PHOTO-THERMAL STUDIES IN JAPANESE PLUMS

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

Heat waves, occurring towards or during the harvesting window of Japanese plum cultivars, hamper production of premium quality plums in the Western Cape Province of South Africa by causing sunburn in the presence of high irradiance. In addition, high respiration rates initiated by high temperatures are thought to deplete internal fruit oxygen and trigger anaerobic respiration with subsequent accumulation of ethanol, resulting in internal damage. Damage that is not apparent at harvest can manifest during cold storage. No information is available on temperature thresholds for thermal damage of the peel and flesh of Japanese plums. In apples, maintaining high stem water potential (SWP) and applying shade netting were reported to alleviate sunburn. Summer pruning is a common practise in Japanese plums, but the timing may affect fruit quality and sunburn incidence. Research in this regard as it pertains to plums is lacking. The main objective of this study was to fill this information gap.

'African Delight' plums from exposed, upper canopy positions were larger, advanced in maturity but more susceptible to sunburn. Delaying summer pruning predisposed fruit to sunburn and did not enhance fruit quality. Early summer pruning decreased sunburn, increased fruit size, red colour and total soluble solids (TSS). Abstaining from pruning reduced sunburn but decreased overall fruit quality. Fruit that developed sunburn received >50% photosynthetic photon flux (PPF) of full sun on average while average fruit surface temperature (FST) exceeded 35 °C. Shade net during the hottest part of the season attenuated PPF, and subsequently, decreased FST and sunburn. Deficit irrigation late in the season elevated canopy temperature, FST and sunburn in 'African Delight' and 'Laetitia' while SWP, flesh firmness, TSS and gas exchange decreased. The increased heat load could be attributed to diminished evaporative cooling as a result of reduced transpiration. Excessive irrigation did not lower FST and sunburn compared to the control.

There were no notable heat waves during the 2012/13 season so in subsequent seasons we assessed fruit respiration rate under simulated heat wave conditions at different fruit maturities in the laboratory. Increases in ethanol at harvest and internal damage after cold storage were higher in more mature fruit treated at 30 °C and 40 °C but tended to decline at 45 °C in 'Laetitia' due to curing. In 'Fortune', more mature fruit were consistently more susceptible to internal heat damage. No symptoms of internal heat damage were observed in 'African Delight' possibly due to this cultivar's high peel permeability that prevented accumulation of threshold ethanol levels.

In conclusion, plum producers should adopt early summer pruning practices and incorporate shade

nets to reduce sunburn. However, the potential of shade nets and potential negative effects on reproductive development requires further evaluation over the entire growing season. Low SWP increases FST and sunburn possibly due to canopy heating and loss of convectional cooling, explaining why excessive irrigation did not reduce sunburn. High temperature treatments can potentially be used for curing against cold storage enhanced heat damaged if used with methods that circumvent external peel damage.

OPSOMMING

Hittegolwe tesame met hoë vlakke van irradiasie kort voor of gedurende die plukvenster van Japanese pruimkultivars veroorsaak sonbrand en belemmer daardeur produksie van premium kwaliteit pruime in die Wes-Kaap Provinsie van Suid-Afrika. Verder word geglo dat hoë temperature interne suurstof in die vrug kan uitput deur respirasie te versnel. Lae interne suurstofvlakke kan anaerobiese respirasie aktiveer met gevolglike akkumulasie van etanol en gepaardgaande interne skade. Skade mag moontlik eers na koue opberging manifesteer. Die drempeltemperature vir skade aan die skil en vleis van Japanese pruime is onbekend. In appels is gerapporteer dat deurlopende hoë stamwaterpotensiale (SWP) en aanbring van skadunette sonbrand kan verminder. Somersnoei is 'n algemene praktyk in Japanese pruime, maar die tydsberekening daarvan kan vrugkwaliteit en die voorkoms van sonbrand affekteer. Navorsing oor bogenoemde aspekte makeer vir pruime en die hoofdoelwit van hierdie studie was daarom om die kennisgebrek aan te spreek.

