on the internal quality of fruit. Chemical protectants are also being introduced to combat the sunburn problem. They are naturally occurring metabolites that are sprayed on trees to protect fruit from excessive temperature and sunlight (Racskó and Schrader, 2012). Tocopherols, abscisic acid (ABA) and anti-transpirants are some of the chemical protectants that are available for use (Yuri et al., 2010).

Shade netting over tree canopies reduces incident sunlight on the fruit surface and fruit surface temperature through the reduction of the transmission of direct solar irradiation through the net (Dussi et al., 2005). Even though shade netting has been found to reduce and limit the incidence of sunburn (Gindaba and Wand 2005; 2007), it also alters the physiological processes of the fruit and the tree due to the reduction in the levels of solar irradiation. For example, photosynthetic capacity of leaves appears to be down-regulated in trees under shade netting (Gindaba and Wand, 2007).

2.4. RED COLOUR OF APPLES

2.4.1. *Importance of red colour*

Anthocyanins impart various colour hues (varying from red to blue) in the fruit peel in which they accumulate (Macheix et al., 1990). Colour is a critical fruit quality factor used in marketing as it plays an essential part in the attractiveness of fruit to the consumer (Senthilkumar and Vijayakumar, 2014). Colour can be used to distinguish between cultivars and to judge maturity (Steyn, 2012). In apples, the red blush on the skin is an important part of the grading standards used during packing. It is also perceived to be an indicator of the quality of a fresh fruit since consumers identify red skin colouration with better taste and flavour (King and Cliff, 2002). Red colour pigments (anthocyanins) also serve as photoprotective light screens in the vegetative tissues in which they accumulate (Hoch et al., 2003; Li and Cheng, 2009; Ma and Cheng, 2003; Steyn et al., 2002).

The biggest impact of insufficient red colour development in red and blushed apples is the financial loss that orchards incur, since fruit are downgraded (Steyn, 2012). Sunburn together with insufficient red colour at harvest remain the biggest abiotic factors increasing culling of red and blushed apples in orchards in South Africa.

2.4.2. Red colour development in apples

Anthocyanins are responsible for red colouration of red and blushed apples (Senthilkumar and Vijayakumar, 2014). Anthocyanin synthesis depends on carbon products produced during photosynthesis and glucose metabolism. Anthocyanins are synthesised in the epidermal and hypodermal cells (Lancaster et al., 1994). According to Saure (1990), there are two peaks of anthocyanin formation in apples: the first occurs during cell division in the fruit whilst the second occurs during the ripening associated peak in biosynthesis in red cultivars. The visible apple peel colour is caused by visual blending of chlorophylls, carotenoids and the phenolic pigments (anthocyanin, flavonols and proanthocyanidins) located in the vacuole of peel tissue (Lancaster et al., 1994). The background green or yellow colour is due to the chlorophylls and carotenoids pigments.

The concentration of anthocyanins relative to that of chlorophyll and carotenoids pigment in the fruit peel results in the different shades of red colour seen on red and blushed apple cultivars. Variations that exist between the shade and pattern of reddening of blushed apples depend on the genotype, developmental and environmental factors (Lancaster and Dougall, 1992; Saure, 1990). Relationships between pigment composition and colour measurement are complex, and there is little evidence to ascertain the extent to which differences in pigment composition can be seen by the eye as colour variations (Knee, 1972).

2.4.3. Physiology and biochemistry of red colour development in apples

There is a marked *de novo* biosynthesis of anthocyanins leading to formation of red colour in apples at maturity (Senthilkumar and Vijayakumar, 2014). The biosynthesis of anthocyanins in fruit peel and other plant tissues is one of the most thoroughly studied plant secondary metabolic pathways (Gould et al., 2008). In apple peel, biosynthesis and accumulation of anthocyanin is genetically determined (Saure 1990; Dussi et al., 1995), although also influenced by climate, and may increase exponentially during the ripening of most blushed and red cultivars. The biosynthesis of anthocyanin begins with the conversion of phenylalanine to cinnamic acid by the enzyme phenylalanine ammonia lyase (PAL) (Lancaster and Dougall, 1992). Harborne (1988) details the subsequent reactions in which coumaroyl-CoA is produced from the initial cinnamic acid and to the production of the first flavonoid molecules of the flavonoid pathway. Through hydroxylation, methylation, acylation, glycosylation the flavonoid structures are modified to produce the unstable

leucoanthocyanidins and anthocyanidins (Harborne, 1988). Red colour development in apples and pears is regulated by the expression and activity of UDPGalactose (Ju et al., 1999). UDPG is responsible for stabilising the leucoanthocyanidins and anthocyanidins by glycosylation. The degree of anthocyanin accumulation in apple peel is highly dependent on the stage of fruit maturity and other developmental factors (Ritenour and Khemira, 2007).

Studies have shown that anthocyanins offer protection to various vegetative tissues (Hoch et al., 2003; Hughes et al., 2007) when factors such as excess light induce pigment bleaching and disrupt cellular metabolism. Smillie and Hetherington (1999) suggested that anthocyanins protect photosynthetic tissues by shading chloroplasts from blue-green light. Fruit are known for their higher sensitivity to light and temperature stress and anthocyanins have been found to reduce photobleaching and decrease photoinhibition in the fruit peel (Li et al., 2008; Steyn et al., 2009). According to Steyn (2009), anthocyanin accumulation in immature fruit and in response to low temperatures could be for a photoprotective function. In red and blushed apple cultivars, poor red colour at harvest is attributed to insufficient anthocyanin accumulation during the ripening-associated peak in synthesis.

2.4.4. Environmental factors affecting red colour development in apple peels

The influence of environmental conditions on the appearance and degree of red colour on fruit cannot be underestimated. Light and temperature are the most important environmental factors affecting the expression and development of red colour in apples (Saure, 1990). Together, light and temperature affect the regulatory complex that controls the anthocyanin biosynthesis in fruit (Lin-Wang et al., 2011).

Light

Light is a prerequisite for anthocyanin synthesis in apples (Saure, 1990) and shows a positive correlation to anthocyanin accumulation in pears (Steyn et al., 2005). It regulates the expression of genes in the anthocyanin biosynthetic pathway by inducing transcription factors (Vimolmangkang et al., 2014). Vimolmangkang et al. (2014) found that various light treatments modified the activities and expression of about 736 genes which played major roles in red colour pigmentation in apples.

Visible light stimulates and increases activities of the anthocyanin biosynthetic pathway (Ritenour and Khemira, 2007). hu Dong et al. (1995) found that the induction of anthocyanin biosynthesis

in ‘Royal Gala’ apple peel could be stimulated by white light, and that the addition of UV light greatly increased the formation and accumulation of anthocyanins. However, the extent of the influence of light is dependent on cultivar (Saure, 1990) and the quality of light (Dussi et al., 1995). UV-B with either white or red light works synergistically in inducing red colour synthesis, while blue and far-red light are less effective in inducing red colour synthesis in apples (Ritenour and Khemira, 2007). On the other hand, high irradiance is required for the induction of anthocyanin synthesis and accumulation in plant tissues (Steyn et al., 2002). Ritenour and Khemira (2007) found that apples require a certain minimum amount of light for anthocyanin production and this minimum is influenced by cultivar and stage of development. In one study, apples did not colour sufficiently when exposed to less than 40% of full sunlight (Ritenour and Khemira, 2007).

Temperature

Studies have shown that red colour development in all apple cultivars benefits from low temperatures (Lancaster et al., 1994; Saure, 1990; Steyn, 2012). Induction of anthocyanins during biosynthesis is triggered by low temperatures (Curry, 1997). Curry (1997) and Reay (1999) showed that anthocyanin synthesis in apples is induced at low temperatures of 2 and 4 °C, respectively, followed by irradiation at higher temperatures (25 and 20°C in their respective studies). Gouws and Steyn (2014) identified two different optimums 19-25 °C and 16-22 °C for colour development in two different seasons after induction at 4°C. Saure (1990), however, reports that induction occurs at temperatures of <12°C. Favourable effects on anthocyanin accumulation in apples are generally observed in the field when cool night temperatures are followed by warm sunny days (Jackson, 2003; Reay, 1999; Uota, 1952). While some researchers report that cool night temperatures of 2-5°C (Jackson, 2003) followed by warm sunny days are favourable for red colour development, others such as Gurnsey and Lawes (1999) noted that cool night temperatures only need to be below 18°C from a few weeks before harvest to enhance apple fruit colour. However, the benefits of cool night temperatures can be nullified by high day temperatures following the cool night (Ritenour and Khemira, 2007). It appears that the climatic conditions experienced during fruit development can affect the potential to synthesise anthocyanins (Gouws and Steyn, 2014; Reay and Lancaster, 2001). PAL is an important enzyme critical for the regulation of the flavonoid and anthocyanin biosynthesis (Jaakola, 2013). Lower temperatures up-regulate PAL activity, leading to increased anthocyanin levels; however, high temperatures reduce PAL

activity and anthocyanin accumulation by increasing the levels of a PAL-inactivating system (Faragher, 1983).

Accumulation of cyanidin and UDP-sugars, which are responsible for anthocyanin development in the apple peel, is hindered under high temperatures (Ban et al., 2009). This generally indicates that high temperatures are detrimental to anthocyanin development and accumulation. Li et al. (2012) found that low temperatures induce anthocyanin accumulation, whilst Lin-Wang et al. (2011) showed that anthocyanin concentration in maturing apples is reduced in warm climates. Lin-Wang et al. (2011) found that ‘Mondial Gala’ apples accumulated more anthocyanins under the cooler New Zealand climate than under the hot climate of Spain. Studies showed extensive anthocyanin development in ‘Cripps’ Pink’ apples when fruit were in cool but clear weather conditions following a cold front (Roberts, 2009). Low or mild day temperatures are important for anthocyanin synthesis, development and accumulation in the apple peel (Curry, 1997; Gouws and Steyn, 2014).

2.4.5. Orchard management practices influencing red colour development

In the orchard, all activities are intertwined, and tend to affect one another. Mineral nutrition, for instance, can affect pigmentation of plant tissues. A surplus in nitrogen fertilisation is associated with a reduction in the percentage of well-coloured fruit at harvest, and its deficiency is known to favour anthocyanin pigmentation in vegetative tissues (Steyn et al., 2002). Urea application also increases the chlorophyll and carotenoid concentrations in fruit peel (Reay and Lancaster, 2001) and reduces anthocyanin concentrations on the blush side of the fruit at maturity (Reay et al., 1998). Crop load can also affect peel pigment accumulation; higher crop loads are associated with a lower percentage of blushed fruit (Jakopič et al., 2013). Thus, fruit thinning can increase total red colour in fruit peel. Pruning of leaves and branches when done appropriately can aid light penetration through the tree canopy, thereby aiding light incidence on the less exposed fruit surfaces. This light exposure can enhance red colour formation of fruit (Jung and Choi, 2010).

Various orchard practices aimed at resolving other orchard problems may also enhance or reduce red colour in apple fruit. Sunburn is one such problem: Practices that are aimed at ameliorating the incidence and impact of sunburn can in turn influence the development of red colour in apples. The use of shade nets as a sunburn control measure reduces the amount of light that reaches the fruit surfaces. This can result in poor colour formation in red and blushed apple cultivars (Gindaba

and Wand, 2005; 2007). On the positive side, the use of EC systems (Racskó and Schrader, 2012) and the use of particle films (e.g. Surround[®]) on the fruit surface (Glenn, 2009) as sunburn control has in some trials enhanced the colour of red and blushed apple cultivars. The former method reduces fruit surface temperatures and the latter reduces the impact of excessive light on fruit surfaces.

Since fruit are more prone to heat stress and fruit surface temperatures can rise by about 10 – 15°C above air temperature, EC has become a chemical-free means to reduce heat stress and sunburn in orchards in many parts of the world (Evans, 1993). The use of EC systems to prevent damage and improve fruit quality has been studied by several researchers (Van den Dool, 2006; Evans, 2004; Iglesias et al., 2002; Parchomchuk and Meheriuk, 1996). EC reduces apple peel temperatures by more than 5 °C (Parchomchuk and Meheriuk, 1996; Wünsche et al., 2001; Gindaba and Wand, 2005; 2007). The benefits of EC have been compared to those of other temperature-ameliorating technologies, and it has been proven to be highly effective in modifying the tree microclimate (Gindaba and Wand, 2005) whilst reducing sunburn. In many locations, EC is the main method used to improve red colour and other fruit quality attributes (Parchomchuk and Meheriuk, 1996; Iglesias et al., 2000; 2005).

Under South African conditions, some of these technologies have not yielded similar results in terms of fruit colour development. Gindaba and Wand (2005; 2007) found no marked improvement in apple colour with the application of EC and particle film (Surround[®]) technologies when compared to the control treatment. They found that technologies that reduce irradiance reaching fruit were successful in reducing sunburn but were detrimental to colour development of blushed apples. Steyn (2003) also found that the benefits of EC on final red colour of ‘Rosemarie’ pears was lost under prolonged application.

2.5. ROLE OF ROOTSTOCK IN SUNBURN AND RED COLOUR DEVELOPMENT

As discussed earlier, rootstocks may confer or modify expression of traits and qualities of the scion (Jensen et al., 2003; Robinson et al., 2003; Autio, 1991). Rootstock also affects the appearance and severity of sunburn on the fruit (Racskó and Schrader, 2012). Generally, sunburn injury is more prevalent in plantings with smaller trees than in more vigorous plantings with bigger trees.

Trees on dwarfing rootstocks produce smaller trees with less foliage which exposes most fruit to direct solar radiation (Parchomchuk and Meheriuk, 1996, Middleton et al., 2002). Racskó et al. (2011) found that fruit from trees on seedling and semi-vigorous rootstocks were less sunburnt compared to the more dwarfing M9 rootstock in South African and Hungarian orchards. However, there are indications that exposed fruit on dwarfing rootstocks can acclimatise to light and temperature conditions which reduces their susceptibility to sunburn damage. In a trial in South Africa, the apples from trees on a range of Geneva rootstocks had less sunburn compared to the semi-vigorous industry standard M793 (Costa, 2011; Racskó et al., 2011). ‘Golden Delicious’ apples on Geneva rootstocks of widely differing vigour, canopy size, crop load and thus light exposure of the fruit (with the possible exception of G222) generally had less fruit sunburn than M793 and other non-Geneva rootstocks. This suggests that fruit on Geneva rootstocks may have acclimatised to the high light and temperature conditions, or that the rootstocks may have modulated the scion physiology in a way that resulted in the lower incidence of sunburn.

Rootstocks have been found to influence red colour development of fruit. Roberts et al. (2008), found that the more dwarfing clonal quince rootstock and the dwarfing OHxF pear rootstock enhanced red colour of ‘Forelle’ pears compared to other clonal pear rootstocks. The fruit used in the study were fully exposed to sunlight. Therefore, the effect of dwarfing rootstocks on red colour was not related to canopy size and shading effects. This supported the earlier results of Autio and Southwick (1993), who found that the dwarfing M9 rootstock produced more red fruit than the semi-vigorous rootstock M7. This indicates that this effect may be transmitted through the graft union, via altered pigment gene expression in the scion, rather than being attributable to differences in light interception or maturity resulting from different canopy structures.

2.6. CONCLUSION

The South African apple growing regions pose unique challenges to growers. The high temperature conditions experienced during summer increase the occurrence of sunburn and insufficient red colour of blushed apples. To maximise yield and profitability, producers are adopting the use of more dwarfing rootstocks. This gives rise to trees with smaller canopies and less foliage, which expose a greater proportion of developing apples to direct sunlight and high peel temperatures. Such exposure is expected to improve red colour development, but increase the proportion of fruit

on the tree prone to sunburn. However, findings from the literature and ongoing local field trials on the new Geneva rootstocks present differing opinions, since acclimatisation to high light and high temperature from early fruit development may decrease the susceptibility of fruit to sunburn at later stages of fruit development compared to fruit that were not acclimatised. In addition, the use of dwarfing rootstocks with more exposed canopies may influence the temperature optimum of red colour (anthocyanin) synthesis in apple peels during development, reflecting the conditions experienced during the course of the season. Thus, the use of a range of rootstocks to test the ability of apple peel to withstand photothermal stress and to develop red colour before harvest provides a useful model for research. In addition, this can provide valuable information to guide rootstock choices for the apple industry.

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3. PAPER 1: SENSITIVITY OF APPLE (*Malus domestica* Borkh.) FRUIT PEEL TO HIGH IRRADIANCE AND TEMPERATURE AS INFLUENCED BY A RANGE OF ROOTSTOCKS

ABSTRACT

Exposure of developing apple fruit to high irradiation can result in high sunburn incidence in warm production areas such as South Africa. Previous studies suggest that the peel of exposed fruit on more dwarfing rootstocks may acclimate to high irradiation and temperature. The aim of this study was to determine the innate sensitivity of peel photosystems of ‘Rosy Glow’ (RG) and ‘Golden Delicious’ (GD) apples on a range of current and new rootstocks to high ambient irradiance and temperature, and the manifestation of visible damage on the exposed peel. ‘Rosy Glow’ apples on eight rootstocks varying in vigour from dwarfing to vigorous (Cepiland, MM109/M9, G3007, G222, M7, M793, G778 and MM109) and on own roots at a rootstock trial at Paardekloof, Witzenberg valley, were used during the 2014-2015 and 2015-2016 seasons. ‘Golden Delicious’ apples on seven semi-dwarfing to vigorous rootstocks (Cepiland, G007, G222, M7, M793, G228 and G778) at a rootstock trial at Bo-Radyn, Villiersdorp, were used in the 2015-2016 season. M793 is the current industry standard. In 2015-2016, fruit were picked separately from inner (shaded) and outer (sun-exposed) canopy positions. Two sets of fruit, one set for the photothermal stress trial and the other for the peel damage trial, were subjected to five durations (1-5 hours) of exposure to ambient high irradiance and high temperature. For the photothermal stress trial, peel photosystem damage was assessed using chlorophyll fluorescence measurements (maximum light use efficiency of photosystem II - F_v/F_m). Visible peel damage was assessed immediately after each exposure duration using a “sunburn” score (chart). Sunburn assessment and F_v/F_m measurements were repeated over the course of the following 24, 48, 72 and 96 hours. Damage to peel photosystems as indicated by reduced F_v/F_m values, and visible peel damage, occurred after all exposure durations in both cultivars. Duration of exposure, the recovery period, and previous shading or exposure in the canopy were the dominant influences on photothermal stress and visible peel damage. Apples exposed to photothermal stress for one hour showed a general recovery over the five-day period, whereas apples exposed for two hours and longer did not recover fully. Visible damage increased progressively over the course of five days in both cultivars. This progression in ‘Rosy Glow’ apples was faster from day two onwards on fruit from sun-exposed positions, whilst

damage was more severe on day one in apples from shaded positions, followed by a slower progression until day five. There was no indication that rootstocks influenced the sensitivity of apple photosystems to photothermal stress. However, there was a three-way interaction for visible peel damage between rootstock, canopy exposure and post-stress period in ‘Rosy Glow’ apples. A slightly lower sensitivity to peel damage was identified in G3007 and a higher sensitivity in M793. G778 also showed sensitivity to visible peel damage, more so in fruit from shaded canopy positions. Results generally indicate that all the rootstocks confer similar sensitivity to high irradiance and temperature.

Keywords: Chlorophyll, Fluorescence, Photosystem, Rootstock, Sensitivity, Vigour,

1. INTRODUCTION

A large proportion of the South African apple production occurs in the Western Cape, a region with poor, shallow mountain soils (Costa and Stassen, 2008) and extreme variability over a relatively small scale due to the diversity of its geology and topography (Midgley et al., 2016). The region is also known for warm and dry summer weather with high incident solar irradiation, and insufficient winter chill accumulation to overcome dormancy in some production areas. These pose challenging conditions for the efficient production of apples of high quality (Costa and Stassen, 2008). A high cull percentage is the single biggest inefficiency in South African apple orchards and significantly decreases potential profitability. In production areas with high incident irradiation such as South Africa, sunburn is considered the single most significant abiotic quality defect, with producers sometimes estimating losses as high as 30-50% (Gindaba and Wand, 2005; Wand et al., 2006). It is also an important cause of cullage in some other apple-growing areas globally (Racskó and Schrader, 2012).

Sunburn is caused by intense or excess solar irradiation which may induce sunburn directly (Schrader et al., 2001; Wünsche et al., 2004; Yuri et al., 2004) or in combination with high fruit surface temperatures (Racskó and Schrader, 2012). Sunburn in apples is classified into three distinct categories: sunburn browning, sunburn necrosis and photo-oxidative sunburn (Schrader et al., 2001). In its mild form, sunburn is often seen as a golden-bronze patch on the sun-exposed surface of the fruit, whilst in its severe form a necrotic lesion is formed on the damaged fruit

surface (Felicetti and Schrader, 2008a; Schrader et al., 2001). Sunburn browning is considered to be the most prevalent and costly (Felicetti and Schrader, 2009). Fruit surface temperatures of 46 to 49°C in the presence of light (UV and visible) are known to induce sunburn browning. Sunburn necrosis, which leads to the thermal death of peel cells, occurs when peel surface temperatures reach $52 \pm 2^\circ\text{C}$ (Schrader et al., 2001; Schrader et al., 2003a).

Once fruit surface temperatures reach a threshold, peel damage occurs, beginning with damage to biological systems (photosystems). The combined action of UV-B radiation and heat stress causes oxidative stress and production of reactive oxygen species (ROS) in the epidermal and sub-epidermal tissues of fruit (Felicetti and Schrader, 2008a; Yuri et al., 2010). This damage occurs due to changes in the efficiency of photochemistry and heat dissipation. Depending on the level of stress and the sensitivity of the photosynthetic apparatus, photosystems can recover from the initial damage over time (Song et al., 2001; Wand et al., 2008). However, damage to the photosystems often manifests as fruit peel browning (Racskó and Schrader, 2012; Song et al., 2001; Wand et al., 2008). When confronted with photothermal stress, plant tissues employ mechanisms such as acclimation to protect the photosystems. Acclimation to high photosynthetic photon flux (PPF) induces some tolerance against subsequent damage to photosystems by photothermal stress (Racskó and Schrader, 2012). Photosystems of apples previously exposed to high PPF are known to be better acclimated and less prone to damage than less exposed or shaded fruit (Awad et al., 2001; Ritenour et al., 2001; Merzlyak et al., 2002; Ma and Cheng, 2003).

The exposure of developing apples to high solar irradiation and temperature is dependent on tree size and structure, which are strongly influenced by rootstock. Bigger trees on more vigorous rootstocks develop larger canopies and more foliage (Costa and Stassen, 2008; 2011) and provide more shade to a higher proportion of developing fruit (Parchomchuk and Meheriuk, 1996; Racskó et al., 2005a). More dwarfing rootstocks give rise to trees with smaller canopies and less foliage, and a higher proportion of fruit are exposed to direct sunlight and higher peel temperatures. Such fruit are generally more prone to developing sunburn (Racskó et al., 2005b, 2009; Wünsche et al., 2004). However, they may also be better acclimated to the prevailing conditions compared to fruit from more shaded canopy positions in larger canopies (Racskó and Schrader, 2012). Felicetti and Schrader (2008b) indicated that maturing fruit that are exposed to gradual seasonal increase in solar irradiation may initiate biochemical and physiological adjustments (also termed acclimation)

and thus reduce susceptibility to photothermal stress and sunburn, compared to fruit developing in shaded or semi-shaded positions. However, for fruit to acclimatise to elevated levels of solar irradiation and temperature, these factors should be high but remain sub-lethal to the fruit peel (Racskó and Schrader, 2012).

There has been demand for new rootstocks in South African orchard systems, specifically to allow for the wider introduction of high density planting and resultant increased yield efficiency and improved fruit quality, whilst accommodating the environmental challenges (Costa and Stassen, 2011; Voigt and Stassen, 2014). The challenging edaphic and climatic conditions have restricted the number of rootstocks that can be used for commercial production, and the introduction of more dwarfing rootstocks is proceeding more slowly than elsewhere (Voigt and Stassen, 2014). However, since the introduction of the new rootstocks from the Geneva range, preliminary results indicate that they are significantly more yield efficient and precocious than the standard rootstock M793 (Voigt, 2014). While some Geneva rootstocks have been commercialised in the last three years, most of the rootstocks are still under evaluation (Voigt, 2014). In a study involving 11 rootstocks, Racskó et al. (2011) found that ‘Golden Delicious’ apples on the dwarfing M9 rootstock, the vigorous Northern spy rootstock and the semi-vigorous M793 rootstock developed more sunburn than apples on other rootstocks evaluated under South African conditions. It is therefore imperative that the sensitivity of the new rootstocks be evaluated against current industry standards in South Africa, to provide valuable information to guide rootstock choices for the apple industry and for individual sites with high sunburn incidences.

The aim of this study was to determine the sensitivity of peel photosystems of ‘Rosy Glow’ and ‘Golden Delicious’ apples on a range of dwarfing to vigorous rootstocks to photothermal stress (exposure to high ambient irradiance and temperature), and the manifestation of visible damage (“sunburn”) symptoms on the exposed peel.

2. MATERIALS AND METHODS

2.1. Plant material

The trials were conducted on the farms Paardekloof in the Witzenberg Valley (33°15'40"S 19°15'55"E) and Bo-Radyn in the Villiersdorp area (33°59'S, 19°18'E). The soils in these two

areas are both of a sandy-loam type high in luvisols and cambisols. Both farms are located in the Western Cape province of South Africa, which has a Mediterranean-type climate.

At the Paardekloof site, an apple industry rootstock evaluation trial was established in 2010 using 'Rosy Glow' (RG) budded onto various new dwarfing, semi-vigorous and vigorous apple rootstocks from the Geneva range as well as M793 (the industry standard), M7 and MM109. The trees were planted at two sites; 1) a more "dwarfing" planting consisting of four blocks of ten rootstocks of varying vigour from dwarfing to semi-vigorous; and 2) a more "vigorous" planting of eight rootstocks ranging from semi-vigorous to very vigorous. The two sites were managed separately and slightly differently according to the different vigour ranges; for example, tree spacing at the more dwarfing planting was 1.25 m, whereas at the more vigorous planting it was 1.5 m. At both sites 'Royal Gala' was used as a pollinator. The rootstocks Cepiland, G3007, 'Rosy Glow' own roots (originally incorrectly identified as G6210), Lancep, MM106, MM109 with an M9 interstem (hereinafter referred to as MM109/M9), RN29 and M7 were included at the more dwarfing planting, whilst the rootstocks G934, G228, G778, M25, MM109 and Maruba were included at the more vigorous planting. G222 and M793 were also included at both sites as internal controls. The trial was laid out in a randomised complete block design consisting of five (more dwarfing planting) and four (more vigorous planting) blocks with three trees in each treatment plot. In some treatment plots, only one or two trees were available due to the prior identification of 'Rosy Glow' scion rooting and nursery mistakes.

At the Bo-Radyn site, an apple industry rootstock evaluation trial was established in 2000 using 'Golden Delicious' (GD) budded onto various new semi-vigorous and vigorous rootstocks from the Geneva range (G007, G189, G222, G228, G239, G253, G707, G778 and G934), as well as Cepiland, M9, M7 and M793. 'Royal Gala' and 'Granny Smith' served as pollinators. The rootstocks were randomised in three blocks with three trees per treatment plot. Both Paardekloof and Bo-Radyn orchards were managed according to standard commercial practices.

2.2. Ecophysiological measurements

Ecophysiological measurements were conducted on trees *in situ* on 5 March 2015, 8 January 2016 and 10 March 2016 at both the more dwarfing and more vigorous rootstock sites at Paardekloof. Leaf and fruit surface temperatures were measured using an infra-red thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA). All measurements (leaf and fruit surface temperature, leaf

stomatal conductance and fruit peel colour) were conducted between 09:00 and 12:00 on the eastern side of the planting row. Measurements were taken on leaves and fruit fully exposed to direct sunlight. Stomatal conductance of shoot leaves was measured using a porometer (AP4, Delta-T Devices, Cambridge, UK). Fruit peel colour (hue angle) was measured on the equator of the blush side of sun-exposed fruit using a chromameter (NR-3000; Nippon Denshoku, Tokyo, Japan). Measurements were conducted on one leaf and one fruit at a height of 1.5 m on three trees per treatment plot.

2.3. Photothermal stress and peel damage in ‘Rosy Glow’ (RG)

2.3.1 General materials and treatments

‘Rosy Glow’ apples from seven selected rootstocks at the more dwarfing site (G3007, G222, ‘Rosy Glow’ own roots, Cepiland, M7, MM109/M9, M793) and two rootstocks at the more vigorous site (G778, MM109) were used for Trials 1 and 2. Fruit from the nine rootstocks were harvested separately from four blocks, transported from the trial site to the Department of Horticultural Science, Stellenbosch, where they were stored in a cold room (-0.5 °C) for one night. The following morning, the apples were kept at room temperature (ca. 25 °C) for two hours after removal from the cold room, before being exposed to high ambient irradiance and air temperature on a warm and clear day. Whole fruit were laid out on trays with portions of previously exposed fruit surface free from blemish placed face up, and trays were transferred to an outside open area on the premises of the Department of Horticultural Science, Stellenbosch. Photosynthetic photon flux (PPF) was measured using a light meter (LI-189, LI-COR Inc., Lincoln, Nebraska, USA) and air temperature was measured with a thermometer.

