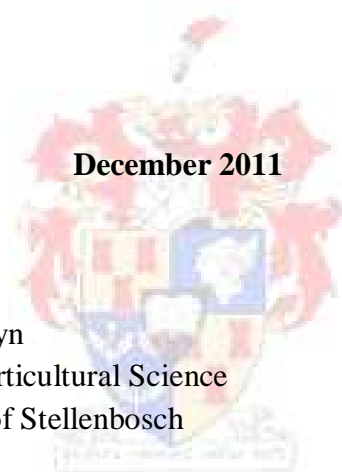


STUDIES TOWARDS UNDERSTANDING SUNBURN IN APPLE

By

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DECLARATION

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SUMMARY

Research was carried out to increase the current knowledge on sunburn development and its control in apples. In addition to its chief causes, viz. high irradiance and high temperatures, water stress has long been thought to contribute to sunburn susceptibility. Certain fruit chemical and textural characteristics have also been suspected to increase sunburn, while it is not clear how factors such as crop load affect sunburn in relation to other fruit quality parameters. In red and blushed cultivars, sunburn could be underestimated due to masking by the red colour overlay.

Half and total irrigation water were withheld for two weeks in ‘Cripps’ Pink’ apples while in a concomitant trial, mulching with vermicompost (plus a thin layer of woodchips), woodchips, plant compost and black geotextile were used to regulate plant water by reducing soil evaporative loss. Sunburn increased with an increase in moisture stress. Mulching had no effect on plant water status, but it generally enhanced plant photochemistry, reducing fruit surface temperature (FST) and sunburn.

Chemical and textural characteristics of exposed, but non-burned ‘Cripps’ Pink’ apples were compared with that of fruit with induced and naturally occurring sunburn. Flesh firmness, total soluble solids (TSS), and titratable acidity (TA) of induced and naturally occurring sunburnt fruit did not differ from each other, but were significantly different from the non-burnt fruit. Based on this, we concluded that sunburn induces textural and compositional changes in sunburnt fruit rather than some fruit being predisposed to develop sunburn due to their texture and chemical composition. Differences in heat stress tolerance, flesh texture and chemical composition of sun exposed and shaded fruit sides seem to relate to their light exposure history.

The effect of crop load on sunburn and fruit quality were assessed on a fruit cluster level in 2008-09 and on a whole tree basis in 2009-10 in 'Cripps' Pink'. In 2008-09, sunburn, red colour and the proportion of first grade fruit decreased with an increase in number of fruit per cluster. Crop load did not have an effect on sunburn and fruit colour in 2009-10 although the lowest thinning severity seemed to increase sunburn severity while the proportion of first grade fruit was highest for the highest thinning severity. Fruit number per cluster had no effect on fruit size, while at the whole tree level, fruit size decreased with increasing crop load. Flesh firmness, starch breakdown, TA and TSS were neither affected by the number of fruit per cluster nor the crop load of the tree as a whole.

Green 'Granny Smith' and 'Golden Delicious', blushed 'Royal Gala', 'Fuji', 'Braeburn' and 'Cripps' Pink', and full red 'Topred' were used to assess how red colour (anthocyanins) masks superficial sunburn browning and bleaching. The greater reduction in superficial sunburn, but not in sunburn necrosis in red and blushed compared to green cultivars with increasing red colour a month towards harvest seemed to confirm the effect of masking.

OPSOMMING

Hierdie navorsing is verrig ter uitbreiding van die huidige kennis oor die ontwikkeling en beheer van sonbrand in appels. Addisioneel tot die hoof oorsake van sonbrand, naamlik hoë irradiasie en hoë temperatuur, word waterstres gesien as bydraend tot sonbrandsensitiwiteit. Daar word ook vermoed dat sekere chemiese en teksturele eienskappe van die vrug sonbrand kan verhoog terwyl daar nie duidelikheid is oor hoe faktore soos oeslading sonbrand relatief tot ander vrugkwaliteitparameters kan affekteer nie. Die omvang van sonbrand in rooi en bloskultivars kan dalk onderskat word vanweë maskering deur die oorliggende rooi pigmentasie.

Besproeiing is vir twee weke gehalveer of gestop in ‘Cripps’ Pink’ appels. Sonbrand het toegeneem met ‘n toename in vogstres. ‘n Deklaag van vermikompos (plus ‘n dun laag houtstukkies), houtstukkies, plantkompos en ‘n swart geotekstiel is toegedien om die plant waterstatus te reguleer deur evaporasie vanuit die grond te beperk. Die deklae het geen effek op die plant waterstatus gehad nie, maar het wel die blaar fotochemie bevorder en terselfertyd die vrugoppervlaktemperatuur verlaag en sonbrand verminder.

Die chemiese en tekturele eienskappe van sonligblootgestelde ‘Cripps’ Pink’ appels sonder sonbrand is vergelyk met vrugte met geïnduseerde sonbrand en vrugte met sonbrand wat natuurlik ontwikkel het. Vrugvleisfermheid, totale oplosbare vastestowwe (TOV) en titreerbare suur (TS) van vrugte met natuurlik en geïnduseerde sonbrand het nie onderling verskil nie, maar het wel betekenisvol verskil van vrugte sonder sonbrand. Ons het gevolglik afgelei dat sonbrand teksturele en komposisionele veranderinge teweeg bring eerder as dat sekere vrugte

gepredisponeer word om sonbrand te ontwikkel vanweë hul tekstuur en chemiese samestelling. Dit kom voor dat verskille in hittestres toleransie, tekstuur en chemiese samestelling tussen die sonligblootgestelde en skadu kante van appels verband hou met hul verskil in sonligblootstelling.

Die effek van oeslading op sonbrand en vrugkwaliteit in ‘Cripps’ Pink’ is ge-evalueer op trosvlak in 2008-09 en op ‘n per boom basis in 2009-10. Sonbrand, rooi kleur en die proporsie eersteklas vrugte het afgeneem met ‘n toename in die aantal vrugte per tros. Op ‘n per boom basis is rooi kleur en sonbrand egter nie deur oeslading geaffekteer nie. Die laagste vlak van vruguitdunning het wel oënskynlik die graad van sonbrand verhoog terwyl die hoogste vlak van vruguitdunning tot ‘n toename in die proporsie eersteklas vrugte gelei het. Die aantal vrugte per tros het geen effek op vruggrootte gehad nie terwyl vruggrootte afgeneem het met oeslading op ‘n heelboombasis. Vrugvleisfermheid, styselafbraak, TOV en TS is nie deur aantal vrugte per tros of die oeslading per boom geaffekteer nie.

Groen ‘Granny Smith’ en ‘Golden Delicious’, rooiblos ‘Royal Gala’, ‘Fuji’, ‘Braeburn’ en ‘Cripps’ Pink’, en volrooi ‘Topred’ is gebruik om die maskering van oppervlakkige sonbrandverbruining en –verbleiking deur rooi antosianien te bestudeer. Oppervlakkige sonbrand het minder toegeneem in die maand voor oes in die rooi- en bloskultivars as in die groen kultivars terwyl vrugkleur skynbaar nie die aanwesigheid van die meer sigbare sonbrandnekrose geaffekteer het nie. Maskering speel dus ‘n rol in die aanwesigheid van sigbare sonbrand.

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DEDICATION

Dedicated to my daughter Gwyneth, my pride and joy.

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GENERAL INTRODUCTION

Fruit producers strive to adopt production practices that produce high yields of good quality fruit that sells for the highest attainable prices. Good quality fruit sold for fresh market consumption is often judged on appearance. Due to high summer temperatures and high irradiation, high quality apple production in the Western Cape of South Africa is hindered by sunburn. About 20 % of first grade fruit in this province is downgraded solely due to sunburn (Deciduous Fruit Producers Trust, 2010). Losses due to sunburn in South Africa are up to four times greater than reported in Washington State, USA (Racskó et al., 2010).

Our study aimed at providing a clearer understanding of the physiology of sunburn, its influence on fruit quality and how certain cultural practices can be manipulated to minimize its development. Therefore, our literature study focused on reviewing factors influencing sunburn and the physiology of processes associated with sunburn in apples, especially how they affect fruit surface temperature. The review also includes a discussion on current sunburn control strategies and their shortcomings.

Changes in fruit chemical and textural characteristics such as increased fruit flesh firmness, dry matter content, reduced relative water content and titratable acidity have been consistently associated with sunburnt fruit (Racskó et al., 2005; Schrader et al., 2009). Although not documented, there appears to be a growing perception in the South African apple industry that these characteristics, particularly higher sugar levels, may render the fruit more susceptible to sunburn. Sugar levels rise in fruit towards harvest and sunburn susceptibility concomitantly

increases towards harvest. For a clearer understanding, in this study we compared chemical and textural characteristic of normal fruit with that of fruit with induced and naturally occurring sunburn.

Water stress can predispose fruit to sunburn or aggravate existing damage (Barber and Sharpe, 1971; Schrader et al., 2003). The evaporation of water from the fruit peel surface extracts energy, which in turn cools the peel surface. Water stress will therefore negate evaporative cooling, resulting in an increase in fruit temperature (Woolf and Ferguson, 2000). If the fruit radius is large, the fruit would even be hotter (Smart and Sinclair, 1976), because absorbed radiation is a function of fruit radius and convective heat loss. This explains why fruit surface temperatures can be 8-12°C higher than air temperature (Wünsche et al., 2001). We manipulated irrigation over a two week period and compared sunburn development for normally irrigated trees with those that received half the normal water delivery and those not completely irrigated over this period. Since soil water can be lost by direct evaporation (Gill and Jalota, 1996), reducing this water loss can also improve plant water status, reducing moisture stress and susceptibility of the fruit to sunburn. Mulching is one of the most practical ways to considerably conserve soil moisture by reducing soil surface evaporation (Himelick and Watson, 1990). In addition to assessing the effect of manipulating irrigation on sunburn development, we simultaneously at a different site, assessed the effect of different mulches on plant water status and subsequent sunburn development.

The effect of crop load on sunburn in apples is not well documented and available literature is inconsistent. The number of fruit in a cluster can influence how fruit shade each other from light

(Awad et al., 2001). In this case, sunburn development therefore would be a function of fruit position in a cluster and how much it is exposed to light. However, factors relating to the crop load of the whole tree also seem to play an important role in sunburn development. The crop load of a given tree can determine the crop to shoot ratio (Van den Ende, 1999), the extent of water usage and the competition for assimilates (Jones et al., 1992) and general tree performance. Trees with heavy crop loads therefore have a greater likelihood of experiencing moisture stress, little foliage and more sun exposed fruit than lightly cropping trees. We therefore assessed the effect of fruit number per cluster on sunburn independent of crop load by thinning to different cluster levels on the same tree in one season. In the next season, trees were then thinned to different severities to assess the effect of crop load on sunburn on the entire tree.

Three types of sunburn, viz. sunburn necrosis, sunburn browning and photo-oxidative bleaching have been characterized (Felicetti and Schrader, 2008; Schrader et al., 2001, 2003). Sunburn browning results in a golden-bronze discoloration of fruit peel subjected to high irradiance at high temperatures between 46 to 49 °C. Higher temperatures exceeding 52 °C completely damage the photosynthetic system, killing epidermal and sub-epidermal tissue to result in the dark sunken necrotic spots characteristic of sunburn necrosis. Photo-oxidative bleaching occurs at temperatures as low as 30 °C. It affects non-acclimated shaded fruit which are suddenly exposed to the sun and can manifest within 24 hours as bleached or white patches on the fruit peel.

Manifestation and visibility of sunburn symptoms is a function of prevailing environmental conditions, cultivar susceptibility and peel colour of the fruit (Racskó, 2010). We considered that

anthocyanin accumulation towards harvest in red and blushed cultivars may mask moderate sunburn browning and sunburn bleaching. Apart from increasing the proportion of the crop that can be marketed, masking may also result in an underestimation of the inherent sunburn susceptibility of red and blushed compared to green cultivars. We subsequently conducted a study to determine the extent of masking in red and blushed cultivars.

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LITERATURE REVIEW

Introduction

Sunburn is one of the major factors hindering apple production in the Western Cape of South Africa, due to above optimal temperature and irradiance characteristic of this growing region with its Mediterranean-type climate. Depending on the season, sunburn can decrease class one exportable fruit by 20-50 per cent in the orchard and packhouse, and a further 10 per cent at the export destination (Bergh et al., 1980). Class one apples have an average net export realisation of R5 881 per ton, while on the local market, the price per ton is R3 656 (Deciduous Fruit Producers Trust, 2010). Sunburnt fruit is normally downgraded to the processing industry, with a low net realisation of R534 (Deciduous Fruit Producers Trust, 2010). Therefore, sunburn is a major challenge that greatly decreases profit margins in apple production. To effectively predict and control sunburn, it is important to have a clearer understanding of the underlying causes of the disorder. The emphasis in this review will be on factors influencing sunburn and the physiology of processes associated with sunburn in apples, especially with regards to fruit surface temperature and appearance. Current sunburn control strategies and their shortcomings will also be discussed.

Significance of light in plants

Solar energy is a vital requirement for all photoautotrophic plants. However, only light energy that falls within the photosynthetically active radiation (PAR) range (400-700 nm) is necessary for the assimilation of carbon dioxide in photosynthesis (Salisbury and Ross, 1992). PAR constitutes a small part of the continuous electromagnetic spectrum. Light is not transmitted or

absorbed in a continuous stream, but in discrete units called photons, whose energy can be measured in quanta (Berrie et al., 1987). Like PAR, each part of the electromagnetic spectrum has a specific wavelength. The amount of energy or photons possessed by light at a particular point of the spectrum is inversely proportional to the wavelength (Berrie et al., 1987). Light of a shorter wavelength is therefore more energetic than light of a longer wavelength.

Ultraviolet (UV) radiation, especially between 280-320 nm (UV-B), can be detrimental to plant tissue (Förschler et al., 2003). Prolonged exposure to UV radiation can result in solar injury, which degrades chlorophyll and damages the plant photosynthetic system (Glenn et al., 2008). Due to a smaller zenith angle and thinner stratospheric ozone layer, plants in tropical regions are subjected to higher levels of UV than those in temperate regions at similar altitudes (Madronich et al., 1998).

Light absorption in plant takes place in chloroplasts by photosynthetic pigments (Raven and Johnson, 1999). Chloroplasts have a mixture of pigments, namely chlorophyll *a*, chlorophyll *b*, xanthophylls and carotenes (Berrie et al., 1987; Salisbury and Ross, 1992; Taiz and Zeiger, 2002). Chlorophyll *a* is the major photosynthetic pigment (Berrie et al., 1987), but both chlorophyll *a* and chlorophyll *b* absorb mainly violet-blue (400-470nm) on one side of the visible spectrum and red light (640-700) on the other side (Raven and Johnson, 1999). In the middle of the visible spectrum, the pigments reflect mostly green, which is perceived as the colour of the plants. Carotenoids absorb blue and green light and reflect red, orange and yellow and thus give the orange-yellow colour where they are predominant.

To maximize the light harvesting efficiency, a group of chlorophyll molecules, or photosynthetic unit, absorbing at different wavelengths, work together and channel the absorbed energy to one reaction centre (Salisbury and Ross, 1992). A photosynthetic unit can contain up to 250-350 chlorophyll molecules. In one photosynthetic unit, all absorbed energy is transferred to a reaction centre with a single chlorophyll *a* molecule absorbing at 700 nm. This chlorophyll molecule is called P700 and it operates what is known as Photosystem I (PS I). Another chlorophyll molecule that absorbs light at 680 nm operates Photosystem II (PS II). These photosystems enhance each other for maximal photosynthesis (Berrie et al., 1987; Salisbury and Ross, 1992).

Upon absorption of light, the chlorophyll molecules become excited and highly reactive and unstable (Berrie et al., 1987). The absorbed light energy displaces one electron of an outermost lone pair of the chlorophyll molecule into a higher energy orbit. As a result, the chlorophyll molecule becomes unstable. The molecule can only be stabilized upon regaining another electron in the outer orbit or, if the displaced electron returns to rejoin its initial partner in the molecule (Berrie et al., 1987). Alternatively, the displaced chlorophyll electron may join a molecule of a different substance. The chlorophyll pigment, from which the electron is lost, becomes the electron donor, and the new substance which receives the electron, becomes the electron acceptor. The chloroplast is characterized by a system of electron donors and acceptors and the light reaction of photosynthesis is chiefly involved with electron transport (Salisbury and Ross, 1992). This produces energy rich adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH₂) used in the light independent stage of photosynthesis to fix carbon dioxide into carbohydrates required for plant growth (Berrie et al., 1987).

Light and fruit colour: The external appearance of fruit is a crucial factor in consumer preference. Fruit peel colour is one of the most important factors in consumer fruit quality perception (Andris and Crisoto, 1996). The perceived apple peel colour is attributed to chlorophyll in chloroplasts, carotenoids in chromoplasts and anthocyanins in vacuoles. These pigments reflect light of various wavelength and their different compositions distribution and concentrations in different cultivars determine the ultimate fruit colour (Lancaster et al., 1994). ‘Granny Smith’ and ‘Golden Delicious’ apples are green because chlorophyll predominates in their peel. However, ‘Golden Delicious’ attains a yellow colour during ripening due to chlorophyll degradation with concomitant carotenoid synthesis (Knee, 1971; Gorski and Creasy 1977). Red and blushed cultivars owe their red colour to the presence of anthocyanins.

Anthocyanin synthesis and subsequent red coloration of red and blushed apple cultivars is stimulated by light (Saure, 1990). It is apparent that irradiance quality and quantity vary with distance into the interior of tree canopies (Warrington et al., 1996). Fruit positioned on outer canopy can be subjected to higher irradiance of mostly UV and red light and less far red light compared to fruit in interior positions (Proctor et al., 1975). Therefore for sufficient red colour formation, the position of the fruit is important as it determines the amount of light the fruit will receive (Awad et al., 2000). Visible and UV light are both important in fruit red colour development, with violet-blue and UV-B light being the most effective, while far red is least effective (Ritenour and Khemira, 1997).

Effects of high irradiation on plant growth: The rate of photosynthesis increases with increasing light levels (Bidwell, 1979; Salisbury and Ross, 1992). At low light levels, photosynthesis is

limited by the light-dependant electron transport. At high light levels, the photons absorbed by the chlorophyll molecules cannot all be used in photosynthesis (Demmig-Adams, 1990; Ma and Cheng, 2003). This excess energy reaching the photosynthetic reaction centers has to be regulated to avoid tissue photo-chemical damage (Hausladen and Alscher, 1993). Under conditions of inadequate regulation of the excess energy, damage occurs to photosynthetic membranes, particularly the thylakoids, caused by the formation of excited oxygen free radicals (Wahid et al., 2007).

Significance of high temperatures on plant growth

Temperature is very important in determining plant growth rates (Woolf and Ferguson, 2000). An increase in temperature generally increases the rate of photosynthesis and subsequently an increase in the growth rate of the plant. (Bidwell, 1979; Salisbury and Ross, 1992; Taiz and Zeiger, 2002). However, very high temperatures may result in heat stress. Wahid et al. (2007) define heat stress as an increase in temperature exceeding a certain threshold, over a period of time long enough to result in damage to plant growth and development. Plant growth and development are affected due to a direct impediment of photosynthesis. Thylakoids in the photosynthetic chloroplasts are sensitive to high temperatures (Wise et al., 2004) and heat stress can distort their structural arrangement, disrupting grana stacking and functionality (Karim et al., 1997). As the PSII is located on the thylakoid membranes, the whole system is therefore greatly slowed down or almost stopped by heat stress. In addition, enzymes and proteins are generally denatured by heat and the fluidity of membranes increases (Wahid et al., 2007).

Excess heat is thought to dissociate or inhibit oxygen evolving complexes (OEC) which are responsible for the movement of electrons from split water molecules to PSII. Under heat stress conditions, proline, instead of water, donates electrons to PSII (De Ronde et al., 2004). Active oxygen species (AOS) increase with increasing temperatures (Camejo et al., 2006). These AOS are the singlet oxygen ($^1\text{O}_2$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and the superoxide radical (O^{2-}). The AOS are responsible for the peroxidation of membrane lipids, distorting membrane functions (Wahid et al., 2007). In addition, AOS result in the degradation of chlorophyll *a* and chlorophyll *b* (Karim et al., 1997). This further breaks down the photosynthetic system, impeding plant productivity.

Effect of heat stress on plant organs: Since high temperatures directly affect physiological processes of plant growth, dry matter production is greatly reduced in organs of heat-stressed plants, resulting in reduced yields (Giaveno and Ferrero, 2003). Root and shoot growth are generally inhibited. Noticeable pre-harvest effects of heat stress on plant organs include burnt foliage, leaf senescence and abscission as well as sunburn and discolouration of fruit (Ismail and Hall, 1995; Wahid et al., 2007).

Plant protective mechanisms against photo-thermal stress

High irradiance in combination with high temperatures (photothermal stress) cause the formation of highly reactive and hazardous AOS in plant tissues (Merzlyak and Solovchenko, 2002; Wünsche et al., 2004). These radicals undergo a series of oxidative reactions affecting cell lipids, proteins and membrane integrity, ultimately killing the cell (Hausladen and Alschner, 1993; Wünsche et al., 2004). Therefore plants developed mechanisms of dissipating this excess energy

(Demmig-Adams and Adams, 1992; Lambers et al., 1998; Niyogi, 1999). Plants can protect themselves from solar damage by synthesizing secondary pigments (Demmig-Adams and Adams, 1992), antioxidants, scavenging enzymes (Demmig-Adams, 1990; Jansen et al., 1998; Noctor and Foyer, 1998) or by dissipating excess energy through the xanthophyll cycle (Müller et al., 2001).

Xanthophyll cycle: When plants are subjected to potentially injurious light and temperature, the xanthophyll cycle is activated and associated with the light harvesting complexes of photosynthesis and lipids in the thylakoid membranes (Wahid et al., 2007). The xanthophyll cycle is an important mechanism used by plants to dissipate the excess energy that would otherwise cause oxidative cell damage (Gilmore, 1997). It involves the reversible interconversion of three forms of xanthophylls, namely violaxanthin, antheroxanthin and zeaxanthin. This is attained by the addition or subtraction of an epoxide group (Demmig-Adams and Adams, 1992; Long and Humphries, 1994). Under high irradiance or in combination with high heat load, violaxanthin is converted to antheroxanthin and rapidly to zeaxanthin. Zeaxanthin is the de-epoxidised form and is responsible for protection of the photosynthetic organelles against excess energy damage by dissipating it as heat. This process can be reversed in the dark. The amount of xanthophylls converted to zeaxanthin under intense irradiance is higher than under conditions of moderate to low irradiance (Demmig-Adams, 1990).

When plants are subjected to high irradiance, the total quantity of all the xanthophylls in the cycle also increases. Under conditions of low irradiance, the xanthophyll cycle carotenoids contribute as low as 5-15 % of the total photosynthetic membrane carotenoid content whereas

exposure of plants to higher irradiance could increase the proportion to about 40 %. (Demmig-Adams, 1990). Therefore acclimation of plants to light up-regulates the xanthophyll cycle pool, enabling sun leaves to dissipate more excess energy than shade leaves. Under both high temperature and high irradiance conditions, xanthophylls molecules result in decreased membrane fluidity and a reduced risk of the peroxidation of lipids (Havaux, 1998).

Protective secondary pigments and antioxidants: Secondary pigments and antioxidants scavenge and quench free radicals caused by excess heat and light energy before they cause damage in plant tissue (Wang et al., 1997). Noctor and Foyer (1998) described antioxidants as compounds with the ability to react with AOS rendering them harmless without itself being converted to destructive radicals. The major antioxidants found in fruit skin are polyphenols, including flavonoids of which anthocyanins form part, β -carotene, ascorbic acid and tocopherol (Rice-Evans et al., 1997).

Anthocyanins have the ability to modulate the quality and quantity of light before interception by chloroplasts (Smille and Hetherington, 1999). Hatier and Gould (2009) indicated that light filtered by anthocyanins is not incident on chloroplasts, although the fate of this diverted light is yet to be established. Merzlyak and Chivkunova (2000) attributed the photoprotective properties of anthocyanins primarily to their ability to modify light, as they cannot directly be involved in free radical reactions in the chloroplasts, due to their vacuolar localization. The anthocyanins in apples were reported to actively absorb light in the green-orange part of the spectrum (500-600 nm), precisely between the chlorophyll and carotenoid absorption range (Merzlyak and Chivkunova, 2000). However, in vegetative tissue, red anthocyanins were reported to

predominately absorb green and ultraviolet light (McClure, 1975). By absorbing in the blue-green range, the anthocyanins reduce the quantity of visible light incident on chloroplasts, protecting them against photodestruction (Smillie and Hetherington, 1999). Merzlyak and Chivkunova (2000) also reported increased visible light absorption with increased anthocyanin accumulation in 'Zhigulevskoe' apples. Li and Cheng (2009) recently showed that bud mutation red 'Anjou' pear peels, which have a higher anthocyanin content, had a higher photoprotective capacity under high light than normal green 'Anjou' peels. In addition, Steyn et al. (2009) also demonstrated the photoprotective capacity of anthocyanins in immature apple and pear peels by noting a reduction in photoinhibition under stressful low temperature conditions that normally induce photoinhibition. Although Li and Cheng (2009) recently found that anthocyanins also reduce photoinhibition at high temperatures, it must be noted that anthocyanin concentrations generally correlate negatively with temperature and high temperatures also increase anthocyanin degradation (Steyn et al., 2004). Hence, the potential beneficial effect of anthocyanins is limited under high temperature conditions. In fact, the additional absorbance of light in peel containing high anthocyanin concentrations may increase the heat load and thereby exacerbate photothermal stress (Schroeder 1965; Smart and Sinclair 1976).