'African Delight' pruime van blootgestelde posisies aan die bokant van die blaredak was groter en meer ryp, maar meer onderhewig aan sonbrand. Die uitstel van somersnoei het vrugte meer vatbaar gemaak vir sonbrand sonder om vrugkwaliteit te verbeter. Vroeë somersnoei het sonbrand verminder asook vruggrootte, rooi kleur en totale oplosbare vaste stowwe (TOVS) verhoog. Geen somersnoei het sonbrand verminder, maar het algemene vrugkwaliteit verlaag. Vrugte wat sonbrand ontwikkel het, was blootgestel aan gemiddeld >50% fotosintetiese fotonvloei (PPF) van vol sonlig terwyl hul gemiddelde vrugoppervlaktemperatuur (FST) 35 ºC oorskry het. Die aanbring van skadunet gedurende die warmste deel van die seisoen het PPF verminder en gevolglik FST en sonbrand verminder. Tekort besproeiing laat in die seisoen het blaredak temperature, FST en sonbrand in 'African Delight' en 'Laetitia' verhoog terwyl SWP, vleisfermheid en gaswisseling verlaag is. Die verhoogde hittelading kon toegeskryf word aan verminderde evaporatiewe verkoeling as gevolg van die verlaagde transpirasie. Oormatige besproeiing het nie FST verlaag of sonbrand verminder nie.

Daar was geen noemenswaardige hittegolwe gedurende die 2012/13 seisoen nie en daarom is vrugrespirasie by verskillende vrugryphede in daaropvolgende seisoene onder gesimuleerde hittegolf kondisies in die laboratorium ondersoek. Toenames in etanol by oestyd en interne skade na koue opberging was hoër in meer volwasse vrugte wat blootgestel was aan 30 ºC en 40 ºC maar het afgeneem by 45 ºC in 'Laetitia' vanweë kruisbeskerming teen koue deur die hitteblootstelling (*curing*). Meer volwasse 'Fortune' vrugte was deurlopend meer vatbaar vir interne hitteskade. Geen interne hitteskade simptome is in 'African Delight' waargeneem nie, moontlik vanweë die hoë

permeabiliteit van hierdie kultivar se skil wat akkumulasie van drempelvlakke etanol voorkom.

Ten slotte kan aanbeveel word dat pruimprodusente vroeë somersnoei toepas en van skadunette gebruik maak om sonbrand te verminder. Die potensiaal van skadunette en moontlike negatiewe effekte op reproduktiewe ontwikkeling benodig egter verdere evaluasie oor die hele groeiseisoen. Lae SWP verhoog FST en sonbrand moontlik deur opbou van hitte in die blaredak en verminderde konveksie verkoeling. Dit verklaar hoekom oormatige besproeiing nie sonbrand verminder het nie. Hittebehandeling kan moontlik gebruik word om vrugte te beskerm teen interne hitteskade wat tydens koue opberging te voorskyn kom indien eksterne skilskade voorkom kan word.

PUBLICATIONS AND CONFERENCE PRESENTATIONS FROM THIS DISSERTATION

Peer reviewed publication

Makeredza, B., M. Jooste., E. Lötze., M. Schmeisser and W. J. Steyn. 2016. Canopy factors influencing sunburn and fruit quality of Japanese plums (*Prunus salicina* Lindl.). 2016. Acta Hort. 1228: 121-128.https//doi 10.17660/ActaHortic.2018.1228.18

Poster presentation

Makeredza, B., M. Jooste., E. Lötze., M. Schmeisser and W. J. Steyn. 2016. Canopy factors influencing sunburn and fruit quality of Japanese plums (*Prunus salicina* Lindl.). 2016. Proceedings of the XI International Society for Horticultural Society Symposium on Integrating Canopy, Rootstocks and Environmental Physiology in Orchard Systems, Aug 2016, Bologna, Italy. (Poster).

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DEDICATIONS

This thesis is dedicated to my daughter Skylar

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This dissertation was written according to the language and style specifications of the *Journal of the American Society for Horticultural Science*. Each chapter represents an individual paper and therefore some repetition between chapters was inevitable.

GENERAL INTRODUCTION AND OBJECTIVES

Japanese plums (*Prunus salicina* Lindl.) are deciduous fruit of the *Rosacea* family that are produced for fresh consumption. They are reported to be native to China (Jensen, 1988; Byrne et al., 2000), but were extensively developed in Japan before being introduced to the rest of the world. Through breeding efforts, Japanese plums have been adapted to a range of soil and climatic conditions, enabling them to be cultivated in many subtropical to temperate regions of the world (Byrne et al., 2000).