2.3.2. Trial 1: Photothermal stress

2015: On 24 March 2015, 20 sun-exposed apples free from blemish were randomly collected from each rootstock (four blocks, five exposure periods, single fruit replicate), giving a total of 180 apples. They were stored overnight at -0.5°C at the Department of Horticultural Science, Stellenbosch. The rootstocks used were: MM109/M9, RN29, Cepiland, G222, ‘Rosy Glow’ own roots, G3007, M793, G778 and MM109. Fruit were placed out in the sun on the premises on the next warm and clear day (25 March 2015) with a PPF of around 2100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from 11:00 to

15:30 (but decreasing to around $1700 \mu\text{mol m}^{-2} \text{s}^{-1}$ after 15:30) and ambient air temperature of $24.0\text{-}26.6^\circ\text{C}$ (Appendix A, Figure 1, Table 1).

Fruit were exposed for five different exposure periods (1, 2, 3, 4 and 5 hours) starting at 11:00 and ending at 16:00. A single fruit was used for each exposure period per rootstock and per block. After each exposure period (12:00, 13:00, 14:00, 15:00 and 16:00), the temperature of exposed fruit surface (which were marked with a circle on the equator of the fruit) was measured using a hand held infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA). Following the end of each exposure period the fruit were taken into the laboratory and covered with black cloth for a dark adaption period of 30 minutes. Fruit peel chlorophyll (*a*) fluorescence within the marked circle was measured with a FSM 1 fluorimeter (Fluorescence Monitoring System 1, Hansatech, Norfolk, UK) and the following measurements recorded: maximum (F_m) and minimum (F_o) fluorescence and maximum light use efficiency $F_v/F_m = (F_m - F_o)/F_m$. Maximum light use efficiency (F_v/F_m) was first measured on dark adapted and undamaged apple peels under laboratory conditions before fruit were exposed to the photothermal stress. Measurements were repeated after 24, 48, 72 and 96 hours in the laboratory to assess potential recovery of the photosystem. The value $F_v/F_m > 0.7$ is used in this paper to indicate a high level of recovery of stressed photosystems.

2016: Methods described above for 2015 were repeated with the following change: a total of 360 ‘Rosy Glow’ apples were collected on 13 March 2016 (40 fruit per rootstock) with 20 fruit each collected from 2 different canopy positions (sun-exposed and shaded positions). The previously exposed side of whole fruit from both shaded and sun-exposed canopy positions, were placed out in the sun on a warm and clear day on 14 March with a PPF of around $2100 \mu\text{mol m}^{-2} \text{s}^{-1}$ from 11:00 to 15:30 (but decreasing to around $1750 \mu\text{mol m}^{-2} \text{s}^{-1}$ after 15:30) and ambient air temperature of $25.0\text{-}29.5^\circ\text{C}$ (Appendix A, Figure 1, Table 2).

2.3.3. Trial 2: Visible peel damage (“sunburn”)

2015: Methods described above for Trial 1 in 2015 were repeated on the same days with the following change: The middle of the previously exposed side of each fruit surface, placed face up, was covered with a black sticker of 19 mm diameter (Self-adhesive labels, Pyrotec packmark, Cape Town, South Africa) to increase the fruit surface temperature underneath the sticker, before the fruit were laid out in the sun. After 1, 2, 3, 4 and 5-hour exposure periods, black stickers were

removed and temperature of the covered area was measured using a hand held infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA). Visible peel damage (“sunburn”, acknowledging that the symptoms in picked fruit can differ from those on-tree) was assessed using the Schrader and McFerson system for ‘Fuji’ apples (Schrader et al., 2003b). The system classifies sunburn into six categories from 0 (no sunburn) to 5 (sunburn necrosis) (Appendix A, Figure 2). Sunburn development post-treatment was repeatedly assessed on the same fruit after 24, 48, 72 and 96 hours in the laboratory.

2016: Methods described above for Trial 2 in 2015 were repeated with the following change: A total of 360 ‘Rosy Glow’ apples was collected on 13 March 2016 (40 fruit per rootstock) with 20 fruit each collected from sun-exposed and shaded canopy positions. The previously exposed side of whole fruit was placed out in the sun on 14 March as described above.

2.4. Photothermal stress and peel damage (“sunburn”) in ‘Golden Delicious’ (GD)

2.4.1 Materials and treatments

‘Golden Delicious’ apples from two dwarfing rootstocks (G007 and Cepiland), two semi-vigorous rootstocks (G222 and M7) and three vigorous rootstocks (M793, G228 and G778) were used for Trials 1 and 2. Fruit from the seven rootstocks were harvested separately on 3 February 2016 from three blocks. All other methods were as for ‘Rosy Glow’ in 2016. The photothermal stress trial (Trial 1) and the sunburn trial (Trial 2) were conducted on 4 February 2016 under PPF of around $2200 \mu\text{mol m}^{-2} \text{s}^{-1}$ from 11:00 to 15:30 (but decreasing to around $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ after 15:30) and ambient air temperature of 28.4-31.4°C (Appendix A, Figure 1, Table 1). A modified version (Appendix A, Figure 3) of the Schrader and McFerson system was used to score damage on ‘Golden Delicious’ apples.

2.5. Peel phenolic and pigment analysis

On 24 March 2015, five sun-exposed ‘Rosy Glow’ apples free from blemish were randomly collected from the eastern side of the row on each of the nine rootstocks from four blocks, giving a total of 180 apples. Fruit were stored at -0.5°C in the laboratory at the Department of Horticultural Science, Stellenbosch. On 25 March 2015, apple peels were thinly removed around the middle (equator) of the entire circumference of the fruit and any remaining cortex tissues

scraped off, after which the peel was freeze-dried and stored at -80°C . The peel was later milled into frozen fine powder using liquid nitrogen and used for pigment analysis.

Total phenolics were extracted from 100 mg frozen milled peel samples in 80% ethanol using Folin-Ciocalteu's phenol reagent, and a standard curve created with gallic acid (Slinkard and Singleton, 1977). The total concentration was determined by measuring absorbance at 750 nm with a spectrophotometer (UV-visible light spectrophotometer, Cary 50Bio, Varian Ltd, Walton-on-Thyme, London, UK), using the coefficients from the standard curve.

Anthocyanin was extracted from 2 g of milled peel samples in methanol (with 1% $3\text{ mol}\cdot\text{l}^{-1}$ HCl) and kept in the dark at 4°C . The solution was stirred for one hour and the extract was centrifuged at 10 000 rpm for 10 min at 4°C . The total anthocyanin concentration was determined by measuring absorbance at 520 nm with a spectrophotometer (Cary 50Bio, Varian Ltd, Walton-on-Thyme, London, UK). The anthocyanin absorbance at 520 nm was corrected for the presence of chlorophyll by subtracting absorbance at 653 nm [$\text{Abs}_{520\text{nm}} - (0.24 \times \text{Abs}_{653\text{nm}})$] (Murray and Hackett, 1991).

Chlorophyll was extracted from 0.5 g milled peel samples in 3 ml acetone at 4°C by stirring with a magnetic stirrer for 24 hours. The resulting extract was centrifuged at 10 000 rpm for 15 min, and the supernatant filtered through a $0.45\ \mu\text{m}$ filter. Chlorophyll concentration was determined by measuring absorbance at 470, 645 and 662 nm with a spectrophotometer (Cary 50Bio, Varian Ltd, Walton-on-Thyme, London, UK). The concentration of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid concentrations were determined according to Lichtenthaler (1987), with 100% acetone as the blank.

2.6. Fruit maturity assessment

'Rosy Glow'

Fifty fruit were harvested from three trees per rootstock planting plot, on 9 April 2015, seven days before commercial harvest. Of these, 25 fruit each were harvested from the western and eastern sides of the rows. On 5 April 2016, a day before commercial harvest, 30 fruit (15 fruit each from the western and eastern sides of the rows) were harvested from three trees per rootstock plot. Fruit were stored overnight in the fruit maturity indexing and quality assessment laboratory of the Department of Horticultural Science, Stellenbosch University, and assessed the following day.

During both seasons, percentage foreground red colour intensity and ground colour were assessed using colour intensity and ground colour charts (Unifruco Research Services, Bellville). Sunburn incidence and severity was assessed using the Schrader and McFerson system for 'Fuji' apples (Appendix A, Figure 2). Percentage starch conversion was estimated using the iodine test with a starch conversion chart (Unifruco Research Services, Bellville). A composite juice sample was prepared from 20 pooled fruit by cutting a slice on both sides of each fruit from both eastern and western sides of the row and blending the pieces in a liquidizer (AEG Electrolux, Type JE-107 no. 91100085/ PNC 950075206, P.R.C). The juice was used in determining total soluble solids (TSS (%)) and total malic acid equivalent acidity (TA). TSS was measured using a calibrated hand-held refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan). TA was measured using an automated titrator (Tritino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland) by titrating 5 g of juice with 0.1 M NaOH to a pH of 8.2. In the 2016 season, fruit firmness was determined on opposite equatorial cheeks by means of a penetrometer (Fruit texture Analyzer, Guss Instruments, Strand, South Africa) with an 11-mm plunger.

'Golden Delicious'

Twenty fruit were harvested from each of the seven rootstocks per block on 3 February 2016, 14 days prior to commercial harvest. The same methods described for 'Rosy Glow' were used to measure the following parameters: ground colour, firmness, starch conversion, TSS and TA.

2.7. Statistical analysis

2.7.1. Ecophysiological, peel phenolic and pigment concentration and maturity index data

Ecophysiological data and peel phenolic and pigment concentration data were subjected to a two-way ANOVA using a XLSTAT statistical package (Addinsoft 1995-2016, Version 2016.03.30882).

Maturity index data was subjected to a two-way ANOVA with rootstock and side (east or west) as treatments using XLSTAT statistical package (Addinsoft 1995-2016, Version 2016.03.30882).

2.7.2. Photothermal stress and peel damage ("Sunburn")

2015: Fruit surface temperature data were subjected to a 2-factor analysis of variance (ANOVA) with exposure (hours of stress duration) and rootstock as factors. Both chlorophyll fluorescence

and sunburn data were subjected to a 3-factor repeated measure ANOVA with exposure, rootstock and day (repeated factor) as factors using STATISTICA (StatSoft Inc. version 13.1).

2016: Fruit surface temperature data, chlorophyll fluorescence and sunburn data were analysed as in 2015 with canopy position (sun-exposed and shaded canopy positions) as an additional factor. All means were separated using LSD of 5% but were presented only when the F-statistic was significant at 0.05.

3. RESULTS

3.1. Ecophysiological measurements

In 2015 there were no significant differences between the different rootstocks for all measured parameters at the more dwarfing rootstock site (Table 1). Similarly, there were no significant differences between the various rootstocks at the more vigorous site except hue angle (Table 1). Fruit from rootstocks M25 and G228 had significantly lower hue angles (redder colour) than fruit from rootstocks G222 and Maruba (Table 1). Fruit from rootstock G778 and G934 also recorded significantly lower hue angles than those recorded from the Maruba rootstock.

There were no significant differences between the various rootstocks at both the dwarfing and more vigorous rootstock site in January and March 2016 for all parameters measured (Table 2).

3.2. Fruit surface temperatures after stress treatments

2015:

No significant interaction between rootstock and exposure hours for fruit surface temperature occurred in trials 1 and 2 in ‘Rosy Glow’ in 2015. Therefore, main effects are presented (Table 3).

Trial 1: The highest fruit surface temperatures (FST) were recorded following 1, 2 and 3 hours of exposure to the stress conditions with a gradual decrease after 4 hours of exposure and a stronger decrease after 5-hour exposure (Table 3). The decrease in FST at 5-hour exposure can be ascribed to the decrease in ambient air temperatures and PPF during the fourth and fifth hours of exposure (Appendix A, Table 1 and Figure 1). Fruit from the rootstock Cepiland recorded higher surface temperatures in trial 1 compared to all other rootstocks except M7 (Table 3). Temperatures recorded on fruit from rootstock M7 were also significantly higher than those recorded on fruit from M793, Rosy Glow own roots and MM109.

Trial 2: Fruit surface temperatures increased from 1 to 3 hours of exposure to the stress condition before decreasing to the least recorded FST at 5-hour exposure. This trend was observed for both covered and uncovered fruit surfaces. Average FST of covered fruit surfaces was about 3.1°C higher than average temperatures measured on uncovered fruit surfaces. Lower FST was recorded on uncovered fruit surfaces of rootstock Cepiland which was significantly lower than FST on all rootstocks except fruit from rootstocks M793 and MM109 (Table 3). The FST recorded on both M793 and MM109 was also significantly lower than that recorded on all other rootstocks except rootstock G222. FST for covered fruit surfaces was significantly lower on fruit from rootstock Cepiland than fruit from all other rootstocks except rootstock MM109 and M793. FST of fruit from MM109 was also lower than all other rootstocks except MM109/M9, M793 and G222 (Table 3).

2016:

There was no significant interaction between rootstock, exposure hours and canopy position for recorded temperatures for both ‘Rosy Glow’ and ‘Golden Delicious’ fruit in trials 1 and 2 (Figure 1 & 2). Significant interaction between exposure hours and canopy position is therefore discussed for both trials. No differences were found between rootstocks.

Rosy Glow’:

Trial 1: FST increased steadily from 1- to 4-hour exposure before decreasing at 5-hour exposure period for both sun-exposed and shaded fruit (Figure 1a). The two canopy positions reacted in the same pattern for the first 4 exposure periods with significantly higher temperatures recorded on shaded apples than sun-exposed apples. The response turned around at the 5-hour exposure period, showing higher FST on sun-exposed than on shaded fruit (Figure 1a).

Trial 2: There was no clear pattern of temperature differences between sun-exposed and shaded fruit (both covered and uncovered surfaces). Highest FST was measured at 4-hour exposure period on both covered and uncovered fruit surfaces of shaded apples (Figure 2a).

‘Golden Delicious’

Trial 1: Sun-exposed fruit experienced a gradual increase in FST from one hour exposure before peaking above 50°C at 3-hour exposure. FST recorded on sun-exposed fruit was higher than shaded at 2- and 3-hour exposures and lower than shaded fruit at 1-hour exposure (Figure 1b).

Trial 2: FST increase was progressive from 1- to 3-hour exposure for covered surface of both sun-exposed and shaded fruit before decreasing at 4- and 5-hour exposure periods (Figure 2b). There was generally higher FST on covered surfaces of shaded fruit than sun-exposed fruit at all exposures except the 5-hour exposure. There was however no clear pattern for FST recorded on uncovered fruit (Figure 2b).

3.3. Photosystem damage and recovery and development of sunburn damage

'Rosy Glow'

2015

Trial 1: There were no significant differences in damage to photosystems attributable to the different rootstocks (data not presented). However, there was significant interaction between the duration of exposure to high light and temperature ("Exposure hours"), and the subsequent change (recovery) in damage to the photosystem (F_v/F_m) over the next four days ("Days") (Figure 3).

Damage to the photosystems occurred at all exposures (Figure 3). However, a significantly higher F_v/F_m value was recorded at 5-hour exposure as compared to 3- and 4-hour exposures and 1- and 2-hour exposures on day 1 (Figure 3). A general recovery from the initial photodamage to the apple peel was observed over the subsequent days with peel damaged by 1-hour exposure almost fully recovering from the damage ($F_v/F_m > 700$). The initial rate of recovery was greater in fruit exposed for 1 and 2 hours but similar for 3-, 4- and 5-hour-exposure. Recovery of 2-hour exposed apples peaked just a little below the F_v/F_m value of 0.7. Apples exposed to 3, 4 and 5 hours did not fully recover from the initial damage to the photosystem; however, there was little difference between final readings of 4- and 5-hour exposure duration periods. Anomalous data observed on day 3 may be possibly due to accidental exposure of fruit to light in the laboratory; fruit were moved across the laboratory on this day.

Trial 2: No rootstock related factors accounted for the visible peel damage (sunburn) observed, for both the covered and uncovered surfaces of 'Rosy Glow' fruit (data not presented) (Figure 4).

Peel damage (sunburn) of covered fruit surfaces on day 1 increased with increasing exposure to the photothermal stress (Figure 4a). Peel damage on 5-hour exposed fruit was significantly different from all other treatments on day 1, but not different to 3- and 4-hour exposed fruit on day 5. 5-hour exposed fruit showed 100% damage (mostly cooked and a few necrotic symptoms of

fruit peels) immediately after removal from the stress condition. Most 3- and 4-hour exposed fruit showed variations in fruit peel browning with a few showing symptoms of cooked fruit peel. However, by day 5, damage recorded on most fruit with 2-, 3- and 4-hour exposures was similar to that recorded on fruit with 5-hour exposure. It is important to note that, due to identification of bruises and sunburn symptoms on some of the fruit pretreatment, previously shaded sides of some sun-exposed apples were used in the same trial.

There was progressively more visible peel damage with increasing duration of exposure for uncovered apple surface (Figure 4b). Peel browning was mostly observed on 5-hour exposed apples and a few 2- and 3-hour exposed apples after removal from the stress condition. 5-hour exposed apples showed immediate and highest levels of damage (browning score of 3) which continued to increase until the fifth day. Final damage levels were similar for 4- and 5-hour exposures, slightly lower for 2- and 3-hour exposures, and significantly lower for 1-hour exposure for uncovered apple surfaces (Figure 4b).

2016

Trial 1: There was no significant difference in the F_v/F_m between the various rootstocks (Figure 5) (data not presented); however, there was a significant interaction between canopy position, exposure hours, and days (recovery) which is discussed below (Figure 5).

Damage to peel photosystems occurred at all exposures immediately after the stress exposures (Day 1) with relatively less damage occurring at three-hour exposure for both shaded and sun-exposed apples (Figure 5). Generally, lower F_v/F_m (more damage) was measured for shaded fruit than sun-exposed fruit for all exposures except at 3-hour exposure (Figure 5). Final F_v/F_m readings for day 5 were generally lower for sun-exposed fruit than for shaded fruit except for 5-hour exposure. One-hour exposed apples showed an almost full recovery on day 5 ($F_v/F_m > 0.7$) for both shaded and sun-exposed apples. Final F_v/F_m readings for 2-hour exposed apples were below 0.6 for both sun-exposed and shaded apples, >0.5 for 3-hour exposed shaded apples, and less than 0.4 for 3-hour exposed sun-exposed apples. Final F_v/F_m readings for 4- and 5-hour exposed fruit were mostly around 0.3 (Figure 5).

Trial 2: There was no significant interaction between rootstock, canopy position, exposure hours, and day (recovery) for both covered and uncovered apple surface for trial 2 (Figure 6). Significant

interactions between rootstock, canopy position and day, and between canopy position, exposure hours, and day are therefore discussed for both covered and uncovered apple surfaces (Figure 6).

Visible peel damage increased rapidly on covered and uncovered fruit surfaces from 1- to 5-hour exposure for shaded than on sun-exposed fruit on day 1 (Figures 6a and b). Peel damage on all apples was progressively greater with increasing exposure duration over the 5-day period (Figure 6a and b) for both covered and uncovered apple surfaces. Greater peel damage was recorded on shaded apples at 2-, 3-, 4- and 5-hour exposures on day 1. Peel damage was generally greater on covered surfaces (mostly cooking of peel surfaces with a few cases of peel browning) of sun-exposed apples on the subsequent days, with a few exceptions especially for the 4- and 5-hour exposure period where differences between shaded and sun-exposed fruit evened out (Figure 6a).

After 4- and 5-hour exposures on days 1, 2 and 3 for uncovered apple surfaces (Figure 6b), shaded apples showed greater peel damage than sun-exposed apples. However, there was greater peel damage to sun-exposed fruit than shaded fruit for all exposure periods on days 4 and 5, with the difference being largest at shorter duration stress exposure (1-3 hours), a trend which already began on day 2. There was a gradual increase in visible peel damage for all exposure periods for the first 3 days which became rapid on days 4 and 5 (Figure 6b). There was greater peel damage on covered apple surfaces than uncovered apple surfaces at all exposure periods on each day.

There was progressive and rapid increase in visible peel damage for the two canopy positions (shaded or sun-exposed) for all nine rootstocks over the course of five days for both covered and uncovered (exposed) apple surfaces (Figure 7a and b). There was more damage on both covered and uncovered fruit surfaces of shaded apples than sun-exposed apples on day 1 for all rootstocks. Peel damage on covered fruit surfaces was greater on sun-exposed apples than shaded apples on day 2 to 4 (Figure 7a). Differences in peel damage on covered surfaces between the canopy positions were reduced on day 5. However, in some rootstocks such as G3007, G222 and 'Rosy Glow' own roots, damage on exposed apples remained higher than on shaded apples.

For uncovered fruit surfaces, observed peel damage was similar for both shaded and sun-exposed apples on days 2 and 3, before becoming greater on sun-exposed apples on day 4 (Figure 7b). Peel damage evened out on day 5 in apples from Cepiland, MM109/M9 and G3007 but remained higher on sun-exposed apples from rootstocks G222, M7, M793, 'Rosy Glow' own roots and MM109

(Figure 7b). Peel damage on covered fruit surfaces was greater and peaked higher than uncovered apple surfaces on day 5 for all rootstocks (Figure 7a and b).

'Golden Delicious'

2016

Trial 1: There were no significant differences in damage to apple peel photosystem attributable to rootstock and canopy position (shaded and sun-exposed) (Figure 8). Significant interaction between exposure hours and day (recovery) is therefore discussed below.

There was significant interaction between the exposure period to high light and temperature ("Exposure"), and the subsequent change (recovery) in damage to the photosystem (F_v/F_m) over the next 4 days ("Days") (Figure 8). Damage to the photosystems occurred at all exposures with more significant damage at 5-hour exposure as compared to 2-, 3- and 4-hour exposures (Figure 8). There was a general steady decrease of F_v/F_m from the post-treatment levels on day 1 for all exposures over the subsequent days, except for 1-hour exposure. Apple peels exposed for 1 hour showed a general recovery with F_v/F_m values ending at a little over 0.6. Final F_v/F_m readings were significantly lower for 2-, 3-, 4- and 5-hour exposures (Figure 8).

Trial 2: There were no significant differences in visible damage to 'Golden Delicious' apple peels attributable to rootstock (Figure 9) (data not presented). Therefore, the significant interaction effects between canopy position, exposure hours and day (recovery) for visible peel damage of both covered and uncovered apple surfaces is discussed.

Visible peel damage was lower for both shaded and sun-exposed fruit after 1-hour exposure, but rapid and severe after 2-, 3-, 4- and 5-hour exposures on both covered and uncovered apple surfaces (Figures 9a and b). Peel damage of uncovered surfaces after 1-hour exposure was low on days 1 and 2 before becoming more severe on days 3, 4 and 5, especially in shaded fruit (Figure 9a). Visible damage for 2-, 3-, 4- and 5-hour exposures for days 2, 3, 4 and 5 reached values close to the highest sunburn chart score (5) (Figure 9a).

In uncovered apple surfaces, there was a gradual increase of visible damage from 1-hour exposure to 3-hour exposure with a constant peak visible damage recorded for 4-hour and 5-hour exposures

for all days (Figure 9b). There was more damage to shaded apples with 1- and 2-hour exposures on day 5.

3.4 Fruit quality and maturity

'Rosy Glow'

2015: There was no significant rootstock-side (east or west) interaction for all external 'Rosy Glow' fruit qualities on both the dwarfing and vigorous rootstock site (Table 4). Thus, only the main effects are presented.

At the more dwarfing rootstocks site, fruit from the vigorous rootstock M793 recorded the lowest percentage foreground red colour which was significantly different from all other rootstocks except Cepiland and 'Rosy Glow' on own roots (Table 4). These latter two rootstocks also had less red colour than MM109/M9, G3007, G222 and MM106. Ground colour of fruit from the rootstock MM106 was significantly greener than background colour of fruit from rootstocks G222 and G3007. G222 fruit were less green compared to fruit from all other rootstocks, except G3007. There was no significant difference between the various rootstocks for sunburn score and percentage sunburn for all rootstocks.

At the more vigorous rootstock site, G222 and G228 fruit produced less green colour than rootstocks G778 and M25. The ground colour recorded on fruit from G228 and G222 was not significantly less green than those recorded on the remaining rootstocks. Fruit from G228 and M25 had a higher sunburn score than those from all other rootstocks except G222 and G934. However, sunburn score of fruit from the latter rootstocks were not significantly lower than sunburn score on G222 and G934. In terms of total percentage sunburn (percentage of fruit with all types of sunburn), no significant differences were identified between the rootstocks (Table 4).

At the more dwarfing rootstock site, Lancep, Cepiland, RN29 and G222 recorded significantly higher starch conversion values than all other rootstocks except MM109/M9 and G3007. Starch conversion values for MM109/M9 were also higher than M793 and M7, whilst the value for G3007 was also higher than for M793. (Table 5). Highest total soluble solutes (TSS) was recorded for rootstock RN29 which was significantly higher than all other rootstocks except Lancep, MM109/M9, and G222. TSS for these three rootstocks was also higher than those recorded for MM106, M7 and 'Rosy Glow' own roots. The titratable acidity (TA) values for fruit from Cepiland

and RN29 were significantly higher than those for fruit from MM109/M9, G3007, G222 and M793. The TA values for Lancep, MM106 and M7 were also higher than those for G3007 (Table 5).

At the more vigorous rootstocks planting site, TSS values for the rootstocks G228, G222, M793, G934 and MM109 were significantly higher than the value for rootstock G778 but not higher than those for the M25 and Maruba rootstocks (Table 5). TA recorded in fruit from the Maruba rootstock was significantly higher than the values for rootstocks G222, G778 and M25 (Table 5). TA for G934 was also higher than the values for G778 and G222. However, there was no significant difference in starch conversion between all rootstocks at the more vigorous rootstock site.

2016: No significant rootstock-side (east and west) interaction occurred for both more dwarfing and more vigorous rootstock sites, thus only main effects of rootstock are presented below (Table 6).

At the dwarfing rootstock site, fruit from ‘Rosy Glow’ own roots recorded significantly lower percentage foreground colour than all other rootstocks except M793. Fruit from rootstock G222 produced less green ground colour than fruit from all rootstocks except G3007. The ground colour recorded for G3007 was also less green than that for MM 109/M9, MM106, M7, M793 and ‘Rosy Glow’ own roots (Table 6). Sunburn score and total percentage sunburn were not significantly different between the rootstocks at the more dwarfing site.

At the more vigorous rootstock site, redder colour was recorded on G222 than all rootstocks except M793, G28 and M25. The red colour recorded on the latter three rootstocks was also significantly higher than the value for Maruba. (Table 6). Less green ground colour was recorded on fruit from rootstock G222 than all rootstocks except G228 and G934. The green colour recorded on G228 was less than M793 and Maruba, whilst that of Maruba was greener than the values for all rootstocks except M25 and M793. A higher sunburn score was recorded on fruit from rootstock G228 than all other rootstocks except G222 and G934. The sunburn score of G222 was higher than those for MM109 and Maruba, whilst the sunburn score of G934 was also higher than that for Maruba (Table 6). There was a higher incidence of sunburn of class 2 and 3 on apples from rootstock G228 than on apples from MM109, M25 and Maruba. The class 2 and 3 sunburn on G222 and G934 was also higher than that on MM109 and Maruba.

Additionally, fruit from rootstock MM106 and Lancep recorded higher starch conversion values than fruit from all rootstocks except Cepiland and M793 at the more dwarfing rootstock site (Table 7). The starch conversion value for Cepiland was significantly higher than the values for MM109/M9, RN29, M7, G3007 and ‘Rosy Glow’ own roots, whilst the lowest starch conversion measured in fruit from ‘Rosy Glow’ own roots was lower than for Lancep, Cepiland, G222, MM106 and M793. Higher TSS values were recorded for RN29 and G222 than for M7, M793 and ‘Rosy Glow’ own roots. TSS for G3007 was also higher than that for M7. TA values measured for fruit on rootstocks Cepiland, MM106 and M7 was significantly higher than those measured on fruit from rootstocks G3007, RN29 and ‘Rosy Glow’ own roots (Table 7). The TA value for Lancep was also higher than G3007, RN29 and ‘Rosy Glow’ own roots.

There was, however, no significant difference in the TSS of fruit between all rootstocks at the more vigorous rootstock site (Table 7). Starch conversion values for fruit from rootstock M25 were significantly higher than fruit from all other rootstocks except Maruba. Starch conversion for the Maruba rootstock was higher than that for G222, G228 and G778. Starch conversion for the rootstock G222 was also higher than that for G778. The TA of fruit from rootstock Maruba was significantly higher than the values for rootstocks G778, G222 and G228. TA of rootstock M25 was also higher than the values for G222 and G228 (Table 7). Fruit from G222 had the lowest TA of all rootstocks except G228 and G778.

‘Golden Delicious’

2016: Fruit from the rootstocks Cepiland, M793 and G228 were significantly firmer than fruit from the rootstocks G007 and G778, but were not significantly different from the values for rootstocks G222 and M7 (Table 8). No significant differences were found between rootstocks for starch conversion, total soluble solids and titratable acids measured on fruit (Table 8).

3.5. Peel phenolic and pigment analysis

No significant differences were identified between rootstocks in concentrations of total phenolics, total carotenoids and anthocyanin pigments in the ‘Rosy Glow’ peel (Table 9). There were, however, significant differences in the concentrations of total chlorophyll between the different rootstocks. There was a higher total chlorophyll concentration in the fruit peel for rootstocks MM109 and M793 than all other rootstocks except M7, ‘Rosy Glow’ own roots and G778. The

peel chlorophyll concentration in M7 and 'Rosy Glow' own roots was also higher than for Cepiland, MM109/M9, G3007 and G222, whilst the concentration in G778 was higher than for Cepiland and G3007 (Table 9).