Ascorbic acid and polyphenols can protect plants against oxidative destruction caused by excess heat and light energy through their antioxidant properties (Rice-Evans et al., 1997). Ascorbic acid is the ultimate electron donor in processes that quenches hydrogen peroxide, the hydroxyl radical and the superoxide anion. It is also involved in the synthesis of other antioxidants such as zeaxanthin and α -tocopherol (Foyer, 1993). Ascorbic acid also works in association with glutathione to prevent cell oxidative damage by hydrogen peroxide (Hausladen and Alscher,

1993). The key antioxidants such as glutathione and ascorbate are higher in sun-exposed fruit than in fruit grown in the shade. Sun-exposed fruit are therefore better acclimated to heat and light stress than shaded fruit (Ma and Cheng 2003). Antioxidant enzymes are compounds that catalyze reactions that quench AOS or they are directly involved in the radical quenching processes (Noctor and Foyer, 1998; Jansen et al., 1998). Important scavenging enzymes are superoxide dismutase (SOD), glutathione reductase and ascorbate peroxidase (Jansen et al., 1998).

Acclimation of leaves and fruit to light environment: Plants can make morphological and biochemical changes to suit the light environment to which they are constantly exposed to (Anderson and Osmond, 1987; Demmig-Adams et al., 1995). Depending on the prevailing light exposure, plants can modify their photosynthetic system to maximize use of available light in carbon assimilation (Ma and Cheng, 2003). Sun leaves for instance allocate more nitrogen (N) to electron chain transport and enzymes involved in carbon fixing for increased photosynthetic capacity, thereby making the best use of the prevalent light. Shade leaves, however, apportion more N into light harvesting photosynthetic units to capture more light and maximize photosynthesis (Evans, 1996).

Morphologically, plants can acclimate to high irradiance by adopting inclined leaf angles (Lovelock and Clough, 1992). Steep leaf angles reduce the solar radiation interception by the leaf, resulting in less energy in excess of that which can be utilized in carbon assimilation (Valladares and Pearcy, 1997). Shade leaves are normally arranged at right angles to the incident solar radiation for maximum interception.

Acclimation of plants to high temperatures: Plants can, to a certain extent combat the negative effects of high temperatures by increasing evapotranspirative water loss (Demmig-Adams et al., 2008). This is not effective if heat stress is accompanied by water stress as the water lost through evapotranspiration has to be immediately replaced through absorption by the roots. A reduction in plant water potential might result in stomatal closure by the plant as a way to conserve water which otherwise might be lost through evapotranspiration (Demmig-Adams et al., 2008). Plants that grow in hot, dry and arid areas have evolved structurally (Berrie et al, 1987) , adopting smaller, lobed or spined leaves that enhance convective cooling especially in the absence of evapotranspirational cooling (Demmig-Adams et al., 2008).

Biochemically, plants respond to high temperatures by synthesizing heat-shock proteins (Wahid et al., 2007). This is a new set of proteins produced at the expense of normal proteins. They are rapidly produced and can constitute a major proportion of plant proteins within as little as 30 min after heat shock (Kimple and Key, 1985). Heat shock proteins' thermo-protection involves protecting cells against denaturation of important enzymes and nucleic acids. Zhang et al. (2003) demonstrated an increase in heat shock proteins of 'Royal Gala' apples exposed to 45 °C between 2-6 h. This increased their photo-thermal tolerance when exposed to sunlight three days later, resulting in less sunburn than untreated fruit.

Chlorophyll Fluorescence

Apple fruit peel have a photosynthetic system that is comparable to that of leaves although the chloroplasts are not as densely distributed as they are in leaves (Blanke and Lenz, 1989; Aschan

and Pfanz, 2003). Apart from driving photochemical processes, the absorbed light by chloroplasts in fruit peel can be dissipated as heat or re-emitted as light, in what is known as chlorophyll fluorescence (DeEll and Toivonen, 2003). Chlorophyll fluorescence therefore gives an indication of light re-emitted out of the total absorbed light which was neither used in photosynthesis nor dissipated as heat because the processes occur competitively (Maxwell and Johnson, 2002). Since the PSII photosynthetic system thylakoid membranes are sensitive to heat and excessive light (Weis and Berry, 1988), their damage would result in a reduction in photosynthesis and an increase in chlorophyll fluorescence. Therefore, the magnitude of photo-thermal damage can be expressed as a function of the efficiency of the chlorophyll apparatus in the fruit peel and this can be measured by chlorophyll fluorescence (Manuel et al., 2001). Chlorophyll fluorescence can thus reliably be used to determine heat and light stress on apple fruit surfaces.

Sunburn: The effect of high light and high temperature.

Sunburn has been characterized into three distinct types, namely sunburn browning, sunburn necrosis and photo-oxidative sunburn (Schrader et al., 2003). Sunburn browning is the most predominant and costly to apple producers (Felicetti and Schrader, 2008). It results from the simultaneous subjection of the apple fruit to high irradiance and high temperatures. Fruit skin temperatures of 46-49 °C in the presence of light, particularly UV-B are required for manifestation of the symptoms of sunburn browning (Schrader et al., 2003). The symptoms appear as yellow, brown, tan or golden bronze-discoloured patches on the affected fruit peel. Although sunburn browning affects the fruit surface and its subsequent appearance, it does not cause fundamental damage to the epidermal tissue and can be seen as an adaptive process.

Sunburn necrosis is mainly caused by excessively high temperatures ($52 \pm 1^\circ\text{C}$) (Schrader et al., 2001). The extreme heat causes thermal death of epidermal and sub-epidermal tissue causing the formation of necrotic spots on the affected fruit area. Sunburn necrosis alters the structure of membranes and thylakoids and there is evidence of electrolyte leakage. Photo-oxidative sunburn occurs when initially shaded fruit is suddenly exposed to high levels of irradiation (Schrader et al., 2008). This sunburn type may occur at temperatures below 30°C and UV-B does not seem to be important in causing this form of sunburn. Symptoms first appear as white discolouration around affected fruit peel. Affected fruit surfaces may undergo subsequent browning or necrosis.

Sunburn and fruit quality: Increased fruit flesh firmness, dry matter content and reduced relative water content and titratable acidity (TA) have been reported in sunburnt fruit (Racskó et al., 2005). Schrader et al. (2009) confirmed that TA generally decreased with increasing sunburn whereas Volz et al. (1995) confirmed increased flesh firmness of 'Royal Gala' apples with increased irradiance. In addition, Schrader et al. (2009) reported an increase in soluble solids with increasing sunburn. Consistent with that, Schrader et al. (2009) generally noted increased starch breakdown with increasing severity of sunburn browning. This could also account for the increased TSS associated with sunburnt fruit. Racskó et al. (2005) postulated that sunburnt cells lose water, resulting in firmer flesh and an increase in TSS.

Crop load and sunburn: A heavy crop load increases the crop to shoot ratio. This can result in less vegetative cover and an increase in fruit exposure to light. Under high light and high temperature conditions, heavily cropped trees are therefore more susceptible to sunburn than

lightly cropped trees. Heavy cropping also causes branch bending and breakage which might result in exposure to light of previously shaded fruit (Bergh, 1980; Hirst et al., 1990). Heavy bearing branches should be trained or propped to maintain fixed positions (Van Den Ende, 1999). Branch movement and resultant sudden exposure of previously shaded fruit to light renders them susceptible to sunburn (Schrader et al., 2008). Van den Ende (1999) indicated that minimum sunburn is obtained if fruit, particularly bi-coloured and red cultivars are thinned to one or two per cluster. Wünsche et al. (2004) did not find significant crop load effects on sunburn in 'Fuji' apples.

Plant water relations and sunburn: Fruit borne on trees performing sub-optimally are prone to sunburn, particularly those which are stressed for water (Van Den Ende, 1999; Schrader et al., 2003; Brown, 2009). Concomitant water stress has been reported to exacerbate the development of sunburn (Barber and Sharpe 1971; Schrader et al., 2003). The evaporation of water from the fruit peel surface extracts energy, which in turn cools the peel surface. Bulky plant tissue such as fruit have reduced evaporative cooling potential compared to leafy tissue (Woolf and Ferguson, 2000). Water stress will therefore result in an increase in fruit temperature (Woolf and Ferguson, 2000) and if the fruit radius is large, the fruit would even be hotter (Smart and Sinclair, 1976), because absorbed radiation is a function of fruit radius and convective heat loss. This explains why fruit surface temperature can be 8-12 °C higher than the air temperature (Wünsche et al., 2001). Therefore, at ambient air temperatures of 30-36 °C, fruit become predisposed to the risk of sunburn considering that the fruit temperature would be between 38-48 °C. The risk increases with concomitant moisture stress.

Under moisture stress, the evaporative cooling potential of trees is greatly reduced (Mastalerz, 1977; Berrie et al., 1987). Coupled with the fact that the fruit does not have effective energy dissipation mechanisms (Jones, 1981; Blanke and Lenz, 1989) as they have lower chloroplast concentrations per unit area compared to leaves (Aschan and Pfan, 2003), this would lead to higher fruit temperatures which in turn contribute to higher sunburn incidence and severity.

Fernandez-Marin et al. (2009) indicated that drought stress conditions in plants could initiate the xanthophyll cycle. The xanthophyll cycle is a response mechanism to get rid of excess light that might cause damage in plants (Müller et al., 2001). The activation of the xanthophyll cycle under drought stress could be an acknowledgement that the plant experiences excess light energy that otherwise might be manageable under normal plant water statuses. Quick et al. (1992), Garcia-Plazaola et al. (1997) and Werner et al. (1999) confirmed increased xanthophyll activity due to photoinhibition caused by water stress.

Photoinhibition in plants is a measure of light energy absorbed in excess of that which can be utilized in carbon assimilation (Demmig-Adams et al., 1995). Photoinhibition increases as adverse environmental factors limit photosynthesis (Manuel et al., 2001). Düring (1999) reported that the quantum yield of water-stressed grape vines decreased at lower irradiance compared to well-watered ones. Closure of stomata, triggered by water stress, limits carbon dioxide availability in mesophyll cells, which results in excessive excitation of the photosynthetic apparatus, particularly photosystem II (PSII). This results in photoinhibition. To protect themselves against photoinhibition, plants make use of non-photosynthetic chemical energy dissipation and the xanthophyll cycle. Failure of these processes to dissipate the excess energy

would result in bleaching of leaves and fruit parts due to breakdown of chlorophyll (Lambers et al., 1998).

Although little work has been done to relate plant water status, excess light and heat to sunburn incidence and severity, maintenance of high water potential within apple trees seems crucial in minimizing the susceptibility of fruit to thermal injury and subsequently sunburn development. Cultural practices such as mulching conserve soil moisture by considerably reducing soil surface evaporation (Huang et al., 2008).

Soil water status and mulching: Plant water stress under natural rainfall conditions and, in some cases, even under irrigated conditions, can be attributed to several soil factors (Cassel, 1983). Of these factors, the amount of available water retained by the soil for plant uptake is the most important. Cassel (1983) defines available water as the quantity of water between the extremes of field capacity and permanent wilting percentage. The available water of a soil is greatly affected by its water holding capacity, which in turn is affected by its texture, structure and organic content (Bidwell, 1979).

Soil texture is the relative proportions of silt, sand and clay in a soil. Soils largely composed of large sand-sized particles (0.05-2mm) have large pores and drain excessively. Soils with particles smaller than 0.05mm diameter have numerous smaller pores which retain more water than soils made up of large particles (Bidwell, 1979).

Soil structure refers to the arrangement of individual soil particles, pores and their continuity and ability to retain and infiltrate water (Lal, 1991). It can be related to soil texture and clay content. The structural units or aggregates might be strong, resisting collapse upon exertion of pressure or might be weak and therefore susceptible to compaction. Soil structure is important because it influences root growth and activity (Bronick and Lal, 2005). It is thought that roots grow by extending preferentially into areas of low mechanical impedance (Drew, 1979) in addition to hydrotropic responses. Therefore, a soil with a good structure makes it possible for deeper rooted plants to extract water from considerable soil depth (Cassel, 1983).

Mulches improve soil structure, water infiltration rates, increase soil organic matter levels, control erosion and eliminate weed competition and ultimately conserve moisture (Bruce et al., 1992; Mathews et al., 2002; Huang et al., 2008). Mulches also control temperature fluctuations by absorbing or reflecting large quantities of heat that may otherwise be conducted down the soil (Montague et al., 1998). Soil temperature buffering capacity of woodchips is well documented in many crops. A temperature difference of 8.9 °C was reported in blueberry soil under pine bark mulch compared to bare soil at 5 cm depth (Krewer et al., 1997).

Conserved soil moisture as a result of mulching may subsequently increase plant water potential (Pinamonti, 1998). This coupled with improved soil conditions may enhance shoot growth, which might provide shading to fruit and reduce sunburn. In a newly established apple orchard, Mathews et al. (2002) reported up to 22 % increment in shoot growth and shoot number due to mulching. According to Van Den Ende (1999), annual shoot extension in the range of 20-30 cm uniformly distributed over the tree ensures filtered light reaching the fruit as opposed to adverse

direct sunlight that might cause sunburn. In addition, a higher water potential enables the tree to cool itself through evaporation from lenticels, lowering the fruit surface temperature (Schrader et al., 2003; Brown, 2007).

Current sunburn control strategies

In order to produce good quality fruit, producers have to intervene with cultural practices that attenuate intense solar radiation and lower fruit temperatures to levels that are below the critical threshold for sunburn development. The most common practices adopted by producers to suppress sunburn in the orchards are the use of evaporative cooling, shade net covering and reflective particle film sprays.

Evaporative cooling: In the evaporative cooling system, an overhead sprinkler is used to wet the canopy when the temperature rises above a specific threshold value (Evans, 1993). Under the hot conditions, water evaporates from the fruit surface, cooling the fruit and reducing its surface temperature in the process (Parchomchuk and Meheriuk, 1996). Overhead water application every 30 minutes was reported to lower mean fruit surface temperatures by up to 8.5 °C, a level comparable to air temperature (Wünsche et al., 2001). Reductions in the incidence of sunburn can be very considerable under this system. In South Africa, Kotze et al. (1988) reported a reduction in sunburn of up to 50% in a ‘Granny Smith’ apple orchard. The major disadvantage with this strategy is that it requires large volumes of good quality water and a huge capital expense for installation. Another disadvantage is that water of the appropriate electrical conductivity should be used in order to avoid deposits of salts on leaves and fruit which might be toxic (Andrews, 1995). The water from evaporative cooling sprinklers can also humidify the

canopy, leading to proliferation of pests and diseases (Evans, 1993). In addition, fruit acclimated to evaporative cooling conditions are more sensitive to photo-thermal damage. A breakdown or interruption of the evaporative cooling system would most certainly result in more severe sunburn (Evans, 1993).

Light-reflecting material: Light-reflecting materials can be applied to reduce the absorption of light by the fruit, particularly UV-B light (Glenn et al., 2002). Kaolin, a white clay dust initially used to ward off insects by coating plant surfaces reflects light and is chemically inert (Glenn and Puterka, 2005). The kaolin-based particle film spray (Surround[®] WP, Engelhard Corporation, NJ, USA) was reported to be effective in lowering apple fruit surface temperatures to levels below the critical threshold for sunburn development (Schrader et al., 2001; Wünsche et al., 2001).

In South Africa, Le Grange et al. (2004) reported effective sunburn control by Surround[®] WP on ‘Fuji’, ‘Granny Smith’ and ‘Royal Gala’ apples. However, Erez and Glenn (2004) found no effect of Surround[®] WP on control of sunburn in ‘Cripps’ Pink’ apples. The major shortcoming of this control strategy is that fruit end up with spray residues that are costly and not easy to remove (Erez and Glenn, 2004; Gindaba and Wand, 2005). In addition, Gindaba and Wand (2007), reported reduced stomatal conductance on ‘Royal Gala’ apples due to Surround[®] WP and attributed this to blocked stomatal pores and increased leaf boundary layer resistance caused by resultant roughness of the leaf surface. Sunguard is another kaolin based whitening agent that has had limited success in decreasing sunburn (Sibbett et al., 1991).

Raynox is a lipid soluble spray that easily binds with fruit skin cuticle (Schrader et al., 2008) and is not visible. Therefore, unlike kaolin based particle sprays, it does not affect selective fruit picking based on colour and the cost and time of removing spray residues at harvest are minimal. Schrader et al. (2008) reported that Raynox protects the fruit by reflecting mostly UV-B and to a less extent UV-A and PAR. A single application of Raynox reflected about 20 % UV-B and a double application also doubled the reflection of UV-B. Over a period of seven years, Raynox consistently decreased sunburn by up to 50 % in the Washington State (Schrader et al., 2008). However, under South African conditions, Raynox alone did not significantly reduce fruit surface temperature compared to untreated control (Gindaba and Wand, 2008). As it is only effective in reducing only irradiance, Gindaba and Wand (2008) observed that the use of Raynox was effective in combination with treatments that also reduce temperature, such as evaporative cooling.

Shade netting: Shade nets are used to cover tree canopies in regions with adverse hot and sunny climates where they diffuse intense solar radiation and increase crop productivity (Al-Helal and Abdel-Ghany, 2011). The light scattering effect of shade nets has significant applications in fruit quality homogeneity and sunburn control. By lessening the intensity of solar radiation reaching the fruit, shade nets subsequently lower the fruit surface temperature (Parchomchuk and Meheriuk, 1996; Jifon and Syvertsen, 2003; Gindaba and Wand, 2005). In a two-season trial in South Africa, a 20 % black shade net was reported to have reduced the incidence of sunburn in ‘Fuji’ and ‘Braeburn’ apples in both seasons, only in the first season for ‘Royal Gala’ and no effect on ‘Cripps’ Pink’ in both seasons (Smit, 2007). The shade nets decreased red colour

development in 'Fuji' and 'Braeburn', whereas 'Cripps' Pink' and 'Royal Gala' were not affected.

Although fruit temperatures under shade netting can be 10 °C cooler, with a reduced UV-B exposure (Gindaba and Wand, 2005), these fruit end up having low antioxidants and heat shock proteins. These fruit therefore have a low heat tolerance especially if exposed during transport from shaded orchards to the cold rooms in open bins (Smit, 2007).

Light attenuation is affected by net colour and density (Scott, 1989; Shahak et al., 2004). Al-Helal and Abdel-Ghany (2011) described net density or solidity as the area covered by net threads, divided by the entire area covered by the net. Gindaba and Wand (2005) reported reduced photosynthetic capacity and poor red colour development due to 20% shade netting.

Black and white nets have been reported to impair the quality of light reaching the plants which resulted in undesirable vegetative growth at the expense of fruit quality (Solomakhin and Blanke, 2007). Red/white and white nets resulted in better fruit quality (high sugar and high sugar: acid ratio), firmer fruit and better colour development in 'Fuji' apples than under red/black and green/black hail nets (Solomakhin and Blanke, 2009). Compared to the control, the green/black and red/black nets reduced PAR by 18-20 % whereas the white and red/white nets reduced it by 12-14 %. It was observed that white nets allowed more PAR to reach the plants and with coloured nets, blush colour improved with an increase in the replacement of black nets by white ones. Therefore, improper choice of net colour and density for particular growing conditions might reduce quality and productivity.

The other disadvantage of shade nets is that they reduce fruit set (Middleton and McWaters, 2002). Although the reasons for increased fruitlet drop under shade net is not quite clear, modulated light levels under nets could stimulate vegetative and reproductive growth competition, as suggested by Bepete and Lakso (1998). In addition, shade nets are very expensive to install and maintain (Dussi et al., 2005) and could prove costly to the farmer, particularly if they carry out regular thinning programs, as they might end up with below normal crop load.

Other cultural practices: The choice of rootstock can have a bearing on the incidence and susceptibility of trees to sunburn. Dwarfing rootstocks result in trees with less dense canopies. (Wünsche et al., 2004). Fruit borne on such canopies are more exposed to light than the vigorous and semi-vigorous rootstock and are therefore are more susceptible to sunburn (Wünsche et al., 2000). Canopy manipulation by cultural practices such as tree training and pruning can be used to suppress sunburn. Shading of fruit by leaves will protect them against sunburn, but if the shade is intense, then red colour development would be compromised (Moran and Rom, 1991). Pruning methods should therefore ensure that the fruit should receive filtered light. Fruit should be allowed to acclimatize to light by consistent and continual removal of water shoots (Van Den Ende, 1999). Care should be taken not to remove water shoots just prior to harvest as this suddenly exposes unconditioned fruit to light (Van Den Ende, 1999).

Fouché (2009) reported benefits of summer pruning in vigorous ‘Granny Smith’ orchards in terms of improved light distribution, with subsequent good green colour with no marked increase

in sunburn. However, attempts to head one year old shoots in the outer canopy to stimulate vegetative re-growth and thereby shading to reduce sunburn, were not successful.

Cultivars such as 'Braeburn' and 'Fuji' are known to have little foliage in the upper canopy, thus resulting in serious sunburn. This can be rectified by proper training of the young trees (Van Den Ende, 1999). 'Granny Smith' apples have what is known as the type IV bearing habit whereby fruit is mostly borne on the terminal ends of medium and long shoots (Lauri et al., 1995). Towards ripening, branches bend under the weight of fruit which expose previously shaded fruit to the sun.

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MULCHING DECREASES, WHILE MOISTURE STRESS INCREASES SUNBURN DEVELOPMENT IN APPLES

Abstract

Experiments were carried out to assess the effect of mulching and moisture stress on plant water relations and subsequent sunburn development in 'Cripps' Pink' apples. Mulching treatments of woodchips, plant compost, vermicompost (with an added thin layer of woodchips to prevent it from being blown away, but referred to as vermicompost), a woven black geotextile polytex and a no mulch control were applied after full bloom in two orchards on sandy loam and clay loamy soils, respectively in 2008-09 and 2009-10 seasons. In 2009-10, irrigation was manipulated in a concurrent trial at a different site to ascertain the role of moisture stress in sunburn development. Normal irrigation, half irrigation and no irrigation treatments were applied for 14 days starting on 15 March 2010. Plant water potential, fruit surface temperature (FST), sunburn incidence and sunburn severity were measured in both trials. Stomatal conductance, transpiration and net CO₂ assimilation rate were measured to determine tree performance for the mulching trial. Sunburn was categorized into browning, necrosis or bleaching in the manipulated irrigation trial. In 2009-10, fruit were tagged for progressive sunburn incidence and sunburn severity assessments in both trials and stem water potential was taken instead of leaf water potential. Mulching did not appear to have a significant effect on plant water status, but enhanced plant performance by increasing photochemistry and gas exchange, with vermicompost in particular, more consistently reducing FST and sunburn compared to the control. Sunburn incidence and sunburn severity did not differ significantly a month before harvest, but increased and significantly differed at harvest, with mulching

treatments being generally lower than the no mulching control. Trees in loamy clay generally performed better than those in sandy loamy. Stem water potential decreased and FST, sunburn incidence and sunburn severity increased linearly with a decrease in irrigation level in the manipulated irrigation trial. Sunburn browning was more prominent under lower moisture stress while necrosis increased with increasing moisture stress. Mulch, particularly vermicompost, enhances tree performance and has the potential to become an important cultural control measure against sunburn although the effects might not be related to plant water relations. Concomitant moisture stress indeed aggravates sunburn development under conditions conducive for its development.

Introduction

The external appearance of fruit is a crucial factor in consumer preference (Gamble et al., 2006). Sunburn, a disorder of the fruit peel caused by high temperature in combination with high irradiance (Glenn et al., 2002; Schrader et al., 2003), negatively affects the appearance of apples in warm production areas such as the Western Cape of South Africa. Depending on irradiance level, temperature and the duration of the photothermal stress (Gindaba and Wand, 2007; Schrader et al., 2003), affected fruit show a golden-bronze sub-lethal or necrotic discolouration of the fruit skin (Racskó et al., 2005; Schrader et al., 2003).

Three types of sunburn have been categorized by Schrader et al. (2009) according to the causative environmental conditions. Sunburn browning is caused by high temperatures in the range of 46-49 °C in the presence of solar radiation. Extreme heat in the order of 52 ± 1 °C causes death of epidermal and sub-epidermal cells resulting in sunburn necrosis. Photo-oxidative

bleaching, which is the third type of sunburn, results when a previously shaded fruit is suddenly exposed to light, and may occur at much lower temperatures (<30 °C).

Losses due to sunburn are therefore due to the downgraded appearance of the fruit. In addition, the sun-exposed areas soften quickly in storage resulting in further losses (Arndt, 1992). Subsequently, sunburn can decrease class one exportable fruit by 20-50 percent in the orchard and packhouse, and a further 10 percent at the export destination (Bergh et al., 1980).