The annual world plum production is approximately 11.8 million tonnes. With over 6.7 million tonnes, China is the biggest producer, holding over 50% of the world market. The US and Romania follow producing just over 400 thousand tonnes each while Serbia and Chile produce around 300 thousand tonnes (FAOSTAT, 2019). The UK and Germany are the biggest importers of fresh plums, supplied by China, Spain, USA and South Africa.

In the southern hemisphere, South Africa is the third largest producer of plums after Chile and Argentina, producing over 75 thousand tonnes of Japanese plums annually (FAOSTAT, 2019; HORTGRO, 2015). The south western parts of the Western Cape Province with its Mediterranean-type climate are the major Japanese plum growing area of South Africa. Production is earmarked for the export market to European countries that pay premium prices during their winter.

To meet the consumer and export market quality expectations, it is important to harvest within the optimum harvest window of each particular cultivar. The plums are therefore harvested mature enough to ripen in transit to the distant market (Jooste, 2012). Low temperature storage is the most effective way to delay postharvest ripening and deterioration of plums, and to schedule ripening according to marketing needs. However, various factors such as climate and seasonal variance can affect the ultimate quality of fruit long before harvest (Kays, 1999).

The major plum production region in the Western Cape Province falls within 33-34°S latitude. Being of a Mediterranean-type, the climate is characterised by high irradiance and high summer temperatures with heat waves a common occurrence during the maturation period of some of the most important cultivars (De Kock, 2015). Weather conditions of high temperature exceeding 35 °C and persisting for about three days or more are considered as heat waves. The heat waves are more prominent in January and February, the hottest part of the season (De Kock, 2015: Jooste, 2012) during which 60% of the total plums produced are harvested and processed for export (HORTGRO, 2017).

A wide range of fruit subjected to high irradiance and high temperatures exhibit external defect symptoms of photo-thermal damage known as sunburn (Kossuth and Biggs, 1978; Wade et al., 1993; Schrader et al., 2001). In plums, sunburn appears as a brown to yellow discolouration on the fruit surface. Severe cases result in necrotic patches and cracking of the fruit peel. Thermal stress that is not apparent at harvest can manifest in cold storage as internal damage in plums (De Kock, 2012). The damage can either manifest as pitburn or gel breakdown. Pitburn appears as a dark brown discolouration of the fruit mesocarp, and is more prominent around the pit (Amiot et al., 1997). Symptoms of gel breakdown may initially appear as a gelatinous breakdown in the mesocarp flesh around the pit which develops a dark discolouration over time (Candan et al., 2008). The symptoms are often observed when fruit is moved to shelf life conditions after cold storage.

It is speculated that the high temperatures result in an increase in respiration rate, depleting internal fruit flesh oxygen while increasing carbon dioxide (Cheng et al., 1998). This causes anaerobic respiration with subsequent internal development of heat damage in the fruit. The magnitude or extent of anaerobic respiration would be related to the amount of ethanol evolved from the fruit sap.

High losses in plums have been reported due to both pre-harvest related and externally appearing sunburn, and cold storage manifesting internal heat damage in the Western Cape Province (Kapp and Jooste, 2006). In 2011, loses due to internal heat damage were in the order of R10 million for the 'Fortune' plum cultivar. Thus it is very important to investigate how high pre-harvest temperature stress affects fruit quality at harvest, determine its post-harvest implications, and the physiological changes associated with the heat stress.

The major pre-harvest factors affecting interception of irradiance by the fruit are canopy size, training or trellising system and row orientation with respect to the position of the sun (Jackson, 1980). Outer canopy fruit are usually exposed to photosynthetic photon flux (PPF) higher than 2000 µmol m^{-2} s^{-1}, which steeply decreases within the canopy and can be lower than 20 µmol m^{-2} s^{-1} for shaded innermost fruit of the canopy (Ördög and Molnar, 2011). As a result, temperature disparities greater than 20°C between exposed and shaded fruit have been reported (Corelli-Grappadelli, 2003).