4. DISCUSSION

Rootstocks can confer new attributes to the scion plant (Jensen et al., 2003). These attributes may modify or improve the qualities that are observed in scion organs such as the fruit. However, rootstocks affect the scion fruit in two ways: 1) they may affect expression of genes in the scion and this may confer unique attributes to the scion fruit (Robinson et al., 2004) or 2) determine tree size which creates the micro climate within which the fruit develops and matures (Parchomchuk and Meheriuk, 1996; Racskó et al., 2005b). The micro climate provided by the canopy may in turn enhance or hinder expression of already existing fruit attributes such as colour of blushed apple cultivars. Vigorous and semi-vigorous rootstocks produce bigger trees with large canopies, which provide a cooler microclimate for developing fruit by reducing the amount of solar radiation and light penetration and distribution within the tree canopy. On the other hand, the use of dwarfing rootstocks with smaller canopies and less foliage enhances light distribution within the canopy and exposes a greater number of fruit to solar radiation, with positive effects on red colour development. When confronted with changes in their environment, plants acclimate. Acclimation in plants is a response to changes in their environment that causes phenotypic alterations due to altered gene expression. Fruit developing within different canopy structures will acclimate when gradual changes in light or temperature occur in their environment. In the case of smaller trees on dwarfing rootstocks, where most fruit are exposed to the sun, acclimation may enable fruit to reduce its susceptibility to damage by excess solar radiation and high temperature (Racskó and Schrader, 2012).

In these trials, rootstock did not contribute to the response of RG and GD apple peel photosystems to stress induced by natural high light and temperature conditions, as measured by maximum light use efficiency of photosystem II (F_v/F_m). Duration of exposure, the length of the recovery period and exposed or shaded canopy position (for RG apples in 2016) were responsible for the damage and recovery potential of the apple peel photosystems. Similarly, duration of exposure and the days after exposure were the principal factors affecting visible peel damage observed on RG and GD apples with rootstock only playing a role in visible damage observed on RG apples in 2016.

Damage to photosystems occurred at all exposure periods. However, RG and GD apples with 1-hour exposure attained a higher level of recovery at the end of the recovery periods in 2015 and 2016. RG apples with one hour exposure recorded final F_v/F_m values above a value at which photosystems can be considered almost fully recovered, whilst GD apples with one hour exposure showed a general recovery from initial damage over the recovery period and peaking below the full recovery value (>0.70). Ma and Cheng (2004) found that previously shaded apple peels exposed to sudden high PPF and high temperature recovered over a period of 10 days. Prolonged exposure of apple peels beyond two hours of high light and temperature conditions resulted in irreparable damage to photosystems (Ma and Cheng, 2004). We identified similar trends in our trials: RG peels exposed beyond three hours (in 2015) and two hours (in 2016) did not attain the F_v/F_m value (>0.70) at which they can be considered almost fully recovered from the photothermal stress. Photosystems of GD apples with exposures of two hours and beyond did not recover from initial damage. However, there was some recovery for RG apples following 2-, 3-, 4- and 5-hour exposures over the recovery period in both years.

Song et al. (2001) indicated that damage to apple peel photosystems with F_v/F_m readings of <0.3 was a good indicator of peel damage, which manifested as browning in apples peels. We observed this when fruit peels of similar temperatures were placed under constant heat conditions for varying exposure periods. Wand et al. (2008) also identified flesh and skin browning in apples when F_v/F_m readings of <0.2 were recorded on apples. Schrader et al. (2001) established that exposing apple fruit to air temperatures above 30°C could lead to FST reaching above 45°C in previously sun-exposed fruit. That, according to these researchers, was the minimum temperature required for sunburn browning to occur in the presence of light for 60 minutes. Further, Schrader et al. (2003b) also indicates that FST of about 52°C for 10 minutes, results in sunburn necrosis. In these trials, FST of $36\text{--}43^\circ\text{C}$ was recorded on RG apples in 2015 with corresponding low F_v/F_m readings (within $0.5 - 0.62$) after all exposure durations. At these temperatures, there were full recoveries for apples with 1- and 2-hour exposures and near full recoveries for 3- and 5-hour exposures at day 5 in 2015. However, visible peel browning was observed on uncovered fruit surfaces under such conditions even though FST was lower than the thresholds stated above (Figure 4b). Since FST was only measured at the end of the exposure period, it is possible that FST may have exceeded the levels recorded here at some point during the exposure. This is possible because fruit were not exposed to constant light and temperature conditions. In 2016, FST

of about 38-48°C was recorded on RG apples from sun-exposed canopy positions with fruit from shaded positions recording FST within 37-57°C. F_v/F_m readings at these temperatures were also lower (< 0.5) except at 3-hour exposure in 2016 for both shaded and sun-exposed apples. Of these, only apples under 1-hour exposure period achieved full recovery at day 5 (>0.7) (all fruit from both shaded and sun-exposed canopy positions). Although there was recovery for 1-hour exposed RG apples, peel browning was observed on most of the fruit (from both shaded and exposed canopy positions), with some uncovered surfaces of RG apples from sun-exposed canopy positions showing severe browning symptoms on day 5 (Figure 6b).

For GD apples, FST measurements were between 46-52°C for sun-exposed and 46-50°C for shaded GD apples, with only 1-hour exposed fruit showing recovery at the end of the trial. No GD apples exposed beyond two hours recovered, with final F_v/F_m readings below 0.3. Under such conditions, visible peel damage (peel browning) was observed on GD apples after two hours of exposure. Peel damage after three hours was severe with peels showing symptoms of cooking and necrosis after removal from the stress condition. The results indicate that longer duration of stress at higher temperatures induces irreparable damage to the photosystems and affects their ability to fully recover. This is confirmed by studies by Song et al. (2001) and Wand et al. (2008), who reported similar findings. Wand et al. (2008) found that temperatures of 45-47°C for longer than 2-hour exposure to heat stress resulted in reductions of F_v/F_m up to about 0.2. They identified that there may be partial or no recovery depending on the duration of exposure and the cultivar used. This is consistent with our findings on GD apple peel which showed no recovery beyond two hours of exposure to natural high light and temperature conditions with corresponding visible damage to the apple peel.

Sunburn necrosis (dark brown or black necrotic spot on the fruit peel) occurs on apple peels when FST reaches $52 \pm 1^\circ\text{C}$ (Schrader et al., 2001). According to Schrader et al. (2001), the symptoms (necrotic symptoms) may appear within a period of one to four days after the initial exposure when FST reaches $52 \pm 1^\circ\text{C}$ for only 10 minutes. This damage occurs regardless of the presence or absence of light; therefore, sunlight is not considered as directly involved in its induction (Racskó and Schrader, 2012). Song et al. (2001) reported that postharvest heat treatments of 46°C applied to fruit often result in visible peel damage after one hour. Wand et al. (2008) found the critical temperature for permanent injury to apple fruit peel photosystems exposed to heat stress in the

dark to be around 48 – 53°C. They also identified that permanent damage occurred at FST of about 42-47°C depending on the length of the duration. Similarly, in this study, FST recorded on covered fruit surfaces was between 46-52°C and 47-53°C for sun-exposed and shaded RG apples, respectively, whilst FST of 47-53°C and 48-55°C was recorded on sun-exposed and shaded GD apples, respectively. Browning symptoms were observed on covered RG surfaces under one and two hour exposures, whilst severe browning and a few necrotic symptoms were observed after 5-hour exposure (Figure 6a). However, by day 5, peel damage was very severe for all fruit showing mostly necrotic symptoms after 2-hour exposure for both shaded and sun-exposed fruit (Figure 6a; Appendix A, figure 6). For GD apples, severe damage occurred (mostly cooking symptoms with few incidences of necrosis) on fruit exposed beyond 3-hours immediately after removal from the stress condition (Figure 9a). By day 5, damage was severe for all fruit beyond 2-hour exposure (Appendix A, Figure 5).

The differences in the response of RG and GD apples to the photothermal stress conditions employed in this study may be due to several factors. Several studies have reported differences in susceptibility of different cultivars to sunburn damage in different apple growing regions (Carb'o et al., 2005; Gindaba and Wand, 2005; Racskó et al., 2005a; van den Ende 1999). Accumulation of fruit pigments in the apple peel serve photoprotective functions through either the screening of solar radiation or playing the role of antioxidants (Racskó and Schrader, 2012). Since the pigments could help mitigate the effects of photothermal damage during high light and temperature stress conditions, the presence or lack of these pigments and compounds would affect the response of each cultivar differently. Li and Cheng (2009) found that, while the light use efficiency (F_v/F_m) of green 'Anjou' pears was negatively affected by exposure to high light and temperature stress, red 'Anjou' pears, on the other hand, possessed a high thermal tolerance when exposed to the same stress conditions. Steyn et al. (2009) also found that the extent of reduction in light use efficiency (F_v/F_m) correlated with hue angle measured on fruit. They identified that reduction in (F_v/F_m) increased with decreasing red colour of pears. The responses of both GD and RG apple peel photosystems in both years confirm these findings. The initial higher damage to RG photosystems after short duration exposure of 1-hour in 2015 and the recovery patterns of RG apple photosystems following 3-, 4- and 5-hour exposures (2015 and 2016) may be an artefact of measuring F_v/F_m in fruit with low chlorophyll levels. Chlorophyll levels are much higher in GD than in RG apples. Therefore, one is likely to obtain much more reliable F_v/F_m data in GD peel

than in RG. One possible way to get around this problem will be to do chlorophyll fluorescence assessments during early fruit development of red and blush apple cultivars while chlorophyll levels are still high.

Damage to the fruit peel photosystem, as measured by maximum light use efficiency of PSII, is always greater in previously shaded fruit in studies where both previously exposed and shaded peels of apples were measured after exposure to the same stress condition (Chen and Cheng, 2007; Chen et al., 2008; Li and Cheng, 2009; Wand et al., 2008). In this trial, more damage to photosystems was observed on shaded RG apples at 1-, 2-, 4- and 5-hour exposures than sun-exposed RG apples after removal from the stress conditions on day 1 in 2016. However, by day 3 differences between shaded and sun-exposed apples became inconsistent. Higher F_v/F_m readings were recorded on shaded apples at 1- and 5-hour exposures on day 3, 3-hour exposure on day 4, and all exposure periods on day 5. The final differences in F_v/F_m readings for shaded apples were not significantly higher for fully recovered 1-hour exposed and non-recovered 5-hour exposed apples. However, final F_v/F_m readings on shaded fruit were significantly higher than sun-exposed fruit at 2-, 3- and 4-hour exposures. Since previously exposed sides of both shaded and sun-exposed fruit were used in this trial, it is possible that previous exposure to high light and temperature under field conditions may have acclimated both sets of peels to the sudden exposure to the high light and temperature stress. The differences in damage to sun-exposed and shaded apples immediately after the stress condition and the difference observed at the end of the recovery period, may be due to different levels of protection induced in the peels by acclimation experienced on the field. It is possible that apples from the sun-exposed canopy positions may have benefitted from a short-term protection from previous acclimation, whilst previous acclimation to high light and temperature stress of the exposed sides of shaded fruit may have induced a form of protection which enabled them to recover better from the initial damage induced on the peels than fruit from sun-exposed positions. Ma and Cheng (2004) assert that fruit from shaded canopy positions can overcome damage if the peel enhances non-photochemical quenching and its antioxidant system to acclimate to a new high light environment. This is possible because peels in the most shaded positions can be exposed to sun flecks when solar beams pass through a gap in a layer of the canopy (Racskó and Schrader, 2012). Such exposures induce acclimation in the previously non-acclimated fruit against subsequent exposure to solar rays.

Initial damage to shaded apples at 1-, 2- and 3-hour exposures indicates that those peels may have initiated a defensive mechanism during the exposure to the stress condition on day 1. Activities of heat stress proteins, xanthophyll cycle and the ascorbate–glutathione pathway found in plants are known to be upregulated in response to stress (Kotak et al., 2007; Wang et al., 2004; Ritenour et al., 2001). The heat stress proteins function in sustaining plant tissues under duress and serve as protein chaperones during and after stress periods. The xanthophyll cycle and the ascorbate–glutathione pathway minimise the photooxidative damage to plant tissues by working to dissipate excess heat energy that might damage photosystems (Ma and Cheng, 2004). These biological mechanisms may have provided some protection against rapid development of necrosis on days 2 to 3 on RG apples in 2016. However, it seems the protection offered was eventually not sufficient to prevent acceleration of damage by day 5 and the defense mechanism appears to have been more successful in uncovered peels, which were not heated as much as covered peels.

There was a progressive increase in damage from day 1 to 5 on all rootstocks with damage being severe on day 5 for both covered and uncovered RG apples from the two canopy positions in 2016. Generally, more visible peel damage was recorded on shaded than sun-exposed apples on day 1 for most rootstocks. On covered peels, more visible peel damage was observed on sun-exposed than shaded apples from day 2 to 4 before evening out on day 5 for most rootstocks, with a few exceptions. For uncovered peel surfaces, peel damage from day 2 to 3 was similar for most rootstocks before becoming significantly greater in sun-exposed peels on day 4. Final visible peel damage evened out for shaded and sun-exposed apples of Cepiland, MM109/M9 and G3007, with the remaining rootstocks scoring more damage on sun-exposed apples except rootstock G778. This is a further indication that the acclimation of sun-exposed apples used in this trial may have provided a protection against immediate damage when fruit were exposed to the natural high light and temperature stress condition. The rootstock G778 recorded significantly more visible damage on shaded than sun-exposed covered peels on day 5 whilst the opposite was observed for rootstocks G3007 and G222. The slightly lower sensitivity identified in rootstock G3007 and a higher sensitivity in M793 in this study provides an indication of a direct rootstock effect on sensitivity of RG apples to peel damage. However, this was the only direct rootstock effect observed. Therefore, rootstock effects on sunburn most likely relates more to vigour and canopy size.

In both years, there were distinct effects of rootstock on maturity as indicated by TSS, starch conversion and TA at both planting sites for RG apples. In 2015, RN29 showed advanced maturity at the more dwarfing site; however, this was not significantly different from the maturity of fruit for G222, MM109/M9 and Lancep. At the more vigorous site, G778 showed delayed maturity compared to all other rootstocks. In 2016, fruit from rootstocks RN29 were more mature than those on M7, M793 and RG own root fruit. This result is corroborated by the full harvest fruit quality (colour) and maturity data presented by Sibozza and Steyn (2015) and Sibozza et al. (2016). Sunburn damage at harvest was significantly higher in RG apples on the semi-vigorous rootstock G228 in 2015 and 2016; this rootstock also recorded the highest incidence of sunburn browning (sunburn class 2-3) in 2016. Song et al. (2001) suggested that maturity of apples can influence their response to heat stress. They deduced that less mature apples may be more resistant to heat stress than more mature fruit. Although we identified differences in maturity between the rootstocks, we did not find enough evidence to identify maturity-related effects of RG apples on sensitivity of RG apples to heat stress.

Work by Costa (2011) and Costa and Stassen (2011) on GD apples on Geneva rootstocks found distinct differences between Geneva rootstocks compared to the South African industry standard M793 in terms of yield efficiency and some quality parameters at harvest. Full harvest data from Sibozza and Steyn (2015) and Sibozza et al. (2016) have also shown better performance of some new rootstocks such as G778 in terms of yield when compared to the industry standard M793. It is encouraging that the new rootstocks used in this trial and the dwarfing rootstock in general are doing at least as well as the industry standard in relation to sensitivity to photothermal stress.

5. CONCLUSION

Based on the results, there was no indication of rootstock influence on the sensitivity of apple peels to high light and temperature and the subsequent occurrence of visible peel damage on the apple peel. Sensitivity of photosystems and subsequent damage observed on fruit from all rootstocks indicate that the new rootstocks and the dwarfing rootstocks in general are performing as well as the industry standards. Since previous evaluations of some of the new rootstocks show better results in terms of yield than current standards, this will make them a better choice in future orchards. However, the indirect effect of canopy size on sunburn damage on the entire tree also need to be considered before any recommendations can be made.

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TABLES AND FIGURES.

Table 1. Leaf and fruit ecophysiological parameters for ‘Rosy Glow’ apple at the more dwarfing and more vigorous rootstock sites, measured *in situ* on 5 March 2015. N=5 at the more dwarfing site and N=6 at the more vigorous site except stomatal conductance where N=3. Ambient air temperature at start of measurement was 27.8°C. Means in columns were separated by LSD at 5% when $Pr < 0.05$.

Rootstock	Leaf temperature (°C)		Fruit surface temperature (°C)		Stomatal conductance (mmol m ⁻² s ⁻¹)		Hue (°)	
More dwarfing site								
Lancep	25.3	ns	29.8	ns	82.0	ns	82.8	ns
Cepiland	24.4		31.4		87.8		78.6	
MM109/M9	25.5		30.3		84.9		76.2	
G3007	24.7		30.4		77.2		78.4	
RN29	24.7		31.1		71.7		71.5	
G222	25.1		31.3		86.8		72.5	
MM106	26.3		31.1		91.6		73.4	
M7	24.9		31.1		97.6		82.3	
M793	25.9		31.4		83.1		85.9	
‘Rosy Glow’ own roots	25.7		31.2		100.1		86.0	
<i>Pr=f</i>	0.916		0.990		0.174		0.593	
More-vigorous site								
G222	27.9	ns	36.9	ns	80.8	ns	86.9	ab
M793	29.3		36.6		61.3		85.3	abc
G228	27.8		35.8		68.0		79.4	c
MM109	28.3		35.8		73.2		85.5	abc
G778	27.6		35.5		66.4		81.4	bc
M25	27.6		34.5		72.1		78.9	c
G934	27.9		35.6		55.2		82.3	bc
Maruba	27.8		35.4		80.8		89.5	a
<i>Pr=f</i>	0.751		0.432		0.870		0.043	

Table 2. Leaf and fruit eco-physiological parameters for ‘Rosy Glow’ at the more dwarfing and more vigorous rootstock sites, measured *in situ* on 8 January and 10 March 2016. N=5 at the more dwarfing site and N=6 at the more vigorous site. Ambient air temperature at start of measurement was 28.4°C and 26.8°C on 8 January and 10 March, respectively. Means in columns were separated by LSD at 5% when $Pr < 0.05$.

Rootstock	Leaf temperature (°C)		Fruit surface temperature (°C)		Stomatal conductance (mmol m ⁻² s ⁻¹)		Hue (°)									
	Jan	March	Jan	March	Jan	March	Jan	March								
More dwarfing site																
Lancep	27.4	ns	25.4	ns	28.6	ns	30.5	ns	172.4	ns	133.5	ns	133.5	ns	80.7	ns
Cepiland	27.0		26.5		32.1		31.7		144.9		129.7		129.7		76.2	
MM109/M9	28.2		27.1		31.7		33.6		163.1		118.3		118.3		76.1	
G3007	27.6		26.8		31.8		34.0		149.9		128.7		128.7		88.1	
RN29	26.5		27.1		30.4		32.4		160.0		123.8		123.8		75.6	
G222	26.4		26.7		31.8		33.8		150.1		131.3		131.3		69.5	
MM106	25.0		26.3		28.8		32.0		158.8		141.8		141.8		73.9	
M7	26.4		25.6		30.6		30.5		175.0		142.3		142.3		75.0	
M793	24.9		26.5		28.5		30.6		189.3		121.3		121.3		73.9	
‘Rosy Glow’ own roots	24.9		25.9		29.0		32.8		186.7		134.3		134.3		69.5	
<i>Pr=f</i>	0.362		0.942		0.168		0.664		0.630		0.757		0.757		0.763	
More vigorous site																
G222	25.5	ns	23.7	ns	30.0	ns	29.3	ns					113.3	ns	80.1	ns
M793	24.7		23.8		28.5		28.4						110.7		71.7	
G228	27.3		22.8		30.0		27.4						111.0		75.9	
MM109	24.9		22.4		28.6		27.0						111.2		75.0	
G778	25.1		23.9		29.3		30.0						109.4		77.1	
M25	25.3		24.2		26.9		28.9						111.1		77.8	
G934	25.2		24.4		28.9		27.9						109.5		73.7	
Maruba	24.2		24.5		26.3		28.9						113.2		73.8	
<i>Pr=f</i>	0.528		0.273		0.116		0.413						0.462		0.859	

Table 3. Fruit surface temperature (°C) of ‘Rosy Glow’ apples from various rootstocks during Trial 1 and Trial 2 in 2015 after different durations of exposure (hours) to high light and high temperature. Means in columns were separated by LSD at 5% when $Pr < 0.05$.

Rootstock	Trial 1:		Trial 2: Sunburn			
	Photothermal stress		Uncovered surface	Covered surface		
Cepiland	42.8	a	43.1	c	46.9	c
MM109/M9	41.6	bc	45.0	a	48.2	ab
G3007	41.7	bc	45.1	a	49.2	a
G222	41.5	bc	44.6	ab	48.5	ab
M7	42.1	ab	45.2	a	49.0	a
M793	41.2	c	43.3	bc	47.4	bc
G778	41.8	bc	44.8	a	48.6	a
‘Rosy Glow’ own roots	41.3	c	45.3	a	48.6	a
MM109	41.2	c	43.3	bc	47.4	bc
Exposure						
1	43.2	a	41.9	d	46.5	c
2	43.4	a	45.1	b	49.9	a
3	43.6	a	46.5	a	49.6	a
4	41.9	b	45.4	b	48.1	b
5	36.4	c	43.2	c	46.4	c
<i>Pr=f</i>						
Exposure	<0.000		<0.000		<0.000	
Rootstock	0.019		<0.000		0.002	
Exposure*Rootstock	0.794		0.912		0.453	

Table 4. Fruit external quality of ‘Rosy Glow’ apples from the more dwarfing and more vigorous rootstock sites at harvest in 2015. N=5 for the more dwarfing site and N=6 for the more vigorous site. Values are means for fruit harvested on the eastern and western side of the row. Percentage foreground colour and background colour were assessed using the industry charts for ‘Pink Lady’. Sunburn was scored according to the Schrader and McFerson chart with 0 indicating no damage and 5 indicating necrosis. Means in columns were separated by LSD at 5% when $P < 0.05$.

Rootstock	% Foreground colour (chart)*		Background colour (chart)**		Sunburn score (Chart)		% Sunburn (all categories) ***		% Sunburn (2-3 Score)		% Sunburn (4-5 Score)	
More dwarfing site												
Lancep	24.7	ab	3.52	bc	1.21	ns	73.7	ns	31.1	ns	2.1	ns
Cepiland	22.7	bc	3.43	bc	1.13		82.4		32.8		0.8	
MM109/M9	26.6	a	3.48	bc	1.21		75.1		27.2		1.6	
G3007	26.9	a	3.61	ab	1.03		71.6		22.8		0.4	
RN29	25.0	ab	3.49	bc	1.10		80.8		21.2		1.6	
G222	26.9	a	3.70	a	1.02		75.2		14.8		1.6	
MM106	27.2	a	3.41	c	1.03		68.7		20.1		0.0	
M7	24.3	ab	3.48	bc	0.91		80.8		16.4		0.4	
M793	20.5	c	3.51	bc	0.92		69.4		17.2		0.8	
‘Rosy Glow’ own roots	22.7	bc	3.50	bc	0.93		76.0		11.2		0.0	
<i>Pr=f</i>												
Rootstock	0.002		0.004		0.372		0.739		0.539		0.369	
Side	0.271		0.452		0.172		0.523		0.478		0.246	
Rootstock*Side	0.432		0.535		0.808		0.782		0.832		0.768	
More vigorous site												
G222	36.4	ns	3.71	a	1.06	ab	82.9	ns	24.3	ns	1.0	ns
M793	34.7		3.67	ab	0.95	b	74.5		19.3		0.0	
G228	35.4		3.70	a	1.21	a	74.8		20.9		1.3	
MM109	35.9		3.65	ab	0.95	b	70.1		15.6		1.7	
G778	33.7		3.63	bc	0.96	b	66.8		16.2		0.0	
M25	37.1		3.59	c	1.12	a	72.1		21.0		1.3	
G934	36.9		3.65	ab	1.08	ab	59.6		21.5		0.7	
Maruba	32.9		3.65	ab	0.97	b	65.7		15.0		1.0	
<i>Pr=f</i>												
Rootstock	0.053		0.002		0.003		0.137		0.299		0.349	
Side	0.306		0.401		0.080		0.324		0.381		0.459	
Rootstock*Side	0.148		0.908		0.225		0.768		0.649		0.786	

*Estimating the percentage blush on fruit using colour charts (from 5 to 100 full blush)

** Background colour: comparing the green side of fruit with the colour chart (0.5 = green to 5 = yellow)

***Percentage of fruit with all types of sunburn scored using the Schrader and McFerson system (0= No Sunburn to 5= necrosis)

Table 5. Fruit maturity of 'Rosy Glow' apples from more dwarfing and more vigorous rootstock sites at harvest in 2015. N=5 for the more dwarfing site and N=6 for the more vigorous site. Values are for pooled samples of fruit harvested from both the eastern and western side of the row. Means in columns were separated by LSD at 5% when $Pr < 0.05$.

Rootstock	Starch conversion (%)	TSS (% Brix)	Titrateable acidity (% malic acid eq.)
More dwarfing site			
Lancep	57.3 a	15.6 ab	0.84 abc
Cepiland	54.8 a	15.3 bc	0.89 a
MM109/M9	49.9 ab	15.6 ab	0.82 bcd
G3007	49.0 abc	15.2 bcd	0.76 d
RN29	57.8 a	16.1 a	0.89 a
G222	55.9 a	15.6 ab	0.82 cd
MM106	41.0 bcd	15.1 cd	0.86 abc
M7	39.6 cd	14.9 cd	0.86 abc
M793	36.6 d	15.1 bcd	0.85 bc
'Rosy Glow' own root	42.8 bcd	14.6 d	0.83 abcd
<i>Pr=f</i>	0.001	0.004	0.019
More vigorous site			
G222	56.8 ns	15.6 a	0.81 d
M793	49.9	15.3 a	0.86 abc
G228	52.8	15.6 a	0.86 abc
MM109	49.5	15.3 a	0.87 abc
G778	45.2	14.6 b	0.82 cd
M25	48.7	15.1 ab	0.84 bcd
G934	47.9	15.4 a	0.89 ab
Maruba	48.5	15.1 ab	0.91 a
<i>Pr=f</i>	0.196	0.019	0.006

Table 6. Fruit external quality of ‘Rosy Glow’ apples for more dwarfing and more vigorous rootstock sites at harvest in 2016. N=5 for the more dwarfing site, N=6 for the more vigorous site. Values are means for fruit harvested on the eastern and western side of the row. Sunburn was scored according to the Schrader and McFerson system with 0 indicating no damage and 5 indication necrosis. Means in columns were separated by LSD at 5% when $Pr < 0.05$.

Rootstock	% Foreground colour (chart)*		Background colour (chart)**		Sunburn score (chart)		% Sunburn (all categories) ***		% Sunburn (2-3 Score)		% Sunburn (4-5 Score)	
More dwarfing site												
Lancep	72.1	a	3.61	bc	0.93	ns	61.5	ns	17.3	ns	2.6	ns
Cepiland	76.2	a	3.62	bc	0.74		58.7		11.3		0.6	
MM109/M9	70.7	a	3.51	c	0.81		52.0		14.0		2.7	
G3007	76.8	a	3.80	ab	0.92		60.0		20.7		1.3	
RN29	75.7	a	3.61	bc	0.91		62.7		14.7		5.3	
G222	76.7	a	3.83	a	1.05		68.0		10.0		1.3	
MM106	75.0	a	3.52	c	0.69		46.0		10.0		1.3	
M7	70.6	a	3.54	c	0.91		57.1		16.3		3.7	
M793	70.4	ab	3.51	c	0.68		41.9		9.9		2.6	
‘Rosy Glow’ own roots	63.7	b	3.53	c	0.71		54.5		13.5		0.0	
<i>Pr=f</i>												
Rootstock	0.004		<0.0001		0.066		0.216		0.233		0.361	
Side	0.798		0.688		0.986		0.408		0.968		0.835	
Rootstock*Side	0.668		0.984		0.527		0.937		0.898		0.901	
More vigorous site												
G222	77.8	a	3.83	a	1.13	ab	66.0	ns	29.7	ab	7.1	ns
M793	72.9	ab	3.51	cd	0.84	bcd	58.6		19.6	abc	0.0	
G228	73.1	ab	3.71	ab	1.22	a	68.2		33.0	a	1.1	
MM109	68.6	bc	3.52	bc	0.86	cd	53.1		14.5	c	0.6	
G778	68.7	bc	3.52	bc	0.83	bcd	53.9		18.9	abc	2.8	
M25	73.3	ab	3.53	bcd	0.82	bcd	52.0		16.7	bc	1.1	
G934	70.6	bc	3.63	abc	1.01	abc	61.0		29.3	ab	2.3	
Maruba	66.9	c	3.34	d	0.62	d	48.9		11.7	c	0.6	
<i>Pr=f</i>												
Rootstock	0.009		< 0.000		0.012		0.500		0.041		0.233	
Side	0.866		0.943		0.345		0.561		0.233		0.111	
Rootstock*Side	0.921		0.987		0.993		0.437		0.827		0.803	

*Estimating the percentage blush on fruit using colour charts (from 5 to 100= full blush)

** Background colour: comparing the green side of fruit with the colour chart (0.5 = green to 5 = yellow)

***Percentage of fruit with all types of sunburn scored using the Schrader and McFerson system (0= No Sunburn to 5= necrosis)

Table 7. Fruit maturity of 'Rosy Glow' apples from more dwarfing and more vigorous rootstock sites at harvest in 2016. N=5 for the more dwarfing site, N=6 for the more vigorous site. Values are for pooled samples of fruit harvested from both the eastern and western side of the row. Means in columns were separated by LSD at 5% when $Pr < 0.05$.