Concomitant water stress has been reported to exacerbate the development of sunburn (Barber and Sharpe 1971; Schrader et al., 2003). The evaporation of water from the fruit peel extracts energy, which in turn cools the peel surface and bulky plant tissue such as a fruit have reduced evaporative cooling potential compared to leafy tissue (Woolf and Ferguson, 2000). Water stress will therefore result in an increase in fruit temperature (Woolf and Ferguson, 2000) and if the fruit radius is large, the fruit would even be hotter (Smart and Sinclair, 1976), because absorbed radiation is a function of fruit radius and convective heat loss. That is why fruit surface temperature can be 8-12 °C higher than the air temperature (Wünsche et al., 2001). Therefore at ambient temperatures of 30-36 °C, fruit become predisposed to the risk of sunburn considering that the fruit temperature would be higher than the air temperature and the risk becomes even higher with moisture stress.

Evaporative cooling through transpiration is instrumental in reducing plant surface temperature (Berrie et al., 1987; Woolf and Ferguson, 2000). Plants under moisture stress have a lower evaporative cooling capacity and therefore have higher leaf and fruit surface temperatures

(Woolf and Ferguson, 2000). The fact that fruit do not have effective heat dissipation mechanisms (Jones, 1981; Blanke and Lenz, 1989), leads to even higher fruit temperatures, which in turn contribute to higher sunburn incidence and severity.

Increased sunburn under moisture stress could also be due to a direct physiological impairment of the fruit photoprotective mechanisms. Fernandez-Marin et al. (2009) indicated that drought stress conditions in plants could trigger the xanthophyll cycle and Mabhaudhi and Modi (2010) indicated that moisture stressed plants have increased free radicals. The xanthophyll cycle is a response mechanism to get rid of excess energy or free radicals that might cause damage in plants (Müller et al., 2001). The activation of the xanthophyll cycle under drought stress could be an indication that the plant is struggling with light energy that otherwise might be manageable at a normal plant water status. Quick et al. (1992), Garcia-Plazaola et al. (1997) and Werner et al. (1999) confirmed increased xanthophyll activity due to photoinhibition caused by water stress.

Photoinhibition in plants is a measure of light energy absorbed in excess of that which can be utilized in carbon assimilation (Long and Humphries, 1994). As adverse environmental factors limit photosynthesis, photoinhibition increases (Manuel et al., 2001). Düring (1999) reported that the quantum yield of water-stressed vines decreased at lower irradiance levels compared to well-watered ones. Closure of stomata triggered by water stress limits carbon dioxide availability in mesophyll cells, which results in excessive excitation of the photosynthetic apparatus, particularly photosystem II (PSII). This results in photoinhibition. To protect themselves against photoinhibition, plants make use of non-photosynthetic chemical energy dissipation such as the

xanthophyll cycle. Failure of these processes to dissipate the excess energy would result in bleaching of leaves and fruit, due to breakdown of chlorophyll (Lambers et al., 1998).

Although little work, has been done to relate plant water status, excess light and heat to sunburn incidence and severity, maintenance of high water potential within apple trees seems crucial in minimizing the susceptibility of fruit to thermal injury and subsequent sunburn development. Cultural practices such as mulching conserve soil moisture by considerably reducing soil surface evaporation (Himelick and Watson, 1990).

The objective of this study was to separately investigate the effect of different mulches and moisture stress on plant water relations and how this would in turn affect sunburn development in apples. Our hypothesis was that moisture-stressed apple trees are more susceptible to sunburn due to increased photoinhibition and lower rates of evaporative cooling through the lenticels of the fruit.

Materials and Methods

Experiment 1

Plant material

The experiment was carried out at Lourensford Estate, Somerset West, South Africa (34° 4' S, 18° 53' E) using 'Cripps' Pink' apples on M793 rootstock. The tree spacing was a 4m x 1.5 m in a North East by South West row orientation, trained to an informal central leader on a three wire training system.

Treatments and experimental design

Four different mulching treatments were applied after full bloom in October. These were woodchips (from ornamental trees), plant compost (20 % mushroom straw compost and 80 % municipal green waste), vermicompost (and a thin layer of woodchips on top to prevent the vermicompost from being blown away by the wind, hereinafter referred to as vermicompost) and a black woven (and therefore penetrable) geotextile polytex (PT110, Spilo, Paarl, SA). Plant compost was supplied by Bionamix Pvt Ltd, Klapmuts, SA and vermicompost, by Worm Works Purveyors Pty Ltd., Simmondium, SA. Vermicompost consisted of variable wheat straw and cow manure mixture, obtained from a commercial dairy farm. The fifth treatment was a control without mulching. Treatments were randomized in six blocks with four trees per plot and two trees as buffers between plots to eliminate between-plot interference. In the 2008-09 season, about 75 L of mulching material was spread out evenly over each 6 m² plot. The quantity of woodchips was doubled in 2009-10 in accordance with commercial standards (150 L). The experiment was conducted in two different orchards on the same farm with two different soil textures, i.e., sandy loam, hereinafter called light loam and heavy silt loam, hereinafter called heavy loam. Fertilizer was applied according to commercial standards and trees were irrigated by microjets at the rate of 5 mm h⁻¹ for three hours, twice a week.

Gas exchange: Stomatal conductance, transpiration and net CO₂ assimilation were measured using an infrared gas analyser, LI-6400 (Li-Cor, Lincoln, Nebraska, USA) at 380 μmol·mol⁻¹ CO₂. Light was set at 1500 μmol·m⁻²·s⁻¹ with a leaf temperature of 23°C. Measurements were

taken from two healthy leaves, one from either side of the row, of two trees in the middle of the four-tree plots. Data were averaged per plot.

Fruit surface temperature (FST): FST was measured on ten sun-exposed apples from trees in the middle of the four-tree plots either side of the row using a hand held infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA). Measurements were carried out on typically hot days, 26 and 27 Mar. for the light and heavy loam respectively between 1300 HR and 1400 HR, for both orchards, in the 2008-09 season. In the 2009-10 season, measurements were carried out on 1 and 2 Apr. for light and heavy loam respectively during the same period of the day as the previous season.

Plant water potential: In 2008-09, the plant water status was determined by measuring leaf water potential using a pressure bomb (Model 600, PMS Instrument Co, USA). At noon, three healthy and mature, shaded leaves at a height of about 1.5 m were selected per plot. Due to enormous variation in leaf water potential in 2008-09, it was decided to measure stem water potential in 2009-10 as this measurement was considered to be more stable and representative of the plant water status (Tsuda and Tyree, 2000). To measure stem water potential, two mature inner canopy leaves closer to the trunk were each enclosed in a black polythene bag lined with an outer silver reflective tape for at least an hour to allow the leaves to reach an equilibrium with stem water potential. Still in the bags, the leaves were cut and water potential measured using the pressure bomb.

Sunburn incidence and severity: In 2008-09, sunburn incidence was measured counting the number of fruit with sunburn in a 50-fruit sample per plot, two weeks before harvest (08 Apr. 2009). Twenty five fruit from either side of the row were randomly selected from shoulder height of the two middle trees. The same fruit was assessed for sunburn severity. Sunburn severity was rated using a 0-5 scale developed by Schrader and McFerson (Schrader et al, 2003) where 0 represented no sunburn and 5 the most severe form. In 2009-10, sunburn incidence and severity was assessed on 10 tagged, sun-exposed fruit per plot a month before harvest and again at harvest. At harvest (between 23-30 Apr. 2010), sunburn incidence and severity were evaluated in the laboratory on a sample of 20 fruit per replicate that were randomly sampled from harvested fruit.

Experiment 2

Plant material

The experiment was conducted during the 2009-10 season at Welgevallen Experimental Farm, Stellenbosch, South Africa (33° 55' S, 18° 53'E) using 'Cripps' Pink' apple planted in 1998 on M793 rootstock. The tree spacing was 4 m x 1.5 m in a North East by South West row orientation. The Western Cape province of South Africa where the experiment was conducted has a Mediterranean-type climate with hot, dry summers that necessitates the use of irrigation.

Treatments and experimental design

Irrigation was manipulated to attain three treatments, i.e., full irrigation, hereinafter called control treatment, half the irrigation amount, hereinafter called half irrigation and no water for

two weeks, hereinafter called no irrigation, starting on 15 Mar. 2010. The normal irrigation at Welgevallen at the onset of the experiment was 6 mm h^{-1} delivery for 2.5 h twice weekly (Tuesday and Friday). The half irrigation and no irrigation treatments were attained by exchanging normal spray nozzles (6 mm h^{-1}) with nozzles that gave half the normal delivery (3 mm h^{-1}) and stoppers (0 mm h^{-1}), respectively. Microjet sprayers were placed 0.5 m on either side of trees. Treatments were randomized in eight blocks with three trees per plot and two buffer trees between plots to avoid treatment interference. The blocks were laid out on flat non-sloping land to avoid the effect of run-off.

Measurements

Measurements of stem water potential, soil water content, and sunburn were taken on days 0, 7 and 14. These days represented the day of setting up the experiment, a week after and two weeks after, respectively. Fruit surface temperature was measured on day 0 and day 14. At harvest, a sample of 15 fruit was taken from each tree for maturity assessment in the laboratory.

Eight sun-exposed fruit without sunburn were tagged per tree on the western side of the row at the onset of the experiment and sunburn severity assessed on days 7 and 14 as well as at harvest as described in Experiment 1. Tagged fruit was culled to one fruit per cluster. Total sunburn incidence was also assessed on a per tree basis at harvest. All fruit with sunburn at harvest were further graded into the three sunburn classes, i.e. bronzing, bleaching and necrosis, according to Schrader et al (2003).

Stem water potential and fruit surface temperature were measured as described in Experiment 1. Relative soil moisture content (RSMC) was determined gravimetrically by weighing the differences between fresh and oven dried auger drawn soil samples at a depth of 45 cm. The relative soil water content was expressed as a percent of the fresh soil weight.

Blush colour was measured using the Pink Lady colour chart (Topfruit Pvt. Ltd., Paarl, SA), where a value of 1 denotes the worst (green) coloured fruit and 12 the best (reddest). Flesh firmness was measured with a flesh texture analyser (Guss electronic model GS 20, Strand, SA). Percentage starch conversion was measured using the starch conversion chart (Unifruco Research Services, Bellville, SA). The juice for measuring the total soluble solids (TSS) and titratable acidity (TA) of the apples was prepared by crushing and extraction of juice from pooled apple pieces in a blender. A hand held refractometer (Model N1, Atago, Tokyo, Japan) was used to measure TSS. TA was determined by titrating 0.1M NaOH to a pH of 8.2 with an automated titrator (Model 719 S, Metrohm AG, Hersiau, Switzerland). TA was expressed as percentage of malic acid ($\text{g } 100 \text{ g}^{-1}$ juice).

Statistical analysis

Data were subjected to Analysis of Variance (ANOVA) by General Linear Methods using SAS version 9.1.3 (SAS Institute Inc. 2003, Cary, USA). Where significant differences occurred ($p \leq 0.05$), means were separated by the Least Significant Difference (LSD). Single degree of freedom, orthogonal, linear and quadratic contrasts for irrigation level were fitted.

Results

Experiment 1

Gas exchange: In 2008-09, the net CO₂ assimilation rate (*A*) did not differ significantly between mulching treatments, but was significantly ($p=0.0011$) higher for all mulches compared to the control treatment in heavy loam soil (Table 1). In light loam, vermicompost had a significantly ($p=0.0067$) higher *A* than the other mulching treatments and the control treatment (Table 2). In 2009-10, the control had significantly lower *A* than the mulching treatments in both soil types, with the vermicompost treatment consistently having the highest net CO₂ assimilation rate (Table 3 and 4).

In 2008-09, stomatal conductance (*g*) for trees in heavy loam soil with vermicompost, wood chips and plant compost were significantly ($p=0.0058$) higher than that of the geotextile and control treatments (Table 1). In light loam, although vermicompost had a slightly higher *g*, there were no significant differences ($p=0.1250$) between treatments (Table 2). In 2009-10, vermicompost, woodchips and plant compost had significantly ($p=0.0001$) higher *g* than the geotextile and the control treatments in heavy loam soil (Table 3), while in light loam soil (Table 4), vermicompost and woodchips resulted in significantly ($p=0.0337$) higher *g* than the geotextile, plant compost and control treatments.

In 2008-09 in heavy loam soil, vermicompost had significantly ($p=0.0075$) higher transpiration (*T*) than the other mulching treatments which in turn had significantly higher *T* than the control (Table 1). In light loam soil (Table 2), *T* was significantly ($p=0.0146$) higher for vermicompost than for other mulching and the control treatment, which did not differ significantly. In 2009-10,

vermicompost and the control had significantly the highest and lowest T , respectively, in both soil types, with the other mulching treatments being intermediate (Tables 3 and 4).

Fruit surface temperature (FST): FST did not differ significantly between treatments in heavy loam soil ($p=0.0643$) in 2008-09 season (Table 1). Under light loam conditions, FST of the control was significantly higher ($p<0.0001$) than other treatments except plant compost, while vermicompost had the lowest FST (Table 2). In the 2009-10 season, vermicompost and the control significantly had the lowest and highest FST respectively in both soil types (Tables 3 and 4).

Water potential: There were no significant differences in leaf water potential between treatments in both soil types in 2008-09 (data not shown). There also were no significant differences in stem water potential in 2009-10 (data not shown).

Sunburn incidence and severity: In 2008-09, the average sunburn incidence ranged between 3.7-8% in heavy loam soil, whereas for light loam soil, it ranged between 4.3-11.3% (Tables 1 and 2). There were no significant differences found in sunburn incidence ($p=0.2617$) and severity ($p=0.7358$) from fruit on trees on heavy loam soil. Significant differences were, however, observed in both sunburn incidence ($p=0.0254$) and severity ($p=0.0367$) in light loam soil. The vermicompost treatment had a lower sunburn incidence than the control and woodchips, while plant compost did not differ significantly from any of the treatments (Table 2). The control had more severe sunburn compared to mulching treatments although it did not differ from geotextile.

Of the mulching treatments, vermicompost had the lowest sunburn severity that, however, was not significantly lower than any of these treatments (Table 2).

In 2009-10 sunburn incidence ranged between 1.7-3.8 % in heavy loam soil whereas for light loam it was 3-5% (Tables 3 and 4). Sunburn incidence and severity increased in the last month before harvest in both soil types (Tables 3 and 4). Sunburn incidence and severity did not differ significantly between treatments a month before harvest. At harvest in both soil types, sunburn incidence did not differ significantly between mulching treatments. Mulching did, however, significantly reduce sunburn incidence compared to the control treatment except for plant compost in heavy loam (Table 3). In heavy loam soil, vermicompost and woodchips had significantly ($p=0.0296$) lower sunburn severity than the plant compost and the control treatments. Geotextile did not differ significantly from other treatments (Table 3). In light loam soil, vermicompost had significantly lower sunburn severity than woodchips and control whereas the plant compost and geotextile did not differ significantly from other treatments (Table 4).

Experiment 2.

Relative soil moisture content (RSMC): The RSMC markedly declined from day 0 to day 14 for the half irrigation and no irrigation treatments, with the half irrigation treatment having a higher RSMC value than the no irrigation treatment throughout the period. The control treatment did not show a declining trend and the RSMC was consistently higher than the two water stress treatments (Fig. 1). However, significant differences in moisture content were only apparent at day 14 ($p=0.0003$). Soil from the control treatment had the highest moisture content, whereas the

no irrigation treatment had the lowest moisture content while the half irrigation treatment had intermediate moisture content.

Stem water potential: The water potential at day 0 ranged from -1.69 MPa to -1.87 MPa between treatments. The range widened throughout the two week period, with the water potential of the no irrigation treatment declining at a faster rate than that of half irrigation treatment whereas that of the control remained within the initial range (Fig. 2). There was a linear relationship at day 14 between stem water potential and level of irrigation ($p < 0.0001$). A reduction in irrigation resulted in a reduction in stem water potential. The stem water potential of the three treatments were significantly different ($p < 0.001$) with the control and no irrigation treatment having the highest and lowest water potentials respectively.

Fruit surface temperature (FST): FST at day 0 was not significantly different between treatments. There was a significant linear relationship ($p < 0.0001$) between irrigation level and FST at day 14. A decrease in irrigation level resulted in an increase in FST. FST of all treatments were significantly different, with the no irrigation treatment having the highest average FST of 36.7 °C (Fig. 3). The FST of the control and half irrigation treatment were 31.6 °C and 34.0 °C, respectively. The average air temperature during FST measurement on day 14 was 25.9 °C. The maximum air temperature attained between day 0 and harvest time was 35.0 °C.

Fruit quality: Fruit firmness and TSS had a significant linear relationship ($p = 0.0004$ and $p < 0.0001$, respectively) with irrigation level (Table 5). A decrease in irrigation increased both fruit firmness and TSS. Firmness did not differ significantly between the no irrigation and the

half irrigation treatments, but both were significantly higher than the control. There were significant differences between all treatments for TSS. The no irrigation treatment had the highest TSS followed by the half irrigation treatment and the control.

There was a significant quadratic relationship ($p < 0.0001$) between irrigation level and TA. The half irrigation treatment had a higher, but not significantly different TA than the control. Completely withholding water significantly decreased TA. There was a significant quadratic effect ($p < 0.0001$) on the TSS: TA ratio between the irrigation treatments. The no irrigation treatment had a significantly higher TSS:TA ratio compared to the half irrigation and control treatments. The half irrigation and control treatments did not differ significantly for TSS:TA ratio. There were no significant treatment effects for starch degradation, the extent and intensity of red colour, and background colour.

Sunburn: Sunburn incidence amongst tagged fruit was significantly lower for the control treatment than for the two water stress treatments at day 7 and 14, and at harvest (Table 6). There was a significant linear relationship between irrigation level and sunburn incidence ($p < 0.001$, $p = 0.0002$ and $p = 0.0006$, for day 7, day 14 and harvest respectively). A decrease in irrigation level resulted in an increase in sunburn incidence, but the water stress treatments did not differ significantly from each other at all three dates. For the total fruit at harvest, the same linear trend was observed ($p < 0.0009$) with the two water stress treatments showing significantly greater sunburn incidence ($p = 0.0032$) compared to the control treatment (Table 7).

Sunburn browning was the most predominant sunburn type in all treatments (Tables 6 and 7). There was a significant linear relationship between sunburn browning ($p=0.0016$ and $p<0.0001$ for tagged fruit and total fruit respectively) and sunburn necrosis ($p=0.0015$ and $p<0.0001$ for tagged fruit and total fruit respectively) with irrigation level for tagged fruit and total fruit at harvest (Tables 6 and 7). An increase in moisture stress resulted in an increase in sunburn necrosis whereas sunburn browning decreased. The no irrigation treatment had significantly lower sunburn browning and significantly higher sunburn necrosis than both the half irrigation and control treatments for tagged and total fruit at harvest (Tables 6 and 7). No significant differences were observed between treatments for sunburn bleaching.

Sunburn severity for both tagged fruit and total fruit at harvest also showed a linear relationship with irrigation level (Table 8). A reduction in irrigation level resulted in an increase in sunburn severity. For the tagged fruit, sunburn severity did not differ significantly in the two water stress treatments, but was significantly greater ($p=0.0005$) than the control treatment at day 7 (Table 8). At day 14, all treatments were significantly different ($p<0.0001$), with control and no irrigation treatments having the least and most severe sunburn, respectively. Sunburn severity for the tagged fruit at harvest was significantly ($p<0.0001$) greater in the no irrigation treatment compared to the half irrigation and control treatments. For all fruit at harvest, the half irrigation treatment did not significantly differ with the control for sunburn severity. However, the no irrigation treatment had a significantly higher ($p<0.0001$) sunburn severity.

Discussion

Differences in sunburn incidence and severity brought about by different mulching treatments were not related to tree water status. Leaf water potential and stem water potential measured in the first and second seasons, respectively, did not differ significantly between treatments in both soil types. This was contrary to part of our hypothesis, where we expected mulching to reduce evaporative soil moisture loss, thereby increasing the water potential of mulched trees compared to the control. However, when irrigation was manipulated in Exp. 2, there was a linear relationship at day 14 between stem water potential and level of irrigation. A reduction in irrigation resulted in a reduction in stem water potential.

The effect of mulching on plant water potential could have been obscured by shading of the soil surface by the plant canopy. Todd et al. (1991) reported a 75% reduction in evaporative moisture loss due to canopy shading under dryland maize whereas under reduced irrigation, mulching and canopy shading contributed equally. This was further confirmed by Adams et al. (1976) and Unger and Jones (1981) who reported that soil shading by the plant canopy almost had the same effect as plant residues in terms of reduction of evaporative soil moisture loss. The effect of mulching on plant water status could also have been masked by the fact that mature apple leaves are capable of osmotic regulation to maintain leaf turgor pressure (Lakso, 1979). Notable reductions in leaf water potential can be observed only under severe moisture deficits (Faust, 1989). The more mature the leaves, the more they are capable of osmotic regulation. It is therefore possible that greater differences in soil moisture content as influenced by different mulching treatments are required for significantly detectable differences in plant water potential, as was the case with direct irrigation manipulation. In addition, by irrigating at the rate of 5mm

h^{-1} for three hours twice a week we also suspected that this might have amounted to over irrigation with the subsequent effects of also obscuring treatment effects on plant water relations.

Faster root growth, respiration and nutrient uptake due to warmer soil temperature (Kasper and Bland, 1992) directly affect shoot physiology such as gas exchange or photochemistry (Boone et al., 1998). DeLucia et al. (1991) reported stomatal closure to be a major factor limiting net carbon assimilation at low soil temperatures in Scots pine seedlings. In our study, mulching improved the photochemical performance of leaves, which could be attributed to modification of the soil micro-climate and soil properties. Mulching increases and stabilizes soil diurnal and seasonal temperatures (Stratton and Rechcigl, 1998). Stable temperatures in the first 20-30 cm depth in soils promote root development and faster plant growth (Haynes, 1980). Hill (1966) found that the root systems of peach trees under mulch were characterized by a prevalence of small feeder roots within 15 cm of the soil surface and a uniform distribution of larger roots lower in the soil profile.

Vermicompost mulching consistently gave a higher net carbon assimilation rate than the control in both seasons and soil types although it did not differ significantly from other mulches in sandy loam in the first season, and with woodchips in heavy loam in the second season. Woodchips and plant compost were better than the no mulching control in heavy loam while the geotextile gave varied responses in different seasons and soil types. Generally though, it does appear that organic mulches improved photochemical efficiency. Vermicompost significantly increased stomatal conductance and transpiration compared to the control, except in light loam in the first season. Woodchips and plant compost also increased stomatal conductance while the geotextile

increased transpiration compared to the control in the heavy loam. Maeda-Martinez (1989) and Otsuki et al. (2000) reported enhanced CO₂ supply to stomata of lower leaves of small plants due to a favourable micro-climate induced by plastic film mulch.

From findings of this study, it seems evident that higher photosynthetic and gas exchange activities were associated with lower FST. This was in agreement with part of our hypothesis where we expected high evaporation rates to lower fruit temperatures by evaporative cooling. Except in heavy loam in 2008-09, vermicompost significantly decreased FST compared to the control. Vermicompost was also the treatment with the most consistent effect on photosynthesis, stomatal conductance and transpiration. As was the case with photochemistry, other mulching treatments had a less consistent effect on FST. Generally though, FST was never significantly higher in any mulching treatment compared to the control.

In the manipulated irrigation trial, moisture stress undoubtedly accounted for an increase in FST, which in turn increased sunburn incidence and severity. The control, with normal irrigation, resulted in fruit with the lowest average FST, whereas completely withholding water gave the highest average FST. Consistently, deficit irrigation of half the normal irrigation rate gave an average FST that was statistically higher than that of the control, but lower than where there was no irrigation at all.

It appears that the plant water status plays an important role in heat dissipation mechanisms that affect fruit surface temperature. Transpiration is one cooling mechanism that involves the plant water potential (Salisbury and Ross, 1992). Withholding soil moisture made water less easily

available to the plant roots, which subsequently absorbed less water. The resultant increase in tension of xylem causes a decrease in the water potential of the fruit peel and leaves (Berrie et al., 1987). This minimizes the rate of water loss by evaporation from mesophyll cells, reducing transpiration. If the cells of the fruit peel attain a certain critical low water potential value, this decreases the vapour pressure deficit with air, thereby resulting in slower evaporation through the lenticels. Under high irradiance and high temperature, fruit surface temperature would become considerably higher than ambient temperature.

Saudreau et al. (2008) reported that fruit temperature is a function of heat exchange between itself and the surrounding plant canopy microclimate, with radiation, evaporation and convection being the components. Of these components, evaporation and convection are greatly depended on the plant water status. Sepulcre-Cantó et al. (2006) demonstrated how water potential affected canopy temperature of olive trees using infra-red sensors. Well watered trees with a higher water potential had a lower canopy temperature than trees under deficit irrigation with a lower water potential. Therefore, if adverse environmental conditions prevail, particularly those conducive to sunburn development, fruit on the water-stressed trees would heat and burn faster than those with a higher water potential. The high FST observed in water stressed plants can thus be partly attributed to the warm microclimate in the canopy, in addition to lower evaporational cooling from lenticels due to smaller vapour pressure deficit between the fruit and the atmosphere.