Production practices such as summer pruning, if done properly, can improve light penetration into the canopy (Rom, 1991) while giving adequate shading against radiant heating of the fruit. In addition, shade nets can be used to attenuate incoming radiation on exposed fruit. In South Africa, Smit (2007) reported a reduction in sunburn on 'Fuji' and 'Braeburn' apples using a 20% black

shade net. To our knowledge, there has not been any previous research on the use of nets to control sunburn in Japanese plums even though nets are considered one of the best strategies in apples.

While working with apples, Wünsche et al. (2001) concluded that sunburn severity is a function of cultivar, growing area and orchard management practices. Tree water management is one of the most important orchard practices that have an effect on fruit surface temperature (Makeredza, 2013). A decrease in plant water potential is associated with decreased rate of transpiration (Álvarez et al., 2011). Decreased transpiration might increase canopy temperature and subsequently fruit surface temperature through reduced convectional heat loss to the environment (Colaizzi et al., 2012). While fruit transpiration towards harvest is negligible in fruit such as apple (Lang 1990), it is considerable in stone fruit such as peaches (Morandi et al., 2010). Therefore in peaches, heat loss from the fruit surface is greatly affected by rate of transpiration. While we are not aware of the extent to which transpiration is important in plum fruit, we speculate that water deficit might predispose fruit to sunburn and all heat induced quality disorders. Mupambi (2017) indicated that water deficit impairs ability of fruit peel to cope with photo-thermal stress in apples.

The objectives of this study were divided and addressed in three Chapters. In Chapter 1, the objectives were to investigate the role of pre-harvest climatic factors, particularly as they interacted with the tree canopy, in affecting general fruit quality and manifestation of external and internal damage in Japanese plums. The relevant climatic conditions were temperature and light. For a clearer understanding of the effects of these factors, orchard light manipulation through summer pruning and shade net incorporation were studied at different tree canopy positions (lower, mid and upper) and row side.

Experiments in Chapter 2 were inspired by previous studies in apples. These indicated that plant water status is important in photo-thermal tolerance. We therefore set out to have an understanding of the relationship and underlying physiology of photo-thermal damage in Japanese plums in relation to plant water status, especially under water limitation. The Western Cape Province constantly experiences drought and water has become a scarce resource (Western Cape Government, 2018). The generated data in this study would therefore be valuable as it would give indications of how water restrictions might impact on plum quality. In addition, the effects of irrigation in excess of normal farmer practice on fruit thermo-tolerance were also investigated.

The objectives of experiments in Chapter 3 focussed on biochemical physiology at the fruit level in relation to high pre-storage temperatures that prevail just before or during harvesting. Exposure to the high temperatures may initiate internal heat damage, which may be more prominent during cold

storage. However, manifestation of internal heat damage symptoms can be unpredictable, particularly if fairly mild weather conditions prevail with no notable heat waves. That was the case in the 2012/13 growing season, and no internal heat damage was observed. In subsequent seasons, temperature treatments were administered under simulated conditions in growth chambers.

Biochemical and physiological aspects studied with respect to the disorders and fruit quality were ethylene evolution and respiration rate, ethanol evolution, and anti-oxidant capacity (glutathione and ascorbic acid concentrations). These were investigated at both early and advanced harvest maturities for the susceptible cultivars Laetitia and Fortune. This is due to the fact that the susceptibility of plums to cold storage induced internal heat damage seems to increase with advanced maturity.

The broader perspective and overall objective of this study was to fill the knowledge gap with regards to external and internal heat damage in plums, drawing from findings on apple research. Not much research and publications are available on plums. Our literature review therefore inevitably relied heavily on apple research and covered a broad area due to the nature of the wide ranging aspects that we addressed in this study.

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LITERATURE REVIEW

Introduction

The Western Cape Province is the major producer of deciduous fruit in South Africa. Japanese plums (*Prunus salicina* Lindl.) are among some of the deciduous fruit cultivated in the province. Although they constitute about 6% of the total land cultivated to fruit in the province (HORTGRO, 2017), production is steadily increasing, now attaining over 80 000 tonnes annually (DAFF, 2015). Production targets EU fresh consumption markets, as well as Russia, USA and parts of Asia. As such, the South African plum industry invested in a consumer awareness campaign of South African plums targeting all these export markets (NAMC, 2014). This has seen the rise of plum production in the country by 26% from 2009 to 2013. The production and market distribution of plums in this growth period is illustrated in Figure 1.