Rootstock	Starch conversion (%)	TSS (% Brix)	Titrateable acidity (% malic acid eq.)
More dwarfing site			
Lancep	67.7 a	15.2 abc	0.75 ab
Cepiland	67.0 ab	15.4 abc	0.77 a
MM109/M9	58.7 cde	15.1 abc	0.74 abc
G3007	55.3 de	15.4 ab	0.70 c
RN29	57.7 cde	15.7 a	0.70 c
G222	61.0 bcd	15.9 a	0.74 abc
MM106	68.5 a	15.1 abc	0.78 a
M7	57.7 cde	14.5 c	0.76 a
M793	64.1 abcd	14.7 bc	0.74 abc
'Rosy Glow' own roots	53.4 e	14.7 bc	0.70 c
<i>Pr=f</i>	<i><0.0001</i>	<i>0.029</i>	<i>0.006</i>
More vigorous site			
G222	54.4 cd	15.8 ns	0.68 d
M793	55.7 bcd	15.1	0.76 abc
G228	49.9 de	15.1	0.73 cd
MM109	60.1 bc	14.8	0.77 abc
G778	46.8 e	15.0	0.73 bcd
M25	68.9 a	15.4	0.80 ab
G934	56.5 bcd	15.2	0.76 abc
Maruba	62.2 ab	14.7	0.81 a
<i>Pr=f</i>	<i>< 0.0001</i>	<i>0.212</i>	<i>0.007</i>

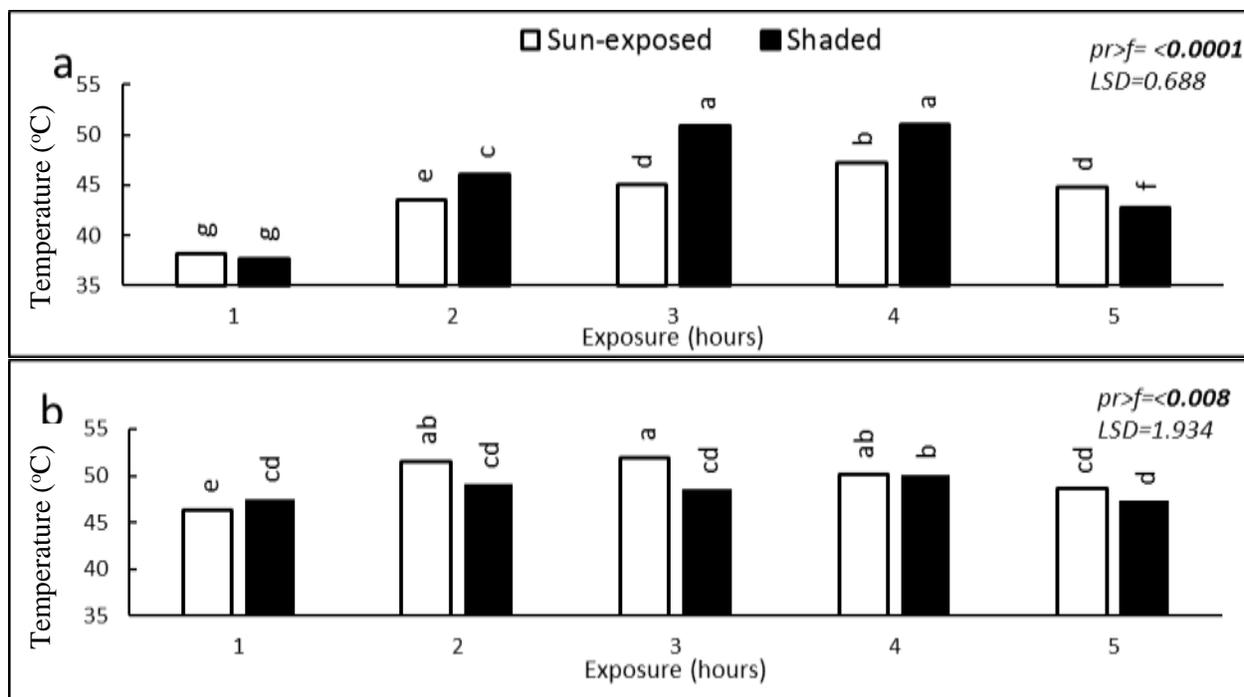
Table 8. External quality and maturity of ‘Golden Delicious’ apples on various rootstocks in 2016. N=3. Means in columns were separated by LSD at 5% when Pr < 0.05.

Rootstock	Ground colour (chart)*	Firmness (kg)	Starch conversion (%)	TSS (% Brix)	Titrateable acidity (% malic acid)
Cepiland	1.97 ns	8.86 a	3.5 ns	11.8 ns	0.48 ns
G007	1.89	8.15 b	4.2	11.2	0.52
G222	2.10	8.41 ab	0.0	11.7	0.50
M7	2.04	8.47 ab	0.0	11.7	0.45
M793	1.93	8.81 a	0.8	11.6	0.49
G228	1.95	8.68 a	4.5	11.3	0.48
G778	2.02	8.18 b	0.4	11.4	0.48
<i>Pr=f</i>	0.288	0.043	0.556	0.827	0.887

* Ground colour: comparing the green colour of fruit with the colour chart (0.5 = green to 5 = yellow)

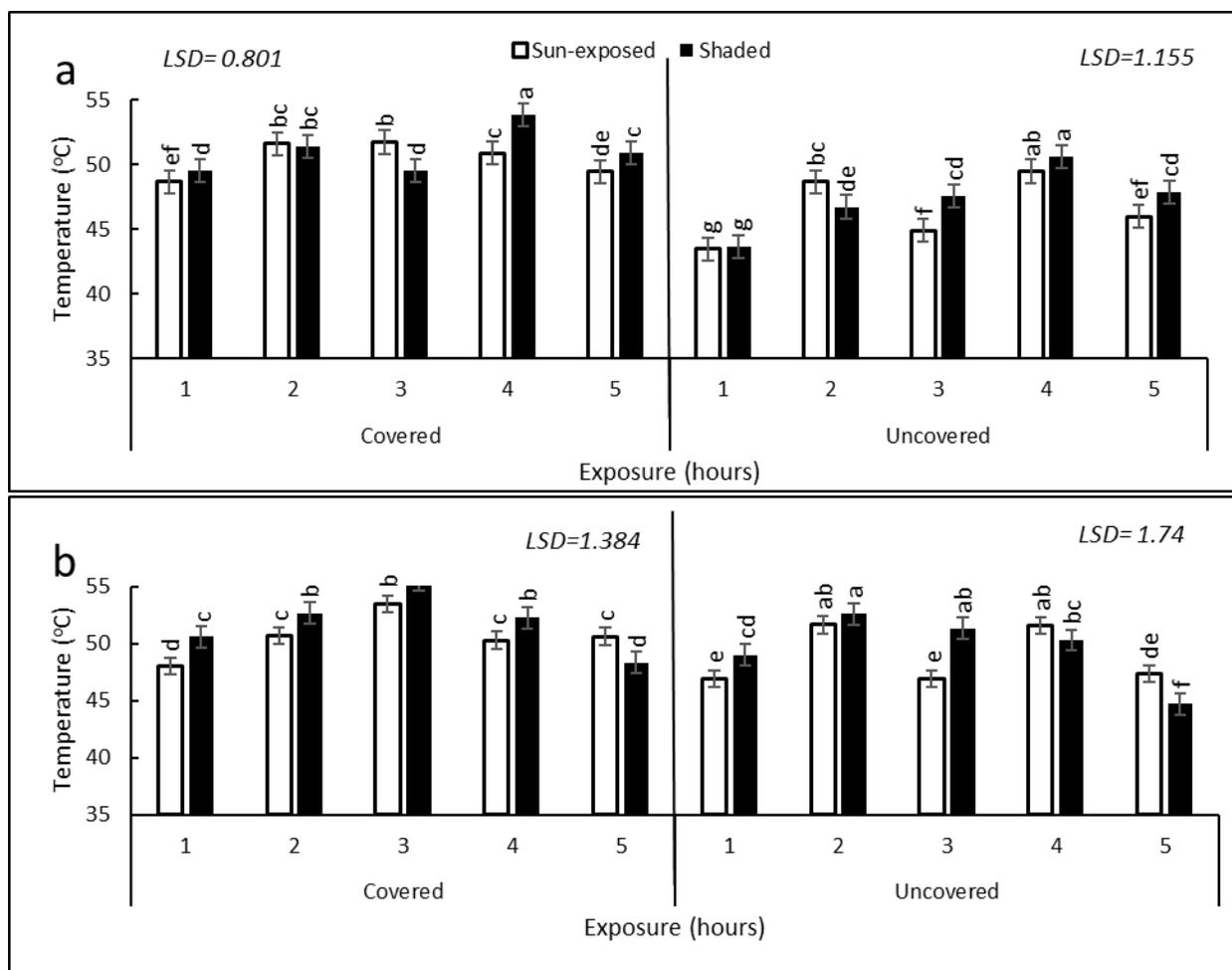
Table 9. Sun-exposed apple fruit peel total phenolics, total chlorophylls, carotenoids and anthocyanin content from fruit collected on 5 March 2015, four weeks before normal harvest. Means in columns were separated by LSD at 5% when $Pr < 0.05$.

Rootstocks	Total Phenolics (mg/100g FW)	Total Chlorophylls (μ g/g FW)	Total Carotenoids (μ g/g FW)	Anthocyanins (μ g/g FW)
Cepiland	62.1 ns	11.3 d	2.7 ns	38.6 ns
MM109/M9	86.1	11.5 cd	2.7	39.3
G3007	89.4	11.2 d	2.3	36.8
G222	71.5	11.4 cd	3.1	40.6
M7	71.1	12.1 ab	2.3	39.9
M793	67.5	14.0 a	2.2	37.4
'Rosy Glow' own roots	58.0	13.1 ab	2.3	43.0
MM109	86.9	13.7 a	2.2	43.2
G778	51.0	12.8 abc	2.2	38.2
<i>Pr >f</i>	0.366	0.001	0.271	0.973



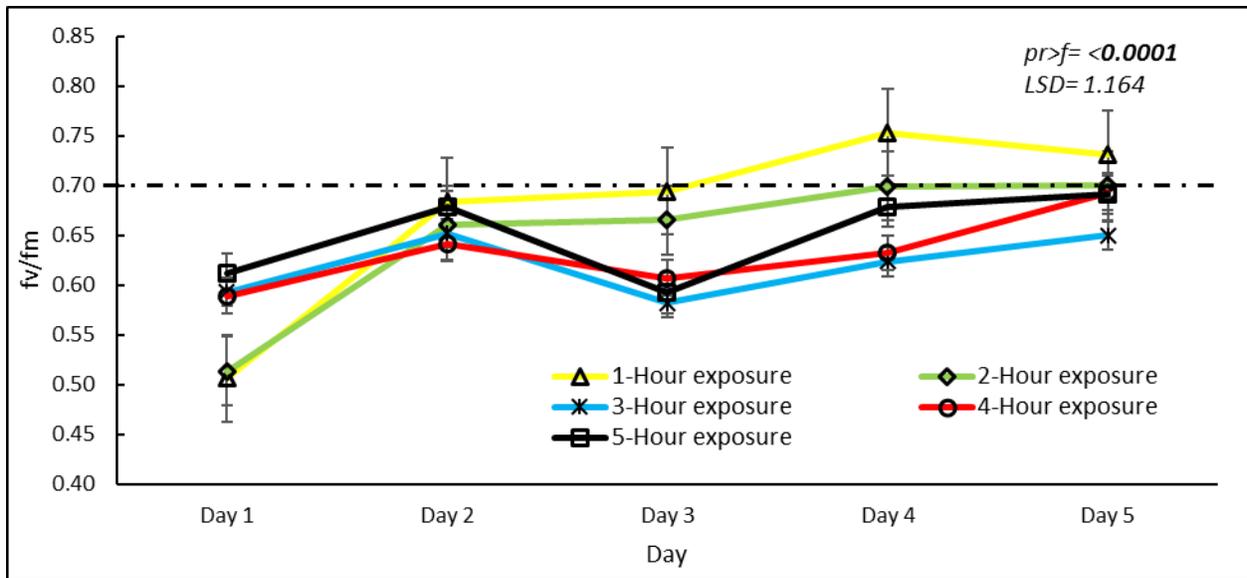
Effect	<i>pr=f</i>	
	'Rosy Glow'	'Golden Delicious'
Rootstock	0.959	0.684
Exposure hours	< 0.0001	< 0.0001
Canopy position	< 0.0001	0.007
Canopy position*Exposure hours	< 0.0001	0.008
Rootstock*Exposure hours	0.517	0.261
Canopy position*Rootstock*Exposure hours	0.069	0.861

Figure 1. Interaction between canopy position (sun-exposed and shaded) and exposure (hours of stress treatment) for fruit surface temperature recorded during Trial 1 in 2016 for (a) 'Rosy Glow' apples and (b) 'Golden Delicious' apples.



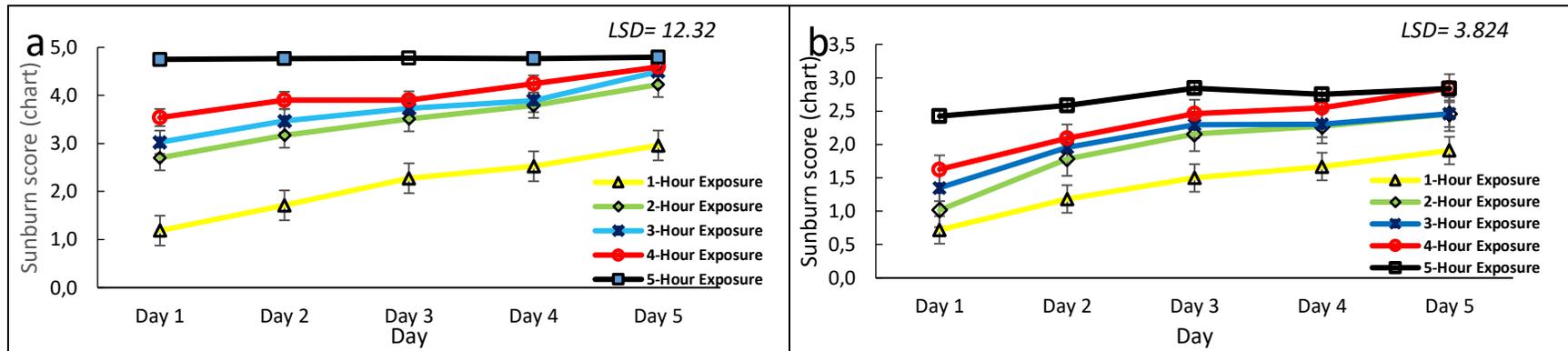
Effect	<i>pr=f</i>			
	Rosy Glow'		Golden Delicious'	
	Covered surface	Uncovered surface	Covered surface	Uncovered surface
Rootstocks	0.288	0.085	0.535	0.178
Exposure hours	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Canopy position	0.002	0.004	0.000	0.000
Exposure hours *Canopy position	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Rootstock*Exposure hours	0.651	0.863	0.318	0.932
Rootstock*Exposure hours *Canopy position	0.963	0.925	0.416	0.995

Figure 2. Interaction between canopy position (shaded and sun-exposed) and exposure (hours of stress treatment) for fruit surface temperature recorded during Trial 2 in 2016 on covered and uncovered surfaces of (a) 'Rosy Glow' apples and (b) 'Golden Delicious' apples. Covered surface denotes portion of fruit surface covered with black sticker which was removed immediately prior to measurement.



Effect	<i>pr=f</i>
Rootstock	0.730
Exposure hours	0.000
Day	<0.000
Exposure hours *Day	<0.000
Rootstock*Exposure hours *Day	0.658

Figure 3. Interaction between duration of exposure (hours of stress treatment) and days (day 1 being the trial day with four days thereafter) on the recovery of photosystems (F_v/F_m) in 'Rosy Glow' apples during Trial 1 in 2015. The dotted black line at $F_v/F_m = 0.7$ denotes a value at which photosystems can be considered to have recovered almost fully. Vertical bars denote 0.95 confidence intervals.



Effect	<i>pr=f</i>	
	Covered Surface	Uncovered Surface
Rootstock	0.645	0.883
Exposure hours	<0.000	<0.000
Day	<0.000	<0.000
Exposure hours *Day	<0.000	<0.000
Rootstock*Exposure hours *Day	0.612	0.510

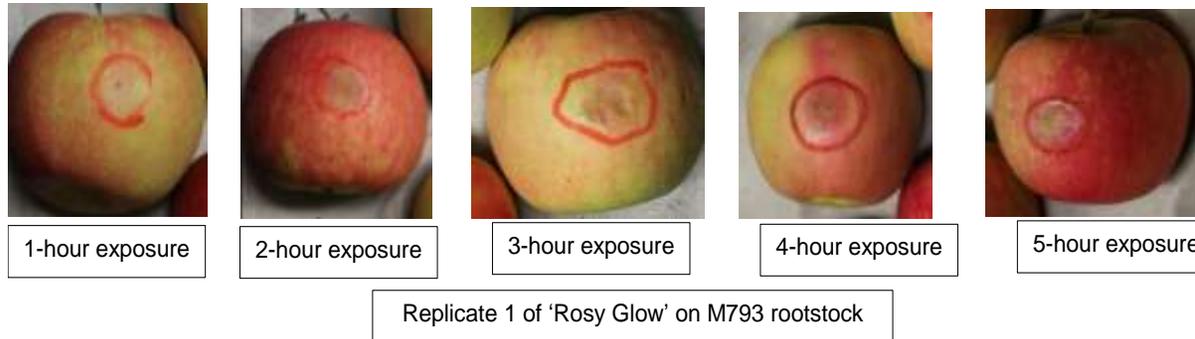
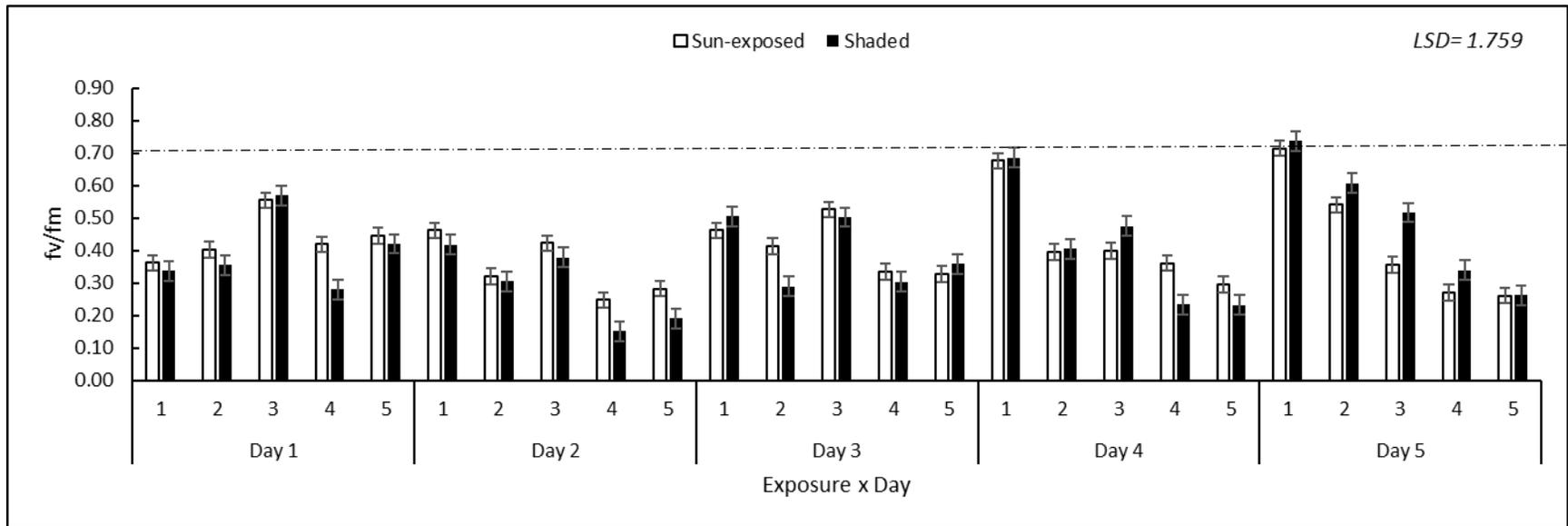
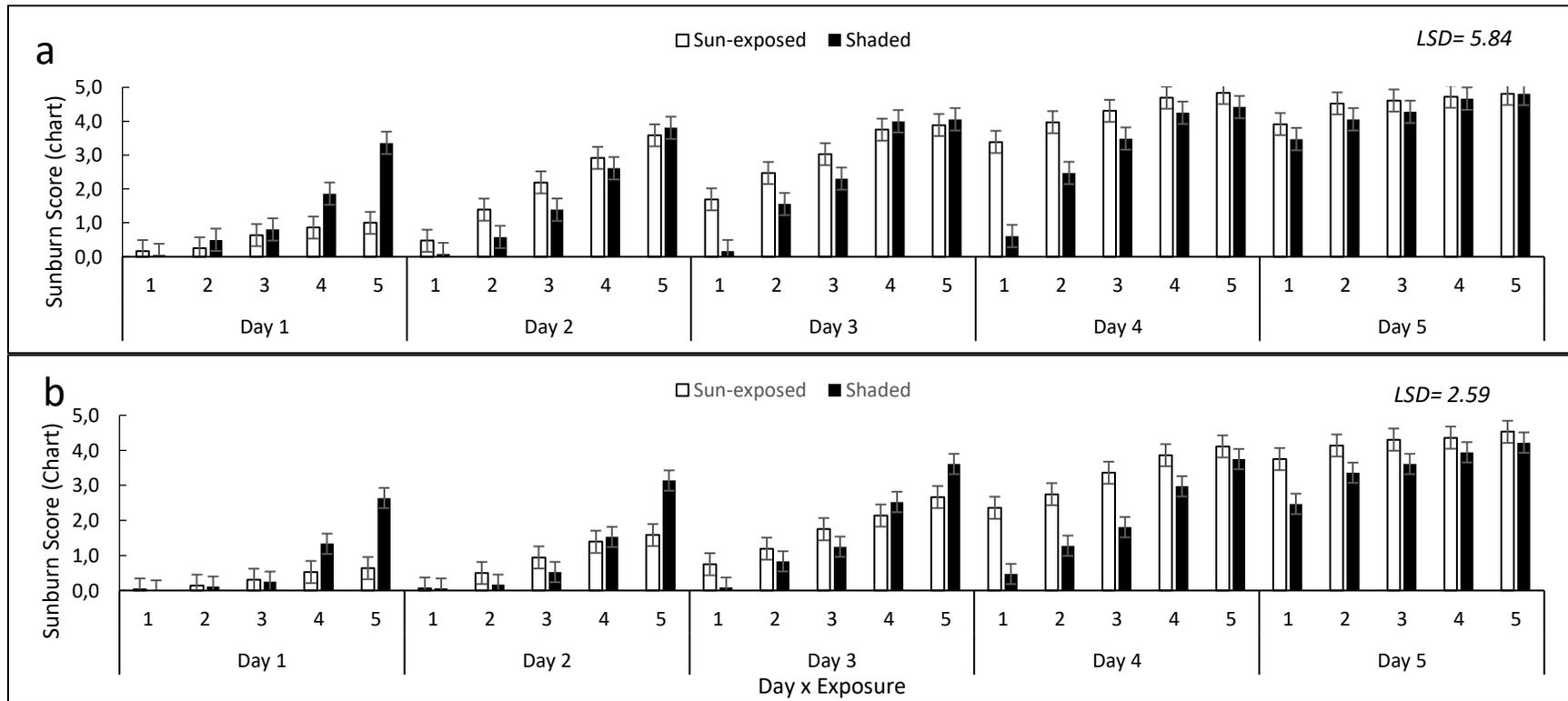


Figure 4. Interaction between duration of exposure (hours of stress treatment) and days (day 1 being the trial day with four days thereafter) on the development of visible peel damage severity on (a) covered and (b) uncovered surfaces of ‘Rosy Glow’ apples exposed to high light and high temperature during Trial 2 in 2015. Vertical bars denote 0.95 confidence intervals. Sunburn was scored according to the Schrader and McFerson system with 0 being no sunburn and 5 being necrosis. The photographs show visible peel damage of ‘Rosy Glow’ on M793 rootstock from block 1 at 1-hour, 2-hour, 3-hour, 4-hour and 5-hour exposure to high light and high temperature on day one.



Effect	<i>pr=f</i>
Rootstock	0.403
Exposure hours	<0.000
Canopy position	0.483
Day	<0.000
Exposure hours *Canopy position*Day	0.030
Rootstock*Canopy position*Day	0.658
Rootstock*Exposure hours *Canopy position*Day	0.913

Figure 5. Interaction between canopy position (shaded and sun-exposed), exposure (hours of stress treatment) and days (day 1 being the trial day with four days thereafter) on the recovery of photosystems (F_v/F_m) in 'Rosy Glow' apples during Trial 1 in 2016. Vertical bars denote 0.95 confidence intervals. The dotted black line at $F_v/F_m = 0.7$ denotes a value at which photosystems can be considered to have recovered almost fully.



Effect	<i>pr=f</i>	
	Covered Surface	Uncovered Surface
Rootstock	0.427	0.982
Exposure hours	<0.000	<0.000
Canopy position	<0.000	<0.000
Day	<0.000	<0.000
Exposure hours *Canopy position*Day	<0.000	0.001
Rootstock*Canopy position*Day	0.002	0.004
Rootstock*Exposure hours *Canopy position*Day	0.698	0.915

Figure 6. Interaction between canopy position (sun-exposed and shaded), exposure (hours of stress treatment) and days (day 1 being the trial day with four days thereafter) on the development of visible peel damage on (a) covered and (b) uncovered surfaces of ‘Rosy Glow’ apples during Trial 2 in 2016. Sunburn was scored according to the Schrader and McFerson system with 0 indicating no damage and 5 indicating necrosis. Vertical bars denote 0.95 confidence intervals.