FST in Exp. 1 did not differ significantly between mulching treatments and the control in heavy loam soil in 2008-09, and neither did sunburn incidence and severity. In light loam and all soil types in 2009-10, geotextile, woodchips and plant compost gave varied FST while vermicompost

and the control consistently maintained the lowest and highest FST extremes respectively. Consequently, vermicompost and control always had the lowest and highest sunburn incidence and severity respectively while other mulching treatments either varied between or did not differ with these extremes. Overall, this observed relationship confirms the direct effect of high FST on sunburn development.

In the manipulation of irrigation (Exp. 2), there was a linear relationship between sunburn severity and irrigation level. An increase in sunburn necrosis was observed with an increase in water stress. This could also be the effect of FST which increased with a reduction in the plant water potential. This type of sunburn is manifested when FST reaches 52 ± 1 °C (Schrader et al., 2003). However, in our studies, the no irrigation treatment had the highest average FST of 36.7 °C at day 14, whereas the control had the lowest of 31.6 °C. Sunburn necrosis for these treatments was 35.7% and 5.4 % respectively. The air temperature on this day was relatively low, averaging 25.9 °C over the measurement period. The maximum air temperature attained during the experimental period was 35.0 °C. Under normal circumstances, FST is reported to exceed air temperature by about 10-15 °C (Bergh, et al., 1980; Woolf and Ferguson, 2000; Wünsche et al., 2001). It is therefore possible that in our study, for treatments where trees were water stressed, FST could have even been elevated to levels conducive to development of the most severe form of sunburn, viz. necrosis.

Sunburn browning requires a lower threshold FST of 46 °C under conditions of high light (Schrader et al., 2003). This was consistent with our findings whereby a higher proportion of sunburn browning was observed in less water stressed trees that had lower FST. The third type of

sunburn, photo-oxidative bleaching, did not seem to be affected by treatment differences, although it was slightly higher in the no irrigation treatment. Photo-oxidative bleaching results when a shaded fruit is suddenly exposed to the sun and can occur at peel temperatures below 30 °C (Felicetti and Schrader 2008). This sudden exposure can be due to branch breakages, defoliation or in response to increased fruit weight as fruit grow.

In addition to sunburn, the fruit quality parameters affected by moisture manipulation and displaying a linear response to irrigation level were TSS, firmness and TA. TSS increased with an increase in moisture stress. A high fruit TSS content and flesh firmness have consistently been reported to be associated with sunburn occurrence (Ebel et al., 1993; Racsko et al., 2005; Schrader et al., 2009). This is in agreement with our findings because moisture stress increased sunburn occurrence, which subsequently could have increased TSS and flesh firmness. This is further supported by Kilili et al. (1996) who reported a higher TSS concentration in response to withholding irrigation in 'Braeburn' apples. Racsko et al. (2005) postulated that sunburnt cells die and lose water, resulting in firmer flesh. We can also speculate that reduced fruit hydration due to withholding of water could also have contributed to firmer fruit.

There were no significant differences in starch conversion to sugars, which we expected to be higher in water-stressed and sunburnt fruit. Kramer (1983) ascertained that reduced irrigation enhances starch to sugar conversion. However, as expected, TA was significantly lower in the no irrigation treatment, although there were no differences between control and half irrigation treatments. In grapes, sun exposed berries had higher temperatures and reduced TA (Kliewer and Lider, 1970) with an increase in temperature of about 10 °C halving the acidity (Coombe, 1987).

Therefore in our study, increased FST due to water stress could have been responsible for reduction in TA in the no irrigation treatment. However, moisture stress has been reported not to directly affect TA (Drake et al., 1981; Kilili et al., 1996).

Plant moisture status indeed plays a significant role in sunburn development and it is also an important factor affecting fruit quality. Increasing moisture stress increased the incidence and severity of sunburn. In this study, fruit on moisture-stressed plants displayed higher fruit surface temperatures with subsequent higher sunburn incidence and severity. Therefore, the most severe form of sunburn, sunburn necrosis was highest where moisture was completely withheld. Increased water stress resulted in firmer fruit with a high total soluble solids and low titratable acidity. Starch breakdown and fruit colour were not affected by the plant moisture status. Mulch, particularly vermicompost, enhances tree performance and has the potential to become an important cultural control measure against sunburn even though the effects might not be related to plant water relations.

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Table 1. The effect of mulching on gas exchange, fruit surface temperature and sunburn in ‘Cripps’ Pink’ apples planted in heavy silt loam soil (heavy loam) soil during the 2008-09 season at Lourensford Estate.

Treatment	A	g	T	FST^z	Sunburn incidence (%)	Sunburn severity
Control	12.03 b ^y	0.32 b	3.37 c	35.9 ns	8.0 ns	1.93 ns
Vermicompost	14.16 a	0.41 a	4.60 a	34.9	3.7	2.33
Geotextile	13.38 a	0.31 b	3.98 b	36.0	6.7	2.13
Woodchips	13.79 a	0.38 a	4.21 ab	35.2	5.7	1.78
Plant compost	14.42 a	0.38 a	3.94 bc	35.5	6.7	1.37
<i>p value</i>	<i>0.0011</i>	<i>0.0058</i>	<i>0.0075</i>	<i>0.0643</i>	<i>0.2617</i>	<i>0.7358</i>

^z Average air temperature during measurement was 28.5°C.

^y Means followed by the same letter in a column are not significantly different at 0.05 level of significance.

ns-Not significant.

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

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

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

FST-Fruit surface temperature (°C).

Table 2. The effect of mulching on gas exchange, fruit surface temperature and sunburn in ‘Cripps’ Pink’ apples during 2008-09 season in sandy loam (light loam) soil at Lourensford Estate.

Treatment	A	g	T	FST ^y	Sunburn	
					Incidence (%)	Severity (%)
Control	11.21 b ^z	0.295 ns	3.24 b	35.5 a	11.3 a	3.32 a
Vermicompost	12.96 a	0.359	4.46 a	30.4 c	4.3 c	1.52 b
Geotextile	11.48 b	0.304	3.89 ab	33.6 b	6.7 bc	2.32 ab
Woodchips	11.52 b	0.304	3.44 b	33.6 b	10.0 ab	1.99 b
Plant compost	11.77 b	0.314	3.36 b	34.1 ab	8.0 abc	1.92 b
<i>p value</i>	<i>0.0067</i>	<i>0.1250</i>	<i>0.0146</i>	<i><0.0001</i>	<i>0.0254</i>	<i>0.0367</i>

^z Means followed by the same letter in a column are not significantly different at 0.05 level of significance.

FST-Fruit surface temperature ^y Average ambient temperature during time of measurement was 28.5 °C.

ns-Not significant.

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

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

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

Table 3. The effect of mulching on gas exchange, fruit surface temperature and sunburn in ‘Cripps’ Pink’ apples during 2009-10 season in heavy silt loamy (heavy loam) soil at Lourensford Estate.

Treatment	A	g	T	FST^z	Sunburn Incidence^y	Sunburn Incidence^x	Sunburn Severity^w	Sunburn Severity^v
Control	16.5 c ^u	0.34 b	3.57 c	39.8 a	1.5ns	3.8 a	1.13 ns	1.75 a
Vermicompost	20.2 a	0.42 a	4.75 a	32.9 d	0.7	1.7 b	0.61	0.92 b
Geotextile	15.3 c	0.33 b	4.05 b	37.7 ab	0.8	1.8 b	0.67	1.31 ab
Woodchips	19.2 ab	0.42 a	3.84 bc	34.2 cd	0.5	2.3 b	0.83	1.06 b
Plant compost	18.3 b	0.40 a	4.03 b	36.7 bc	1.0	2.7 ab	1.5	1.69 a
<i>p value</i>	<i><0.0001</i>	<i>0.0001</i>	<i><0.0001</i>	<i>0.008</i>	<i>0.4307</i>	<i>0.0010</i>	<i>0.3401</i>	<i>0.0296</i>

FST-fruit surface temperature. ^z Average air temperature during time of measurement was 28.7°C.

^y Sunburn incidence (%) a month before harvest.

^x Sunburn incidence (%) at harvest.

^w Sunburn severity a month before harvest.

^v Sunburn severity at harvest.

^u Means followed by the same letter in a column are not significantly different at 0.05 level of significance.

ns- Not significant.

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

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

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

Table 4. The effect of mulching on gas exchange, fruit surface temperature and sunburn in ‘Cripps’ Pink’ apples during 2009-10 season in sandy loam (light loam) soil at Lourensford Estate.

Treatment	A	g	T	FST^z	Sunburn Incidence^y	Sunburn Incidence^x	Sunburn Severity^w	Sunburn Severity^v
Control	12.40 c ^u	0.30 b	3.01 b	39.45 a	1.0 ns	5.0 a	0.78 ns	2.29 a
Vermicompost	17.32 a	0.38 a	4.32 a	35.13 b	0.7	3.0 b	1.00	1.39 b
Geotextile	14.15 b	0.30 b	3.68 ab	40.37 a	1.0	3.0 b	0.67	2.00 ab
Woodchips	13.25 bc	0.34 ab	3.20 b	39.41 a	1.3	3.0 b	1.67	2.67 a
Plant compost	14.09 b	0.32 b	3.61 ab	38.78 ab	1.0	3.3 b	1.50	2.05 ab
<i>p value</i>	<i>0.0030</i>	<i>0.0337</i>	<i>0.0753</i>	<i>0.0628</i>	<i>0.9124</i>	<i>0.0450</i>	<i>0.5827</i>	<i>0.0418</i>

FST-Fruit surface temperature. ^z Avergae ambient temperature was 29.2 °C.

^y Sunburn incidence (%) a month before harvest.

^x Sunburn incidence (%) at harvest.

^w Sunburn severity a month before harvest.

^v Sunburn severity at harvest.

^u Means followed by the same letter in a column are not significantly different at 0.05 level of significance.

ns-Not significant.

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

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

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

Table 5. Effect of moisture stress between 15 and 29 March 2010 on fruit quality and maturity of ‘Cripps’ Pink’ apples at Welgevallen Experimental farm at harvest.

Treatment	Starch (%)	Foreground colour	Background colour	Firmness (kg)	Blush coverage (%)	TSS (° Brix)	TA (%)	TSS:TA
Control	41 ns	2.89 ns	3.21 ns	8.29 b ^z	23 ns	13.2 c	0.60 b	22.2 b
Half irrigation	39	3.21	3.18	9.58 a	27	14.5 b	0.63 b	23.2 b
No irrigation	40	3.08	3.17	9.93 a	25	16.5 a	0.43 a	38.2 a
<i>Contrasts</i>								
<i>Treatment</i>	<i>0.8613</i>	<i>0.5727</i>	<i>0.5806</i>	<i>0.0010</i>	<i>0.2610</i>	<i><0.0001</i>	<i><0.0001</i>	<i><0.0001</i>
<i>Linear</i>	<i>0.6940</i>	<i>0.5428</i>	<i>0.3033</i>	<i>0.0004</i>	<i>0.4268</i>	<i><0.0001</i>	<i><0.0001</i>	<i><0.0001</i>
<i>Quadratic</i>	<i>0.7050</i>	<i>0.3886</i>	<i>0.8711</i>	<i>0.1525</i>	<i>0.1517</i>	<i>0.3063</i>	<i><0.0001</i>	<i><0.0001</i>

^zMeans in the same column followed by the same letter are significantly different at 0.05 level.

ns-Not significant.

Table 6. Effect of moisture stress between 15 and 29 March 2010 on sunburn incidence of tagged ‘Cripps’ Pink’ apples at Welgevallen Experimental farm.

Treatment	Day 7	Day 14	Sunburn incidence (%)			
			Sunburn browning	Sunburn necrosis	Sunburn bleaching	Total sunburn
Control	15 b ^z	30 b	94 a (39) ^y	5 b (1.6)	3 ns (1.2)	41 b
Half irrigation	42 a	56 a	92 a (58)	6 b (3.8)	2 (1.3)	64 a
No irrigation	45 a	61 a	75 b (55)	25 a (18)	0	73 a
<i>Contrasts</i>						
<i>Treatment</i>	0.0001	0.0004	0.0026	0.0024	0.3927	0.0019
<i>Linear</i>	<0.0001	0.0002	0.0016	0.0015	0.1792	0.0006
<i>Quadratic</i>	0.0231	0.0649	0.0810	0.0756	1.0000	0.2964

^zMeans in the same column followed by the same letter are significantly different at 0.05 level.

^yFigures in brackets represent the proportion of sunburn out of the total tagged fruit while those outside brackets represent the proportion out of total sunburnt fruit.

ns Not significant.

Table 7. Effect of moisture stress on sunburn incidence (all fruit at harvest) of ‘Cripps’ Pink’ apples at Welgevallen Experimental farm.

Treatment	Sunburn browning (%)	Sunburn Necrosis (%)	Sunburn bleaching (%)	Total Sunburn (%)
Control	94 a ^z (8.4)	5 b (0.5) ^y	1 ns (0.09)	9 b
Half irrigation	87 a (13)	12 b (1.8)	1 (0.02)	15 a
No irrigation	59 b (10.6)	36 a (6.5)	5 (0.90)	18 a
<i>Contrasts</i>				
<i>Treatment</i>	<0.0001	0.0001	0.1921	0.0032
<i>Linear</i>	<0.0001	<0.0001	0.1007	0.0009
<i>Quadratic</i>	0.0454	0.0751	0.4397	0.5585

^zMeans in the same column followed by the same letter are significantly different at 0.05 level.

^y Figures in brackets represent the proportion of sunburn out of the total tagged fruit while those outside brackets represent the proportion out of total sunburnt fruit.

ns Not significant.

Table 8. Effect of moisture stress between 15 and 29 March 2010 sunburn severity at harvest of ‘Cripps’ Pink’ apples at Welgevallen farm.

Treatment	Tagged fruit			Total fruit
	Day 7	Day 14	Harvest	Harvest
Control	0.20 b ^z	0.56 c	0.95 b	1.76 b
Half irrigation	0.53 a	1.06 b	1.47 b	2.04 b
No irrigation	0.70 a	1.61 a	2.44 a	2.95 a
<i>Contrasts</i>				
<i>Treatment</i>	0.0005	<0.0001	<0.0001	<0.0001
<i>Linear</i>	<0.0001	<0.0001	<0.0001	<0.0001
<i>Quadratic</i>	0.4766	0.9015	0.3540	0.0740

^z Means followed by the same letter in a column are not significantly different at 0.05 level of significance.

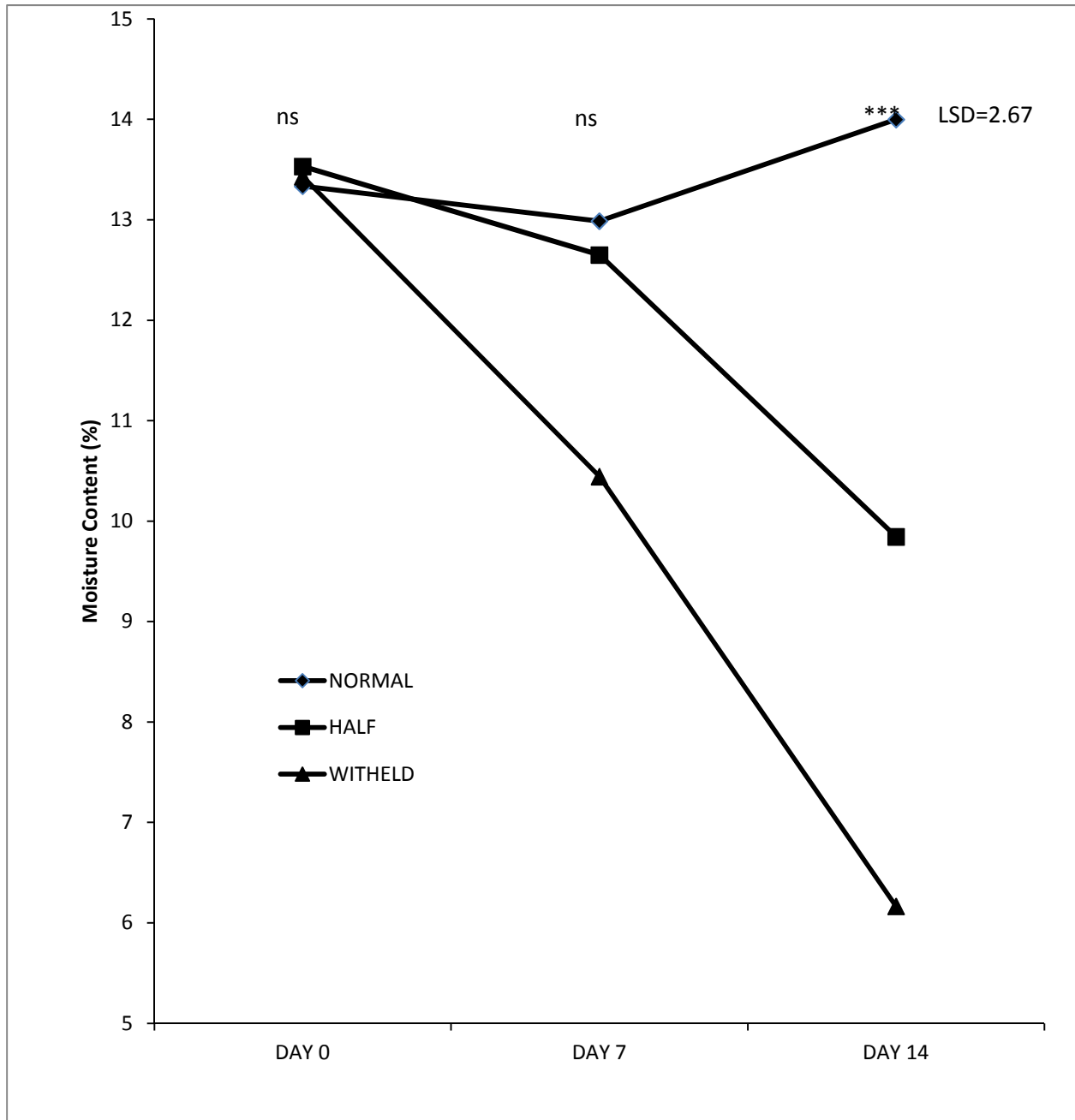


Figure 1. The effect of irrigation on soil moisture content over a period of two weeks in Cripps' Pink apple orchard at Welgevallen Experimental farm in the 2009-10 season. ***= $p < 0.0001$

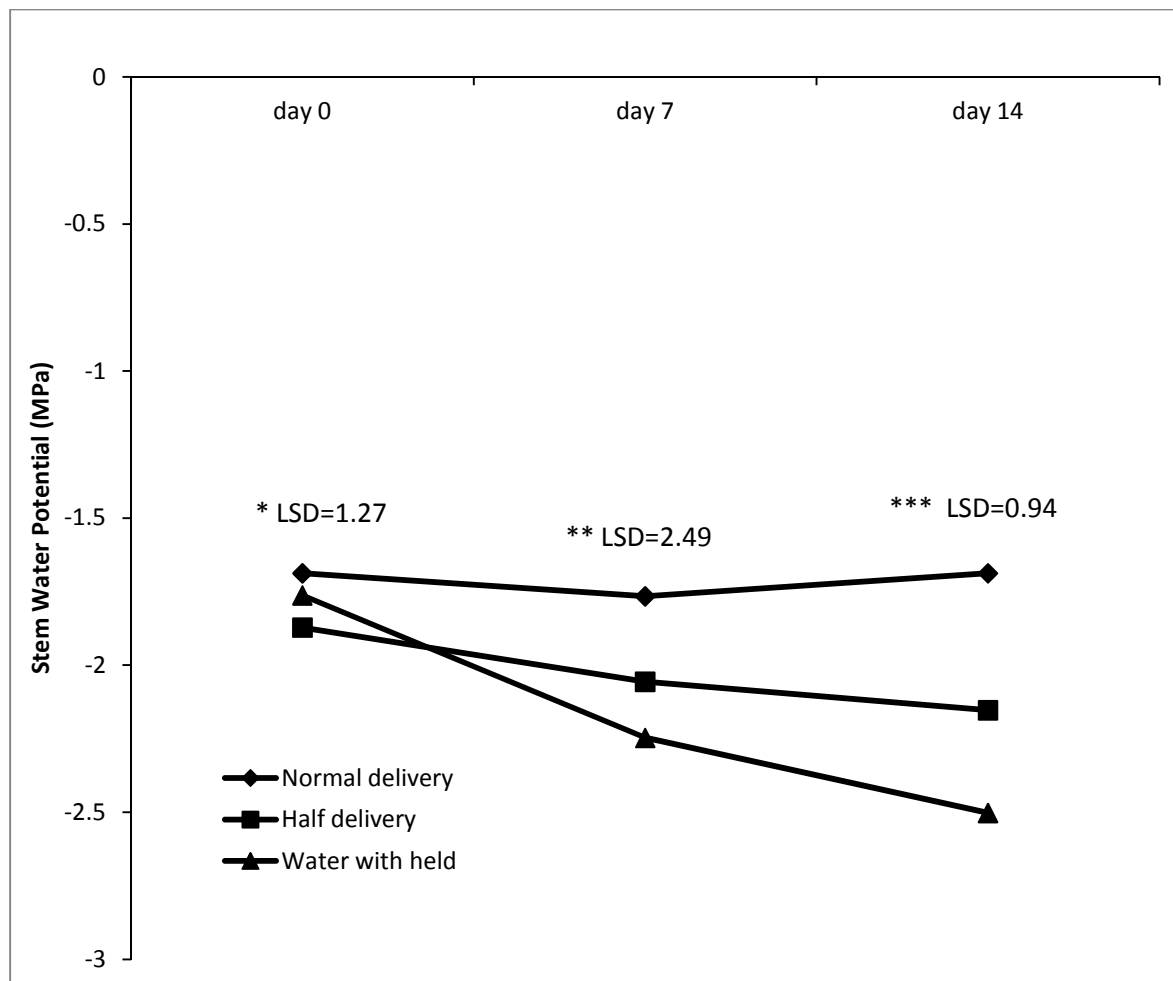


Figure 2. The effect of drought stress on stem water potential of apple trees over two weeks at Welgevallen Experimental farm in the 2009-10 season. *= $p < 0.05$, **= $p < 0.001$, ***= $p < 0.0001$.

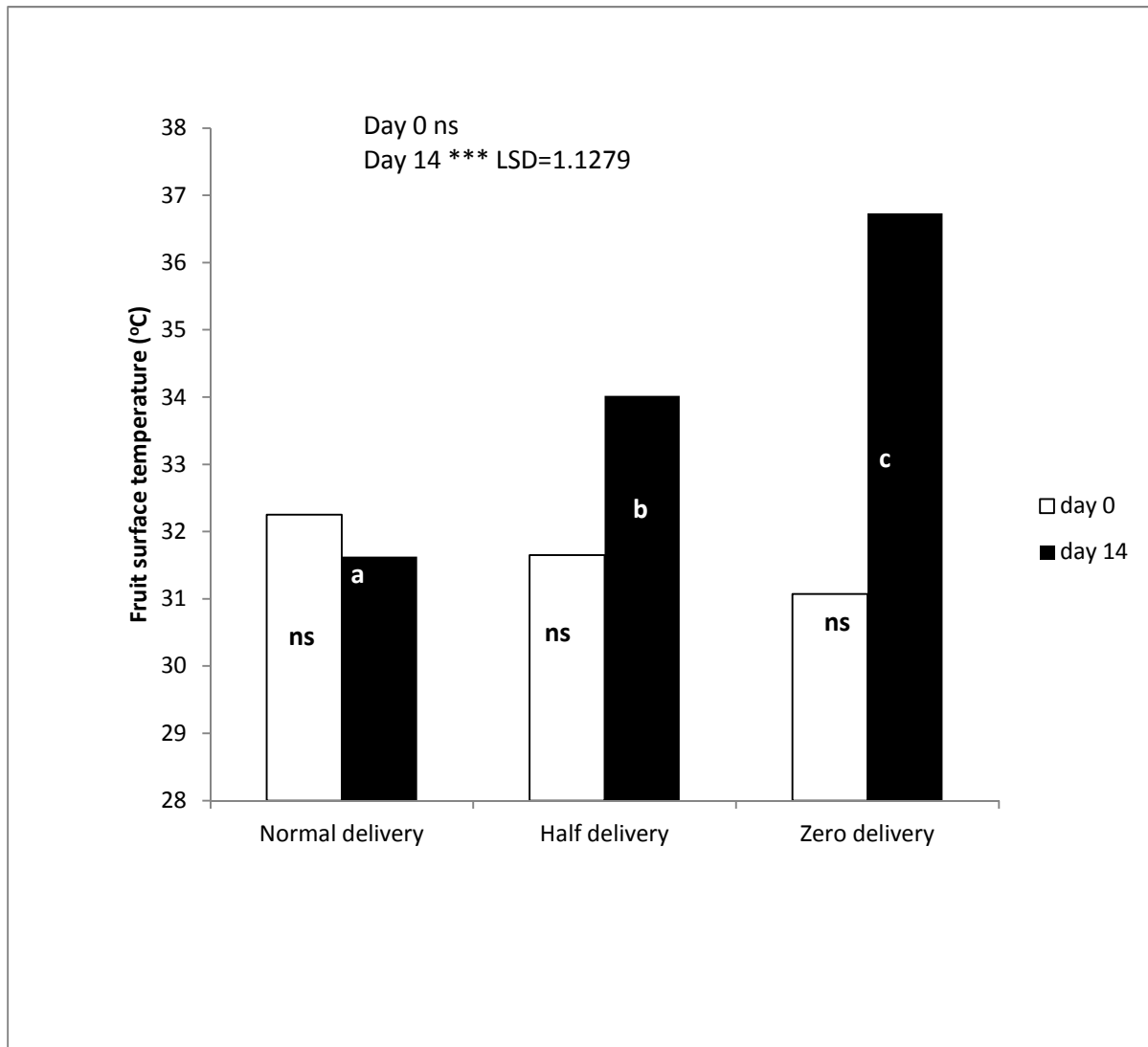


Figure 3. Effect of drought stress on average fruit surface temperature of Cripps' Pink apples at Welgevallen Experimental farm in the 2009-10 season. Air temperatures on day 0 and day 14 were 26.5°C and 25.9°C respectively. ***= $p < 0.0001$. Treatments with different letters differ significantly at 0.05 level.