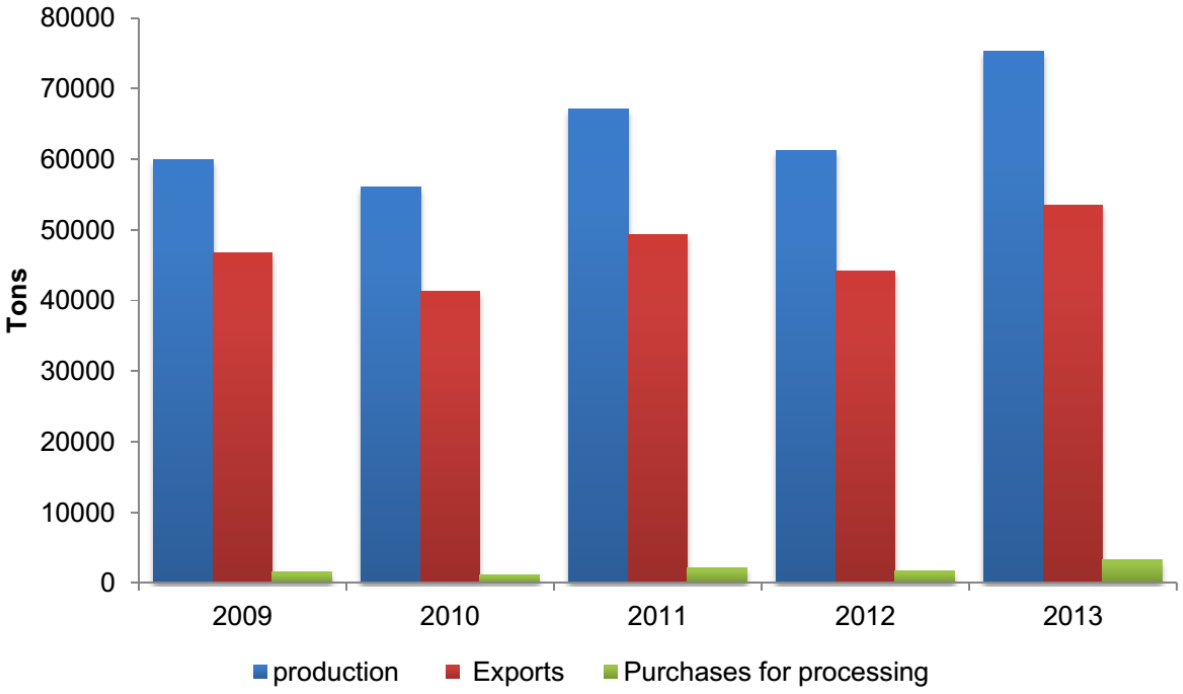

Figure 1. South African plum production and market distribution: Source: NAMC 2014; Quantec database

A wide range of plum cultivars are cultivated in South Africa. Most of these cultivars were developed and bred locally. The most widely grown plum cultivar is Laetitia (HORTGRO, 2017), a locally bred cultivar released in 1985 (Fruits Unlimited, 2014). It colours to a bright red hue, with a yellow flesh. It has a semi-clinging stone and is harvested in late January. Songold, another local cultivar, is the second most widely planted cultivar (HORTGRO, 2017) although it was developed long before 'Laetitia' in 1970 (Fruits Unlimited, 2014). It is yellow-green when mature but might be slightly yellow-red with yellow flesh when fully ripe. Picking time is early February. 'African Delight' is a fairly new South African cultivar. Released in 2008, it has already made a mark on

the export market because of its excellent eating quality, owing to its high sugar content (Von Mollendorf et al., 2008). It is an oblong red fruit with yellow flesh and a cling stone. It can be harvested from mid-February. A breakdown of the important plum cultivars according to area planted in South Africa is shown in Table 1.

Table 1. Breakdown of plum cultivars according to area (hectares) planted in South Africa from 2010-2015. Source: HORTGRO, 2017.