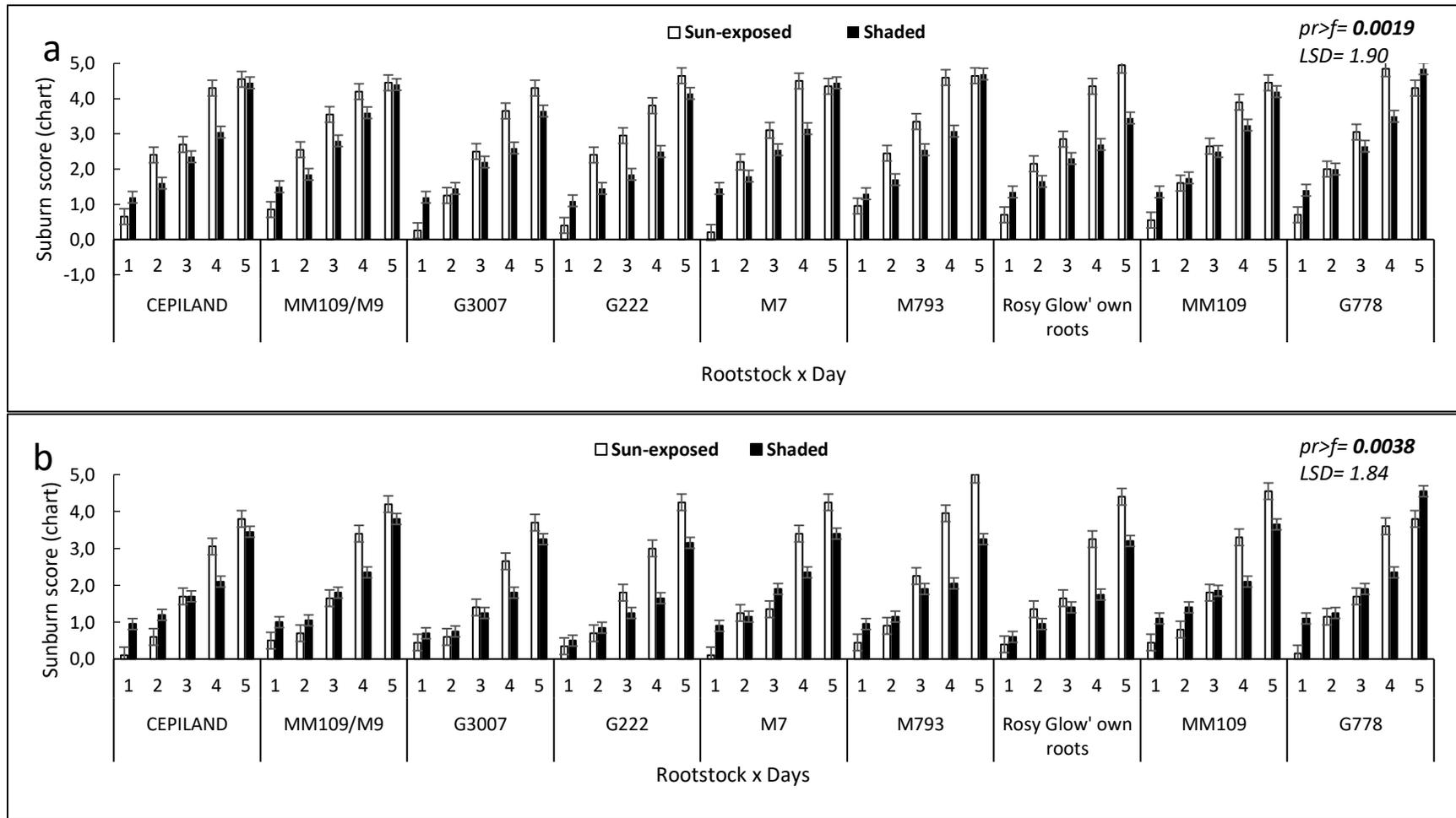
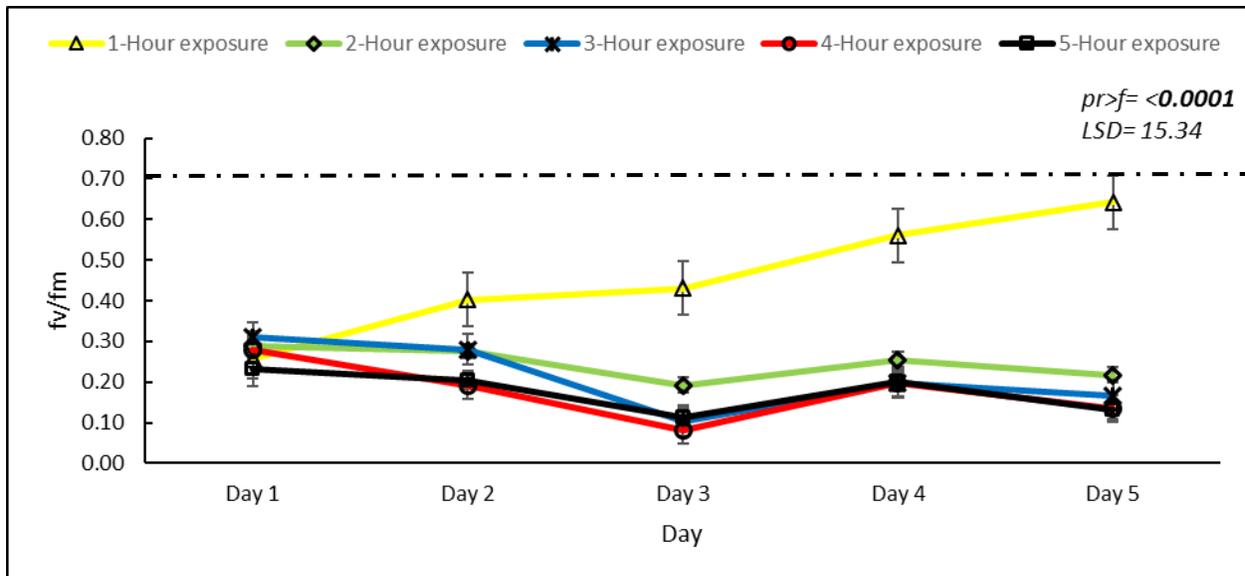
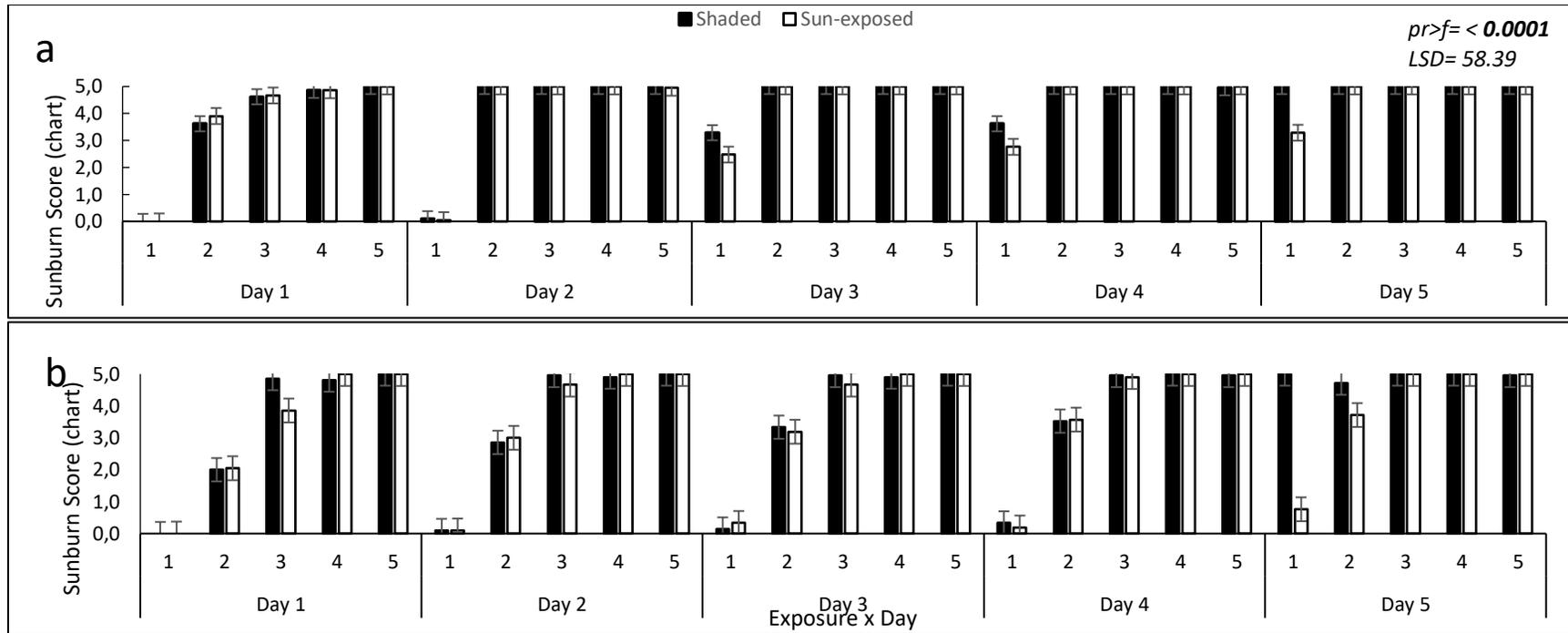


Figure 7. Interaction between rootstock, canopy position (sun-exposed and shaded) and recovery days on visible peel damage on (a) covered and (b) uncovered surfaces of ‘Rosy Glow’ apples during Trial 2 in 2016. Sunburn was scored according to the Schrader and McFerson system with 0 indicating no damage and 5 indicating necrosis. Vertical bars denote 0.95 confidence intervals.



Effect	<i>pr=f</i>
Rootstock	0.743
Exposure hours	<0.000
Canopy position	0.349
Day	0.004
Exposure hours *Day	<0.000
Exposure hours s*Canopy position*Day	0.932
Rootstock*Canopy position*Day	0.477
Rootstock*Exposure hours *Canopy position*Day	0.937

Figure 8. Interaction between exposure (hours of stress treatment) and days (day 1 being the trial day with four days thereafter) on the recovery of photosystems (F_v/F_m) in 'Golden Delicious' apples during Trial 1 in 2016. The dotted black line at $F_v/F_m = 0.7$ denotes a value at which photosystems can be considered to have recovered almost fully. Vertical bars denote 0.95 confidence intervals.



Effect	pr=f	
	Covered surface	Uncovered surface
Rootstock	0.427	0.982
Exposure hours	<0.000	<0.000
Canopy position	<0.000	<0.000
Day	<0.000	<0.000
Exposure hours *Canopy position*Day	<0.000	<0.000
Rootstock*Canopy position*Day	0.508	0.229
Rootstock*Exposure hours *Canopy position*Day	0.428	0.371

Figure 9. Interaction between exposure (hours of stress treatment), days (day 1 being the trial day with four days thereafter) and canopy position (sun-exposed and shaded) on the development of visible peel damage severity on (a) covered and (b) uncovered surfaces of ‘Golden Delicious’ apples during Trial 2 in 2016. Sunburn was scored according the Schrader and McFerson system with 0 indicating no damage and 5 indicating necrosis. Vertical bars denote 0.95 confidence intervals.

4. PAPER 2: EFFECT OF NINE APPLE ROOTSTOCKS OF VARYING VIGOUR ON THE ABILITY OF ‘ROSY GLOW’ APPLE (*Malus domestica* Borkh.) PEEL TO DEVELOP RED COLOUR UNDER DIFFERENT TEMPERATURE CONDITIONS

ABSTRACT

Poor colour of red and blushed apples at harvest remains one of the most important challenges facing the South African apple industry. This problem is attributable to above-optimal temperatures during peak red colour development in ripening fruit. Differing exposure to solar radiation with accompanying levels of fruit surface temperature during fruit development could lead to acclimation and differing responses of colour development (anthocyanin synthesis) to temperature. Rootstocks of varying vigour give rise to canopies with greater or lesser shading and may reduce fruit surface temperature of inner canopy fruit, providing a comparison for the study of such potential acclimation. However, rootstocks may also exert an innate influence on the development of red colour of fruit. ‘Rosy Glow’ on four dwarfing (Cepiland, MM109/M9, G3007, RN29) and four semi-vigorous to vigorous (G222, M793, M7, MM109) rootstocks, as well as the vigorous ‘Rosy Glow’ own roots were assessed for the development of red colour four weeks before commercial harvest in response to six different temperature treatments. Fruit were kept in the dark at 4°C for 72 hours to induce anthocyanin synthesis, before peel discs cut from the green side of fruit were subjected to temperature treatments ranging from 16°C to 31°C for 72 hours, together with moderately high visible light (between 550-650 $\mu\text{mol m}^{-2} \text{s}^{-1}$) to stimulate anthocyanin synthesis. The hue angle (indicating green/yellow to red colour) of peel discs was measured pre-treatment and post-treatment, and the absolute change (decrease) in hue angle determined. Generally, the lowest red colour development was observed under the 16°C temperature treatment, except for ‘Rosy Glow’ on own roots where it was high at this temperature. Red colour development for the rootstocks Cepiland, MM109, MM109/M9 and RN29 was consistently low under all temperature treatments. Colour development for the rootstocks G222, G3007, ‘Rosy Glow’ own roots and MM793 showed a good ability to develop red colour at 19°C, 22°C and 25°C. The rootstocks G222 and G3007 and the industry standard rootstock M793 showed the greatest red colour development at the high temperature treatment of 31°C. Results indicate a different optimum temperature range for different rootstocks in the red colour development of ‘Rosy Glow’ apples. Under warm daytime late-season conditions in South African

apple production regions, the new generation G222 and G3007 rootstocks showed the highest potential for good colour development following the induction of anthocyanin synthesis during cool nights, on a par with the current industry standard M793. Red colour development of ‘Rosy Glow’ apple is rootstock related and not related to vigour. Further research is required in different production locations to determine whether growing conditions and seasonal changes may influence the ability of rootstocks to promote colouration in blushed apple cultivars.

1. INTRODUCTION

The South African apple industry is export oriented (HORTGRO, 2016). In the 2014/2015 growing season over 44% of harvested fruit were exported (HORTGRO, 2016). Africa (31%), the United Kingdom (25%) and Far East and Asia (24%) are the major destinations of South African apples (PPECB, 2016). For the European Union, the intensity and quality of the red peel colour, together with fruit size, provides the basis for grading the standards of red and bi-coloured apples. Fruit colour remains a major quality factor in selecting fruit for markets, especially in red and blushed apple cultivars where it is an important consideration for consumers in purchasing fruit (Steyn 2012). This makes colour one of the most widely measured product quality attributes (Reay, 1999; Senthilkumar and Vijayakumar, 2014). As a result, poor red colour development of red or blushed fruit lead to low income for many orchards.

The development of red colour in red and blushed apples is attributed to the accumulation of anthocyanin pigments (Lancaster, 1992; Lancaster et al., 1994), which become prominent towards the end of maturity, at the onset of ripening (Senthilkumar and Vijayakumar, 2014). Plant genetics and developmental factors are the main factors affecting the anthocyanin biosynthetic pathway (Saure, 1990; Vimolmangkang et al., 2014). The process of induction and development of the anthocyanin pigment in apple is entirely dependent on light with an action maximum at 650 nm (Saure, 1990) and affected by temperature (Gouws and Steyn, 2014; Lancaster et al., 1994; Lin-Wang et al., 2011; Steyn, 2009). Light, however, is not a limiting factor to colour development of apples under South African climatic conditions (Pretorius and Wand, 2003), but can become limiting in shaded inside positions within larger canopies. Induction of anthocyanin biosynthesis in apple peel is triggered under low temperatures (Curry, 1997). Cool nights with temperatures of

10°C or lower are required to induce anthocyanins (Curry, 1997) whilst biosynthesis occurs at mild temperatures on detached apples (Curry, 1997; Reay, 1999). Poor red colour at harvest is attributed to insufficient anthocyanin accumulation during the ripening-associated peak in synthesis.

Moderate day temperatures consistently increase the rate of anthocyanin synthesis and colour development in apples (Curry 1997; Gouws and Steyn, 2014; Iglesias et al., 2002; Reay, 1999) whilst high temperatures generally produce poor red colour in apples. Faragher (1983) found that maximum anthocyanin accumulated in matured green ‘Jonathan’ apples at 12°C and maximum anthocyanin accumulation in ripe green fruit occurred between 16°C and 24°C. Green apple peels of various cultivars, harvested a few weeks before commercial maturity, have also been found to accumulate anthocyanin at 21°C - 25°C (Curry, 1997) and 19°C - 25°C (Gouws and Steyn, 2014). Anthocyanin accumulation and red colour development is also known to be cultivar dependent (Saure, 1990). Different cultivars used in the trials of both Curry (1997) and Gouws and Steyn (2014) showed different temperature optima for maximum red colour development. In addition, Gouws and Steyn (2014) found some cultivars to have different optimum temperature ranges for colour development, i.e. a broad temperature range for ‘Early Red One’ and a narrow range for ‘Fuji’ and ‘Cripps Pink’. They postulated that cultivars with a narrower optimum temperature range and a low optimum temperature are more likely to develop poor red colour in warmer production areas. Reaching optimum fruit colour in red and blushed apples remains a big challenge in warm apple producing areas as South African apple producing regions because of prevailing harsh and unpredictable climatic conditions (Reay, 1999; Wand et al., 2005). Inconsistent temperature fluctuations during the peak of anthocyanin synthesis and accumulation frequently result in poor red colour in red and blushed cultivars. However, the responses appear to be cultivar-specific (Saure, 1990). Gouws and Steyn (2014) postulated that climatic conditions experienced by the fruit during fruit development may shift the optimum temperature for red colour development. This can affect the potential anthocyanin synthesis and the final red colour on fruit at harvest.

The South African climate and soil conditions remain a major limiting factor in the selection of rootstocks for apple trees (Costa and Stassen, 2011; Voigt and Stassen, 2014). Rootstocks are selected to maximise higher and earlier production of fruit and ability to efficiently manage pest

and diseases. The industry relies heavily on the semi-vigorous M793 rootstock, as well as semi-dwarfing M7 and more vigorous MM109 to a smaller degree. There are several rootstock evaluation trials currently ongoing to select new rootstocks best suited to the South African apple industry (Costa and Stassen, 2011; Voigt, 2014). Some of the new generation Geneva rootstocks have proven successful compared to current industry standards in terms of yield efficiency, and a small number have been commercialized, with others still under evaluation (Voigt, 2014). Rootstocks are known to modify and influence tree (scion) attributes (Jensen et al., 2003; Robinson et al., 2003). Rootstocks may improve apple fruit quality (Autio, 1991; Autio et al., 2011; Fallahi et al., 2002; 2012) and red colour development in pears (Roberts et al., 2008) and apples (Autio and Southwick 1993). Geneva rootstocks influence and confer unique qualities to the scions (Jensen et al., 2003; Robinson et al., 2003). Generally, rootstocks are believed to influence some internal and external quality parameters in fruit (Castle 1995; Fallahi et al. 2002). Though rootstock may directly influence the innate potential for red colouration in apples (Autio and Southwick, 1993), the effect of rootstock on fruit colour development could also relate to the canopy structure and how this determines the light and temperature environment of the fruit during its developmental stages. Fruit may also acclimate to the growing conditions by adjusting the temperature optimum for anthocyanin synthesis, which may in turn affect their ability to develop better red colour at the later stages of fruit development (Steyn et. al., 2004; Gouws and Steyn, 2014).

As efforts are put in place to select a range of new generation rootstocks that will best suit the South African production environment, it is important to assess how various rootstocks affect the ability of apple peel to develop red colour in a warm production area such as South Africa. Our research focused on determining whether rootstocks of differing vigour influence the ability of ‘Rosy Glow’ apples (a better colouring clone of ‘Cripps Pink’) to develop red colour, under a wide temperature range, four weeks before commercial harvest. We relate this to on-tree fruit surface temperature and red colouration during the same period, and red colouration and fruit maturity at harvest.

2. MATERIALS AND METHODS

2.1. Plant material

'Rosy Glow' (RG) apples were collected from a rootstock evaluation trial site on the farm Paardekloof in the Witzenberg valley (33°15'40"S 19°15'55"E). The soil at this site is sandy-loam and the region has a Mediterranean-type climate typical of the Western Cape Province of South Africa. 'Rosy Glow' is a high-coloured spontaneous, single bud mutation of 'Cripps Pink', which can also be marketed under the Pink Lady trade mark when it meets the quality criteria, and is thought to give better fruit colour in warmer growing areas.

The rootstock evaluation trial was established in 2010 using RG budded onto various new dwarfing, semi-vigorous and vigorous apple rootstocks from the Geneva range as well as M793 (the industry standard), M7 and MM109. The trees were planted at two sites; 1) a more "dwarfing" planting consisting of four blocks of ten rootstocks of varying vigour from dwarfing to semi-vigorous; and 2) a more "vigorous" planting of eight rootstocks ranging from semi-vigorous to very vigorous. The two sites were managed separately and slightly differently according to the different vigour ranges; for example, tree spacing at the more dwarfing planting was 1.25 m, whereas at the more vigorous planting it was 1.5 m. At both sites 'Royal Gala' was used as a pollinator. The rootstocks Cepiland, G3007, 'Rosy Glow' own roots, Lancep, MM106, MM109/M9, RN29 and M7 were included at the more dwarfing site, whilst the rootstocks G934, G228, G778, M25, MM109 and Maruba were included at the more vigorous site. G222 and M793 were also included at both sites as internal controls. The trials were laid out in a randomised complete block design consisting of five (more dwarfing planting) and four (more vigorous planting) blocks with three trees in each treatment plot (10 more dwarfing and 8 more vigorous plots). In some treatment plots, only one or two trees were available due to the prior identification of RG scion rooting and naming mistakes in the nursery. Average monthly maximum and minimum air temperatures recorded at a weather station near the trial site are presented in Figure 1.

For this trial, the dwarfing/semi-dwarfing rootstocks Cepiland, G3007, MM109 with an M9 interstem (MM109/M9) and RN29 were used, as well as the semi-vigorous G222, M7, and the vigorous rootstocks M793, MM109 and 'Rosy Glow' own roots (originally thought to be the dwarfing rootstock G6210). All the rootstocks used in this trial were located on the more dwarfing

rootstock site except fruit from MM109, which were sampled from the more vigorous site. Apples were picked at random before 1100 HR from the eastern side of the row on 11 March 2016. Fifty-four apples were picked from the inner canopy of the nine rootstocks. Only apples with a green side were chosen (showing no red colour development). Fruit were placed on ice in a cooler bag for transport to the laboratory of the Department of Horticultural Science at the University of Stellenbosch, and stored in the dark at 4°C for 72 hours to induce anthocyanin synthesis (Curry, 1997).

On 14 March 2016, 12 peel discs (15 mm in diameter, 5 mm thick) per rootstock were punched from the green side of the apples to yield a total of 108 discs and these were used as follows: 1 peel disc per rootstock per temperature treatment replicated twice. Discs were randomly placed on the 12 Peltier plates (5.0 cm x 6.5 cm) of a 'Celtec' apparatus, constructed according to the design of Burke and Mahan (1993), with an H₂O-moistened filter paper between each disc and the plate. Each Peltier plate was covered with thin (0.5 mm) 100% crystal clear polyethylene wrap (Glad Wrap™, Glad South Africa, Randburg, South Africa). A few holes were made in the plastic with a toothpick to prevent the build-up of CO₂ and, possibly, ethylene, and to reduce the condensation of water on the inside of the plastic.

2.2. Temperature treatments

Set temperatures of 16°C, 19°C, 22°C, 25°C, 28°C and 31°C were randomly assigned to the 12 Peltier plates of the Celtec. The Celtec was stationed in a growth chamber set at 12°C with two overhead lamps (400W High Pressure Sodium; SON-T; Osram GmbH, Munich, Germany) providing irradiance of 550 to 650 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux (PPF) as measured with a quantum meter (LI-189; Li-Cor, Lincoln, Nebraska, USA) at disc level. Disc temperature was measured with an infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA) to ensure that the required temperatures were maintained. Temperatures of peel discs on each plate, as well as the moisture level of the filter paper on which discs were placed, were monitored at frequently (least twice daily) to ensure that they stayed in the required state.

2.3. Colour measurement

Disc hue angle was measured with a chromameter (NR-3000; Nippon Denshoku, Tokyo, Japan) before (pre-treatment) and again 72 hours after placement on the Celtec (post treatment). Hue angle (0° = red-purple, 45° = red, 90° = yellow, 120° = yellow-green) was used to express the differences

in colour development over time. Colour development was recorded as the change in hue angle by subtracting the post-treatment value from the pre-treatment value.

2.4. In-field fruit colour and maturity

These data were collected as part of a larger study of an extended set of rootstocks (as reported in Paper 1) growing at two sites. We did not perform a different statistical analysis for this study on the selected rootstocks but highlight the rootstocks used at the more dwarfing planting and the more vigorous planting.

In-field fruit colour and FST

Fruit surface temperatures and fruit peel colour measurements were conducted on trees *in situ* on 8 January 2016 and 10 March 2016. Fruit surface temperatures were measured using an infra-red thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA). Temperature measurements were taken on the exposed surface of sun-exposed fruit. Fruit peel colour (hue angle) was also measured on the exposed surface of the sun-exposed side of the fruit using a chromameter (NR-3000; Nippon Denshoku, Tokyo, Japan). Measurements were conducted on three fruit per rootstock treatment. All measurements were conducted between 09:00 and 12:00 on the eastern side of the tree row.

Fruit maturity assessment

On 05 April 2016, a day before commercial harvest, 30 fruit (15 fruit each from the western and eastern sides of the rows) were harvested from each rootstock plot. Fruit were stored overnight in the fruit maturity indexing and quality assessment laboratory of the Department of Horticultural Science, Stellenbosch University, and assessed the following day.

Percentage foreground red colour and ground colour were assessed using colour intensity and ground colour charts (Unifruco Research Services). Percentage starch conversion was estimated using the iodine test with a starch conversion chart (Unifruco Research Services). A composite juice sample was prepared from 20 pooled fruit by cutting a slice on both sides of each fruit from both eastern and western sides of the tree row and blending the pieces in a liquidizer (AEG Electrolux, Type JE-107 no. 91100085/ PNC 950075206, P.R.C). The juice was used in determining total soluble solids (TSS (%)) and total malic acid equivalent acidity (TA). TSS was measured using a calibrated hand-held refractometer (TSS 0-32%, Model N1, Atago, Tokyo,

Japan). TA was measured using an automated titrator (Tritino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland) by titrating 5 g of juice with 0.1 M NaOH to a pH of 8.2.

2.5. Statistical analysis

Ecophysiological data were analysed as a two-way ANOVA with the General Linear Models (GLM) procedures of XLSTAT statistical package (Addinsoft 1995-2016, Version 2016.03.30882). Maturity index data were subjected to a two-factor ANOVA with rootstock and side (Eastern or Western) as factors using XLSTAT statistical package (Addinsoft 1995-2016, Version 2016.03.30882). Both ecophysiological and maturity data analyses were conducted for the full set of rootstocks at each site (more dwarfing and more vigorous) – as discussed in Paper 1 – and the rootstocks used in the laboratory trial highlighted in the results table.

Hue angle data was analysed as a one-way ANOVA with the General Linear Models (GLM) procedures of XLSTAT statistical package (Addinsoft 1995-2016, Version 2016.03.30882).

3. RESULTS

3.1. Influence of temperature on colour development

There was rootstock-treatment interaction ($p < 0.0001$) on the change in hue angle of the fruit peel discs (Figure 2a and b). The different rootstocks responded differently to the various temperature treatments (Figure 2b). The highest change in hue angle was observed for the Geneva rootstocks G3007 and G222 and the industry standard M793 under temperature treatments 19°C to 31°C. However, change in hue angle was consistently low for rootstocks Cepiland, MM109, MM109/M9 and RN29 under all temperature treatments (Figure 2b).

The 16°C temperature treatment generally resulted in the lowest average change in hue angle (development of colour from green/yellow to red) measured for all rootstocks, except for ‘Rosy Glow’ own roots (Figure 2b). The highest average change in hue angle for all rootstocks was measured under temperature treatments 19°C to 28°C and sometimes including 31°C.

The highest change in hue angle under the 16°C temperature treatment was measured on peel discs from ‘Rosy Glow’ on own roots, with the lowest change in hue angle measured on peel discs from rootstocks Cepiland, MM109/M9, MM109, G3007 and RN29 (Figure 2a). Under the 19°C temperature treatment, the highest change in hue angle was measured on peel discs from rootstocks M793 and G3007. The lowest change in hue angle under 19°C temperature treatment was measured on rootstock MM109, Cepiland and M7 (Figure 2a).

Under the 22°C temperature treatment, peel discs of rootstock G3007 and ‘Rosy Glow’ on own roots recorded the highest change in hue angle. The lowest hue angle change was measured on peel discs from rootstocks Cepiland, MM109/M9, RN29, MM109 and M7 (Figure 2a). The highest change in hue angle measured under the 25°C temperature treatment was on peel disc from rootstock G222, followed by G3007, M7 and ‘Rosy Glow’ own roots. However, the lowest change in hue angle under the 25°C temperature treatment was measured on peel disc from rootstocks Cepiland, MM109, MM109/M9 and RN29 (Figure 2a). The change in hue angle on peel discs was generally low for all rootstocks under the 28°C temperature treatment, with only rootstocks G3007 and M793 recording a high change (Figure 2a). The lowest change in hue angle under the 28°C temperature treatment was measured on peel discs from rootstocks Cepiland, G222, MM109/M9 and RN29. The highest change in hue angle under the 31°C temperature treatment was measured

on peel discs from the semi-vigorous M793 rootstock. Peel discs from rootstocks G222 and G3007 also recorded a considerable change in hue angle as compared to the remaining rootstocks; however, the lowest change in hue angle under the 31°C temperature treatment was recorded on peel discs from rootstocks Cepiland, M7 and RN29 (Figure 2a).

For rootstock G3007 the highest change in hue was obtained between temperature treatment 19°C and 31°C with higher changes observed at 22°C and 28°C. There was a gradual increase in change in hue on G222 rootstock from 16°C until it peaked at 25°C before decreasing to 31°C. Change in hue for M7 was high only at 25°C. Change in hue for M793 was high under all temperatures except 16°C. There was a general higher change in hue from of 16-25°C but low under 28 and 31°C on fruit from ‘Rosy Glow’ own roots (Figure 2b).

3.2. In-field fruit surface temperature (FST), fruit colour and maturity

For these parameters, we use results from the 2016 in-field and maturity index data presented in Paper 1. In Tables 1-3 we present the results for all rootstocks in the evaluation trial due to the separate statistical analyses conducted between the more dwarfing and more vigorous plantings. We took this approach for the in-field measurements owing to the different planting and management approaches between the two planting sites, which could influence the results.

There were no significant differences in FST and hue angle between RG fruit from the various rootstocks measured *in situ* on 8 January and 10 March 2016 (Table 1).

At harvest, no rootstock-side (Eastern and Western side of tree row) interaction occurred in foreground and ground colour at both the more dwarfing and more vigorous rootstock site, and the side main effect was also not significant whereas the rootstock main effect was significant, and thus only rootstock effects are presented below (Table 2).

At the dwarfing rootstock site, fruit from ‘Rosy Glow’ “own roots” recorded significantly less foreground colour than all other rootstocks except M793. Fruit from rootstock G222 produced less green ground colour than fruit from all rootstocks except G3007. The ground colour recorded on G3007 was also less green than Lancep, Cepiland and RN29 (Table 2). At the more vigorous rootstock site, a greater extent of red colour was recorded on G222 than all rootstocks except M793, G228 and M25. The red colour recorded on the latter three rootstocks was also significantly higher than Maruba (Table 2). Less green ground colour was recorded on fruit from rootstock

G222 than all rootstocks except G228 and G934. The green colour recorded on G228 was less than M793 and Maruba whilst that of G934 was less than Maruba.

Fruit from ‘Rosy Glow’ own roots recorded the lowest percentage foreground red colour, which was significantly different to fruit from all other rootstocks used in this trial (Table 2). Fruit on rootstocks G3007 and G222 possessed more yellow ground colour than those from rootstocks MM109/M9, M7, M793 and ‘Rosy Glow’ own roots (Table 2), but similar to RN29 and Cepiland. The ground colour of fruit from rootstock MM109 was similar to that recorded on MM109/M9, M7, M793 and ‘Rosy Glow’ own roots.