DO CHEMICAL AND TEXTURAL CHARACTERISTICS PREDISPOSE APPLES TO SUNBURN?

Abstract

An investigation was conducted to 1) assess the effect of light exposure history on the heat acclimation of apples and 2) to determine whether differences in the chemical and textural composition of sunburnt fruit compared to fruit without sunburn relate to differences in sunburn susceptibility or reflect changes taking place in response to sunburn development. In 2008-09, the heat tolerance of sun exposed and shaded fruit peel of ‘Cripps’ Pink’ was evaluated by measuring chlorophyll fluorescence (F_v/F_m) at room temperature, after subjecting peel to 30 °C, 35 °C, 40 °C, 45°C or 50 °C for three hours at $550 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and again after a four hour recovery period in the dark. Flesh firmness, total soluble solids (TSS), titratable acidity (TA) and relative water content (RWC) were compared between fruit peel of the two sides at the various temperatures. In 2009-10, textural and chemical characteristics were evaluated on fruit with induced sunburn, naturally occurring sunburn and control fruit. Sunburn was induced by sticking a cluster of four 13 mm black stickers on the sun exposed side of fruit without sunburn three weeks before harvest. A control group of fruit without sunburn was left without stickers while fruit that already displayed sunburn symptoms were also tagged. In 2008-09, the sun exposed sides had a higher F_v/F_m after a four hour recovery from heat stress although the residual photo-inhibition did not differ significantly. Peel exposed to temperatures lower than 40 °C had significantly lower photoinhibition than peel exposed to 45 °C and higher. After the four-hour recovery period, peel previously exposed to 30 °C and 35 °C had lower residual photoinhibition than

peel exposed to 45 °C and higher. It appeared a greater light exposure history might contribute to better heat stress acclimation. The sun exposed side had firmer flesh, higher TSS and lower RWC than shaded side peel. In 2009-10, induced sunburn and sunburn treatments had firmer fruit with lower TA, RWC and higher TSS than control fruit. The magnitude of differences between the sun exposed and shaded fruit sides were consistent, regardless of treatment type. We concluded that the environmental conditions which cause sunburn viz. high irradiance and high temperatures result in the chemical and textural characteristics such as high flesh firmness, TSS and lower TA and RWC normally associated with sunburnt fruit.

Introduction

The major challenge of fruit producers and marketers is to deliver fruit of the highest quality that meets consumer expectation (Harker et al., 2003). In the Western Cape of South Africa, first class apple production is greatly hampered by sunburn (Smit, 2007). It is frequently the largest cause of downgrading fruit in Washington State (Schrader et al., 2003), and accounts for up to 10-50 percent of fruit rejections in South Africa (Bergh et al., 1980). Sunburn is caused by a combination of high light and high fruit surface temperatures. Affected fruit are not visually appealing to the consumers due to discolouration of their epidermal tissue, which however seldom suffers serious damage (Racskó et al., 2005).

Depending on how adverse the environmental conditions causing sunburn are, the symptoms can be classified into three classes (Schrader et al., 2003). Sunburn browning occurs at fruit surface temperatures ranging from 46 to 49 °C under high irradiance. Epidermal tissue is only damaged

when fruit surface temperatures exceed ± 52 °C to cause necrotic patches. The third type of sunburn, i.e. sunburn bleaching, occurs when previously shaded fruit peel are suddenly exposed to high irradiance. This type of sunburn may occur at temperatures below 30 °C. Sunburn browning is the most predominant and most costly to apple growers (Felicetti and Schrader, 2009).

The sensitivity to photothermal damage in apples can be expressed as a function of the efficiency of the chlorophyll apparatus in the fruit peel and this is measured by chlorophyll fluorescence (Manuel et al., 2001). It gives an indication of light re-emitted out of the total absorbed light which was neither used in photosynthesis nor dissipated as heat (DeEll and Toivonen, 2003). The use of absorbed light energy for photosynthesis and dissipation as heat or chlorophyll fluorescence occur competitively (Maxwell and Johnson, 2002). Since the thylakoid membranes of the photosystem II (PSII) photosynthetic system are sensitive to heat and excessive light (Weis and Berry, 1988), their damage would result in a reduction in photosynthesis and an increase in chlorophyll fluorescence. Therefore chlorophyll fluorescence can reliably be used to determine heat and light stress on apple fruit surfaces.

Changes in fruit chemical and textural characteristics have been consistently associated with sunburnt fruit. Increased fruit flesh firmness, dry matter content and reduced relative water content and titratable acidity have been reported in sunburnt fruit (Racskó et al., 2005). In addition, Schrader et al. (2009) reported an increase in soluble solids with increasing sunburn. Racskó et al. (2005) postulated that sunburnt cells die and lose water, resulting in firmer flesh.

The increase in soluble solids is also attributed to the loss of water in the affected tissue (McGlone et al., 2003).

Pering (1989a) indicated that the physical characteristics and the distribution of chemical constituents are not uniform around the circumference of apple fruit. The physical and chemical constituents vary longitudinally from the calyx to the stem end and transversely from the peel towards the core of the apple. Wilkinson and Perring (1964) reported that soluble chemical components such as sugars and acids are found in high concentrations in the periphery of the fruit and dry matter also decreases transversely towards the core of the apple. In mature 'Cox's Orange Pippin', Pering (1989b) reported an increase in dry matter from the calyx to the stem end of the fruit. Constituent gradients may differ between different apple cultivars (Perring, 1988) and may be affected by environmental factors the fruit are exposed to (Clijsters, 1969).

Sun exposed fruit are considered better acclimated to heat and light stress, because they have more heat shock proteins, chlorophyll pigments and thicker cuticular wax layers than shaded fruit (Ma and Cheng, 2003). However, the chemical and textural composition of the fruit in relation to light exposure history and sunburn development has not properly been investigated. In the South African apple industry, the perception has gained ground that the observed higher sugar levels and increased fruit density seemingly associated with sunburn, may not be due to sunburn, but that high sugar levels in some fruit may actually make fruit more susceptible to sunburn. According to this reasoning, there is a causal relationship between the well documented increase in the susceptibility of ripening fruit to sunburn (Schrader et al., 2009), the concomitant increase in sugar levels during ripening, and the higher sugar levels in sun-exposed fruit.

Evidently, there is a need for a clearer understanding of the relationship between sunburn and fruit quality attributes.

The objective of this study was to compare the chemical and textural characteristics, as well as the heat stress acclimation of fruit with different light exposure histories. Sun-exposed and shaded fruit peel were subjected to different levels of heat stress at constant light. Stress tolerance was determined by measurements of maximum quantum efficiency of photosystem II (F_v/F_m). In addition, we sought to have a clearer understanding of textural and chemical characteristics of normal fruit and sunburnt fruit. To achieve this, chemical and textural characteristic of normal fruit were compared with that of fruit with induced and naturally occurring sunburn.

Materials and Methods

Experiment 1: The influence of chemical and textural characteristics on heat sensitivity of sun exposed and shaded fruit sides.

‘Cripps’ Pink’ apples grown at Welgevallen Experimental Farm, Stellenbosch, South Africa (33° 55’ S, 18° 53’E) and planted in 1998 on M793 rootstock were used in the experiment. The tree spacing was 4 m x 1.5 m in a North East by South West row orientation. Shortly before harvest, on 15 Apr. 2009, a random sample of 80 sun-exposed, non-sunburnt fruit were harvested from all parts of the outer tree canopy and taken into the laboratory. These were divided into two groups of forty. One group was for measurements on the sun exposed sides of fruit while the other was for measurements on the shaded side.

Non-destructive measurements of fruit mass, fruit diameter and blush intensity were taken first. Blush colour was measured using the Pink Lady colour chart (Topfruit Ltd., Paarl, South Africa), where a value of 1 denotes the worst (green) coloured fruit and 12 the best (reddest).

Peel disks of 12 mm diameter on 30 mm flesh were punched from the stem end half of the fruit. Fruit peel disks extracted from each of the two groups were further subdivided into five groups of eight disks each. The disks were placed, peel side up, in five distilled-water filled cuvette holders with sixteen holding wells. Each of the five cuvette holders contained adjacently staggered fruit peel disks from both the sun exposed and shaded fruit groups. Peel disks were subjected for three hours to visible light at a Photosynthetic Photon Flux (PPF) of $550 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and varied temperatures of 30, 35, 40, 45 and 50 °C effected by different arrangements of infrared lamps (PAR 38 IR 175R, Philips, Poland). Chlorophyll fluorescence measurements were carried out using a pulse amplitude modulated fluorimeter (FSM 2, Hansatek Instruments, Kings Lynn, UK). The optimal quantum efficiency of photosystem II was measured as F_v/F_m where F_v is variable fluorescence and F_m , maximal fluorescence. To compute F_v , the yield of fluorescence in the absence of photosynthetic active radiation (F_0) was subtracted from maximum fluorescence (F_m), obtained by applying a saturating light pulse at PPF of $10,800 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 0.7 s at room temperature after acclimation of the fruit peels in the dark for an hour, hereinafter called initial readings. F_v/F_m was measured again after 30 min dark adaptation at room temperature following the 3 h exposure to the various temperatures, hereinafter called 3 h heat stress readings and again after a further 4 h recovery period in the dark hereinafter called 4 h recovery readings.

The remainder of the fruit after fruit peel disk extraction was taken for destructive fruit maturity indexing. Starch breakdown was determined using a starch conversion chart (Unifruco Research Services, Bellville, South Africa). The juice for measuring the total soluble solids concentration (TSS) and titratable acidity (TA) of the apples was prepared by crushing and extraction of juice from pooled apple pieces in a blender. A hand held refractometer (Model N1, Atago, Tokyo, Japan) was used to measure TSS. TA was determined by titrating 0.1M NaOH to a pH of 8.2 with an automated titrator (Model 719 S, Metrohm AG, Hersiau, Switzerland). TA was expressed as percentage of malic acid ($\text{g } 100 \text{ g}^{-1}$ juice).

A slice of flesh either on the sun exposed or shaded fruit side, depending on fruit group, was weighed, oven dried and then weighed again. Relative Water Content (RWC) was obtained by subtracting the dry weight from the fresh weight. RWC was expressed as the proportion of fresh weight.

Experiment 2: Effect of inducing sunburn on chemical and textural characteristics

The experiment was carried out at Lourensford Estate, Somerset West, South Africa ($34^{\circ} 4' \text{ S}$, $18^{\circ} 53' \text{ E}$) using ‘Cripps’ Pink’ apple on M793 rootstock planted in 1998. The tree spacing was 4 m x 1.5 m in a North East by South West row orientation. Trees were trained to a central leader on a three wire training system.

On 1 Apr. 2010, twelve fruit with sunburn and twenty four sun exposed fruit without prior sunburn were randomly selected in each of four blocks. Three treatments were applied. The first treatment entailed the induction of sunburn by applying a cluster of four 13 mm black stickers (DIA 13 mm, Tower Muizenberg, SA) on sun exposed apples without prior sunburn per block, randomly selected from the twenty four fruit previously selected. This treatment is hereinafter referred to as induced sunburn. The second treatment, hereinafter referred to as sunburn, was made up of the twelve fruit that had naturally developed sunburn. The last treatment was a control and consisted of the remaining twelve fruit per block that were free of sunburn. At harvest (21 Apr. 2010), 74 % and 12% of induced sunburn and control fruit respectively developed sunburn. The sunburn treatment had 100 % fruit with sunburn. The 12% control fruit that developed sunburn were discarded from analysis. Therefore seven fruit were used per treatment. Fruit surface temperature was measured by a handheld infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, California) and measurements were taken during the hottest part of the day (13:00 hrs to 14:00 hrs) on 8 Apr. 2010.

All fruit quality parameters except for starch breakdown, blush colour and fruit density were measured on both shaded and sun exposed sides of fruit as described in Experiment 1. Fruit density was computed by dividing fruit mass with volume. Fruit volume was obtained by immersing fruit in a cylinder filled to known volume with water. The fruit volume was determined as the difference between the final volume of water rising due to apple displacement and the initial water volume. After blush colour and fruit density measurements, fruit were transversely cut into slightly larger stem end and smaller calyx end pieces. The smaller piece was used for starch breakdown measurements. Flesh firmness, TSS, TA and RWC were measured at

two depths, the outer and inner fruit positions, on both the sun-exposed and shaded fruit sides (Figure 1).

Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA) by General Linear Methods using SAS version 9.1.3 (SAS Institute, 2003, Cary, NC). Where significant ($p \leq 0.05$), means were separated by the Least Significant Difference (LSD).

Results

Experiment 1: The influence of chemical and textural characteristics on photosensitivity of sun exposed and shaded fruit sides.

Fruit size: There were no significant differences in fruit size parameters between fruit sampled for sun exposed and shaded side measurements. Fruit diameter averaged 62.6 and 62.1 mm for the sun exposed side fruit and shaded side fruit respectively ($p=0.4978$). Fruit for sun exposed side measurements had an average weight of 138.6 g while that for shaded side measurements averaged 135.5 g ($p=0.1178$).

Fruit colour: The sun exposed fruit side had significantly greater blush coverage (23%) than the shaded side (5%) ($p < 0.0001$). The background colour did not differ significantly ($p=0.1786$) between the fruit sides. The sun exposed side had a chart value of 2.61 while the shaded side averaged 2.72.

Textural and chemical characteristics: The sun exposed side had significantly firmer flesh ($p < 0.0001$) (9.53 kg) than the shaded side (8.59 kg). TSS were also higher ($p = 0.0047$) on the sun exposed side (14.5 %) than the shaded side (14 %). TA was slightly lower on the sun exposed side than the shaded side, but did not differ significantly ($p = 0.1486$) (0.56 % and 0.59 %, respectively). Starch breakdown did not differ significantly ($p = 0.7237$) between the fruit for sun exposed measurements (21 %) and shaded side measurements fruit (23 %). The sun exposed side fruit had a significantly lower ($p = 0.0142$) RWC, averaging 79 % while the shaded side fruit had 80 %.

Fluorescence: Fruit peel temperature ranges were 32.3 ± 0.23 °C, 37.5 ± 0.25 °C 42.3 ± 0.23 °C, 46.8 ± 0.38 °C and 49.9 ± 0.27 °C at temperatures set for 30, 35, 40, 45 and 50, respectively. There were no significant interaction between temperature and fruit side for initial F_v/F_m prior to heat treatment ($p = 0.9180$), after 3 h at the respective temperatures ($p = 0.8696$) or after 4 h recovery at room temperature ($p = 0.9762$). Initial F_v/F_m for the sun exposed side was significantly higher ($p = 0.0345$) (Table 1) than the shaded side. There were no significant difference in F_v/F_m between the sun exposed and shaded sides after exposure to the various temperatures. However, after 4 h recovery, F_v/F_m for the sun exposed side fruit peel were higher ($p = 0.0127$) than the shaded side.

Fruit peels exposed to 30 °C, 35 °C and 40 °C for 3 h had significantly higher F_v/F_m values than peel exposed to 45 °C and 50 °C (Table 2). Fruits previously exposed to 30 °C and 35 °C had the significantly highest F_v/F_m values after 4 h recovery at room temperature in the dark while those that were kept at 40 °C had higher readings than those at 45 °C and 50 °C.

Fruit previously exposed to 30 °C, 35 °C and 40 °C, displayed significantly ($p < 0.0001$) less photo inhibition (% decrease in F_v/F_m) after the three-hour heat stress, compared to fruit kept at 45 °C and 50 °C (Table 1). The percentage photo inhibition decreased during the 4 h recovery period in fruit exposed to 30 °C and 35 °C, while no meaningful recovery was seen for fruit exposed to higher temperatures (Table 2). After the four-hour recovery period, fruit previously exposed to 30 °C and 35 °C, showed the least residual photo inhibition, while photo inhibition remained very high in fruit exposed to 45 °C and 50 °C. Fruit exposed to 40 °C were intermediate. However, although the sun exposed side showed slightly less photo inhibition than the shaded side, there were no statistical differences in percentage decrease in F_v/F_m between the two sides.

Experiment 2: Effect of inducing sunburn on chemical and textural characteristics

Fruit surface temperature (FST), density and red colour: There were significant differences ($p < 0.0001$) for FST between all treatments. Induced sunburn fruit had the highest FST while the control had the lowest, while sunburn fruit were intermediate (Table 3). The sunburn and induced sunburn fruit did not differ in density, but were significantly ($p = 0.0233$) less dense compared to the control fruit (Table 3). Control fruit and sunburn fruit had significantly higher starch breakdown than fruit of the induced sunburn treatment (Table 3). There were no significant differences ($p = 0.3847$) in red colour between the treatments (Table 3).

Relative water content (RWC) and chemical and textural characteristics: When considering only the fruit sides, chemical and textural characteristics did not differ significantly on the shaded side of the fruit, except for RWC. The control had a significantly higher ($p=0.0121$) RWC than the sunburn and induced sunburn treatments (Table 4). On the sun exposed side, the control fruit had significantly lower TSS ($p=0.0003$), firmness ($p=0.0007$) and TSS:TA ($p=0.0011$) while TA ($p=0.0496$) and RWC ($p=0.0471$) were significantly higher than the sunburn and induced sunburn treatments. Sunburn and induced sunburn did not differ significantly in all parameters (Table 4). Considering only fruit depth, TSS, TSS:TA and firmness of sunburn and induced sunburn fruit were consistently higher than that of control while TA and RWC were lower at all depths (Table 5). Sunburn and induced sunburn treatments had significantly greater differences of firmness ($p=0.0073$) and TSS ($p=0.0221$) between the sun exposed and shaded fruit side compared to the control (Table 6). For TA and TSS:TA, the control had greater differences between the sun exposed side and shaded side compared to both sunburn and induced sunburn treatments (Table 6). With regards to fruit depth, no clearer trends between treatments were observed (Table 6).

Discussion

Thorpe (1974) and Woolf and Ferguson (2000) reported temperature gradients between the shaded and sun exposed sides of apple fruit of up to 15 °C and suggested that this might result in metabolic disparities accounting for the differences in flesh firmness observed between these sides. Consistent with this, the sun exposed sides of fruit sampled during the 2008-09 season for heat sensitivity tests had significantly firmer flesh than the shaded sides. This confirms results of Volz et al. (1995) who reported increased flesh firmness of 'Royal Gala' apples with increased

irradiance. Opara et al. (1997) postulated that textural differences in sun-exposed and shaded sides of fruit are due to variations in cell structure and shape. Observations in 2009-10 indicated that there was less variation in firmness between the sun-exposed and shaded sides of control fruit than there was for the fruit sides of sunburn and induced sunburn fruit. It is not clear whether the greater difference in firmness between the shaded and exposed sides of sunburn apples compared to the difference between the sides of non-burnt apples also relates to cell structural differences.

Differences in calcium content of sun exposed and shaded sides of apples might also account for the differences in firmness between these fruit sides. The sun exposed side has higher calcium content (Woolf et al., 1999). However, our results seem to agree with Racskó et al. (2005) who indicated that sunburn damage would result in loss of water in affected tissue, making it firmer. In 2008-09, the sun exposed side had lower RWC than the shaded side. Woolf and Ferguson (2000) indicated that evaporative water loss around a fruit varies due to differences in permeance. The sun exposed side is more permeable to water (Purvis, 1984) but differences are only substantial when the fruit is detached from the tree (Woolf and Ferguson, 2000). Maguire et al. (1999) also ascertained the variability of water loss around the fruit, but indicated that there was no clear relationship with sun exposure. Regardless of exposure history, as indicated by consistent trends between shaded and sun exposed sides, our findings in 2009-10 indicated that sunburn and induced sunburn fruit had lower RWC than the control.

It appears from our findings that control fruit were different from fruit of sunburn and induced sunburn treatments in terms of the chemical and textural characteristics in the 2009-10 season.

Induced sunburn fruit were indistinguishable from control fruit until when the sunburn induction treatment was effected and yet the sunburn and induced sunburn fruit appeared to have similar chemical and textural characteristics, different from the control fruit. This attests to the fact that chemical and textural characteristics associated with sunburn are not inherent and only concomitantly occur with sunburn development.

Since control and induced sunburn fruit were under similar irradiance, their differences in characteristics can only be attributed to differences in FST. The high FST of induced sunburn fruit subsequently resulted in the manifestation of sunburn symptoms observed in these treatments. Therefore the development of sunburn on the fruit is associated with specific chemical and textural changes. Schrader et al. (2009) noted a correlation in which an increase in sunburn browning resulted in an increase in TSS, flesh firmness and a reduction in TA. Consistent with this in our findings, TSS and flesh firmness of induced sunburn and sunburn treatments were higher than the control while TA was lower. It was apparent that the sun exposed fruit side was the more important side in determining differences in chemical and textural characteristics between control and both sunburn treatments.

Measurements on the shaded fruit side did not give significant differences between control and induced as well as sunburn fruit for TSS, flesh firmness and TA. This further confirms that the development of sunburn in association with high FST, is instrumental in chemical and textural changes during sunburn development. Our results in 2008-09 seem to confirm that in order to combat the effects of high temperatures, thermal protective measures are restricted to the sun exposed side. The sun exposed side had significantly higher F_v/F_m after recovery than the shaded

side. According to Ma and Cheng (2003), sun exposed fruit are better acclimated to heat and light stress because they have more heat shock proteins, chlorophyll and thicker fruit peel waxes than shaded fruit. Sun exposed fruit also have higher levels of anti-oxidants such as ascorbic acid and glutathione than shaded fruit because the levels increase in response to heat exposure (Ma et al., 2008). This was also confirmed by Chen et al. (2008) who reported an up-regulated anti-oxidant system in apples exposed to high light and high temperatures. In addition, sun exposed apple peel have a larger xanthophyll cycle pool size (Ma and Cheng, 2003). The sun exposed fruit therefore can better dissipate excess energy and quench reactive oxygen species (ROS) than shaded fruit. An up regulated anti-oxidant system in the sun exposed side of the fruit also aids in the efficient operation of the xanthophyll cycle (Ma and Cheng, 2003).

However, persistent high FST would affect fruit peel photochemistry and subsequently peel damage, observed as sunburn, appears. From our findings, fruit surface temperatures equal to or higher than 40 °C for three hours would damage the photo system beyond recovery. The normal range for F_v/F_m values (maximum quantum yield of fluorescence) is between 0.7 and 0.8, while the critical value is around 0.6 (Ritchie, 2006). If there is no recovery, F_v/F_m below the critical value indicates damage to the photosynthetic system (Ritchie, 2006). Between 30 and 35 °C, maximum quantum yield was decreased to a critical value. However, a 4 h recovery period was sufficient for fruit peel to revert to normal photosynthetic capacity.

The high temperatures can speed up starch breakdown in affected fruit, resulting in higher TSS concentrations compared to control fruit (Schrader et al., 2009). In grapes, it was demonstrated that TA can be reduced by up to 50 % with a 10 °C rise in temperature (Coombe, 1987). TA of

apples is expected to be lower on the sun exposed side than the shaded side (Woolf and Ferguson, 2000) and sunburnt fruit (Racskó et al., 2005). TA was indeed lower on the sun exposed side in our 2008-09 findings although this did not differ significantly from the shaded side. Computed differences between the fruit sides in 2009-09 indicated that there were wider variations of TA for the control fruit than the for sunburn and induced sunburn treatments.

In the 2009-10 season, the chemical and textural changes associated with sunburn were consistent between treatments, whether the inner or outer flesh were considered. This seems to suggest that fruit depth is less important than fruit side in sunburn development and subsequent chemical and textural changes in a fruit. We expected induced sunburn and sunburn fruit to be denser compared to the control, but our findings were contrary to this. These treatments had significantly less dense fruit compared to the control. Density has reliably been used to predict soluble solids and dry matter content in most fruits (Nelson et al., 1963; Abbot et al., 1997). Further, Asami et al. (1988) and Richardson et al. (1997) reported that fruit density at harvest is positively correlated with both dry matter content and soluble solids in kiwi fruit. Since sugars make up a great proportion of dry matter (Beever and Hoopkirk, 1990) and have greater density than water, fruit density increases with an increase in TSS. Although it was not clear why this was not consistent, we speculate that the reduced water content in sunburnt fruit results in more intercellular air spaces. Fruit density, to a great extent is also determined by intercellular air spaces (Racskó, 2006) in addition to dry matter content.