PLUMS	2010	2011	2012	2013	2014	2015
LAETITIA	625	607	603	597	597	619
SONGOLD	618	602	576	548	541	539
ANGELENO/SUPLUMSIX	273	290	309	329	350	393
SAPPHIRE	395	396	386	364	357	355
AFRICAN DELIGHT	239	293	329	335	336	341
FORTUNE	247	240	257	267	281	318
PIONEER	317	327	305	281	269	247
AFRICAN PRIDE/SUNKISS	243	246	242	242	243	233
RUBY STAR	0	0	0	120	139	197
AFRICAN ROSE	82	113	150	168	175	186
SOUTHERN BELLE	190	191	191	176	177	174
FLAVOR KING	167	173	188	175	173	168
PURPLE MAJESTY	99	113	125	130	135	136
SUN SUPREME	88	110	117	112	112	110
LADY RED	137	137	120	111	107	103
FLAVOR FALL	31	76	86	88	93	101
LARRY ANNE	55	55	69	91	91	92
SOUVENIR	92	95	92	92	92	86
OTHER	569	645	669	668	730	806
TOTAL	**4 466**	**4 708**	**4 814**	**4 895**	**5 000**	**5 205**
% CHANGE (YEAR-ON-YEAR)	**-**	**5%**	**2%**	**2%**	**2%**	**4%**

The Western Cape is characterised by a Mediterranean-type climate. Although this climate largely meets the requirements for successful plum production in South Africa, it also brings challenges in producing fruit of the highest quality. In the growing season, summer days are characterised by clear skies with high irradiance and high temperatures of up to 42°C (Tadross and Johnston, 2012). Heat waves are therefore common and they have a large bearing on tree physiology and ultimate fruit quality (De Kock, 2015). The most direct and most noticeable effects on the fruit are discolourations on the fruit surface due to excessive irradiance in combination with radiant heating (Barber and Sharpe 1971; Thorpe 1974; Smart and Sinclair, 1976, Schrader et al., 2001). This disorder has been extensively studied in many fruit and is defined as sunburn (Racskó and Schrader, 2012).

Sunburn downgrades the fruit quality and market value at harvest. Control measures for sunburn include the use of shade nets to attenuate incoming solar radiation. Shade nets have been considered

one of the best strategies against sunburn in apples (Middleton, 2010). However, we are not aware of any previous research on the use of shade nets on Japanese plums to mitigate sunburn. Another climate ameliorating technique is the use of overhead irrigation to reduce the fruit surface temperature by evaporative cooling. Water stress predisposes fruits to sunburn (van den Ende, 1999; Woolf and Ferguson, 2000), possibly by reduced evapotranspiration due to low stomatal conductance. Great success has been achieved in the reduction of heat stress in cherries and Kakamas peaches by increasing irrigation during heat wave conditions (Kotzé and Bothma, 1989). Therefore maintaining trees at optimal plant water potential would reduce the incidence of sunburn. In addition, cultural practices such as the proper timing of summer pruning vegetative manipulation can improve the light/shade dynamics within the canopy, minimising sunburn.

Plums exposed to high temperature in the absence of irradiance can succumb to two forms of heat damage, namely pitburn and gel breakdown (Maxie and Claypool, 1956; Kapp and Jooste, 2006; De Kock, 2015). The high temperature accelerates respiration, lowering O_2 levels within the fruit. This leads to anaerobic respiration with the ultimate production of ethanol and manifestation of internal heat damage (Bufler and Bangerth, 1982).

Pitburn manifests as dark brown discolourations of the inner mesocarp of the flesh, with severe forms spreading out to the periphery (Amiot et al., 1997). Gel breakdown appears as a dark gelatinous discoloration in the mesocarp flesh around the stone (Candan et al., 2008). Although both forms of the damage can be observed in the orchard after heat waves, damage is more prominent during or after cold storage (Kapp and Jooste, 2006). Improper procedures to remove field heat at harvest aggravate the problem. Stepwise forced air cooling has been reported to minimise the incidence of internal heat damage of ‘Laetitia’ in cold storage (HORTGRO, 2016).