For internal quality parameters at the more dwarfing site, fruit from rootstock MM106 recorded higher starch conversion values than fruit from all rootstocks except Cepiland and M793 at the more dwarfing rootstock site (Table 3). The starch conversion value for Cepiland was significantly higher than those from MM109/M9, RN29, M7, G3007 and ‘Rosy Glow’ own roots whilst starch conversion measured in M793 was higher than ‘Rosy Glow’ own roots. Higher TSS values were recorded for RN29 and G222 than M7, M793 and ‘Rosy Glow’ own roots. TSS for G3007 was also higher than M7. TA values measured for fruit on rootstock Cepiland, MM106 and M7 were significantly higher than those measured on fruit from rootstocks G3007, RN29 and ‘Rosy Glow’ own roots (Table 3). The TA value for Lancep was also higher than G3007, RN29 and ‘Rosy Glow’ own roots. There was, however, no marked difference between the TSS of fruit between all rootstocks at the more vigorous rootstock site (Table 3). Starch conversion values for fruit from rootstock M25 were significantly higher than fruit from all other rootstocks except for the Maruba rootstock. Starch conversion for the Maruba rootstock was higher than G222, G228 and G778. Starch conversion for the rootstock G222 was also higher than G778. The TA content of fruit from rootstock Maruba was significantly higher than rootstocks G778, G222 and G228. TA of M25 was also higher than G222 and G228 (Table 3).

Fruit from ‘Rosy Glow’ own root recorded the lowest values for starch conversion which were significantly lower than those recorded on Cepiland, G222 and M793 (Table 3). Starch conversion values for rootstock MM109 were in the range similar to those recorded on rootstocks G222. For total soluble solids, highest values were measured on fruit from rootstocks G222 and RN29 which were significantly higher than values measured on rootstocks M7, M793 and ‘Rosy Glow’ own root, but similar to those for G3007, Cepiland and MM109/M9 (Table 3). TSS of MM109 fruit

were also similar to those from rootstocks M793, Rosy 'Glow own' roots and M7. Titratable acids values measured in fruit on rootstock Cepiland and M7 were significantly higher than those measured in fruit from rootstocks G3007, RN29 and 'Rosy Glow' own roots (Table 3). TA values for MM109 fruit were similar to those recorded on G3007, RN29 and 'Rosy Glow' own roots.

4. DISCUSSION

Low night and mild day temperatures are considered very important for anthocyanin induction and synthesis, respectively, in apple peel. As such, high temperatures experienced in warm production areas lead to poor red colour development of red and bicoloured apples (Reay, 1999; Gindaba and Wand, 2005). Results from this trial indicate that rootstock attributes may modify and influence the colouring ability of RG apples under differing climatic conditions. Red colour development of peel discs varied between different rootstocks under constant light but different temperature treatments. Apart from the 16°C temperature treatment, all the other temperature treatments (19°C, 22°C, 25°C, 28°C and sometimes also 31°C) favoured colour development of fruit on all rootstocks (with some outliers). This indicates that consistent daytime temperatures between 19 °C and 31°C can generally enhance colour development in RG apples. Gouws and Steyn (2014) reported that some cultivars such as Royal Gala had a broad effective temperature range for colour development. Our results indicate that RG is a cultivar that has a broader optimum temperature range for red colour development compared to 'Cripps Pink' from which it originated. However, the details of this response depend on the rootstock used.

Curry (1997) found that different cultivars had distinct optimum temperatures for anthocyanin accumulation; however, all cultivars assessed had an optimum within 20 - 25°C at which the accumulation of anthocyanin was highest. In a previous study by Gouws and Steyn (2014), using different apple cultivars from two production regions in South Africa, it was also found that temperatures from 19°C - 25°C favoured anthocyanin accumulation and peel colour development. Gouws and Steyn (2014) found that 'Early Red One' developed red colour over a broad temperature range whilst 'Cripps' Pink' had a narrower optimum temperature range for good colour development. In this study, RG which is a mutation of 'Cripps' Pink', showed a broader optimum temperature range colour development and showed considerable colour development under all temperature treatments on different rootstocks. This indicates that the mutation is better

able to develop red colour at a broader temperature range including high temperatures. Faragher (1983) reported the optimum range for red colour development in green mature Jonathan' apples to be in the range of 16 to 24°C. Our study showed that the temperature treatments of 19°C, 22°C, 25°C, 28°C and 31°C generally favoured the greatest colour development of RG on various rootstocks, which indicates that RG apples will produce redder hues in climates experiencing such temperatures during the ripening stage. It is, however, important to note that detached apples have a higher temperature optimum for anthocyanin synthesis, which may be a result of accelerated ripening, than attached fruit (Ritenour and Khemira, 1997). As such, the optimum temperatures for red colour development of RG apples *in situ* should be lower than the temperatures reported in this trial.

Peels from 'Rosy Glow' on own roots showed a greater development of red colour at 16°C compared to all other rootstocks. Fruit on 'Rosy Glow' own root were the reddest at a lower temperature range of 16°C-25°C, whilst red colour development was lower at the high temperatures of 28°C and 31°C. Blush colour at harvest was, however, lower on 'Rosy Glow' own roots than all the other rootstocks. Full harvest maturity data collected on the site by Sibozza et al. (2016) also indicates lower blush on 'Rosy Glow' own roots at harvest. FST and Hue angle measured on all rootstocks prior to the lab trials did not yield any distinct difference between the rootstocks. Therefore, the poor blush colour of 'Rosy Glow' own roots at harvest and the ability of the peels to develop colour under lower temperatures in the laboratory may be due to individual characteristics of the rootstock and may also be maturity related since fruit from this rootstock were less mature at harvest (Table 3).

Unlike the findings by Roberts et al. (2008) and Autio and Southwick (1993), who showed that dwarfing rootstocks (low vigour) produced redder colour in pears and apples, respectively, only one (G3007) out of the four dwarfing rootstocks used in this trial recorded a significantly greater ability to develop red peel colour in the lab. The remaining dwarfing rootstocks (Cepiland, MM109/M9 and RN29) showed a lower ability to produce red colour under all temperature treatments, as did the semi-vigorous MM109. The remaining semi-vigorous rootstocks including 'Rosy Glow' own roots showed high colour development under different temperature treatments. These colour development patterns show that rootstock effect on colour development of RG apples is not transferred via vigour but is rather intrinsic. It is possible that other factors may have

accounted for such observations. Reay and Lancaster (2001) indicated that apart from temperature, factors such as maturity and previous exposure to light are major modifying factors in the accumulation of anthocyanin in ‘Gala’ and ‘Royal Gala’.

Colour development of fruit on ‘Rosy Glow’ own root was generally high within the range of 16°C to 25°C but lower under high temperatures of 28°C and 31°C. High red colour development on rootstock M7 was only attained under 25°C. Similar to Gouws and Steyn (2014), we believe that colour development of fruit on each rootstock is not restricted to a single temperature optimum but that each rootstock may perform well under a certain temperature range. Results from our trials show a narrower range for the rootstock M7 (centred on 25°C), and a broader range (19-31°C) for rootstocks G3007, M793, G222, Cepiland, MM109, MM109/M9 and RN29.

Red colour development in apples is maturity-related (Marais et al., 2001) and highest red colour develops during the ripening of fruit. Previously, Autio (1991) found that there was a rootstock effect on the ripening of ‘Golden Delicious’ apples during a four-year rootstock trial. Costa and Stassen (2008) did not find any marked maturity differences at harvest between a similar set of rootstocks as in this trial, with ‘Golden Delicious’ as the scion. In our study, analyses of RG fruit maturity at harvest in 2016 (April) yielded significant effects of rootstocks. Apples from the more dwarfing rootstocks RN29, G3007 and Cepiland and the semi-vigorous G222 showed more advanced maturity than fruit from other rootstocks in this trial, based on foreground (although not significant) and ground colour and TSS values. This is supported by the full harvest fruit quality (colour) and maturity data presented in Sibozza et al. (2016). Of these four rootstocks mentioned above, only two (G3007 and G222) showed a higher increase in colour development in RG apples in the lab than Cepiland and RN29. This could indicate an inherently high colouring ability in the Geneva rootstocks one month before harvest, with maturation and red colour development peaking later in RN29 and Cepiland. Since the difference between the time of fruit picking for this trial and the time of harvest was about four weeks, harvest maturity may not fully explain the effects of maturity on colour development at the time of this trial. Determining the maturity of RG apples on the various rootstocks prior to the colour development trial would provide a clear indication of whether maturity has a role to play in the innate ability for peel colour development.

5. CONCLUSION

There was no evidence to show that rootstock vigour influenced red colour development in RG apples. The development of red colour of the fruit was distributed across the vigour classes indicating that colour development in this trial was due to rootstock related factors and not specifically to vigour class. The ability of rootstock G3007 and G222 to develop better red colour compared to the other rootstocks used in this trial indicates that colour development of these Geneva rootstocks could be maturity related. The results of this study indicate rootstock-specific optimum temperature ranges for red colour development of blushed apple peels. It was identified that whilst M7 had a narrower temperature optimum (ca. 25°C), all other rootstocks had a broad optimum range (19-31°C) for colour development of RG apples.

Further investigation in different production areas and different seasons is required to verify these findings. For future studies, a wider range of Geneva rootstocks with more replications can be evaluated, taking into account fruit maturity before trials, and trials can be repeated over a period of time during fruit development.

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TABLES AND FIGURES

Table 1. Fruit surface temperature (FST) and hue angle for ‘Rosy Glow’ at the dwarfing and semi-vigorous rootstock sites, measured *in situ* on 8 January and 10 March 2016. N=5 at the dwarfing site and N=6 at the semi-vigorous site. Ambient air temperature at start of measurement was 28.4°C and 26.8°C on 8 January and 10 March, respectively. This data was collected as part of a larger study on a wider range of rootstocks (presented in Paper 1), and is presented separately for each planting site owing to differences in planting and management between the sites, which may influence in-field measurement of FST and hue angle. The rootstocks used in this study are indicated in bold. Means in columns were separated by LSD at 5%.

Rootstock	Fruit surface temperature (°C)		Hue (°)	
	Jan	March	Jan	March
More dwarfing site				
Lancep	28.6 ns	30.5 ns	133.5 ns	80.7 ns
Cepiland	32.1	31.7	129.7	76.2
MM109/M9	31.7	33.6	118.3	76.1
G3007	31.8	34.0	128.7	68.1
RN29	30.4	32.4	123.8	75.6
G222	31.8	33.8	131.3	69.5
MM106	28.8	32.0	141.8	73.9
M7	30.6	30.5	142.3	75.0
M793	28.5	30.6	121.3	73.9
‘Rosy Glow’ own roots	29.0	32.8	134.3	69.5
<i>Pr=f</i>	0.168	0.664	0.757	0.763
More vigorous site				
G222	30.0 ns	29.3 ns	113.3 ns	80.1 ns
M793	28.5	28.4	110.7	71.7
G228	30.0	27.4	111.0	75.9
MM109	28.6	27.0	111.2	75.0
G778	29.3	30.0	109.4	77.1
M25	26.9	28.9	111.1	77.8
G934	28.9	27.9	109.5	73.7
Maruba	26.3	28.9	113.2	73.8
<i>Pr=f</i>	0.116	0.413	0.462	0.859

Table 2. Percentage (%) foreground colour and background colour of ‘Rosy Glow’ apples at the dwarfing and semi-vigorous rootstock sites at harvest in 05 April 2016. N=5 for the dwarfing site, N=6 for the semi-vigorous site. This data was collected as part of a larger study on a wider range of rootstocks (presented in Paper 1), and is presented separately for each planting site owing to differences in planting and management between the sites, which may influence in-field measurement of fruit colour. The rootstocks used in this study are indicated in bold. Values are means for fruit harvested on the eastern and western side of the row. Means in columns were separated by LSD at 5%.

Rootstock	% Foreground colour (chart)*		Background colour (chart)**	
More dwarfing site				
Lancep	72.1	a	3.61	bc
Cepiland	76.2	a	3.62	bc
MM109/M9	70.7	a	3.51	c
G3007	76.8	a	3.80	ab
RN29	75.7	a	3.61	bc
G222	76.7	a	3.83	a
MMI06	75.0	a	3.52	c
M7	70.6	a	3.54	c
M793	70.4	ab	3.51	c
‘Rosy Glow’ own roots	63.7	b	3.53	c
<i>Pr=f</i>				
<i>Rootstock</i>	0.004		<0.000	
<i>Side</i>	0.798		0.688	
<i>Rootstock*Side</i>	0.668		0.984	
More-vigorous site				
G222	77.8	a	3.83	a
M793	72.9	ab	3.51	cd
G228	73.1	ab	3.71	ab
MM109	68.6	bc	3.52	bc
G778	68.7	bc	3.52	bc
M25	73.3	ab	3.53	bcd
G934	70.6	bc	3.63	abc
Maruba	66.9	c	3.34	d
<i>Pr=f</i>				
<i>Rootstock</i>	0.009		< 0.000	
<i>Side</i>	0.866		0.943	
<i>Rootstock*Side</i>	0.921		0.987	

*Estimating the percentage blush on fruit using colour charts (from 5 to 100= full blush)

** Background colour: comparing the green side of fruit with the colour chart (0.5 = green to 5 = yellow)

Table 3. Fruit maturity parameters of ‘Rosy Glow’ apples from dwarfing and semi-vigorous rootstocks at harvest 05 April 2016. N=5 for the dwarfing site, N=6 for the semi-vigorous site. Values are for pooled samples of fruit harvested from both the eastern and western side of the row. This data was collected as part of a larger study on a wider range of rootstocks (presented in Paper 1), and is presented separately for each planting site owing to differences in planting and management between the sites, which may influence measurement of fruit maturity. The rootstocks used in this study are indicated in bold. Means in columns were separated by LSD at 5%.

Rootstock	Starch conversion (%)	TSS (% Brix)	Titrateable acidity (% malic acid eq.)
More dwarfing site			
Lancep	67.7 a	15.2 abc	0.75 ab
Cepiland	67.0 ab	15.4 ab	0.77 a
MM109/M9	58.7 cde	15.1 abc	0.74 abc
G3007	55.3 de	15.4 ab	0.70 c
RN29	57.7 cde	15.7 a	0.70 c
G222	61.0 bcd	15.9 a	0.74 abc
MMI06	68.5 a	15.1 abc	0.78 a
M7	57.7 cde	14.5 c	0.76 a
M793	64.1 abcd	14.7 bc	0.74 abc
‘Rosy Glow’ own roots	53.4 e	14.7 bc	0.70 c
<i>Pr=f</i>	<0.0001	0.029	0.006
More-vigorous site			
G222	54.4 cd	15.8 NS	0.68 d
M793	55.7 bcd	15.1	0.76 abc
G228	49.9 de	15.1	0.73 cd
MM109	60.1 bc	14.8	0.77 abc
G778	46.8 e	15.0	0.73 bcd
M25	68.9 a	15.4	0.80 ab
G934	56.5 bcd	15.2	0.76 abc
Maruba	62.2 ab	14.7	0.81 a
<i>Pr=f</i>	< 0.0001	0.212	0.007

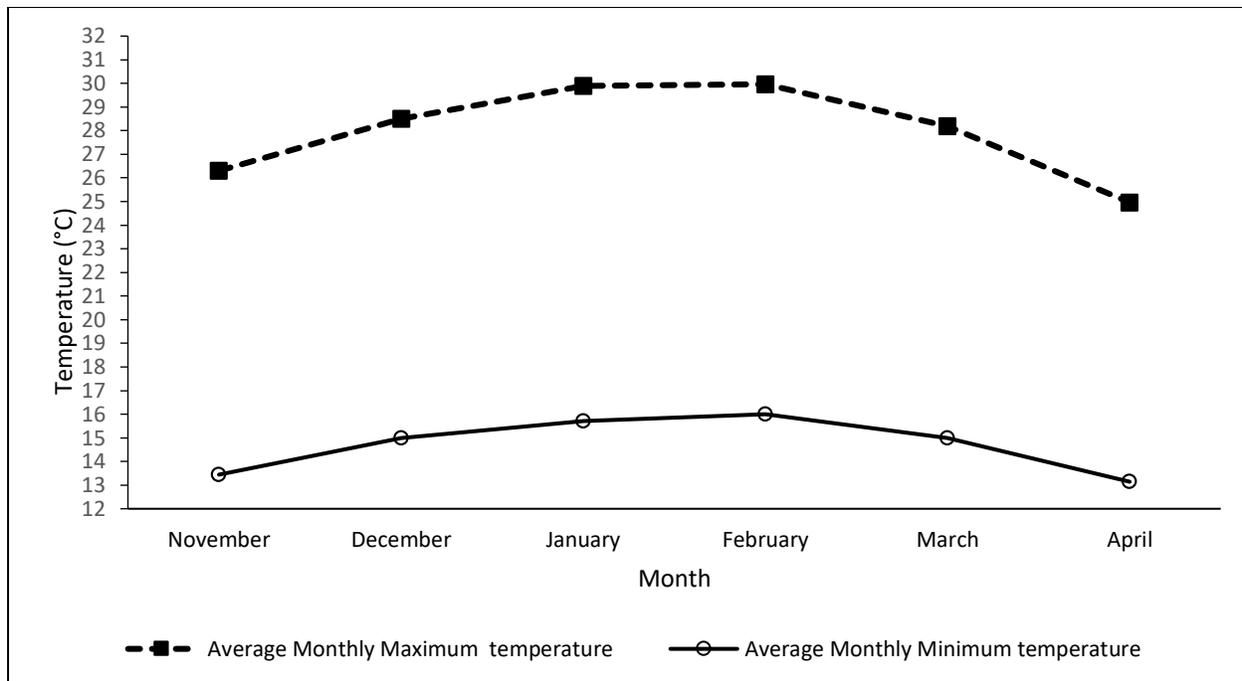


Figure 1. Average maximum and minimum air temperatures recorded for November 2015 to April 2016 at the Paardekloof weather station (latitude: 33° 26' S, longitude: 19° 26' E, altitude 878 m). Data source: Agricultural Research Council.

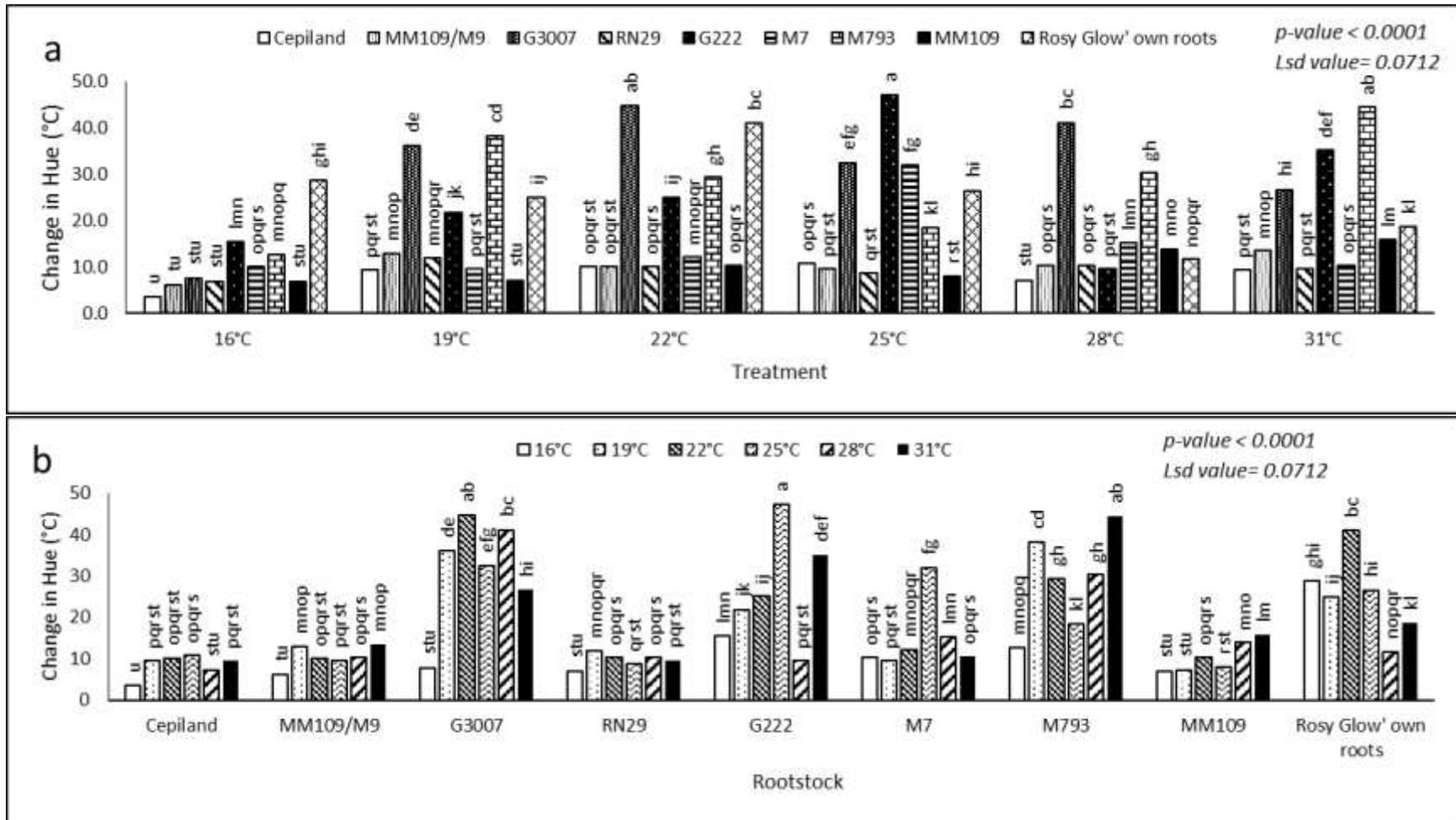


Figure 2. Interaction between 9 rootstocks (Cepiland, G222, G3007, ‘Rosy Glow’ own roots, M7, M793, MM109, MM109/M9 and RN29) and 6 different temperature treatments (15 °C, 18 °C, 22 °C, 25 °C, 28 °C and 31 °C) on the change in hue angle of ‘Rosy Glow’ apple peel discs after 72 hours of exposure to the different temperature treatments. N=2. Letters denote significant difference at 5% confident interval.

5. PAPER 3: RED COLOUR DEVELOPMENT OF ‘CRIPPS PINK’ APPLES UNDER DIFFERENT FRUIT MICROCLIMATIC CONDITIONS

ABSTRACT

Red colour development in apples can be insufficient under South African climatic conditions. During the warm summer months, a cool period followed by another warm period has led to a muted red colour response at harvest. This could suggest an acclimation of anthocyanin biosynthesis to lower temperatures during fruit development leading to a sub-optimal anthocyanin synthesis during a warm pre-harvest period. We tested the hypothesis that acclimation of anthocyanin biosynthesis to reduced peel temperatures during fruit development (simulating cooler weather) negatively influences the colour of ‘Cripps Pink’ apples at harvest. In 2016, we employed four different fruit cooling treatments from mid-January to mid-April, mid-January to mid-February, mid-February to mid-March and mid-March to mid-April, and compared them to a control treatment of no cooling. Peel red colour (hue angle) and relative change in hue angle were monitored over the course of the trial from mid-January to mid-April (harvest). There was no negative effect of cooling on the colour of ‘Cripps Pink’ apples at harvest, indicating a lack of acclimation to reduced peel temperatures. Results indicate that cooling at all periods from mid-January to mid-March was successful in decreasing the hue angle at harvest. However, the benefit of redder peel (hue angle) was reduced when early cooling from mid-January was stopped in mid-February whilst air temperatures were still high. Although the red peel colour of apples under this treatment at harvest was not as good as the red colour under the uninterrupted cooling treatment, it was still better than the control treatment. Uninterrupted cooling (mid-February to mid-April) and cooling from mid-February to mid-March were the most beneficial treatments for red colour at harvest (lowest hue angles). Although these treatments had slightly higher red foreground colour coverage than the other treatments, the differences were not statistically significant. The two treatments incorporating early cooling in mid-January to mid-February led to lower fruit total soluble solids at harvest, whereas the two treatments incorporating late cooling in mid-March to mid-April had the lowest titratable acidity. The findings suggest that cooling of fruit using evaporative cooling did not acclimatise fruit to below optimal temperatures for red colour development under South African weather conditions. However, treatment differences might also be due to decreased heat-induced anthocyanin degradation in treatments that received cooling.

Further research is needed to understand red colour development in apples under variable microclimatic conditions in different production locations.

1. INTRODUCTION

Colour is a critical quality factor used in harvesting and marketing of many fruit. Anthocyanins and other pigments play an essential part in the attractiveness and potential income from fruit (Steyn, 2012; Senthilkumar and Vijayakumar, 2014). Production of blushed apples in the warm climate of South Africa is problematic in some seasons characterised by mid-season cold fronts and subsequent warm periods before harvest, with suggestions that red colour at harvest is compromised by such fluctuations and possible acclimation of the anthocyanin biosynthetic pathway.

Generally, colour development of exposed apple peel is induced under low temperatures (Saure, 1990; Curry, 1997). Temperatures of about 10°C or lower are required for anthocyanin induction in apples (Curry, 1997), whilst subsequent synthesis and accumulation occurs under mild temperatures of about 19 to 25°C (Curry 1997; Gouws and Steyn, 2014). In South Africa, extensive anthocyanin development occurs on tree in ‘Cripps Pink’ apples when weather conditions are cold but clear (Roberts, 2009) and optimal colour development is observed at 22°C on detached apples (Gouws and Steyn, 2014). However, Gouws (2010) found the optimum temperature for colour development of detached ‘Cripps Pink’ peels to increase gradually from 13.91 (\pm 6.12) °C on 16 January, to 20.23 (\pm 0.84) °C (20 February), 21.21 (\pm 0.67) °C (20 March) and 22.34 (\pm 0.46) °C (3 April). High temperatures are detrimental to red colour development. Lin-Wang et al. (2011) found that high temperatures (>30 °C) reduce colouring in blushed apples by downregulating the transcription factors necessary for anthocyanin biosynthesis.

Temperatures above 37°C significantly reduce the colouring of apple peels (Reay 1999). Felicetti and Schrader (2008) noticed that sunburn, which is caused by high temperatures and light, caused a decrease in anthocyanin in ‘Fuji’ apple peel, allowing the increased carotenoid concentrations to become more noticeable. Thus, high temperature may not only limit anthocyanin synthesis but also facilitates the degradation of anthocyanin pigments. This can lead to poor red colour development of apples in warm production areas. Lin-Wang et al. (2011) found that biosynthesis and accumulation of anthocyanins differed when the same cultivar, Mondial Gala, was taken from

two different climatic conditions. In their trials, fruit from the warmer region (Spain) produced much less red colour than those from the colder climate of New Zealand at harvest. High temperatures result in the rapid reduction in anthocyanins. The reduction and low levels of anthocyanin observed in plants growing at elevated temperatures may be due to a combination of a slower rate of biosynthesis and increased catabolism (Steyn et al., 2002; Oren-Shamir 2009).

On a warm day, fruit surface temperature can exceed air temperature by 5°C or more (Racskó and Schrader, 2012). However, evaporative cooling (EC) greatly reduces apple peel temperatures by more than 8°C (Parchomchuk and Meheriuk, 1996; Wünsche et al., 2001). The use of over-tree irrigation systems to provide evaporative cooling for improving fruit quality or preventing damage has been studied by several researchers (Evans, 2004; Iglesias et al., 2002; Parchomchuk and Meheriuk, 1996; Van den Dool, 2006). Although it was found not to be as effective as some other temperature ameliorating technologies in reducing fruit temperature, it was not detrimental to red colour development compared to some technologies which also reduce irradiation when applied over the full season (Evans 1993; Gindaba and Wand, 2005; Iglesias et al., 2000; Unrath and Sneed, 1974). Wand et al. (2004) found early EC application from mid- to late-December to yield good blush formation in ‘Rosemarie’ pears. In some studies, EC improved red colour of apples and it can bring about earlier maturity of fruit (Dussi et al., 1997; Van den Dool, 2006).

Since induction of anthocyanin biosynthesis occurs at low temperatures, cool weather can at any time from December (in South Africa) trigger the start of red colour development in apples. Since the temperature optimum for colour development in ‘Cripps Pink’ apple increases with fruit maturity, reaching about 22°C during the final peak in synthesis, it is possible that acclimation of fruit peel to variable weather conditions during the late stages of fruit development would have a negative effect on peel colour observed at harvest (Willem J. Steyn, Dept. Hort. Sci., Stellenbosch Univ., Matieland, South Africa, personal observation). Our aim in this trial was to study the acclimation response of red colour development in ‘Cripps Pink’ apple peel to reduced peel temperatures at various times during the mid to late season developmental period and the subsequent red colour response at harvest.

2. MATERIALS AND METHODS

Plant materials and experimental design

The trial was carried out in 2016 on ‘Cripps Pink’ apple trees on M793 rootstock and planted in 1998 at Welgevallen Experimental Farm, University of Stellenbosch (33°56'52.5"S 18°52'20.2"E). The region has a Mediterranean-type climate with warm dry summers and cool wet winters. The trees were planted at a spacing of 4 m x 1.5 m in a north-east by south-west row orientation.