Induced sunburn and sunburn treatments had a better balance of sweetness and sour taste because they had significantly higher TSS:TA ratio than the control fruit. Kuhn and Thybo (2001)

indicated that a higher TSS:TA ration is associated with a good balance of sugar and acid for good apple taste. According to Abbot et al. (2004), a low TSS:TA between 19.1 and 24.5 is characteristic of apples with a sour taste. TSS:TA of sunburn and induced sunburn treatments were higher than this range, but the control fruit were close to it.

Chemical and textural characteristics of exposed peel do not predispose this peel to sunburn. In fact, shaded peel is much more sensitive to photo thermal stress than exposed peel. It appears that the chemical and textural characteristics of fruit peel and flesh are a result of the environmental conditions the fruit is exposed to. The exposed sides of fruit differ significantly from the shaded sides in certain chemical and textural attributes, most likely due to the major differences in light exposure. Upon sunburn induction on the exposed sides of previously unblemished fruit, further changes in chemical and textural attributes occurred in the flesh so that it resembled the fruit that developed sunburn without induction. The chemical and textural characteristics of sunburnt fruit are therefore not inherent. The observation that fruit become more prone to develop sunburn during their development may relate less to changes in the chemical and textural attributes of the fruit than to changes in the heat exchange capacity of the fruit, changes in photochemistry, increasing temperatures during the growing season and changes in light exposure due to bending of branches.

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Table 1. Effect of fruit side on peel chlorophyll fluorescence of ‘Cripps’ Pink’ apples from Welgevallen Experimental Farm during 2008-10 season after exposure to heat stress for 3 h at $550 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$

Fruit side	Initial F_v/F_m	F_v/F_m after 3 h heat stress.	F_v/F_m after 4 h recovery	Photoinhibition^y (%)	Residual photo inhibition^x (%)
Shaded	0.80 a ^z	0.38 ns	0.39 a	52.6 ns	50.0 ns
Exposed	0.82 b	0.42	0.47 b	48.7	43.5
<i>p value</i>	0.0345	0.2003	0.0127	0.6410	0.0983

^z Means in the same column followed by the same letter are not significantly different at $P < 0.05$.

^y Percentage difference of F_v/F_m after three-hour heat stress with respect to room temperature F_v/F_m .

^x Percentage difference of F_v/F_m after four-hour recovery period with respect to room temperature F_v/F_m .

ns Not significant.

Table 2. Effect of temperature on fruit peel chlorophyll fluorescence at $550 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of Cripps Pink apples from Welgevallen Experimental Farm during 2008-09 season.

Temperature (°C)	Initial F_v/F_m	F_v/F_m after 3 h heat stress	Final F_v/F_m after 4 h recovery	Photoinhibition (%)	Residual photo inhibition ^x (%)
30	0.81 ns	0.63 a ^z	0.76 a	21.0 b	4.4 c
35	0.81	0.62 a	0.71 a	23.7 b	12.5 c
40	0.82	0.55 a	0.53 b	33.2 b	35.8 b
45	0.83	0.13 b	0.06 c	91.8 a	92.7 a
50	0.80	0.07 b	0.09 c	83.6 a	88.2 a
<i>p value</i>	0.4280	<0.0001	<0.0001	<0.0001	<0.0001

^zMeans in the same column followed by the same letter are not significantly different at $P < 0.05$.

^y Percentage difference of F_v/F_m after three-hour heat stress with respect to room temperature F_v/F_m .

^x Percentage difference of F_v/F_m after four-hour recovery period with respect to room temperature F_v/F_m .

ns-Not significant

Table 3. Effect of naturally occurring and induced sunburn on fruit quality of ‘Cripps’ Pink’ apples at Lourensford Estate during the 2009-10 season.

Treatment	Fruit density(g cm⁻³)	Fruit surface temp. (°C)^y	Blush color^x	Starch breakdown (%)
Control	0.87 a ^z	33.9 a	5.39 ns	76.8 a
Induced sunburn	0.82 b	44.5 b	6.10	72.5 b
Sunburn	0.81 b	40.9 c	5.57	78.6 a
<i>p value</i>	<i>0.0233</i>	<i><0.0001</i>	<i>0.3847</i>	<i>0.0233</i>

^zMeans in the same column followed by the same letter are not significantly different at P<0.05.

^y Measured on 8 Apr. 2010 from 13:00 to 14:00. Air temperature during this period was 28 °C.

^x Blush colour was measured using a colour chart where 1 denotes worst (green) coloured fruit and 12 the best (reddest).

ns-Not significant.

Table 4. The effect of naturally occurring and induced sunburn on chemical and textural characteristics of the sun exposed and shaded fruit sides of ‘Cripps’ Pink’ apples.

Treatment	Sun exposed fruit side					Shaded fruit side				
	RWC ^z (%)	Firmness (kg)	TA (%)	TSS (°Brix)	TSS:TA	RWC (%)	Firmness (kg)	TA (%)	TSS (°Brix)	TSS:TA
Control	80.7 a ^y	8.9 a	0.53 a	14.2 b	26.6 b	79.2 a	8.3 ns	0.42 ns	14.2 ns	33.9 ns
Induced sunburn	77.4 b	9.8 b	0.45 b	15.5 a	35.1 a	75.7 b	8.5	0.42	14.3	34.5
Sunburn	76.9 b	9.7 b	0.45 b	15.6 a	35.3 a	75.9 b	8.3	0.41	14.3	35.4
<i>p value</i>	0.0471	0.0007	0.0496	0.0003	0.0011	0.0121	0.7504	0.0535	0.7575	0.7733

^zRelative Water Content (%)

^yMeans in the same column followed by the same letter are not significantly different at P<0.05.

ns-Not significant

Table 5. The effect of naturally occurring and induced sunburn on chemical and textural characteristics on outer and inner flesh of ‘Cripps’ Pink’ apples.

Treatment	Outer fruit flesh					Inner fruit flesh				
	RWC ^z (%)	Firmness (kg)	TA (%)	TSS (*Brix)	TSS:TA	RWC (%)	Firmness (kg)	TA (%)	TSS (*Brix)	TSS:TA
Control	81.6 a ^y	8.7 b	0.61 a	13.4 b	21.9 b	81.0 a	8.4 b	0.61 a	13.7 b	22.4 b
Induced sunburn	76.2 b	10.0 a	0.43 b	15.5 a	36.4 a	76.8 b	9.3 a	0.42 b	15.1 a	33.0 a
Sunburn	77.0 b	9.7 a	0.44 b	15.6 a	36.5 a	76.0 b	9.2 a	0.41 b	15.5 a	32.3 a
<i>p value</i>	0.0075	<0.0001	<0.0001	<0.0001	0.0011	0.0005	0.0018	<0.0001	<0.0001	<0.0001

^z Relative Water Content (%)

^y Means in the same column followed by the same letter are not significantly different at P<0.05.

Table 6. Computed differences between parameters of sun exposed and shaded fruit sides of naturally occurring and induced sunburn in ‘Cripps’ Pink’ apples at Lourensford Estate during the 2009-10 season.

Treatment	Differences ^z between fruit sides					Differences ^y between fruit depth				
	RWC	Firmness	TA	TSS	TSS:TA	RWC	Firmness	TA	TSS	TSS:TA
	(%)	(kg)	(%)	(° Brix)		(%)	(kg)	(%)	(° Brix)	
Control	1.56 ns	0.65 a ^x	0.12 b	-0.05 a	-7.21 b	4.03 a	-0.04 b	0.057 a	-0.825 b	-4.52 c
Induced sunburn	1.64	1.34 b	0.02 a	1.18 b	0.70 a	-2.28 b	1.16 a	-0.11 b	1.06 a	9.30 a
Sunburn	1.05	1.36 b	0.05 a	1.29 b	-0.20 a	-0.45 ab	0.49 ab	-0.02 ab	-0.01 ab	2.45 b
<i>p value</i>	0.9382	0.0073	0.0170	0.0221	0.0264	0.0419	0.0108	0.0038	0.0085	0.0010

^z Differences were computed by subtracting values of the shaded fruit side from the sun exposed side.

^y Differences were computed by subtracting values of the inner fruit depth from the outer depth.

^x Means in the same column followed by the same letter are not significantly different at P<0.05.

ns –Not significant

SHADED SIDE

SUN EXPOSED SIDE

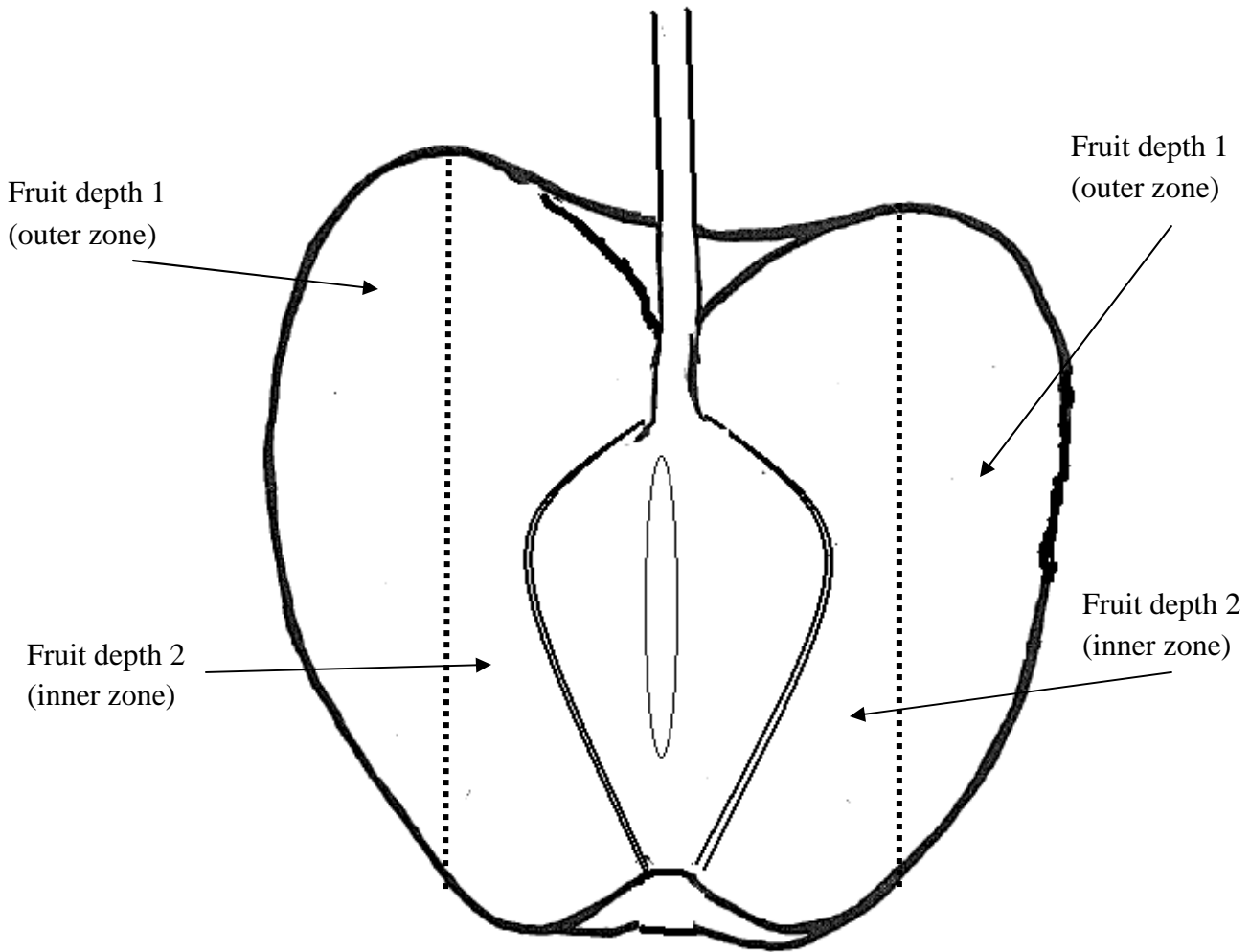


Figure 1. Longitudinal section of apple showing fruit depths taken for maturity indexing parameters in 2009-10 season.

THE EFFECT OF CROP LOAD ON FRUIT QUALITY AND SUNBURN IN 'CRIPPS' PINK' APPLE.

Abstract

The effects of crop load on fruit quality and sunburn were evaluated on 'Cripps's Pink' apples at 1) fruit cluster level and 2) whole tree crop load level in the 2008-09 and 2009-10 growing seasons, respectively. Fruit were thinned to one, two, three and four fruit clusters on the same tree in 2008-09. In 2009-10, the thinning severities were i) three fruit per cluster throughout the entire tree, ii) two fruit per cluster below and three fruit per cluster above 1.8 m, iii) two fruit per cluster throughout the entire tree, iv) one fruit per cluster below and two fruit per cluster above 1.8 m and v) one fruit per cluster throughout the entire tree. At the fruit cluster level, sunburn incidence, sunburn severity, blush coverage and blush intensity decreased with an increase in the number of fruit per cluster. This seems to be due to inter-fruit shading which could have increased due to an increase in fruit number per cluster. The fruit size quality parameters, fruit weight and fruit diameter did not differ significantly whereas for the biochemical and textural parameters, firmness, titratable acidity (TA), relative water content (RWC), total soluble solids (TSS) and starch breakdown, only RWC significantly decreased with an increase in fruit per cluster. The proportion of first grade fruit decreased with an increase in fruit number per cluster. At the whole tree level, greater thinning severities resulted in bigger fruit sizes although there were no significant differences in yield. Blush coverage, blush intensity, TSS, TA, flesh firmness, starch breakdown and total sunburn did not differ significantly between thinning

severities. However, a breakdown of the sunburn types indicated that the lowest thinning severity of three fruit per cluster had a significantly higher proportion of sunburn bleaching than other thinning severities most likely due to exposure of previously shaded fruit as branches bend under the increasing weight of fruit in addition to a decrease in foliage due to competition between shoot and fruit growth. The proportion of sunburn browning and sunburn necrosis did not differ significantly between thinning severities although the $\text{ton}\cdot\text{ha}^{-1}$ of sunburn necrosis were significantly higher at three fruit per cluster compared to greater thinning severities. Overall, the proportion of unexportable sunburn did not differ significantly between treatments. Due to the effect on fruit size, the proportion of first grade fruit was significantly highest and lowest for thinning to one and three fruit per cluster, respectively. Due to inter-fruit shading, increasing the number of fruit per cluster decreases sunburn as well as the extent and intensity of red colour among fruit within the cluster. However, at the whole tree level, sunburn and red blush did not increase at high crop loads, possibly due to increased exposure of fruit.

Introduction

Fruit thinning is an important cultural practice that can be used to manipulate the crop load of a tree. Crop load is very influential in fruit quality determination of deciduous fruit such as apples (Stopar et al., 2002). Lighter crop loads have been associated with improved red colour development, increased fruit size and firmness (Link, 2000). In addition, Palmer et al. (1997) reported that light crop loads advance harvest maturity as evidenced by increased TSS, starch breakdown and fruit background colour. The effect of crop load on parameters such as foreground colour (blush coverage and intensity) and sunburn, which are light dependent

(Arakawa et al., 1985; Awad et al., 2001; Schrader et al., 2003), could be a function of how that particular crop load modifies the light environment of the fruit. Red foreground colour requires adequate light during the day coupled with low night temperatures (Reay, 1999). However, concomitant high irradiance and high temperatures cause sunburn (Schrader et al., 2003).

Sunburn manifests as a bronze-yellow discolouration, bleaching or necrotic patches on the apple fruit peel, depending on the particular combination and severity of inductive conditions, i.e., high irradiance and moderate to high temperature (Schrader et al., 2003). The negative effect of sunburn on the appearance of the fruit results in the downgrading of fruit to lower grades which are sold at lower prices.

The effect of thinning and crop load on sunburn in apples is not well documented and available literature is not clear. There are contrary reports, with some suggesting that crop load has no effect on sunburn (Wünsche et al., 2004a). However, according to Van den Ende (1999), sunburn development can be reduced if fruit, particularly of bi-coloured and red cultivars, are thinned to one or two per cluster. Therefore, a clearer understanding of the relationship between crop load and sunburn is required. It is important to note that the effects of crop load on sunburn might be related to the number of fruit per cluster or on a whole tree basis.

On the cluster level, sunburn development can be a function of fruit position and how the fruit shade each other from light. Awad et al. (2001) reported that fruit in an apple cluster shade each other and attributed the inadequate red colour development in high cropping trees to increased

shading due to the high number of fruit in clusters. Palmer et al. (1997) found the same results in 'Braeburn' apples.

On the entire tree level, crop load directly affects the crop to shoot ratio (Van den Ende, 1999). A heavy crop load increases the crop to shoot ratio, resulting in little foliage and a lot of fruit exposed to the sun. In addition, a heavy crop load results in branch movement and bending in response to increasing fruit weight, exposing previously shaded fruit. Previously shaded fruit are predisposed to sunburn development as they would not have acclimated to photothermal stress (Ma and Cheng, 2003).

Water stress was reported to predispose fruit to sunburn or aggravate existing damage (Paper 1; Ware, 1932; Barber and Sharpe, 1971; Schrader et al., 2003). A tree with a high water potential is capable of cooling itself through transpiration (Brown, 2009). Heavy crop loads increase water and assimilate requirements (Jones et al., 1992). Trees with heavy crop loads therefore have a greater likelihood of experiencing moisture stress than lightly cropping trees. Under adverse environmental conditions conducive to sunburn development, heavily cropped trees are therefore more likely to be affected by sunburn than lightly cropped trees.

We hypothesized that sunburn increases as the crop load increases. The objective of this study was to assess the effect of thinning practice and crop load on the incidence of sunburn caused by factors affecting fruit number per cluster as well as crop load on the entire tree, while keeping in mind other fruit quality parameters that might affect marketable yield. To assess the effect of fruit number per cluster on sunburn independent of crop load, we thinned to four different cluster

levels on the same tree in the first season. In the following season, trees were thinned to five severities to assess the effect of crop load on sunburn on the entire tree.

Materials and Methods

Plant material

The experiments were carried out at Welgevallen Experimental Farm, Stellenbosch (33° 55' S, 18° 53' E) in 2008-09 and Carica Estate, Vyeboom (34° 4' S, 19° 6' E) in 2009-10, South Africa using 'Cripps' Pink' apples. At Welgevallen Experimental Farm, the trees were planted in 1998 on M793 rootstock with a tree spacing of 4 m x 1.5 m in a North East by South West row orientation. At Carica Estate, the trees were planted in 1996 on M793 rootstock and had the same tree spacing and row orientation as that at Welgevallen Experimental Farm. The orchard at Welgevallen farm has a history of low yield, averaging 18 t ha⁻¹ over the last five years whereas the orchard at Carica Estate is high yielding at 110 t ha⁻¹ since 2004.

Treatments and experimental design

In 2008-09, twenty trees were used at Welgevallen Experimental Farm. On the North Western side of each tree, 12 clusters were randomly selected on 28 Nov. 2008 and thinned to give four treatments, namely, four, three, two and one fruit per cluster on the same tree. Hence, each treatment was replicated three times in a random order on each tree. The standard thinning strategy followed at Welgevallen involves hand thinning to two fruit per cluster in late December. Treatments were thus effected before the commercial thinning procedure.

In 2009-10, at Carica Estate, crop load was adjusted on 29 Nov. 2009, making use of five thinning severities: 1) thinning to three fruit per cluster for the entire tree, 2) two fruit per cluster and three below 1.8m of the tree, 3) two fruit per cluster for the entire tree, 4) one fruit per cluster and two below 1.8m of the tree, and 5) one fruit per cluster for the entire tree. The experiment was laid out in a randomized complete block design replicated twelve times with single-tree treatment plots. The tree height was between 3.8- 4 m.

Measurements

In 2008-09 season, all fruit quality parameters were measured on all fruit at harvest on 27 Apr. while in 2009-10 measurements were taken from two 40 fruit samples taken at the two harvesting dates (22 Apr and 07 May 2010). Averages were computed for statistical analysis.

Sunburn severity was measured according to the Schrader and McFerson sunburn severity chart (Schrader et al, 2003), where 0 represented no sunburn and 5 the most severe necrotic sunburn. In the 2009-10 season, sunburn fruit were further categorised into sunburn browning, sunburn necrosis and photo-oxidative bleaching. Each category was expressed as a percentage of the total sunburnt fruit. All fruit were graded into first grade (Pink LadyTM, ≥ 60 mm diameter, $>40\%$ blush coverage, and <2 sunburn score), second grade ('Cripps' Pink', >60 mm diameter, 10-40% blush coverage, and <2 sunburn score) or third grade (any of <60 mm diameter, $<10\%$ blush coverage, ≥ 2 sunburn score). Blush colour was measured using the Pink LadyTM colour chart (Topfruit Pty Ltd, Simondium, South Africa), where a value of 1 denotes the least coloured fruit (green) and 12 the reddest.

Flesh firmness was measured with a flesh texture analyser (Guss electronic model GS 20, Strand, South Africa) on opposite peeled sides of the fruit with an 11mm tip. Starch conversion was measured by applying 1% iodine solution on the transversely cut surface and assessing the percentage starch conversion using the starch conversion chart (Unifruco Research Services, Bellville, South Africa). The juice for measuring the total soluble solids (TSS) and titratable acidity (TA) of the apples was prepared by crushing and extraction of juice from pooled apple pieces in a blender. A refractometer (Model N1, Atago, Tokyo, Japan) was used to measure TSS whereas TA was determined by titrating 0.1M NaOH to a pH of 8.2 with an automated titrator (Model 719 S, Metrohm AG, Hersiau, Switzerland). TA was expressed as percentage of malic acid ($\text{g } 100 \text{ g}^{-1}$ juice). To determine relative water content (RWC), a slice of flesh was weighed, oven dried and then weighed again. RWC, which was expressed as a proportion of fresh weight, was then obtained by subtracting the dry weight from the fresh weight.

Tree vigour, measured only in the 2009-10 season, was determined by measuring the trunk growth increment from first measurement on 30 Nov 2009 until the second measurement on 23 Sep 2010. The second measurement was used to determine trunk cross sectional area (TCSA). The crop density index (CDI) was then calculated by dividing the total number of fruit left on the tree after thinning with TCSA. Treder (2010) indicated that CDI is the most reliable and accurate way to describe thinning intensity as it gives an indication of the quantity of fruit that can be sustained by a tree volume, or TCSA (Bergh, 1990; Treder, 2008).

Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA) by General Linear Methods using SAS version 9.1.3 (SAS Institute, 2003, Cary, NC). Where the treatment effect was significant ($p \leq 0.05$), means were separated by Least Significant Difference (LSD). The CDI at each thinning severity rather than the number of thinned fruit was used to calculate coefficients for fitting linear and quadratic contrasts.

Results

Number of fruit per cluster: RWC (Table 1), blush coverage, and sunburn severity (Table 2) increased linearly with a reduction in fruit number per cluster while blush intensity (Table 2) showed a quadratic trend in 2008-09. Fruit from four fruit clusters had the lowest RWC, while thinning to one fruit resulted in the highest. Two and three fruit clusters were intermediate in level for this variable and did not differ significantly. For blush intensity, one fruit and four fruit clusters had the highest and lowest blush intensity, respectively ($p < 0.0001$), while fruit from two fruit clusters were redder than fruit from three fruit clusters. Three and four fruit clusters had the lowest blush coverage ($p = 0.0122$) although they did not differ significantly from two fruit clusters (Table 2). The three and four fruit clusters had lower sunburn severity ($p = 0.0019$) than one and two fruit clusters. Sunburn incidence decreased linearly ($p < 0.0001$) with an increase in fruit number per cluster (Table 2). Starch breakdown, firmness, TA, TSS (Table 1), fruit weight and fruit size (Table 2) did not differ significantly and no significant trends were observed. There were no significant trends or differences in fruit size and fruit weight although there was a significant linear relationship ($p = 0.0217$) between the percentage of first grade fruit and number of fruit per cluster (Table 2).

The percentage first grade fruit decreased with an increase in fruit number per cluster. First grade fruit was low for all treatments due to small fruit size. When fruit in clusters with more than one fruit were categorised from greatest red colour coverage to least coloured, the reddest fruit in each cluster did not seem to differ from the fruit in the one fruit cluster in terms of blush coverage (Table 3). Sunburn incidence and first grade fruit decreased for the reddest fruit in a cluster, with an increase in the number of fruit. Also, compared to the single fruit cluster, sunburn severity only decreased for the reddest fruit with more than two fruit per cluster whereas blush intensity decreased only up to three fruit per cluster. Fruit size did not differ between fruit clusters (Table 3).

Thinning severity and crop load: In the 2009-10 season, there was a significant linear ($p=0.0035$) trend of the number of fruit left on the tree at harvest (Table 4). Thinning to one fruit per cluster over the entire tree significantly decreased the number of fruit left on the tree at harvest compared to other thinning treatments except for thinning to two and three fruit per cluster over the entire tree. Thinning to one fruit per cluster also resulted in the lowest CDI ($p=0.0047$) although it did not significantly differ with three fruit per cluster over the entire tree. CDI decreased linearly ($p=0.0022$) with an increase in thinning severity (Table 4).

Fruit weight ($p<0.0001$) and size ($p=0.0003$) decreased linearly with an increase in CDI. Yield seemed to increase linearly with an increase in CDI although this trend was not significant ($p=0.0572$). The different thinning severities did not result in significant differences in fruit yield ($p=0.1872$) (Table 4). There were no significant trends and treatment effects on blush colour,

blush intensity (Table 4), TSS, TA, flesh firmness, background colour and starch breakdown (data not shown).