Successful production of plum fruit of the highest possible quality requires a clearer understanding of the effects of high light and temperature and how these factors might interact with the environment to affect general tree physiology and specific fruit biochemical processes. Research in this regard has mostly focused on apples. This review of literature therefore aims to bridge the gap between what is currently known and how this would apply to plums.

Light and plant productivity

Solar radiation is fundamental to plant productivity. Although the radiation reaches the earth surface in a broad spectrum, only a small component affects plant physiology and productivity (Bastías and Corelli-Grappadelli, 2012). The pertinent spectra lie within 200-800 nm. The ultraviolet (UV)

radiation is the most energetic, with UV-B (280-320 nm) and UV-A/B (300-400 nm). Photosynthetically active radiation (PAR) lies within 400-700 nm of the electromagnetic spectrum and is utilised by plants to assimilate carbon dioxide in the process of photosynthesis (Majnooni-Heris, 2014). PAR can be further subdivided into blue light (400-500 nm), green light (500-600 nm) and red light (600-700 nm) (Nobel, 1983). The quantity of PAR available for interception by the plant can be quantified as photosynthetic photon flux (PPF).

The spectral composition of light in the orchard tree canopy is a function of how light can penetrate the canopy, or be scattered by components such as leaves, branches and clouds (Grant, 1997; Corelli-Grappadelli, 2003). Therefore, radiation within the plant canopy is comprised of two components, namely filtered and unfiltered radiation (Bastías and Corelli-Grappadelli, 2012). Filtered radiation is the diffuse light weakened by canopy foliage or scattered by the clouds whereas unfiltered radiation has full spectral strength as it passes through gaps in the canopy (Hardy et al., 2004). Therefore light distribution within the canopy is almost always not uniform.

It is important to note that on cloudy days diffuse radiation can be higher than direct radiation within the plant canopy (Lakso and Musselman, 1976; Hardy et al., 2004). Unlike direct radiation which is unidirectional, diffuse radiation can penetrate the canopy from any direction (Li et al., 2014). This can greatly alter the light balance between outer and inner canopy positions. Light absorption by the leaves accounts for about 80% of incident visible solar radiation (Corelli-Grappadelli, 2003). In fruit production, light absorption can be maximised by manipulating factors such as tree planting density, tree arrangement, orchard design and tree training and pruning systems (Stadler and Stassen, 1985; Stassen et al., 1995; Stassen and Davie 1996).

Carbon assimilation and solar injury

Light absorption and utilisation within a plant is governed by a complex photosystem. It is made up of two reaction centres, namely photosystem I (P700) and photosystem II (P680) (Anderson and Andersson, 1988). Light harvesting pigment complexes (LHCs) absorb light energy, specifically PAR, and channel it to these reaction centres to drive photosynthesis (Taiz and Zeiger, 2002). In addition, the oxygen evolving complex and the electron transport system form part of the complex light absorption and utilisation system.

When LHCs in the photosynthetic organs absorb PAR, it excites chlorophyll molecule *a* into a highly energetic singlet state (Müller et al., 2001). This molecule can revert to ground state when the excitation energy goes through one of several fates. The energy can be channelled to a

photosynthetic reaction centre where it is used for carbon assimilation. It can either be emitted as heat or re-emitted as light of longer wavelength, in what is known as chlorophyll fluorescence (Maxwell and Johnson, 2000). These processes occur competitively and a reduction in one would increase the efficiency of the other two.

PAR in excess of that which can be utilised in photosynthesis results in the inhibition of the process, and in severe cases, damages the photosynthetic apparatus (Baker and Bowyer, 1994). When excessive PAR is absorbed, the singlet chlorophyll *a* can dissipate excitation energy by transforming to a triplet state (Müller et al., 2001; Gill and Tujeta, 2010). The triplet molecule passes on energy to oxygen containing molecules, yielding highly reactive and hazardous singlet oxygen molecules and active oxygen species (AOS). These highly reactive AOS degrade cellular components and macromolecules, including photosynthetic pigments and apparatus (Gill and Tujeta, 2010). The resultant photoinhibition aggravates the stress on the organs as this might further reduce their capability to utilise light.