The trial was a one-way completely randomized design with five treatments allocated to 30 trees in six replicates. Two adjacent rows were used. Treatments were applied to a representative scaffold branch on the western side of the row, at 1.5 to 2 m height on each tree. Cooling treatments were applied to all fruit on the branch, but measurements were carried out on three tagged fruit throughout the trial period.

Treatments

The treatments involved cooling of fruit surfaces by water spraying when air temperatures measured by a thermometer installed at the site exceeded 28°C (Figure 1) (although there were gaps). Fruit cooling involved manual wetting of the whole fruit surface using a hand-held 1.5-liter pressure bottle sprayer, repeated after about 10-20 minutes according to a rotation between the 12 trees treated at any given time. Fruit were dry before the next spray application was made. The wetting was continued for as long as air temperature was still above 28°C.

The treatments evaluated were: no spraying (Control), cooling from 18 January to 15 April (Jan-April), cooling from 18 January to 19 February (Jan-Feb), cooling from 19 February to 15 March (Feb-March) and cooling from 15 March to 15 April (March-April).

Physical measurements

Dry fruit surface temperature (FST) and fruit blush colour measurements were carried out in the centre of the exposed side of three tagged sun-exposed fruit on each representative branch between 1000HRS and 1100HRS on 19 January (a hot day, 40.6°C), 19 February (a mild day, 24.3°C), 15 March (a warm day, 31.5°C) and 15 April (a warm day, 26.7°C) in 2016, however, FST was measured on all tagged sun-exposed fruit after the first cooling of the day had been carried out in 2017 (when surface of fruit under cooling treatments was dry before the onset of the next cooling application). FST was measured with an infra-red thermometer (Raynger MX4, Raytek

Corporation, Santa Cruz, USA). Fruit blush colour (hue angle, 0° = red-purple, 45° = red, 90° = yellow, 120° = yellow-green) was measured with a chromameter (NR-3000; Nippon Denshoku, Tokyo, Japan).

Fruit maturity and quality

Fruit were harvested on 15 April 2016, one day before commercial harvest. Eight fruit per representative branch were harvested, giving 48 fruit per treatment and a total of 240 fruit. Fruit were taken to the fruit evaluation laboratory of the Department of Horticultural Science, Stellenbosch University, Stellenbosch, for analysis of maturity and external quality.

Ground (green-yellow) colour and percentage foreground (blush) colour were measured using colour charts for 'Pink Lady' apples (Unifruco Research Services Ltd, Cape Town, South Africa). Sunburn severity was assessed using the Schrader and McPerson chart for 'Fuji' apples (Schrader et al., 2003) (Figure 2).

A composite juice sample was prepared by cutting a slice from both sides of each fruit and blending the slices in a liquidizer (AEG Electrolux, Type JE-107 no. 91100085/ PNC 950075206, P.R.C). The juice was used in determining total soluble solids (TSS) and titratable acidity (TA). TSS was measured using a calibrated hand-held refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan). Titratable acids (TA) was measured using an automated titrator (Tritino 719S and Sample Changer 674, Metrohm Ltd., Herisau, Switzerland) by titrating 5 g of juice from each sample with 0.1 M NaOH to a pH of 8.2. Percentage starch conversion was assessed using the iodine test and a starch conversion chart (Unifruco Research Services Ltd, Cape Town, South Africa).

Statistical analysis.

Data were analysed using the General Linear Models (GLM) procedures of XLSTAT (Addinsoft 1995-2016, Version 2016.03.30882). FST and colour (hue angle) data measured in the orchard were analysed using ANCOVA with initial measurements on 19 January used as covariates. Percentage change in hue angle data was analysed as a one-way ANOVA. This was done following the identification of treatment differences for data obtained on 19 January. For maturity and quality data, a one-way ANOVA was performed. Means were separated using the LSD at $p < 0.05$.

3. RESULTS

Air temperature

Average daily maximum temperatures recorded during the trial were 33.1°C (range: 26.5-40.6 °C), 30.5°C (range: 24.3 – 37.6°C), 27.8°C (range: 19.6 – 34.2 °C) and 27.2°C (range: 20.3 – 34.3 °C) for the months of January, February, March and April, respectively. Average daily minimum temperatures recorded for the same period were 18.3°C (range: 13.3 – 19.2 °C), 15.5°C (range: 8.7 – 18.1 °C), 14.2°C (range: 6.8 – 17.2 °C) and 12.0°C (range: 7.1 – 14.0 °C), respectively (Figure 1). Cooler daytime periods were observed between: 24 - 27 January, 19 - 22 February, 26 - 31 March and 1 April, whilst cooler night temperatures were recorded on: 26 – 30 January, 27 – 28 February, 28 – 29 March, 3 - 7 April, and 14 - 15 April.

Fruit surface temperature (FST)

There were no significant differences in FST of dry fruit between treatments (Figure 3). This indicates that all fruit were similarly exposed and thus had a similar pre-treatment baseline temperature. Although we did not measure the FST of wet fruit in the 2016 season, previous EC trials on this cultivar in this orchard have shown a significant reduction in FST of 5-7°C during the warmest part of the day (Gindaba and Wand, 2005). In January 2017, the technique was applied to fully sun exposed apples in the same orchard, resulting in FST reductions of 10-11°C when wet peel was drying at air temperatures above 35°C at the trial site (Table 1).

Fruit blush colour development

There were significant differences between treatments in hue angle and percentage change of hue angle on each measurement date (Figure 4 and Figure 5). At the end of the first cooling period on 19 February, the lowest hue angles (<100°) were measured on fruit under cooling treatments Jan-April and Jan-Feb, which were significantly lower than the control but were not significantly different from cooling treatments Feb-March and March-April (Figure 4). The latter two treatments which had not received any cooling at that point did not differ from the control. At the end of the second cooling period on 15 March, the lowest hue angle was measured on apples under cooling treatment Jan-Feb. The mean value was, however, not significantly different from the mean values for the cooling treatments Jan-April and Feb-March, but was significantly different from those for the control and treatment March-April (cooling from March-April had not started at this point) (Figure 4). The hue angles under the Jan-April and Feb-March treatments did not

differ from the control, but were significantly lower than the March-April values. At the end of the third cooling period on 15 April, apples under the cooling treatment Jan-April recorded the lowest hue angle, which was significantly lower than those of the control treatment and cooling treatments Jan-Feb and March-April, but not significantly different from the mean values for the cooling treatment Feb-March (Figure 4). Values were significantly lower in the Jan-Feb treatment compared to the control but the March-April treatment did not differ from the control.

In mid-February, the percentage change in hue angle relative to mid-January was highest in the Jan-April treatment and significantly higher than the control, but was not significantly different from that measured in treatments Jan-Feb, Feb-March and March-April (Figure 5). In March, the percentage change in hue angle relative to mid-February was significantly higher for the Jan-April and the Feb-March treatments compared to the control, Jan-Feb and March-April treatments (Figure 5). The values for the Jan-Feb and March-April treatments were significantly higher than the control treatment. The final mid-April percentage change in hue angle relative to mid-March was highest in the Jan-April treatment which was significantly higher than the control, Jan-Feb and March-April treatments, but was not significantly different from the percentage change in the Feb-March treatment (Figure 5). The percentage change in hue angle of the Feb-March treatment was also significantly higher than the control and March-April treatments but not significantly higher than the Jan-Feb treatment. The Jan-Feb treatment did not differ significantly from the control and March-April treatments.

Fruit maturity and quality

There were no significant differences between treatments for the assessed external fruit quality parameters (Table 2) or starch conversion at harvest (Table 3). There were, however, significant differences between treatments for TSS, TA and the TSS to TA ratio (Table 3). Significantly higher TSS values were found in control apples and those under cooling treatments Feb-March and March-April compared to the cooling treatments Jan-April and Jan-Feb. The highest TA value was recorded in apples under cooling treatment Feb-March; this was significantly higher compared to all other treatments (Table 3). TA values for control fruit and those under cooling treatment Jan-Feb were significantly higher than those from fruit under cooling treatments Jan-April and March-April. The TSS to TA ratio of apples under cooling treatment March-April was significantly higher than that of the control, Jan-Feb and Feb-March treatments. However, TSS:TA was significantly

lower in cooling treatments Jan-Feb and Feb-March compared to the control and Jan-April treatments.

4. DISCUSSION

The colour response (change in hue angle) of the Jan-April cooling treatment at harvest suggests that the cooling of fruit throughout the experimental period did not lead to acclimation of the fruit peel to lower temperatures in a manner which was detrimental to red colour development at harvest. Evaporative cooling can reduce FST by more than 8.5°C on warm days, with some researchers also putting this figure above 10°C when air temperatures were above 30°C (Parchomchuk and Meheriuk, 1996; Wand et al., 2002). Gindaba and Wand (2005) observed that on days when maximum air temperatures were between 34-37°C at the same trial site used in our study, fruit under EC were 3.1-5.8°C cooler than control fruit. Our measurements showed a significant reduction in FST of about 10-11°C when the wet peel was drying at air temperatures above 35°C (Table 1). Apples are known to develop the best colour under optimum temperature conditions of 19-25 °C (Curry, 1997; Gouws and Steyn, 2014). However, Gouws (2010) also found that, under South African climatic conditions, the optimum temperature for red colour development in detached ‘Cripps Pink’ apples gradually increased from 13.91 (\pm 6.12) °C on 16 January, to 20.23 (\pm 0.84) °C (20 February), 21.21 (\pm 0.67) °C (20 March) and 22.34 (\pm 0.46) °C on 3 April. There was a corresponding increase in potential colour development from January through to April. Under the longer conditions of cooling in this trial (as applied in the Jan-April treatment), a negative impact on red colour development would have been observed if the cooling treatment had resulted in acclimating the anthocyanin biosynthetic pathway in the peel to below optimum temperatures. In contrast, the Jan-April treatment was able to enhance colour development (change in hue angle). Although the long-term Jan-April treatment together with Jan-Feb and Feb-March cooling treatments were successful in decreasing the hue angle measured on the ‘Cripps Pink’ apples, there were no significant effects of any of these treatments on the surface coverage (percentage foreground colour) of blush observed on the fruit at harvest. This suggests that anthocyanin accumulated to or was retained at higher concentrations in these treatments whilst the extent of pigment coverage of the fruit surface was not affected.

The early short term treatment (Jan-Feb) also produced fruit with significantly lower hue angle at harvest compared to the control, but with results similar to the later short term cooling treatments (Feb-March and March-Apr). Initial improved colour (low hue angle) of fruit under the Jan-Feb treatment was observed when measurements were conducted on 19 February (Figure 4). This indicates that the treatment kept fruit surface temperature at levels which increased anthocyanin synthesis in the peel or, alternatively, decreased anthocyanin degradation. Fruit under this treatment showed continued positive response (reduction in hue angle) 4 weeks after the treatment was stopped as observed on 15 March. The effects of the Jan-Feb treatment wore off in the second month (15 April) after the treatment application was stopped. Data collected indicate that maximum air temperatures were sometimes above 30 °C in late March 2016 (Figure 1) with FST already above 25°C in the late mornings of 15 and 16 March (Figure 3). High temperatures prevent the accumulation of cyanidin and UDP-sugars in apples (Ban et al., 2009) and reduce phenylalanine ammonia lyase (PAL) activity and anthocyanin accumulation by increasing the levels of a PAL-inactivating system (Faragher, 1983). This results in rapid reduction in anthocyanins at high temperatures. The low levels of anthocyanin observed in ‘Cripps Pink’ fruit under the Jan-Feb treatment at harvest may be due to a reduction in the anthocyanin synthesis capacity of the fruit following the initial boost during treatment application. This may be the result of an increase in FST following the cessation of the cooling treatment. Reduced levels of anthocyanins in plants grown under elevated temperatures is believed to be a combination of a slower rate of biosynthesis and increased catabolism (Oren-Shamir, 2009). Optimum temperatures of about 16-24°C are known to stimulate red colour (anthocyanin) formation in mature attached ‘Jonathan’ apples (Faragher, 1983). However, this depends on the duration of fruit exposure at the optimum temperature. Longer durations within the optimum temperature range would boost red colour development whilst fluctuations between below/above optimal and optimal temperatures will result in lower red colour development and even losses of anthocyanin. From the colour response at harvest, it appears that the boost in red colour development (decreasing hue angle) achieved during Feb-March cooling treatment could not be sustained until harvest (15 April). Nevertheless, its effect improved the hue angle (but not percentage foreground colour) of ‘Cripps Pink’ apples relative to the control, but not as effectively as the change in hue measured on the Jan-April cooling treatment.

Evaporative cooling (EC) has proven successful in reducing FST in trials where it was employed. However, although the majority of EC experiments succeeded in improving fruit colour in blushed apple cultivars, some EC trials did not improve fruit colour as expected when compared to control fruit. Even though EC was successful in reducing air temperature and FST of ‘Jonagold’ apples (Parchomchuk and Meheriuk 1996), they did not identify any colour differences between EC and control treatments. They attributed their finding to the location of the trial and the late start date of cooling. In a study at the same site used in this trial, Steyn (2003) found that application of EC from three to two weeks before harvest improved the colour of ‘Rosemarie’ pears. Our results indicate that the late cooling treatment applied from mid-February to mid-March, was effective in enhancing the red colour (low hue angle) of ‘Cripps Pink’ apples compared to the Jan-April and Jan-Feb treatments at harvest. This treatment (Feb-March) also produced fruit with the best foreground colour and showed less sunburn damage (although foreground colour and sunburn were not significantly different from those for the other treatments).

The cooling treatments applied in this trial did not show any statistically significant effects on the foreground and ground colour, and the incidence and severity of sunburn of ‘Cripps Pink’ apples at harvest (all apples on the treated branch). However, percentage foreground (blush) colour was highest in cooling treatments Jan-April and Feb-March, which aligns with the results presented above for hue angle. The overall percentage sunburn incidence was greater on control fruit (70.8%) and treatment March-April (66.7%), than on the other treatments, and lowest in treatment Feb-March (35.7%). This was particularly noticeable in fruit with sunburn necrosis (classes 4 and 5). However, the differences were not statistically significant and a larger sample size may have been necessary to distinguish clearly between the treatments. Accumulation of anthocyanins is believed to decrease the conspicuousness of superficial sunburn types (e.g. sunburn browning). Makedredza et al. (2015) observed that red and blushed apples showed a considerable decrease in hue angle and an increase in blush coverage towards harvest which masked the appearance of sunburn symptoms observed on such fruit compared to non-blushed cultivars. The high incidence of sunburn recorded on fruit may have resulted in the similar blush coverage observed on ‘Cripps Pink’ apples at harvest despite the differences in hue angle. Generally, ‘Cripps Pink’ apples are regarded as less susceptible to sunburn damage compared to other cultivars such as ‘Granny Smith’ and ‘Golden Delicious’ (Gindaba and Wand, 2005; Schrader *et al.*, 2003; van den Ende, 1999); therefore, the reason for the high incidence and severity of sunburn may be due to the exceptionally

hot season experienced in 2016. In addition, the Stellenbosch area is quite warm compared to the major apple production regions in South Africa and this orchard has a history of high sunburn incidence. Although the cooling treatments may have succeeded in reducing FST, they played no role in mitigating the effects of incident solar radiation. Gindaba and Wand (2005) found that shade nets and kaolin particle application treatments were more effective in reducing sunburn than EC in the same orchard, since they effectively reduced the combined effects of high temperatures and incident solar radiation whereas EC only succeeded in decreasing FST.

The treatments employed in this trial produced different effects on maturity of ‘Cripps Pink’ apples as indicated by TSS and TA. In this trial, it appears that cooling of fruit early in the trial (Jan-April and Jan-Feb cooling treatments) resulted in a reduction in TSS compared to the other treatments. On the other hand, there was a reduction in TA when fruit were cooled throughout the trial period (Jan-April) and from mid-March to mid-April, with fruit under cooling treatment Feb-March recording highest TA values. The timing of the March-April treatment (which may be considered a control due to a relatively low number of cooling days) might have induced a response in the fruit similar to the Jan-April treatment causing them to produce fruit with higher TAs. In literature, there are inconsistencies in TSS and TA results when evaporative cooling is applied to fruit. Whilst Iglesias et al., (2002), reported higher TSS and TA values for ‘Topred Delicious’ apples under EC treatments, Gindaba and Wand (2005), whose trials were conducted on our trial site, found a reduction in TSS in ‘Cripps Pink’ apples under evaporative cooling. Other authors, such as Wand et al. (2002) also report that early maturation was observed in some pear fruit under EC. This suggests that apart from the evaporative cooling applied, other factors such as the timing of the EC application and existing local climate might play a role that affects fruit maturity parameters.

In conclusion, our results suggest that the cooling of ‘Cripps Pink’ apples during fruit development did not result in acclimation of anthocyanin synthesis to lower temperatures in a manner that was detrimental to red colour response at harvest. Cooling treatments Jan-April, Jan-Feb and Feb-March did, however, result in fruit with a redder colour (lower hue angles) at harvest. Our results indicate benefits of fruit cooling (showing as lower hue angle) four weeks after cooling treatment Jan-Feb was stopped compared to the control. However, this benefit becomes muted over time when treatment has stopped, suggesting a gradual reduction in anthocyanin synthesis capacity or increased anthocyanin degradation after initial boost during the cooling period. It appears that the

impact of fluctuations in weather conditions on ‘Cripps Pink’ apple colour at harvest may vary depending on when the cooling occurs and whether the boost to anthocyanin synthesis resulting from the initial cooling is maintained until harvest. The development of red colour of blushed apples under variable microclimatic conditions requires further research and in different production locations.

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TABLES AND FIGURES

Table 1. Average fruit surface temperature (FST) of fully sun-exposed uncooled and cooled apples at air temperatures above 35°C on 17 January 2017 at the trial site. Means represent measurements carried out on five apples.

Time (Hours)	Fruit surface temperature (FST)	
	Uncooled fruit	Cooled fruit
12:00	42.2±0.701	30.8±1.634
12:30	42.5±0.613	31.4±1.227
13:00	44.1±0.578	32.3±1.144
13:30	40.8±0.622	30.0±0.586
14:00	44.6±1.065	33.7±0.618

Table 2. External quality of ‘Cripps’ Pink’ apples at harvest in response to the different fruit cooling treatments. N=6. Fruit were harvested a day before commercial harvest on 15 April 2016. Foreground colour percentage and background colour were measured using industry charts for ‘Pink Lady’ apples. Severity of sunburn was scored using the Schrader and McFerson chart for ‘Fuji’ apples (Figure 2). Means in columns were separated at 5% LSD.

Treatment	Foreground colour (%)^x		Ground colour^y		% Sunburn Class (1)^z		% Sunburn Class (2-3)		% Sunburn Class (4-5)		%Sunburn (all classes ≥1)	
Control	36.0	ns	3.5	ns	8.3	ns	27.1	ns	35.3	ns	70.8	ns
Jan-April	38.9		3.5		18.8		18.1		20.1		56.9	
Jan-Feb	37.6		3.3		18.8		16.7		18.8		54.3	
Feb-March	40.6		3.4		6.3		16.6		12.8		35.7	
March-April	32.2		3.4		25.0		16.7		25.0		66.7	
p-value	0.142		0.723		0.168		0.307		0.708		0.312	

^x Foreground colour percentage: estimation of the total blush on the fruit surface

^y Background colour: comparing the green side of the fruit with the colour chart (0.5 = green background and 5 = yellow background)

^z Severity of sunburn on fruit measured using the Schrader and McFerson chart: Classes range from 0 to 5 (0= no sunburn and 5= necrosis) (Schrader et al., 2003).

Table 3. Maturity and internal quality of 'Cripps' Pink' apples measured at harvest in response to the different fruit cooling treatments. N=6. Fruit were harvested a day before commercial harvest on 15 April 2016. Means in columns were separated at 5% LSD. Abbreviations: TSS = total soluble solids; TA = titratable acidity.

Treatment	Starch conversion (%)	TSS (%Brix)	TA (% Malic acid eq.)	TSS:TA
Control	39.1 ns	16.4 a	0.61 b	27.0 b
Jan-April	28.1	15.4 b	0.56 c	27.8 ab
Jan-Feb	43.9	15.5 b	0.62 b	24.8 c
Feb-March	34.7	16.3 a	0.67 a	24.6 c
March-April	45.2	16.2 a	0.57 c	28.4 a
p-value	0.671	<0.000	<0.000	<0.000

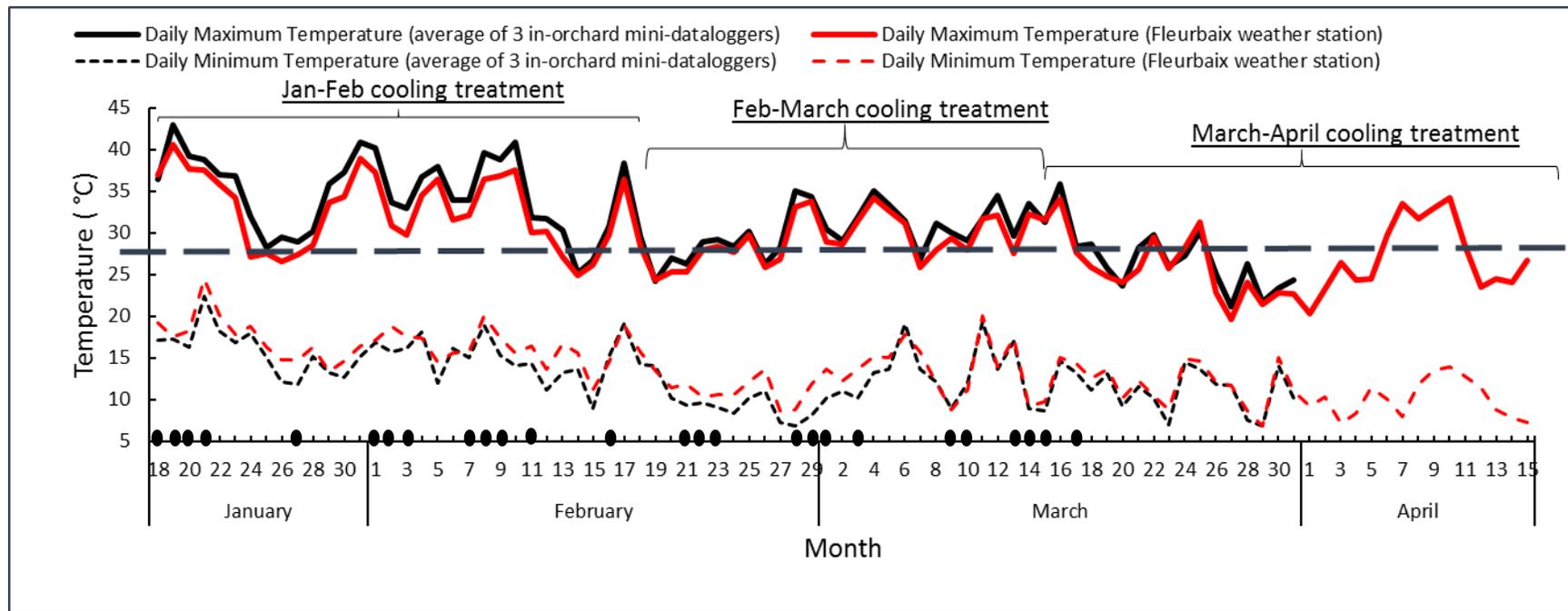


Figure 1. Daily maximum and minimum air temperature recorded within the orchard row ($33^{\circ}56'51.6''S$ $18^{\circ}52'20.1''E$) over the experimental period by three mini-dataloggers, and recorded at the Fleurbaix weather station in Stellenbosch ($33^{\circ}57'01.9''S$ $18^{\circ}50'50.6''E$) from 19 January until 15 April 2016. The three mini-dataloggers were placed at the beginning, middle and end of the orchard row but were not sufficiently shielded from direct irradiation, hence their higher temperature values compared to Fleurbaix, especially on warmer days. Fruit cooling treatments were, however, guided by a separate shaded thermometer in the orchard. The horizontal dashed line indicates the air temperature threshold ($28^{\circ}C$) above which fruit cooling was done on most days with temperatures above this threshold. Black dots on the X-axis indicate dates on which fruit cooling was done.

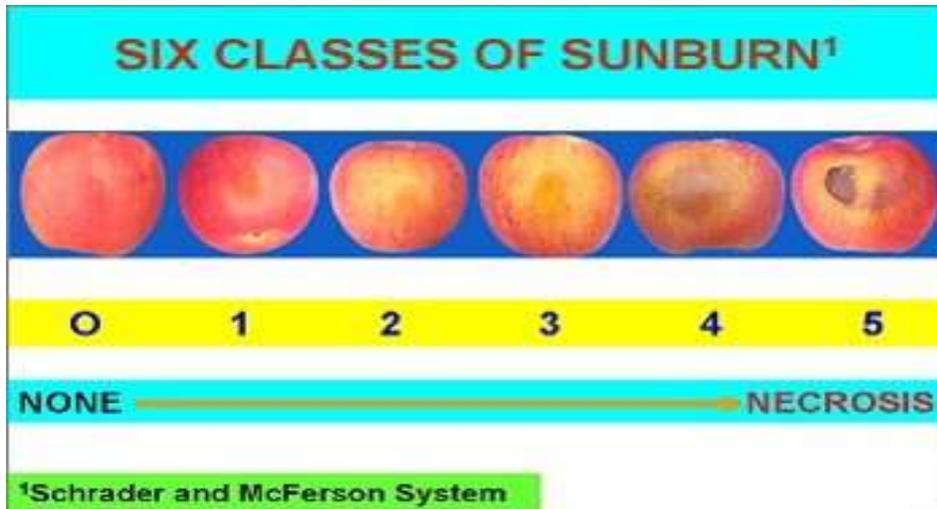
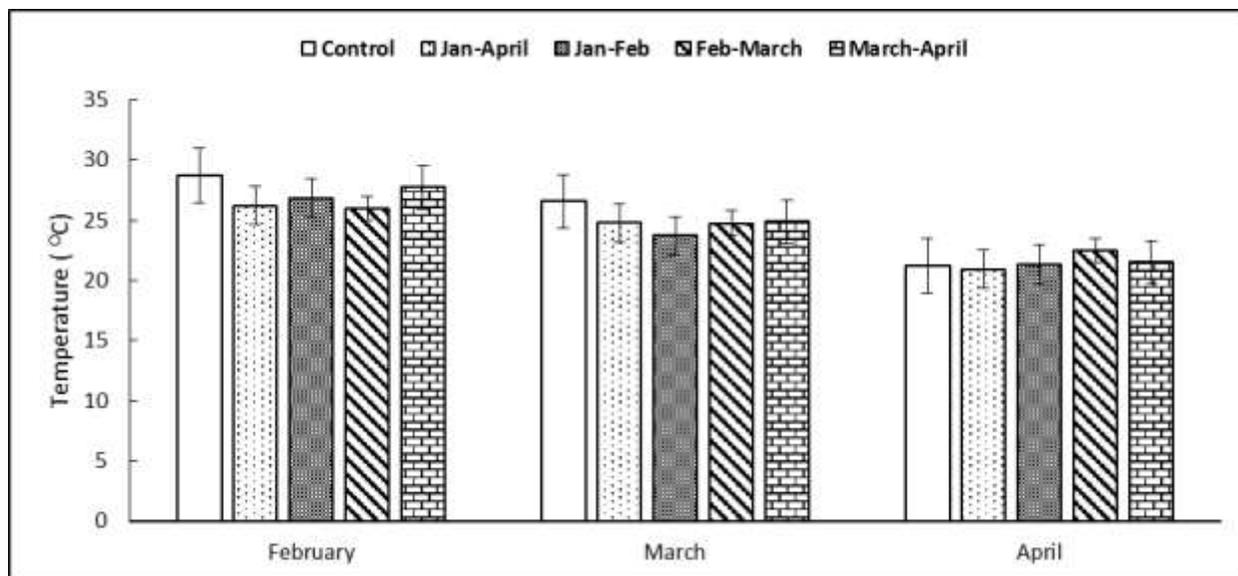
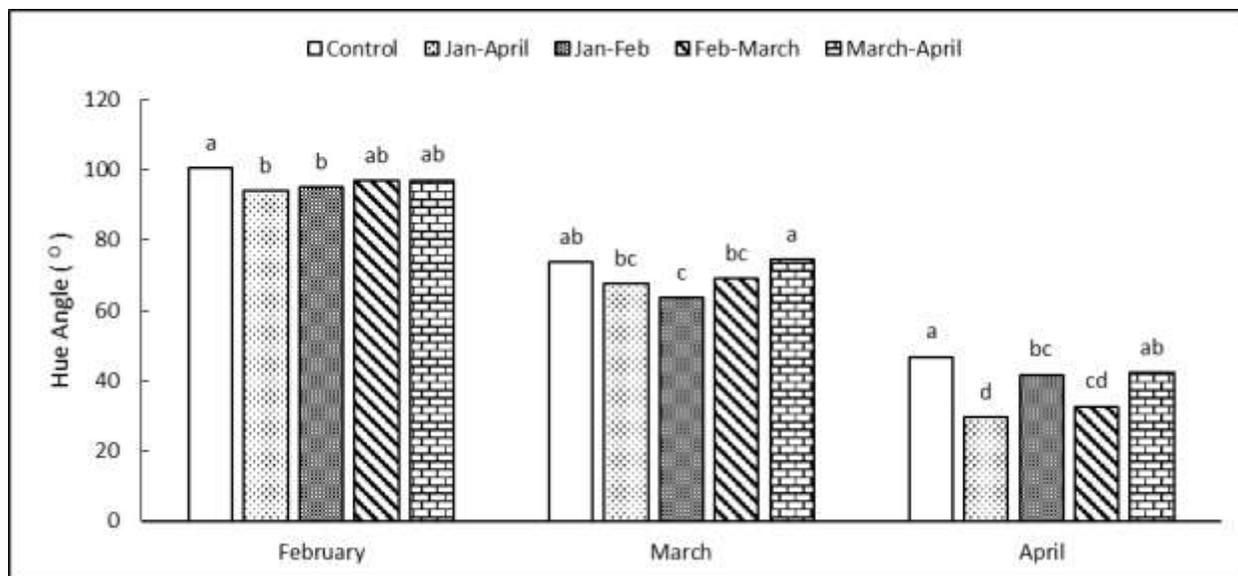


Figure 2. Schrader and McFerson system of sunburn evaluation for 'Fuji' apples (Schrader et al., 2003).