Although not statistically significant ($p=0.0703$), thinning to three fruit over the entire tree seemed to increase, while thinning to one fruit below and two above 1.8 m seemed to decrease sunburn incidence (Table 5). Thinning did not affect the percentage of fruit with un-exportable sunburn ($p=0.2179$) (Table 5). In all treatments, sunburn browning was more prominent than sunburn necrosis and photo-oxidative bleaching (Table 6). Leaving three fruit per cluster seemed to decrease the proportion of sunburnt fruit with sunburn browning compared to higher thinning levels, although the difference was not significant ($p=0.0563$). However, thinning to three fruit per cluster over the entire tree resulted in significantly higher sunburn bleaching ($p=0.0418$) than the rest of the treatments (Table 6). The percentage of sunburnt fruit with sunburn necrosis did not differ between thinning severities ($p=0.6733$), although in actual $\text{ton}\cdot\text{ha}^{-1}$, thinning to three fruit over the entire significantly ($p=0.0045$) increased sunburn necrosis compared to greater thinning severities (Table 6).

The percentage first grade fruit increased linearly ($p=0.0053$) with an increase in CDI (Table 7). Thinning to one fruit per cluster over the entire tree significantly ($p=0.0114$) increased the percentage first grade fruit compared to lesser thinning severities with the exception of thinning to two fruit per cluster over the entire tree (Table 7). The rest of the treatments did not differ significantly. There were indications that leaving three fruit per cluster decreased the proportion of second grade fruit compared to greater thinning severities, but differences were not significant ($p=0.0889$). The most severe thinning treatment decreased the percentage of third grade fruit

compared to the two least severe thinning treatments ($p < 0.0204$). There were no significant differences in $\text{ton}\cdot\text{ha}^{-1}$ of first grade fruit ($p = 0.3040$) between thinning severities, whereas thinning to one fruit per cluster below and two above 1.8 m significantly increased the second grade tonnage, but not significantly more than thinning to two fruit per cluster below 1.8 m and three above ($p = 0.0028$) (Table 7). Thinning to three fruit per cluster resulted in the lowest $\text{ton}\cdot\text{ha}^{-1}$ of second grade fruit although it did not differ with thinning to one or two fruit over the entire tree. In terms of third grade fruit, thinning to two fruit per cluster below and three above 1.8 m gave the highest $\text{ton}\cdot\text{ha}^{-1}$ ($p < 0.0001$), but did not differ from thinning to two fruit over the entire tree, whereas thinning to one fruit per cluster over the entire tree gave the lowest. Thinning to three fruit over the entire tree and thinning to one fruit per cluster below 1.8 m and two above were intermediate.

Discussion

There were no significant differences between yield parameters, such as fruit size and weight, in the 2008-09 season when the effect of fruit number per cluster (one to four) was assessed. Literature indicates that a decrease in fruit numbers increases fruit weight and size due to lower competition for assimilates (Jones et al., 1992; Racskó, 2006). However, in our study, thinning to different cluster levels on the same tree could have obscured treatment effects as there was likely to have been interference or compensatory activity between branches with different treatments. Palmer et al. (1991) reported that fruit weight of 'Cox's Orange Pippin' was linearly related to leaf area per fruit of the whole tree regardless of completely removing fruit on one side of the canopy, alternate branches or uniformly over the whole tree. Therefore, obtaining accurate

treatment effects on size parameters of different treatments on the same tree would be difficult even if thinning was carried out a month and half after full bloom.

Sunburn severity, sunburn incidence, blush coverage and blush intensity decreased with an increase in the number of fruit per cluster. As both parameters are light dependent, we reasoned that this was an effect of fruit shading each other in clusters with more than one fruit. Anthocyanin synthesis and subsequent red coloration of red and blushed apple cultivars is stimulated by light (Saure, 1990). Awad et al. (2000) reported that terminal fruit had greater blush coverage than lateral fruit due to shading of the latter. The higher the number of fruit in a cluster, the more they shade each other and hence the observed reduction in sunburn and red blush development with an increase in fruit per cluster in our findings. In evidence of this, blush coverage of the single fruit per cluster did not differ much from the blush coverage of the reddest fruit in clusters with two, three or four fruit per cluster (Table 3). However, the remaining fruit in clusters with multiple fruit had lower red colour coverage. Inter fruit shading decreased sunburn and red colour intensity in all the fruit in multiple fruit clusters. The negative effect on red colour development caused the linear decrease in the proportion of first grade fruit with an increase in fruit number per cluster. Sunburn severity and incidence, which decreased with an increase in number of fruit per cluster, did not impact drastically on fruit grade determination as sunburn severity generally averaged below the cut off score (<2) for marketable fruit. As fruit size did not differ significantly, blush coverage was therefore the predominant determinant of fruit grades. This was confirmed by the breakdown and categorisation of fruit in clusters according to a blush coverage gradient (Table 3).

In the 2009-10 season, we studied the effect of thinning severity and crop load on sunburn, other fruit quality parameters and yield. Thinning to three fruit per cluster over the entire tree and two fruit per cluster below and three above 1.8 metres, were the lowest thinning severities, as indicated by the lowest number of thinned fruit. We therefore expected these thinning severities to have the highest number of fruit left on the tree at harvest. Although thinning to two fruit per cluster below and three above 1.8 m was consistent with our expectation, thinning to three fruit per cluster over the entire tree was not, and it did not differ statistically with all the thinning severities for the number of fruit left on the tree at harvest. The possible reason for this anomaly could have been the effects of a wind storm experienced in the Grabouw area prior to the second harvest. Considering that Cripps' Pink is a cultivar with short pedicels, having more fruit in a cluster may also increase fruit drop prior to harvest. The lowest fruit weight observed in both thinning to two fruit per cluster below and three above 1.8 m and three fruit per cluster over the entire tree confirm the existence of high fruit numbers during growth and maturation of fruit on the trees. Thinning to two fruit per cluster below and three above 1.8 m gave a high CDI (which did not differ with thinning to two fruit over the entire tree and one fruit below and two above 1.8 m). Thinning to one fruit per cluster over the entire tree consistently resulted in the lowest number of fruit left on the tree at harvest with correspondingly the lowest CDI.

As expected, thinning to one fruit per cluster resulted in significantly bigger fruit size than thinning to two or three fruit over the entire tree. There, however, were no significant differences in yield between thinning severities. As total yield is determined by the number of fruit borne on a tree and their weight (Jimenez and Diaz, 2004), increased fruit size compensated for low fruit number in our findings. McCartney et al. (1996) confirmed that increased fruit numbers reduce

fruit weight and size and vice-versa. However, differences in fruit size were important in determining percentage first grade fruit as there were no significant differences in sunburn and blush coverage. Thinning to one fruit per cluster, which gave the biggest fruit diameter (although not statistically different from thinning to one fruit per cluster below and two above 1.8 m), correspondingly resulted in the highest percentage of first grade fruit. However, this was not reflected in the actual tonnage of first grade fruit harvested.

Thinning intensity did not have an effect on total sunburn or un-exportable sunburn, although thinning to three fruit per cluster over the entire tree seemed to increase sunburn incidence. In a three-season trial on the effects of crop load on 'Ultra Red Gala' apples, Yuri et al. (2011) also reported no significant effects of crop load on sunburn incidence in all seasons. However, we found that thinning to three fruit per cluster over the entire tree significantly increased the proportion of fruit with photo-oxidative bleaching. Forshey and Elfving (1977) suggested that excess metabolites, that are a result of reduced crop load, are channelled into vegetative growth. This increases the leaf to fruit ratio, thereby improving light attenuation in adverse conditions that otherwise might cause sunburn. In addition, higher crop loads induce bending of branches, which further exposes the fruit to light, rendering fruit more prone to sunburn development, especially sunburn bleaching. Shaded apple peel is very susceptible to photo-oxidative stress and sunburn (Wünsche et al., 2004b), photo-oxidative bleaching in particular (Schrader et al., 2008). Sun exposed fruit are better acclimated to heat and light stress because they have higher levels of anti-oxidants such as ascorbic acid and glutathione and a larger xanthophyll cycle pool size than shaded fruit (Ma and Cheng, 2003; Cheng et al., 2008; Ma et al., 2008). In addition, they have more heat shock proteins, chlorophyll and thicker fruit peel waxes (Ma and Cheng, 2003).

We expected an advance in fruit maturity with a decrease in crop load (Wünsche et al., 2005). Fruit on lightly cropping trees normally have better red colour development, yellower background colour, higher firmness (Palmer et al., 1997; Link, 2000) and higher TSS and advanced starch breakdown (Wünsche et al., 2005). Our observations at different thinning severities (data not presented) and at different fruit numbers per cluster did not confirm this expectation. Fruit firmness, starch breakdown, TSS and TA did not differ significantly between clusters with different fruit numbers. The linear trend in which RWC increased with a decrease in fruit per cluster could have resulted from an increase in sunburn incidence. Racskó et al. (2005) indicated that sunburn damage would result in loss of water in affected tissue. There was also no significant relationship between thinning severity and fruit quality parameters. Such inconsistencies on the effects of crop load on fruit quality have however been previously reported. Opara and Tadesse (2000) did not find clear effects of crop load on TSS in ‘Pacific Rose’, whereas Mpelasoka and Behboudian (2002) found no effects on TA in ‘Braeburn’.

It appears that the effects of fruit thinning on sunburn development in apples are not conclusive. Due to inter fruit shading, increasing the number of fruit per cluster decreases sunburn, as well as the extent and intensity of red colour, among fruit within the cluster. However, sunburn may be increased at high crop loads due to increased exposure of previously shaded fruit as branches bend under the increasing weight of fruit and foliage is reduced due to a decrease in shoot growth. Increased sun exposure at high crop loads may increase red colour development even though carbohydrate limitation is generally believed to decrease anthocyanin synthesis (Awad et al., 2001). At the cluster level, fruit number did not significantly affect fruit size, but at the whole

tree level, fruit size decreased with increasing crop load. The effects of crop load on physical quality attributes such as fruit size and weight seemed to be more consistent than on biochemical parameters such as TSS and TA. Reasons for unclear or inconsistent effects of crop load on fruit quality could be attributed to differences in cultivars, differences in times of thinning or thinning intensity and subsequent crop load with its resultant vegetative growth affecting fruit to leaf ratio.

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Table 1. The effect of fruit number per cluster on fruit quality and sunburn of 'Cripps' Pink' apples at Welgevallen farm during the 2008-09 season.

Treatment (Fruit per cluster)	Firmness (kg)	Starch (%)	TSS (%)	TA (%)	RWC^z (%)
One	9.12 ns	28.2 ns	15.2 ns	0.56 ns	81.03 a ^y
Two	8.77	30.1	15.4	0.54	79.2 b
Three	8.93	30.9	15.7	0.54	79.4 b
Four	8.87	28.3	15.6	0.53	77.9 c
<i>Contrasts</i>					
<i>Treatment</i>	<i>0.4036</i>	<i>0.9496</i>	<i>0.4850</i>	<i>0.3630</i>	<i><0.0001</i>
<i>Linear</i>	<i>0.1759</i>	<i>0.9498</i>	<i>0.1290</i>	<i>0.1112</i>	<i><0.0001</i>
<i>Quadratic</i>	<i>0.3173</i>	<i>0.5657</i>	<i>0.5414</i>	<i>0.8869</i>	<i>0.7602</i>

^z RWC relative water content expressed as percentage of total fresh fruit weight.

^y Means in the same column followed by the same letters are not significantly different at p<0.05.

ns-Not significant.

Table 2. The effect of fruit number per cluster on sunburn and marketable yield parameters of ‘Cripps’ Pink’ apples at Welgevallen farm during the 2008-09 season.

Fruit per cluster	Fruit weight (g)	Fruit size (mm)	Blush intensity^z	Blush coverage (%)	Sunburn severity^y	Sunburn incidence (%)	First grade (%)	Second grade (%)	Third grade (%)
One	100.7 ns	59.5 ns	7.67 a ^x	25.0 a	1.46 a	51 a	7 ns	62 ns	31 ns
Two	99.9	60.9	4.71 b	19.6 ab	1.44 a	43 ab	3	64	33
Three	99.0	60.0	2.09 d	17.7 b	0.81 b	30 bc	1	58	41
Four	99.0	60.6	2.90 c	15.0 b	0.44 b	14 c	0	63	37
<i>Contrasts</i>									
<i>Treatment</i>	<i>0.8219</i>	<i>0.3048</i>	<i><0.0001</i>	<i>0.0122</i>	<i>0.0019</i>	<i>0.0010</i>	<i>0.1051</i>	<i>0.9389</i>	<i>0.6550</i>
<i>Linear</i>	<i>0.3673</i>	<i>0.3658</i>	<i><0.0001</i>	<i>0.0013</i>	<i>0.0002</i>	<i><0.0001</i>	<i>0.0217</i>	<i>0.8983</i>	<i>0.3187</i>
<i>Quadratic</i>	<i>0.8060</i>	<i>0.4789</i>	<i><0.0001</i>	<i>0.5303</i>	<i>0.4321</i>	<i>0.6786</i>	<i>0.3716</i>	<i>0.9397</i>	<i>0.6065</i>

^z A value of 1 denotes the least (green) coloured fruit and 12 the reddest (Pink Lady colour chart).

^y 0-5 score, with 0 having no sunburn and 5 the severest (Schrader and McFerson sunburn chart).

^x Means in the same column followed by the same letters are not significantly different at p<0.05.

ns-Not significant.

Table 3. The per-fruit breakdown of external fruit quality parameters within clusters at Welgevallen farm during the 2008-09 season.

	Red blush coverage (%)	Fruit size (mm)	Red blush intensity^z	Sunburn severity^y	Sunburn Incidence (%)	First grade fruit (%)
One fruit per cluster						
Fruit one	25 ± 2	59.5 ± 0.5	7.7 ± 0.3	1.46 ± 0.3	51 ± 8	7 ± 4
Two fruit per cluster						
Fruit one ^x	25 ± 2	59.9 ± 0.4	4.5 ± 0.3	1.48 ± 0.3	39 ± 8	2 ± 0.4
Fruit two	14 ± 1	61.9 ± 0.5	5.0 ± 0.3	1.38 ± 0.3	47 ± 8	3 ± 0.4
Three fruit per cluster						
Fruit one	24 ± 2	60.4 ± 0.5	3.1 ± 0.3	1.05 ± 0.1	31 ± 7	2 ± 1
Fruit two	17 ± 1	58.9 ± 0.5	1.4 ± 0.2	0.88 ± 0.1	36 ± 8	0.5 ± 0.5
Fruit three	12 ± 1	60.7 ± 0.5	1.6 ± 0.2	0.50 ± 0.1	30 ± 7	0.5 ± 0.5
Four fruit per cluster						
Fruit one	22 ± 2	59.4 ± 0.4	3.4 ± 0.3	0.84 ± 0.1	17 ± 6	0
Fruit two	17 ± 1	61.5 ± 0.5	2.9 ± 0.3	0.62 ± 0.1	20 ± 7	0
Fruit three	13 ± 1	61.8 ± 0.5	2.9 ± 0.3	0.23 ± 0.1	10 ± 5	0
Fruit four	8 ± 0.4	59.7 ± 0.5	2.3 ± 0.2	0.10 ± 0.04	8 ± 4	0

^z a value of 1 denotes the least (green) coloured fruit and 12 the reddest (Pink Lady colour chart).

^y 0-5 score, with 0 having no sunburn and 5 the severest (Schrader and McFerson sunburn chart).

^x In clusters with more than one fruit, fruit were numbered from the greatest blush coverage (reddest) to the lowest (greenest).

Table 4. The effect of crop load (thinning intensity) on yield parameters, fruit size and fruit colour of ‘Cripps’ Pink’ apples at Carica Farm during the 2009-10 season.

Treatment	Thinned fruit	Fruit per tree^z	Crop density index^y	Yield (ton·ha⁻¹)	Fruit weight (g)	Fruit size (mm)	Blush intensity^x	Blush coverage (%)
1 fruit per cluster	412 a ^w	535 c	3.8 c	101 ns	128.4 a	65.6 a	5.7 ns	45 ns
1fruit per cluster below 1.8 m and 2 above	273 b	689 ab	4.7 ab	124	121.1 b	64.4 ab	5.5	42
2 fruit per cluster	145 c	593 bc	4.6 ab	104	118.8 bc	63.9 b	5.5	43
2 fruit per cluster below 1.8 m and 3 above	78 d	724 a	5.2 a	120	111.8 d	62.3 c	5.8	45
3 fruit per cluster	48 d	621 abc	4.2 bc	106	114.9 cd	63.1 bc	5.7	45
<i>Contrasts</i>								
<i>Treatments</i>	<i><0.0001</i>	<i>0.0299</i>	<i>0.0047</i>	<i>0.1872</i>	<i><0.0001</i>	<i>0.0003</i>	<i>0.6011</i>	<i>0.2179</i>
<i>Linear</i>	<i><0.0001</i>	<i>0.0035</i>	<i>0.0022</i>	<i>0.0572</i>	<i>0.0048</i>	<i>0.0003</i>	<i>0.9401</i>	<i>0.2175</i>
<i>Quadratic</i>	<i>0.0008</i>	<i>0.9981</i>	<i>0.0912</i>	<i>0.9808</i>	<i><0.0001</i>	<i>0.9787</i>	<i>0.2583</i>	<i>0.6611</i>

^z Number of fruit left on tree at harvest.

^y Number of fruit per trunk cross sectional area.

^x A value of 1 denotes the least (green) coloured fruit and 12 the reddest (Pink Lady colour chart).

^w Means in the same column followed by the same letters are not significantly different at p<0.05.

ns-Not significant.

Table 5. The effect of crop load (thinning intensity) on sunburn incidence of ‘Cripps’ Pink’ apples at Carica farm during the 2009-10 season.

Treatment	Total sunburn (%)	Un-exportable sunburn^z (%)
1 fruit per cluster	19.3 ns	3.3 ns
1 fruit per cluster below 1.8 m and 2 above	16.3	2.8
2 fruit per cluster	17.9	2.6
2 fruit per cluster below 1.8m and 3 above	21.1	2.7
3 fruit per cluster	24.1	5.1
<i>Contrasts</i>		
<i>Treatments</i>	<i>0.0703</i>	<i>0.2179</i>
<i>Linear</i>	<i>0.6626</i>	<i>0.2175</i>
<i>Quadratic</i>	<i>0.4637</i>	<i>0.6611</i>

^zFruit which scored ≥ 2 on the 0-5 Schrader and McFerson sunburn severity chart.

Table 6. The effect of crop load (thinning intensity) on sunburn type distribution of ‘Cripps’ Pink’ apples at Carica farm during the 2009-10 season.

Treatment	Sunburn^z browning (%)	Sunburn necrosis (%)	Sunburn bleaching (%)	Sunburn browning (ton·ha⁻¹)	Sunburn necrosis (ton·ha⁻¹)	Sunburn bleaching (ton·ha⁻¹)
1 fruit per cluster	93 ns	6 ns	2 a	19 ns	1.2 b	0.4 b
1 fruit per cluster below 1.8 m and 2 above	92	5	3 a	19	1.0 b	0.6 b
2 fruit per cluster	91	7	2 a	17	1.3 b	0.4 b
2 fruit per cluster below 1.8m and 3 above	91	6	3 a	23	1.5 b	0.8 b
3 fruit per cluster	86	8	8 b	23	2.1 a	2.1 a
<i>Contrasts</i>						
<i>Treatments</i>	<i>0.0563</i>	<i>0.6733</i>	<i>0.0418</i>	<i>0.4781</i>	<i>0.0045</i>	<i><0.0001</i>
<i>Linear</i>	<i>0.6198</i>	<i>0.7540</i>	<i>0.7534</i>	<i>0.5910</i>	<i>0.6655</i>	<i>0.1360</i>
<i>Quadratic</i>	<i>0.4400</i>	<i>0.6833</i>	<i>0.3070</i>	<i>0.4651</i>	<i>0.7949</i>	<i>0.0222</i>

^z one fruit could have more than one sunburn type.

Table 7. The effect of crop load (thinning intensity) on fruit grade of ‘Cripps’ Pink’ apples at Carica farm during the 2009-10 season.

Treatment	First grade (Pink Lady™) %	Second grade (Cripps’ Pink) %	Third grade %	First grade (ton·ha⁻¹)	Second grade (ton·ha⁻¹)	Third grade (ton·ha⁻¹)
1 fruit per cluster	46 a ^y	33 ns	21 b	47 ns	34 bc	21 d
1 fruit per cluster below 1.8 m and 2 above	37 b	35	28 ab	46	43 a	35 bc
2 fruit per cluster	40 ab	32	28 ab	42	33 bc	39 ab
2 fruit per cluster below 1.8m and 3 above	35 b	31	34 a	42	37 ab	41 a
3 fruit per cluster	36 b	27	37 a	38	29 c	30 c
<i>Contrasts</i>						
<i>Treatments</i>	<i>0.0114</i>	<i>0.0889</i>	<i>0.0204</i>	<i>0.3040</i>	<i>0.0028</i>	<i><0.0001</i>
<i>Linear</i>	<i>0.0053</i>	<i>0.6754</i>	<i>0.0397</i>	<i>0.6452</i>	<i>0.0291</i>	<i><0.0001</i>
<i>Quadratic</i>	<i>0.4394</i>	<i>0.7940</i>	<i>0.4310</i>	<i>0.4629</i>	<i>0.9496</i>	<i>0.0861</i>

^zThe same fruit might have more than one type of sunburn.

^y Means in the same column followed by the same letters are not significantly different at p<0.05.

ns-Not significant.

SUNBURN MASKING BY ANTHOCYANIN IN APPLE PEEL

Abstract

Red colour development towards harvest by production of anthocyanins in apple peel may conceal superficial blemishes. In addition, anthocyanins may potentially reduce sunburn by affording photoprotection or increase sunburn by increasing the fruit surface temperature (FST). We hypothesized that red colour development masks sunburn in apple peel. For the hypothesis to be true, one would expect sunburn browning and bleaching, the two least severe and superficial forms of sunburn, to increase less in the same fruit in the month before harvest in red and blushed compared to green cultivars. At the same time, sunburn necrosis, which is not masked by anthocyanins, should not differ between red and blushed compared to green cultivars. Hue angle, blush coverage, sunburn incidence and sunburn severity were assessed on the same apples a month before harvest and at harvest in the green cultivars, Granny Smith and Golden Delicious, blushed Royal Gala, Fuji, Braeburn and Cripps' Pink, and the full red cultivar Topred. In all cultivars, sunburn browning was the most predominant sunburn type, followed by sunburn necrosis while sunburn bleaching was almost negligible. Sunburn incidence increased towards harvest in all cultivars and the increases were greater in green than in red and blushed cultivars. Although 'Cripps' Pink' and 'Topred' had the highest sunburn incidence at harvest, they had the lowest increase in sunburn incidence towards harvest. Red colour development evidently masked sunburn browning and sunburn bleaching while the incidence of sunburn necrosis was not affected by anthocyanin accumulation. Sunburn severity a month

before harvest and at harvest did not seem to relate to fruit colour although sunburn severity increased more in green cultivars than in red and blushed cultivars. The lower increase in sunburn browning and sunburn bleaching in red and blushed cultivars in the month before harvest compared to green cultivars could be attributed more to the effect of anthocyanin masking than photo-protection because sunburn necrosis did not seem to be affected by the increase in red colour.

Introduction

The low latitude apple production areas of the Western Cape of South Africa are subject to high summer temperatures and high irradiation levels. These environmental conditions are conducive to the development of sunburn (Andrews and Johnson, 1996; Van den Ende, 1999; Schrader et al., 2003), resulting in the downgrading of fruit and lower income for producers.

Almost all cultivars are susceptible to sunburn, although some are seemingly more sensitive than others. Sensitive cultivars include ‘Granny Smith’ (Nel and Dalton, 1982) and ‘Fuji’ (Parchomchuk and Meheriuk, 1996), while ‘Royal Gala’ is less sensitive (Gindaba and Wand, 2007). Schrader et al. (2001) demonstrated that threshold temperature for sunburn development is cultivar dependent and ranged from 47-51°C. ‘Delicious’ and ‘Jonagold’ had the highest and lowest thresholds respectively, while ‘Gala’, ‘Fuji’ ‘Braeburn’ and ‘Golden Delicious’ were intermediate. This differential susceptibility could be a function of the physiochemical properties of fruit skin (Wünsche et al., 2004), but may also relate to indirect factors that may modulate the occurrence of sunburn, such as the ripening period of the fruit, the tree growth form bearing habit

and the accumulation of anthocyanin in the peel towards harvest, that may mask the visible symptoms of sunburn (Evans, 2004; Schrader et al., 2001).

As anthocyanins mostly accumulate in the epidermal and hypodermal tissue of apple peel (Gross, 1987), red colour development may conceal the two superficial forms of sunburn viz. sunburn browning and photo-oxidative bleaching. These two types of sunburn entail the loss of chlorophyll and accumulation of phenolic compounds and carotenoids (in the case of browning) in the hypodermis (Felicetti and Schrader, 2009a; 2009b; Wünsche et al., 2001). Sunburn necrosis, however, is not hidden by red colour development, as it results in the death of epidermal and sub-epidermal tissue, causing the formation of sunken, brown to black necrotic spots on the affected fruit area (Schrader et al., 2003). The ability of apple fruit to produce anthocyanin during ripening differs among cultivars (Saure, 1990; Curry, 1997). It follows that the more intense the pigmentation, the less severe the sunburn browning and bleaching on an affected fruit is likely to appear.