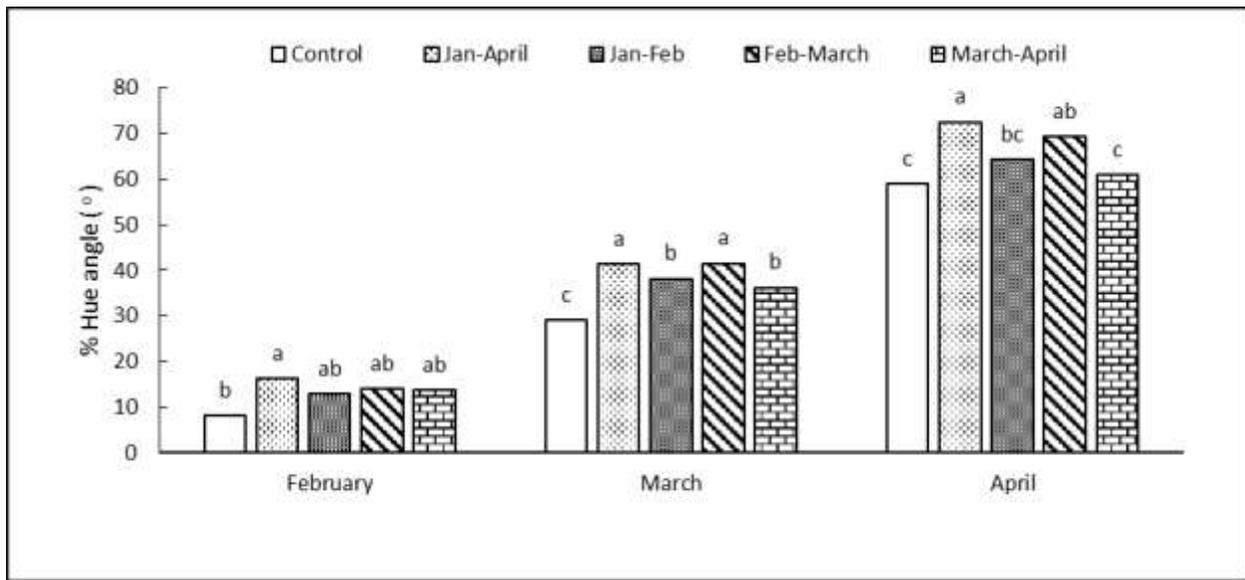
Source	p-values		
	February	March	April
Covariate	0.188	0.001	0.455
Treatment	0.121	0.436	0.333

Figure 3. Dry fruit surface temperature under the various fruit cooling treatments measured at the end of each cooling period in 2016. All measurements were carried out between 10:00HRS and 11:00HRS on 19 January (used as covariate), 19 February, 15 March and 15 April 2016. Means are separated at 5% LSD. (Control- no cooling, Jan-April – cooling from mid-January to mid-April, Jan-Feb – cooling from mid-January to mid-February, Feb-March – cooling from mid-February to mid-March, March-April – cooling from mid-March to mid-April)



Source	p-values		
	February	March	April
Covariate (January)	0.025	0.176	0.871
Treatment	0.045	< 0.000	< 0.000

Figure 4. Hue angle of 'Cripps' Pink' apples under the various fruit cooling treatments, measured at the end of each cooling period on 19 January (used as covariate), 19 February, 15 March and 15 April 2016. Means are separated at 5% LSD.



Source	p-values		
	February	March	April
Treatment	0.010	< 0.000	< 0.000

Figure 5. Percentage change in hue angle on ‘Cripps’ Pink’ apples under the various fruit cooling treatments, measured at the end of each cooling period on 19 January, 19 February, 15 March and 15 April 2016. Means are separated at 5% LSD.

6. GENERAL DISCUSSION AND CONCLUSION

The profitability of apple orchards is affected by quality defects such as sunburn and poor red colour of bi-coloured apples. In warm production regions with high incident solar radiation, such as South Africa, sunburn and poor red colour at harvest account for the highest percentage cullage in orchards. Temperature and sunlight are the primary direct factors that determine the occurrence and severity of sunburn damage (Racskó and Schrader, 2012). High temperatures, on the other hand, also limit anthocyanin synthesis and increases anthocyanin degradation (Marais et al., 2001), which affects the extent of red colour development in blushed apples (Lancaster, 1992; Reay & Lancaster, 2001; Saure 1990). The exposure of apple fruit to high solar radiation and high temperature is highly dependent on the fruit's micro-climate which is dependent on tree size. The tree size is strongly influenced by rootstock, with bigger trees on more vigorous rootstocks providing more shade to a higher proportion of developing fruit due to development of larger canopies and more foliage (Racskó and Schrader, 2012). On the other hand, a higher proportion of fruit on more dwarfing rootstocks are more exposed to direct sunlight and experience higher peel temperatures due to smaller canopies with less foliage. While some researchers (Racskó et al., 2005a, 2009; Wünsche et al., 2004) have found apples on dwarfing rootstock to be more prone to sunburn, evidence suggests that such fruit may be better acclimatized to the high light and temperature conditions making them less susceptible to sunburn damage than shaded fruit on larger canopies (Racskó and Schrader, 2012). Notwithstanding these considerations, dwarfing rootstocks are preferred for high density plantings in modern orchards in most apple growing regions. As efforts are being made to introduce new and improved rootstocks of varying vigour to the South African apple industry, it is important to know how rootstock influences the sensitivity of apple peel to prevailing high light and temperature conditions and how this affects the occurrence of fruit peel damage and the ability to develop red colour. This will help identify rootstocks best suited for the South African apple growing regions and serve as guide for rootstock selection for future orchard plantings. Hence, the overall aim of this study was to better understand the sensitivity of apple peel photosystems and red colour (anthocyanin) biosynthesis to irradiance and temperature, as influenced by rootstocks of differing vigour and the fruit microclimate.

In the first study, sensitivity and visibility of peel damage was attributable to the duration of exposure, the length of the post treatment period and the canopy position of the fruit (Paper 1,

Figures 3-9). Rootstocks did not influence the sensitivity of peel photosystems to damage and their ability to recover from the initial damage after exposure to natural high light and temperature stress condition, as measured by maximum light use efficiency of photosystem II (F_v/F_m). This suggests that rootstocks largely affect the proportion of fruit with sunburn on the tree through their effect on canopy size and fruit exposure to light. A lower maximum light use efficiency (F_v/F_m) was reported for sunburnt apple peels compared to non-sunburnt peels. The results from this study concur with the findings of Chen et al. (2008), Wand et al. (2008) and Song et al. (2001). Damage to peel photosystems occurred after all exposure durations (from one to five hours) under natural high irradiance between 1700-2100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and ambient air temperature between 24 and 28°C. Near-complete recovery was achieved for shorter duration exposures of one and two hours in 'Rosy Glow' apples and one hour in 'Golden Delicious'. The difference between the cultivars was attributable to the higher fruit surface temperature in the 'Golden Delicious' trial. These findings are consistent with previous findings by Wand et al. (2008) and Song et al. (2001), who identified that there may be partial or no recovery of apple peel photosystems exposed to high temperature stress, depending on the stress level and the duration of exposure.

Acclimation is a response of a plant to an environmental change that causes phenotypic alterations with no change in genetic complement (Huner et al., 1998). Racskó and Schrader (2012) define acclimation to sunburn as a series of physiological, biochemical or molecular processes that occur in the fruit to adjust to a gradual increase in solar radiation, which allows the apple to reduce its susceptibility to sunburn damage. The amplitude of response of the acclimated fruit to the solar radiation is reduced since a certain level of protection is already in place. Thus, previously sun-exposed apples are believed to be better protected from sunburn damage than shaded apples due to acclimation. Ma and Cheng (2003) found sun-exposed apples to have higher antioxidant activities which make them better protected against oxidative stress caused by high irradiance than shaded fruit. In South Africa, Wand et al. (2006) found fruit grown under shade netting to have lower F_v/F_m values at 43°C than non-netted fruit, which suggests similar acclimation responses in previously exposed fruit. We compared the photosensitivity of previously exposed sides of apples from the inner canopy (referred to as shaded canopy position) and outer canopies (referred to as sun-exposed canopy position). We argued that there was a form of acclimation in fruit from both canopy positions. It was noted that whilst exposed peels of fruit from sun-exposed canopy positions seem to possess greater protection against immediate damage (on day 1) (Paper 1, Figure

5 and 7) than peels from shaded canopy positions, exposed peels of apples from shaded canopy positions seem to possess greater protection against progressive damage induced which showed as a better recovery of the photosystem (Paper 1, Figure 5) and less visible peel damage observed in some cases on the fifth post treatment day (Paper 1, Figure 6 and 7). However, it is worthy to note that this acclimation was not attributable to rootstock vigour but was distributed across the range of vigour used in the study.

High prevalence of sunburn injury has been reported in high-density plantings with smaller trees because of greater fruit exposure to solar radiation than in traditional (lower density) plantings with larger trees (Parchomchuk and Meheriuk, 1996; Racskó et al., 2005b). Fruit on these trees are more likely to develop sunburn. Several authors have found this assertion to be true (Racskó and Schrader, 2012) with trees on dwarfing rootstocks such as M.9 and M.6 generally being more susceptible to sunburn, compared to semi-vigorous or vigorous stocks (e.g., MM106 and M.793, respectively). However, Costa (2011) found on Geneva rootstocks in South Africa, that sunburn was more noticeable in more vigorous rootstocks in heavy crop years. The findings from this study and the data gathered at harvest in our trial orchard (Siboza and Steyn, 2015; Sibozza et al., 2016) do not concur with such findings. There was no indication of rootstock effect on visible peel damage of ‘Rosy Glow’ apples attributable to vigour and acclimation. Where differences in sunburn incidences were identified among rootstocks, they were distributed across the vigour classes and not linked to the low vigour class. Since sensitivity of apple peel to damage was not influenced by rootstock vigour, any of the rootstocks studied can be recommended for future orchard plantings based on this consideration, and decisions can be made by taking into consideration other parameters (e.g. yield efficiency).

Fruit surface temperatures (FST) above 45°C in previously sun-exposed fruit is the minimum temperature required for sunburn browning to occur in the presence of light for 60 minutes or longer (Schrader et al., 2001). FST of about 52°C for 10 minutes results in sunburn necrosis (Schrader et al 2003a). In this study, FST reached 36-48°C and 46-52°C for uncovered ‘Rosy Glow’ and ‘Golden Delicious’ apples, respectively. Using the Schrader chart (Schrader et al., 2003b), sunburn browning was the most notable sunburn type observed after exposing ‘Rosy Glow’ apples to natural high sunlight and temperature. Damage increased with increasing duration

of exposure. On the subsequent days peel damage became progressively greater, showing mostly symptoms of cooking, with a few cases of peel browning on fruit exposed beyond four hours (Paper 1, Figure 7). Peel damage observed on ‘Golden Delicious’ apples was low with sunburn browning being the most notable symptom after 1-hour exposure in natural high light and temperature conditions. Necrotic symptoms and cooking of fruit surfaces was observed on most ‘Golden Delicious’ apples after 3-hour exposure to natural high light and temperature conditions (Paper 1, Figure 9 and Appendix, figure 5 and 6). Unlike previous studies by Schrader and his colleagues (Schrader et al., 2001, 2003b), where peel damage symptoms were quantified after fruit were exposed to high light and temperature conditions under controlled conditions, fruit in our trials were exposed to natural high light and temperature conditions. As such, other factors (e.g. cloud cover, wind velocity and humidity) could have affected fruit surface temperatures at any time during the exposure period. Nonetheless, FST measured on fruit after the exposure periods were mostly in the range of the thresholds for the various sunburn types with corresponding symptoms observed under this study.

In general, it was found that rootstocks possessed differential innate abilities to influence red colour development of ‘Rosy Glow’ apples under a range of temperatures. Different apple cultivars are believed to have different optimum temperature ranges for red colour development (Curry, 1997; Gouws and Steyn, 2014). Results from this study show that the degree of red colour development of the same apple cultivar under a specific temperature can be influenced by the rootstock used. Whilst we identified that one rootstock (M7) had a narrower optimum range for colour development, all others (Cepiland, MM109/M9, RN29, G222, G3007, ‘Rosy Glow’ own roots, MM109 and MM793) showed a broad optimum range for colour development. Geneva rootstocks G222 and G3007 appear to show the highest potential for good colour development under temperatures of 19-31°C, on par with the current industry standard M793. Rootstock vigour can affect the colouring potential of blush apple and pear cultivars, with rootstocks of lower vigour producing redder fruit than rootstocks of higher vigour (Autio and Southwick, 1993; Roberts et al., 2008). Whilst final harvest data from the trial site clearly showed that ‘Rosy Glow’ apples on dwarfing rootstocks produced more pink blush at harvest than the semi-vigorous and vigorous rootstocks (Siboza et al., 2016), the peel colour development trends of the more dwarfing rootstocks under controlled lab conditions did not confirm this. The lower innate ability of the more dwarfing ‘Cepiland’, ‘RN29’ and MM109/M9 rootstocks is surprising since they showed

good blush colour under field conditions at harvest. Since the colour development between individual apples on the same tree can differ significantly (Awad et al 2001), it is possible that the low number of replications (which may have influenced the variances observed in the Celtec data) used in this trial and the lack of seasonal repetition might have produced some artefacts that influenced the results observed. We therefore recommend that more work is needed in different seasons and different locations to confirm these findings.

Gouws (2010) found the optimum temperature for colour development of detached ‘Cripps Pink’ peels to increase gradually from 13.91 (\pm 6.12) °C in January to 22.34 (\pm 0.46) °C in April when tested under laboratory conditions. It is possible that cooling of fruit during mid-season fruit development might acclimate the anthocyanin biosynthetic pathway to temperatures lower than optimum required for colour development during the final fruit development stage. In this study, evaporative cooling treatments at various periods in the mid- to late-season did not lead to acclimation of anthocyanin synthesis to lower temperatures than the optimum, as shown by the lack of negative impact on the red colour of ‘Cripps’ Pink’ apples at harvest. In fact, the colour of ‘Cripps’ Pink’ apples at harvest benefited from the cooling treatments, with the cooling treatments applied from mid-February to mid-March and for the full period (January to April) being the most effective in terms of changes in hue angle (redness) (Paper 3, Figure 3). Temperature reductions as a result of cooling likely brought the fruit peel into the optimum range for colour synthesis. However, blush colour was not significantly different between the treatments at harvest. Cooling of fruit at any time from mid-January improved the colour (reduced the hue angle) of the ‘Cripps’ Pink’ apples; However, this effect depended on the duration and time of treatment since the benefit of cooling was partially lost sometime after the cooling treatment was stopped whilst air temperatures were still high. From the results obtained, the early cooling treatment (mid-January to mid-February) provided a boost to the anthocyanin synthesis capacity of the peel. We observed a lag period during which the initial boost benefit obtained under previous cooling was maintained when the treatment was stopped. However, it appeared that the benefit of cooling could not be sustained until harvest whilst air temperatures were still high. We propose that a gradual reduction in anthocyanin synthesis capacity occurred after the initial boost under cooling. High temperatures prevent anthocyanin accumulation by increasing the levels of a PAL-inactivating system (Ban et al., 2009; Faragher, 1983). This results in rapid reduction in anthocyanins at high temperatures. The small relative reduction in hue angle measured between mid-March and harvest under the

mid-January to mid-February treatment may be due to a reduction in the anthocyanin synthesis capacity or increased degradation of anthocyanins due to an increase in temperature (Marais et al., 2001) of the fruit peel following the initial boost during treatment application. This may have resulted in poorer colour (hue angle) observed at harvest than would have been expected based on the colour in mid-March. Nevertheless, there was still greater colour (lower hue angle) observed on such fruit than the control treatment. It appears that cooling in the warm period mid-February to mid-March, irrespective of cooling before or after this period, was effective in producing better red colour (hue angle).

Apple fruit peel photosystems appear to benefit from acclimation induced by previous exposure to light and temperature, rendering the peel less susceptible to sunburn damage and poor red colour development. However, this study has shown that peel sensitivity is not influenced by rootstock or associated with rootstock vigour class. The duration of exposure to the high irradiance and temperature condition is the most important factor affecting the damage to peel photosystems, recovery of photosystems and visible peel damage of the apple peel. Red colour development in 'Rosy Glow' apple peel under controlled environments appears to be due to inherent (genetically determined) rootstock abilities rather than related to vigour class. Cooling of fruit during mid- to late-season fruit development using evaporative cooling did not acclimate fruit to lower temperatures that were detrimental to the colour response of 'Cripps Pink' apples at harvest, but were in fact beneficial to colouring. The beneficial impacts of cooling on anthocyanin synthesis were greatest in February-March, with earlier beneficial impacts (January-February) being significantly reduced by harvest. More work is needed on better understanding the effects of rootstocks and acclimation of apples to variable temperatures on colour development of blushed cultivars, in different seasons and locations.

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APPENDIX A: SUPPLEMENTARY DATA OF PAPER 1

Table 1. Average ambient temperature (°C) per minute recorded Stellenbosch University Weather station (33°55'42.84" S, 18°51'55.08" E). For Trial 1 and 2 dates in 2015 and 2016.

Table 2. Maximum light use efficiency (F_v/F_m) on dark adapted and undamaged 'Rosy Glow' and Golden 'Delicious' apple peels under room temperature conditions in 2016 before fruit were exposed to the photothermal stress.

Figure 1. PPF measured with a light meter at trial site (Department of Horticultural Science, Stellenbosch University) on 25 March 2015, 3 February and 14 March 2016 during the period of Trials 1 and 2.

Figure 2. Schrader and McFerson system of sunburn evaluation (Schrader et al., 2003). 0, no sunburn; 1, Sunburn severity 1; 2, Sunburn severity 2; 3, Sunburn severity 3; 4, Sunburn severity 4; 5, Sunburn severity 5 (necrosis).

Figure 3. Sunburn evaluation system of 'Golden Delicious' apples adapted from the Schrader and McFerson system (Schrader et al., 2003).

Figure 4. Golden delicious apples harvested from sun-exposed canopy positions under 1-hour exposure to the photothermal stress condition a) immediately after removal from the photothermal stress b) after 5 days of observation under room temperature conditions.

Figure 5. Golden delicious apples harvested from sun-exposed canopy positions (from block 3) five days after exposure to photothermal stress a) apples under 2-hour exposure to the photothermal stress condition b) apples under 3-hour exposure to the photothermal stress. Black circles indicate area of fruit covered with black sticker.

Figure 6. 'Rosy Glow' apples harvested from block 3 five days after exposure to the stress condition a) apples from shaded canopy positions b) apples from sun-exposed canopy positions. Black circles indicate area of fruit covered with black sticker.

Table 1. Average ambient air temperature (°C) per 30-minute period recorded at the Stellenbosch University Weather station (33°55'42.84" S, 18°51'55.08" E). For Trial 1 and 2 dates in 2015 and 2016.

Time (hours)	Average Temperature 25/03/15	Air Average Temperature 4/02/16	Air Average Temperature 14/03/16
10:00	20.7	26.5	23.7
10:30	21.9	27.6	23.7
11:00	24.0	28.4	25.0
11:30	24.7	29.6	25.9
12:00	25.4	30.1	26.4
12:30	25.4	31.4	27.2
13:00	26.4	30.4	27.8
13:30	26.6	30.0	27.8
14:00	25.3	30.4	28.2
14:30	26.2	30.4	28.3
15:00	26.5	30.1	29.4
15:30	25.7	30.8	29.5
16:00	26.4	30.2	29.0
16:30	26.2	30.7	29.0
17:00	26.4	31.3	28.2

Table 2. Maximum light use efficiency (F_v/F_m) on dark adapted and undamaged ‘Rosy Glow’ and ‘Golden Delicious’ apple peels under room temperature conditions in 2016 before fruit were exposed to the photothermal stress.

Rootstock	Sun-exposed apples	Shaded apples
‘Rosy Glow’		
Cepiland	0.809	0.822
MM109/M9	0.811	0.821
G3007	0.804	0.821
G222	0.815	0.816
M7	0.812	0.814
MM109	0.805	0.812
G778	0.818	0.819
‘Rosy Glow’ own roots	0.814	0.816
MM109	0.817	0.798
Pr=f	0.480	0.823
‘Golden Delicious’		
Cepiland	0.819	0.823
G007	0.822	0.820
G222	0.787	0.817
M7	0.821	0.822
M793	0.789	0.818
G228	0.822	0.827
G778	0.822	0.825
Pr=f	0.231	0.385

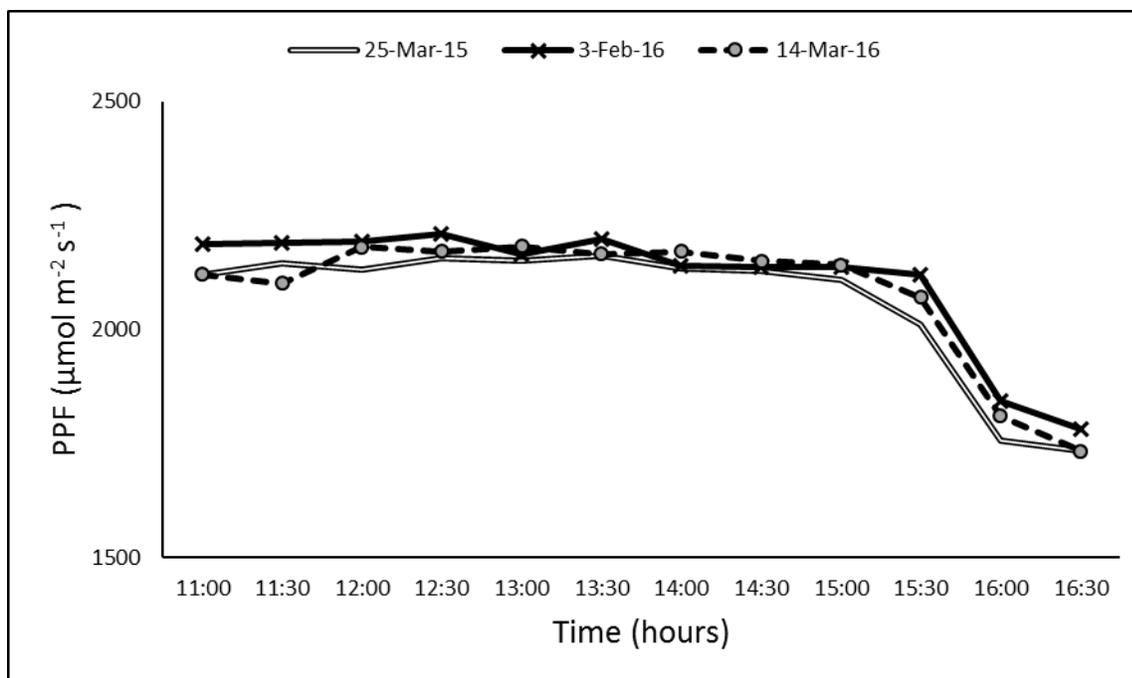


Figure 1. Photosynthetic photon flux (PPF) measured with a light meter at the trial site (Department of Horticultural Science, Stellenbosch University) on 25 March 2015, 3 February and 14 March 2016 during the period of Trials 1 and 2.

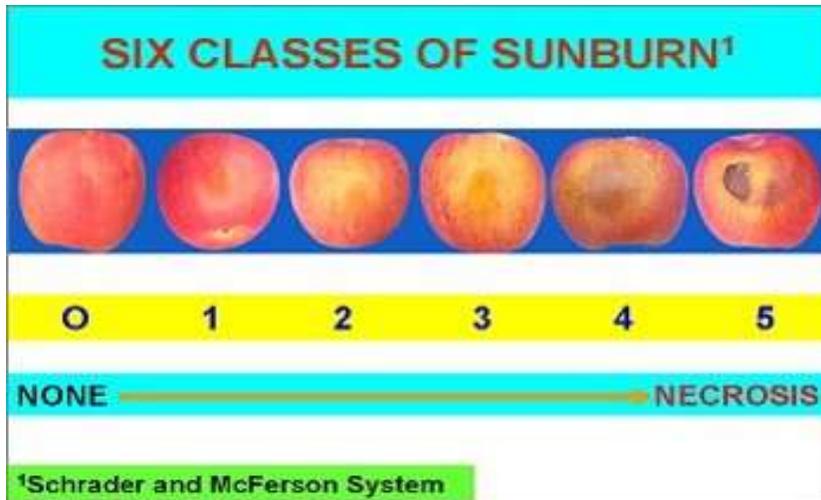


Figure 2. Schrader and McFerson system of sunburn evaluation (Schrader et al., 2003). 0, no sunburn; 1, Sunburn severity 1; 2, Sunburn severity 2; 3, Sunburn severity 3; 4, Sunburn severity 4; 5, Sunburn severity 5 (necrosis).



Figure 3. Sunburn evaluation system of 'Golden Delicious' apples adapted from the Schrader and McFerson system (Schrader et al., 2003).

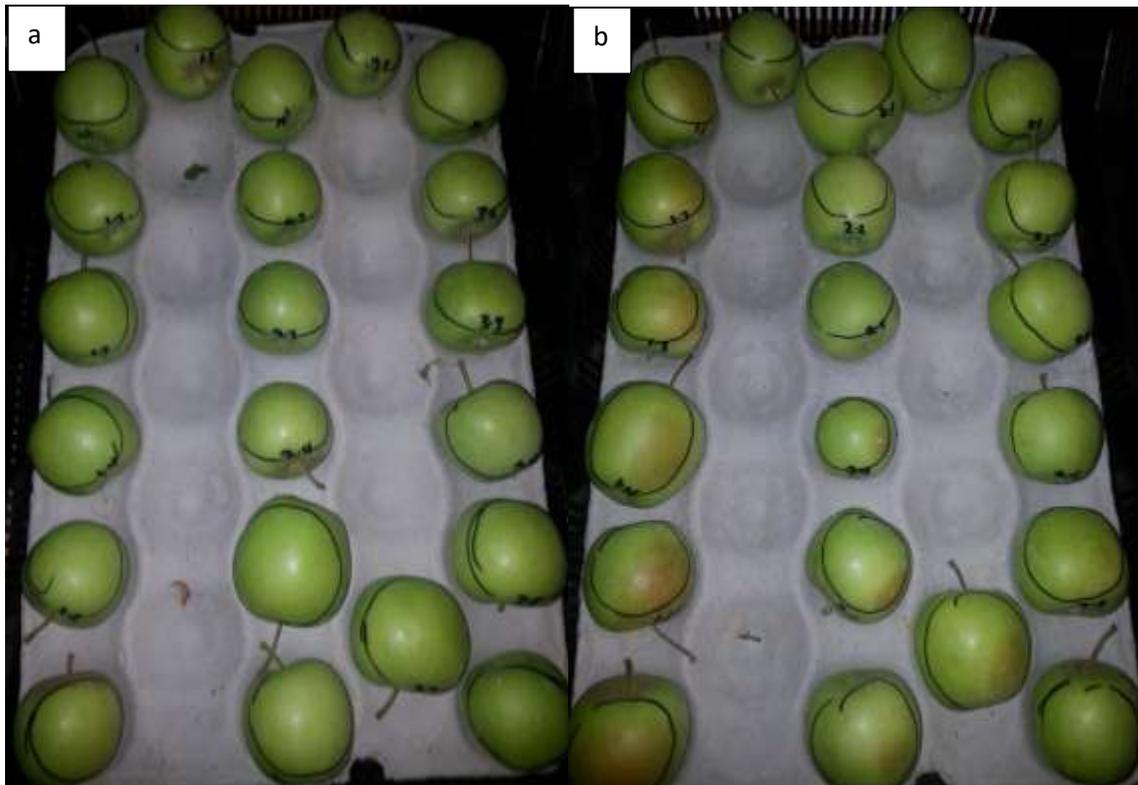


Figure 4. Golden delicious apples harvested from sun-exposed canopy positions under 1-hour exposure to the photothermal stress condition a) immediately after removal from the photothermal stress b) after 5 days of observation under room temperature conditions.



Figure 5. Golden delicious apples harvested from sun-exposed canopy positions (from block 3) five days after exposure to photothermal stress a) apples under 2-hour exposure to the photothermal stress condition b) apples under 3-hour exposure to the photothermal stress. Black circles indicate area of fruit covered with black sticker.



Figure 6. 'Rosy Glow' apples harvested from block 3 five days after exposure to the stress condition a) apples from shaded canopy positions b) apples from sun-exposed canopy positions. Black circles indicate area of fruit covered with black sticker.