Although red colour development may mask underlying sunburn symptoms, some sources argue that the accumulation of additional pigment should increase sensitivity to sunburn. Woolf and Ferguson (2000) reasoned that dark pigmented fruit would attain higher temperatures than green pigmented fruit under the same conditions. Anthocyanin accumulation makes the fruit darker, reducing the reflective capacity (albedo) (Schroeder, 1965; Smart and Sinclair, 1976; Evans 2004). The resultant increased radiant heating due to low albedo, increases fruit surface temperature (Smart and Sinclair, 1976). Since high temperature is intimately involved in the development of sunburn (Schrader et al., 2008), increased fruit surface temperatures should

increase the risk of sunburn. In agreement, Hetherington (1997) reported increased sunburn in purple mango cultivars compared to green ones.

By acting as a superficial light screen attenuating irradiation impinging on chloroplasts, anthocyanins have been shown to reduce short-term photo-inhibition (Gould et al., 1995; Hada et al., 2003). Rabinowitch et al. (1983) argued that green pepper and cucumber cultivars are more predisposed to sunburn development compared to red and blushed cultivars, because their fruit peel contains more chlorophyll which plays a role in the photo-oxidative processes of sunburn.

To fully understand the differential sunburn susceptibility between different apple cultivars, a comparative progressive assessment of sunburn development between these cultivars is necessary. The objective of this study was to investigate the effect of red colour development on sunburn incidence and severity in red, blushed and green apple cultivars. We hypothesized that blushed and red cultivars would show less sunburn browning and bleaching and a lower sunburn severity at harvest compared to non-blushed cultivars due to masking by overlaying anthocyanin, although the presence of anthocyanin should not affect the incidence of sunburn necrosis.

Materials and Methods

Plant material

The experiment was carried out at De Rust Estate (34° 10' S, 19° 4' E) in Elgin, South Africa during the 2009-10 season. Seven apple cultivars were used viz. green 'Granny Smith' and 'Golden Delicious', blushed 'Royal Gala', 'Fuji', 'Braeburn' and 'Cripps' Pink', and the full red 'Topred'.

'Royal Gala', 'Granny Smith' and 'Fuji' were all on M793 rootstock. 'Granny Smith' and 'Fuji' were planted in 2000 at a tree spacing of 4 m x 1.5 m, while 'Royal Gala' was planted in 1987 at a tree spacing of 4 m x 2 m. The remaining cultivars were all on seedling rootstock. 'Topred' and 'Golden Delicious' were planted in 1984, whereas 'Cripps' Pink' and 'Braeburn' were planted in 1991 and all at a tree spacing of 4 m x 2 m. 'Fuji', 'Granny Smith' and 'Cripps' Pink' had a North East by South West row orientation, while 'Royal Gala', 'Braeburn', 'Golden Delicious' and 'Topred' had an East to West orientation.

Treatments and experimental design

Thirty-fruit plots replicated four times were used for each cultivar. Fruit that were fully sun-exposed, but showed no prior sunburn were tagged and marked on 12 Jan. 2010 for repeated evaluation.

Measurements

Sunburn incidence and sunburn severity were evaluated a month before harvest and at harvest repeatedly on the same fruit. Sunburn severity was assessed using a 0-5 scale developed by Schrader and McFerson (Schrader et al, 2003), where 0 represented no sunburn and 5, sunburn necrosis. At harvest, sunburn severity was assessed using the same Schrader and McFerson scale and a 'Granny Smith' sunburn evaluation chart (Deciduous Fruit Board Set A33) for all cultivars. Assessments for a month before harvest were made on 12 Jan. ('Royal Gala'), 03 Feb. ('Topred'), 11 Feb. ('Golden Delicious'), 25 Feb. ('Fuji' and 'Braeburn'), 06 Mar. ('Granny Smith'), and 19 Mar. ('Cripps' Pink'). Fruit were harvested on 11 Feb ('Royal Gala'), 06 Mar.

(‘Golden Delicious’ and ‘Topred’), 30 Mar. (‘Fuji’), 31Mar. (‘Braeburn’), 09 Apr. (‘Granny Smith’), and 23 Apr. (‘Cripps’ Pink’).

At harvest, sunburnt fruit were classified according to sunburn category, viz. sunburn browning, sunburn necrosis and photo-oxidative bleaching (Schrader et al., 2003). Each category was expressed as a percentage of the total sunburn. Sunburn incidence was expressed as the proportion of fruit which showed sunburn symptoms out of the total fruit.

Hue angle and blush coverage were used as fruit colour parameters. Blush coverage was assessed visually at harvest, while hue angle was assessed on the reddest position a month before harvest and at harvest, using a portable colorimeter (CR-400, Minolta Co Ltd, Tokyo, Japan).

Results and discussion

Sunburn incidence increased towards harvest and it was higher in green cultivars than in the red and blushed ones (Table 1). The chlorophyll level in the fruit peel decreases during fruit development (Knee, 1972; Gorski and Creasy, 1977). Therefore, the lower chlorophyll levels towards harvest, coupled with longer periods of exposure to high temperatures and high irradiance, rendered the fruit more susceptible to photo-inhibition (Li and Cheng, 2008; Steyn et al, 2002), with subsequent increases in sunburn.

In general, green cultivars showed a greater increase in sunburn incidence in the month before harvest than red and blushed cultivars (Table 1). Sunburn incidence did not differ significantly between the green cultivars Golden Delicious and Granny Smith. However, for the red and

blushed cultivars, Cripps's Pink and Topred had higher sunburn incidence than Fuji, Braeburn and Royal Gala a month before harvest. At harvest, 'Cripps' Pink' and 'Topred' had higher sunburn incidence than 'Braeburn' and 'Royal Gala', while 'Fuji' had the lowest. Similarly, high increases were noted in 'Golden Delicious' and 'Granny Smith' while 'Topred', 'Fuji' and 'Cripps Pink' had the lowest (Table 1).

For red and blushed apple cultivars, the period toward ripening is marked by intense anthocyanin production in the fruit peel (Saure, 1990). Anthocyanins have fruit peel photo-protection abilities as they can modulate the quality and quantity of light before interception by chloroplasts (Steyn et al., 2002). Anthocyanin concentration can be correlated to fruit colour (Steyn, 2009). Although we did not measure anthocyanin concentrations in our study, hue angle values relate to light reflection (Steyn et al., 2009) and hence provide an indication of anthocyanin concentrations. Red and blushed cultivars showed a considerable decrease in hue angle and an increase in blush coverage towards harvest due to anthocyanin accumulation (Table 2). 'Topred', 'Cripps' Pink' and 'Fuji' had the greatest reduction in hue angle towards harvest. Consistent with this, these three cultivars had the lowest increase in sunburn incidence during the last month of maturation. This seemed to imply that the increase in anthocyanins offered photo-protection (Pietrini and Massacci, 1998) or masked the symptoms of sunburn (Schrader et al., 2001).

In our findings, we noted that red colour development towards harvest masked sunburn browning (Table 1, Figure 1). Contreras et al. (2008) also acknowledged the difficulty of detecting less severe sunburn at harvest in blushed apple cultivars, while red colour disqualifies background colour in maturity indexing of peaches and nectarines (Crisoto, 1994). When present, the severity

of sunburn a month prior to harvest and at harvest did not seem to relate to fruit colour. However, towards harvest, sunburn increased more in severity in green compared to red and blushed cultivars (Table 2). In all cultivars, sunburn browning was the most predominant sunburn type, followed by sunburn necrosis. Sunburn bleaching was almost negligible (Table 1). Symptoms of sunburn necrosis, however, were clearly visible (Fig. 1) and the incidence of necrosis did not seem to relate to fruit colour (Table 1). It is therefore unlikely that the reduction in sunburn severity and incidence towards fruit maturity was due to photo-protection offered by anthocyanins. Although changes in hue angle gave us an indication of anthocyanin accumulation during fruit development in our studies, it appears that for effective photo-protection by anthocyanins, threshold concentrations have to be present in the fruit peel. Merzlyak and Chivkunova (2000) observed little to no photo-destruction of chlorophyll in apples which had anthocyanin concentrations of up to 50 nmol cm^{-2} after illuminating them with photosynthetically active radiation (PAR) of $11,425 \text{ } \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Those with lower anthocyanin concentrations were bleached within 100 min. Therefore in our study, as changes in hue angle of red and blushed cultivars were consistent only with reduced changes in sunburn browning and sunburn bleaching rather than sunburn necrosis, anthocyanins seemed only to be effective in masking superficial symptoms.

Sunburn severity in 'Fuji' and 'Cripps' Pink', which had low sunburn incidence, did not differ significantly from 'Braeburn', which had the highest sunburn browning severity at harvest (Table 2). If the less severe sunburn is masked, the remaining visible sunburn that is averaged would be more severe, contributing to higher sunburn severity. This could explain why blushed cultivars such as Fuji and Cripps' Pink had a lower sunburn incidence than the non-blushed

cultivars, but did not differ significantly from them with regards to sunburn severity. For the non-blushed cultivars, sunburn symptoms are observed against a pale background. This is mostly chlorophyll, the actual pigment acted upon in the photo-oxidative processes of sunburn (Rabinowitch et al., 1983). Therefore the slightest sunburn development is easily noticeable and this amounts to high sunburn incidence among these fruit.

Using the Schrader and McPerson chart for sunburn severity assessment, sunburn browning and bleaching for all cultivars did not seem to differ. However, using the rigorous ‘Granny Smith’ chart with a wider scale and more subtle differences between grades, sunburn severity became amplified. Sunburn browning and bleaching generally were not easy to distinguish into discrete grades for red and blushed cultivars. To become easily noticeable, sunburn symptoms have to become necrotic in blushed cultivars. ‘Braeburn’ and ‘Cripps’ Pink’ had significantly higher sunburn browning and bleaching severity than ‘Royal Gala’ and ‘Topred’. ‘Fuji’ did not differ significantly with either of the two extremes (Table 2). For the non-blushed cultivars, ‘Granny Smith’ had higher sunburn severity than ‘Golden Delicious’. In ‘Golden Delicious’, sunburn browning and bleaching severity could also have been lessened by attainment of a yellow colour during ripening due to chlorophyll degradation with concomitant carotenoid synthesis (Knee, 1971; Gorski and Creasy 1977). Therefore, apart from differences in red colour development in the fruit peel, our findings also seem to suggest the importance of differential sunburn susceptibility among cultivars which could be a function of other inherent physiochemical properties of fruit skin (Wünsche et al., 2004).

In conclusion, red colour masked sunburn browning and sunburn bleaching, reducing the increase in sunburn severity and sunburn incidence in blushed cultivars compared to non-blushed ones. Since sunburn browning was the most predominant form, manipulating factors that enhance red colour development can substantially reduce the proportion of fruit culled due to sunburn in blushed apple cultivars. In addition, results on the differential sensitivity of cultivars to sunburn should be interpreted with caution since the visible presence of sunburn does not necessarily relate to the actual sensitivity.

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Table 1. Sunburn incidence and sunburn type distribution for different apple cultivars at De Rust Estate during the 2009-10 season. Values are means \pm SE.

Cultivar	Sunburn incidence^z (%)	Sunburn incidence^y (%)	Change in sunburn incidence (%)	Sunburn browning^y (%)	Sunburn necrosis^y (%)	Sunburn bleaching^y (%)
<i>Green cultivars</i>						
G. Delicious	46 \pm 7.4	69 \pm 5.9	23 \pm 5.2	95 \pm 5 (66) ^x	0 (0)	5 \pm 5 (4)
G. Smith	38 \pm 2.7	57 \pm 6.7	19 \pm 8.7	70 \pm 5 (40)	24 \pm 8 (14)	7 \pm 7 (3)
Mean	42 \pm 4	63 \pm 6	21 \pm 2	83 \pm 13 (53)	13 \pm 12 (7)	6 \pm 1 (4)
<i>Blushed/Red cultivars</i>						
Cripps' Pink	40 \pm 5	46 \pm 6.7	6 \pm 2.1	82 \pm 7 (38)	13 \pm 5 (6)	8 \pm 5 (4)
Royal Gala	15 \pm 2.8	26 \pm 3.2	11 \pm 2.9	63 \pm 5 (16)	29 \pm 10 (8)	13 \pm 10 (3)
Braeburn	18 \pm 7.5	29 \pm 5.8	11 \pm 3.9	84 \pm 6 (24)	15 \pm 5 (4)	3 \pm 3 (1)
Fuji	13 \pm 3.7	18 \pm 5.0	5 \pm 2.4	87 \pm 8 (16)	21 \pm 8 (4)	0 (0)
Topred	34 \pm 6.4	38 \pm 4.1	4 \pm 3.1	89 \pm 7 (34)	11 \pm 7 (4)	0 (0)
Mean	24 \pm 5	31 \pm 5	7 \pm 2	81 \pm 5 (26)	18 \pm 3 (5)	5 \pm 3 (2)

^zMeasurements taken a month before harvest (Schrader and McFerson scale).

^y Measurements taken at harvest (Schrader and McFerson scale).

^x Proportion of fruit showing a particular sunburn type out of all tagged fruit at harvest.

Table 2. Development of red colour and sunburn severity during the last month before harvest and at harvest for different apple cultivars at De Rust Estate during the 2009-10 season. Values are means \pm SE.

Cultivar	Hue angle (°)		Change in hue angle (°)	Blush coverage (%)	Sunburn severity ^x		Change in severity	G. Smith ^w chart
	Before harvest	At harvest			Before harvest	At harvest		
<i>Green cultivars</i>								
G. Smith	116 \pm 0.2	116 \pm 0.2	0	-	1.1 \pm 0.2	2.6 \pm 0.4	1.5 \pm 0.3	4.9 \pm 0.3
G. Delicious	115 \pm 0.2	112 \pm 0.2	3 \pm 0.2	-	0.4 \pm 0.1	2.0 \pm 0.1	1.6 \pm 0.1	3.2 \pm 0.2
Mean	116 \pm 0.5	114 \pm 2	2 \pm 2		0.8 \pm 0.4	2.3 \pm 0.3	1.6 \pm 0.1	4.1 \pm 2.0
<i>Blushed/Red cultivars</i>								
R. Gala	69 \pm 2.8	48 \pm 3.4	21 \pm 4	29 \pm 2	0.7 \pm 0.2	1.7 \pm 0.1	1.0 \pm 0.2	4.1 \pm 0.2
C. Pink	78 \pm 0.9	36 \pm 2.1	42 \pm 2	35 \pm 2	1.3 \pm 0.1	2.3 \pm 0.2	1.0 \pm 0.1	4.6 \pm 0.2
Topred	81 \pm 1.7	34 \pm 2.0	47 \pm 2	100	1.3 \pm 0.2	1.9 \pm 0.1	0.6 \pm 0.2	4.0 \pm 0.2
Fuji	82 \pm 2.3	52 \pm 4.5	30 \pm 4	26 \pm 2	1.0 \pm 0.2	2.1 \pm 0.3	1.1 \pm 0.3	4.5 \pm 0.3
Braeburn	80 \pm 1.4	59 \pm 1.1	21 \pm 1	38 \pm 1	0.7 \pm 0.2	2.5 \pm 0.2	1.8 \pm 0.2	4.7 \pm 0.3
Mean	78 \pm 2	46 \pm 5	32 \pm 5	46 \pm 13	1.0 \pm 0.1	2.1 \pm 0.1	1.1 \pm 0.2	4.4 \pm 0.1

^x 0-4 sunburn browning and bleaching score, with 0 having no sunburn and 4 being the severest form of that type of sunburn.

^w 1-8 score, with 1 having the least sunburn.



Figure 1. The effect of red colour development on sunburn symptoms on 'Braeburn' apples at De Rust Estate during the 2009-10 season. A1 =fruit 1 a month and half before harvest and A2=fruit 2 a month and half before harvest. B1=fruit 1 at harvest and B2=fruit 2 at harvest.

GENERAL DISCUSSION AND CONCLUSIONS

Plants performing sub-optimally, especially those under moisture stress, are susceptible to sunburn (Schrader et al., 2003). With global warming, water will become more scarce and expensive. Part of this study focused on relating plant water status and cultural practices that maintain high water potential to photo-thermal injury and subsequent sunburn development.

We were able to confirm that an increase in water stress linearly increased sunburn incidence and severity. Moisture stress increased the proportion of sunburn necrosis, the most severe form of sunburn, while sunburn browning was more prevalent under lower moisture stress conditions. The differences in the sunburn type distribution were undoubtedly a function of differences in fruit surface temperature (FST) as affected by plant water status. Not much is known on the fruit surface characteristics with regards to evaporation and the extent to which this evaporation cools the fruit down. We speculate that under moisture stress, just as in the leaves, the low water potential in the fruit peel reduces the vapour pressure deficit with the air, minimizing evaporative water loss through the lenticels. Consequently, fruit under moisture stress would have less evaporative cooling and attain higher FST resulting in a higher incidence and greater severity of sunburn.

Cultural practices that optimize water uptake, consistently maintaining high plant water status, may increase profitability by reducing sunburn culling. To this end, we expected cultural practices such as mulching to considerably conserve soil moisture compared to a no mulch control, by reducing soil surface evaporation (Himelick and Watson, 1990), thereby maintaining a favourable plant water status, which in turn reduces sunburn. We tested this theory in sandy

loam (light loam) and heavy silt loam (heavy loam) orchards. Mulching generally decreased sunburn incidence and sunburn severity compared to the no mulch control, with vermicompost being more consistent than plant compost, geotextile and woodchips. However, leaf and stem water potential did not differ between treatments over the two seasons. In this case, the reduction in sunburn due to mulching was therefore not directly related to tree water status, and could have been a result of possible photochemical enhancement. Leaf stomatal conductance, transpiration and net carbon assimilation were generally higher in mulching treatments with vermicompost being consistent over the two seasons while woodchips, plant compost and the geotextile gave varied responses. The key fruit anatomical features such as stomata and lenticels involved in gas exchange of photochemistry are also involved in evaporative cooling. Enhanced photochemistry through higher stomatal conductance would mean faster gas exchange and possibly a higher rate of transpiration. The high rate of transpiration would cool the fruit down, resulting in a lower FST that we generally observed in mulching treatments. The increase in photosynthesis as a result of mulching could also have been brought about through stabilizing soil diurnal and seasonal temperatures (Stratton and Rechcigl, 1998). Stable temperatures in the upper soil layer promote root development, improve nutrient uptake (Kasper and Bland, 1992), accelerate gas exchange (Boone et al., 1998) and increase plant growth (Haynes, 1980). It is, however, important to have a clearer understanding of the direct physiological relationship between photochemistry and evaporative water loss which results in cooling of the FST.

Apart from the environmental conditions which raise the FST, the extent of sunburn development in different cultivars can be a function of inherent cultivar physiochemical properties of fruit peel (Wünsche et al., 2004). One important aspect of the apple fruit peel with

regards to sunburn development is the ability to produce red colour (anthocyanins) towards harvest. We investigated whether anthocyanins can possibly mask superficial sunburn, such as browning and bleaching in full red 'Topred' and blushed 'Royal Gala', 'Fuji', 'Braeburn' and 'Cripps' Pink' compared to green, 'Granny Smith' and 'Golden Delicious'. In other fruits, red or any other dark colour overlay has been found to make it difficult to assess superficial defects visually (Arpaia et al., 1987). Although, anthocyanins may potentially reduce sunburn through their photo-protective capability, in some cases, the dark anthocyanin pigmentation might reduce the reflective capacity (albedo) of the fruit (Schroeder, 1965; Smart and Sinclair, 1976; Evans 2004), thereby aggravating sunburn. In our study, the increase in sunburn browning and sunburn bleaching in the month before harvest was lower in red and blushed cultivars compared to green ones. Since sunburn necrosis, which results in death of epidermal and sub-epidermal tissue, was not affected by anthocyanins, the reduction in sunburn browning and sunburn bleaching can therefore be attributed to masking of the less severe symptoms by the anthocyanins.

Evans (2004) argued that apples without visible sunburn symptoms, due to masking by red colour, easily suffer internal damage in storage. As sunburnt fruit have always been associated with high total soluble solids (TSS) concentration, low titratable acidity (TA) and a low relative water content (RWC), it may be possible to use non-destructive near infrared determination of TSS and RWC to identify sunburnt fruit as proposed by Jordan et al. (2000) for kiwifruit and successfully used post-storage for 'Royal Gala' by McGlone et al. (2002).

There is a perception in the South African apple industry that the textural and chemical characteristics consistently associated with sunburn, especially the high TSS, could actually be

predisposing apples to sunburn rather than resulting from sunburn development. The perception stems from the fact that towards ripening, TSS increases, while fruit also become more susceptible to sunburn development. We compared chemical and textural characteristic of exposed, non-burned fruit (control) with that of fruit with naturally occurring sunburn and fruit where we induced sunburn by raising the FST. The induced sunburn and sunburn treatments had firmer fruit with lower TA and RWC, and higher TSS concentration than control fruit. This implied that the sunburn induction treatment changed the chemical and textural attributes in the flesh to resemble the fruit that developed sunburn without induction. Hence, the environmental conditions the fruit is subjected to, clearly plays a vital role in determining the chemical and textural characteristics of fruit flesh. Therefore, the chemical and textural characteristics usually associated with sunburn are not inherent in the fruit and are not the cause of sunburn development.

The effect of light exposure history on heat stress acclimation was also investigated. Stress tolerance was determined by measurements of maximum quantum efficiency of photosystem II (F_v/F_m) at various temperatures (30 °C, 35 °C, 40 °C, 45°C and 50 °C). Temperatures of 40 °C and higher were more detrimental to the fruit peel's photosynthetic system while the sun exposed fruit sides were slightly more acclimated to sustain heat stress than the shaded sides. The sun exposed sides had a higher F_v/F_m after a four hour recovery from heat stress although the residual photo-inhibition did not differ significantly. Indications of acclimation to high heat load for the sun exposed sides are evident even at the cellular level, where activities of irradiance protective enzymes (such as catalase, ascorbate peroxidase and superoxide dismutase) are higher (Chen and Cheng, 2007; Ma and Cheng, 2003). It might be beneficial, for natural resistance to sunburn

development, that the fruit undergoes a certain extent of sun hardening or acclimatgion, which confers sunburn tolerance via increased levels of antioxidants and heat shock proteins.

Comparing the sun exposed and shaded fruit sides indicated that the sun exposed sides had firmer flesh, higher TSS, lower TA and lower RWC than the shaded sides. Although both fruit sides had no sunburn, the sun exposed fruit side deals with a greater heat load than the shaded side. This further confirms our findings on the role the environment plays in modifying fruit chemical and textural characteristics. Therefore, changes in the heat exchange capacity of the fruit, changes in photochemistry, increasing temperatures during the growing season and changes in light exposure due to bending of branches can be directly be related to sunburn development and subsequent changes in chemical and textural characteristics. However, our research did not address the issue of whether the increase in sunburn susceptibility towards harvest is related to changes in fruit composition and texture. To test this hypothesis would be very difficult.

The effect of factors such as crop load on sunburn is not well understood. However, crop load is an important factor in fruit quality determination of apples (Stopar et al., 2002). We investigated the effect fruit number per cluster as well as crop load on a whole tree basis on sunburn development while not disregarding the effect of fruit number and crop load on other fruit quality parameters that determine marketable yield. Sunburn incidence, sunburn severity, blush coverage and blush intensity decreased with an increase in the number of fruit (from 1 to 4) per cluster, due to an increase in inter-fruit shading, with an increase in fruit number per cluster. Fruit size parameters were not affected as these are more related to leaf area per fruit of the whole tree (Palmer et al., 1991), rather than different fruit cluster numbers on the same tree.

Other fruit quality parameters such as TSS, TA and flesh firmness were also not affected. In the following season, when thinning severities were conducted on a whole tree basis, thinning to one fruit per cluster resulted in significantly bigger fruit size than thinning to two or three fruit over the entire tree, although there were no significant differences in yield. Flesh firmness, TSS, TA and fruit colour were not affected.

There were no significant differences in sunburn incidence and neither were there differences in proportions of fruit with marketable sunburn browning between the different crop loads. However, the lowest thinning severity, thinning to three fruit per cluster over the entire tree, resulted in higher sunburn necrosis and sunburn bleaching compared to other thinning severities. This could be attributed to increased exposure of previously shaded fruit to the sun as branches bend under the increasing weight of fruit. In addition, the leaf to fruit ratio might be reduced due to a decrease in shoot growth. The proportion of first grade fruit was greatest when fruit were thinned to one fruit per cluster, but there were, however, no significant differences in the actual tonnage harvested. On the other hand, the proportion of first grade fruit at cluster level decreased with an increase in number of fruit per cluster. This was a function of blush coverage decreasing with decreasing light at the cluster level while at the tree level where fruit colour did not differ between thinning treatments, fruit size seemed to be important in determining the proportion of first grade fruit. It appears that conclusions on the effect of crop load on apples are difficult to draw because inter-fruit shading within a cluster would interact to various degrees with factors that relate to the whole tree such as leaf to fruit ratio and branch movement.

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