Plants are exposed to even more energetic and detrimental UV radiation (Förschler et al., 2003; Glenn et al., 2008). Persistent exposure to UV radiation can result in chlorophyll degradation and impairment of the plant photosynthetic system, particularly the PSII (Kulandaivelu and Noorudeen, 1983). In addition, it disrupts the function and structure of cellular nucleic acids. Much of this high energy shortwave radiation is attenuated by stratospheric ozone (Wand, 1995). However, in the past years, the concentration of the stratospheric ozone has been reported to be decreasing at a dramatic rate (Hoffman et al., 1992; Gleason et al., 1993). Plants are therefore increasingly exposed to a greater risk of solar injury. Damage is aggravated when combined with adverse conditions such as high temperatures.

Light utilisation and chlorophyll fluorescence

During photosynthesis, the LHCs absorb PAR, exciting chlorophyll molecule *a* into a highly energetic singlet state (Müller et al., 2001). This molecule can revert to ground state when the excitation energy is channelled to a photosynthetic reaction centre where it is used for carbon assimilation. However, the plant's energy requirements for carbon assimilation are usually smaller than what it actually absorbs (Ritchie, 2006).

To avoid leaf damage, all of the absorbed energy must be utilised or somehow dissipated. Energy not utilised for photosynthesis can be emitted as heat, or re-emitted as light of longer wavelength, in what is known as chlorophyll fluorescence (Maxwell and Johnson, 2000). All these processes,

known as energy quenching, occur competitively and a reduction in one would increase the flux of the others. The utilisation of energy for carbon assimilation is known as photochemical quenching (qP) while heat dissipation is known as non-photochemical quenching (qN). Chlorophyll fluorescence occurs when a chlorophyll molecule cannot pass energy to another molecule because it is already overloaded with energy or it is not joined to it (Lawlor, 1993). Therefore chlorophyll fluorescence is a useful indicator of the dynamics of energy absorption and utilisation in the photosynthetic system.

Components of chlorophyll fluorescence are well described by Ritchie (2006). At qP, where all reaction centres are open, fluorescent emissions increase up to a certain point referred to as the original fluorescence (F_0). Immediately after this is always a rapid increase to a peak fluorescence level (F_m). The progression from F_0 to F_m is termed variable fluorescence (F_v) (Ritchie, 2006). From the maximal Fm, fluorescence rapidly declines before gradually progressing to a stable level, the steady state (F_t). To determine how efficient the light reaction of photosynthesis is, physiologist can assess the ratio of F_v/F_m. This is known as the optimal quantum efficiency. It gives an indication of the ratio of moles of carbon fixed per mole of light photons absorbed by the photosystem. The optimal quantum efficiency is therefore a useful parameter that indicates that leaf photosynthetic tissue has undergone stressful conditions.

To be precise, chlorophyll fluorescence indicates the PSII energy utilisation and how its photosynthetic apparatus is being damaged by excess energy (Maxwell and Johnson, 2000). The rate of photosynthesis can be inferred from how fast electrons flow in the PSII system. Thus the efficiency of PSII photochemistry can be measured. When all PSII reaction centres are all open, there is maximal yield in photochemistry. This results in minimal yield in fluorescence (Butler, 1978). Consistently, fluorescence yield increase to maximum at zero yield of photochemistry when the PSII reaction centres are not open to accept electrons.

In addition to photosynthetic efficiency, chlorophyll fluorescence can be used to determine the extent to which plants tolerate or are damaged by other environmental stresses (Bilger et al., 1995). Environmental stress such as drought stress affect chloroplast metabolism and therefore photosynthetic efficiency (Reddy et al., 2004). This is due to changes in quantum yield brought about by a disruption in the balances of energy generation and utilisation (Foyer and Noctor, 2000).

Altering energy dynamics inevitably results in the dissipation of excess energy in the PSII core and antenna. This is associated with the generation of hazardous AOS. These highly reactive AOS

damage cellular components and macromolecules, including photosynthetic pigments and apparatus (Gill and Tujeta, 2010). In addition to the lower F_v/F_m values, damage to the photosynthetic tissue by environmental stress is indicated by a longer fluorescence peak compared to that of cells not under stress (Ritchie, 2006). This is the evidence that healthy photosynthetic tissue has a higher photochemical quenching capacity compared to damaged tissue. Figure 2 illustrates a comparison of the chlorophyll emission curve for a healthy and a stressed seedling.

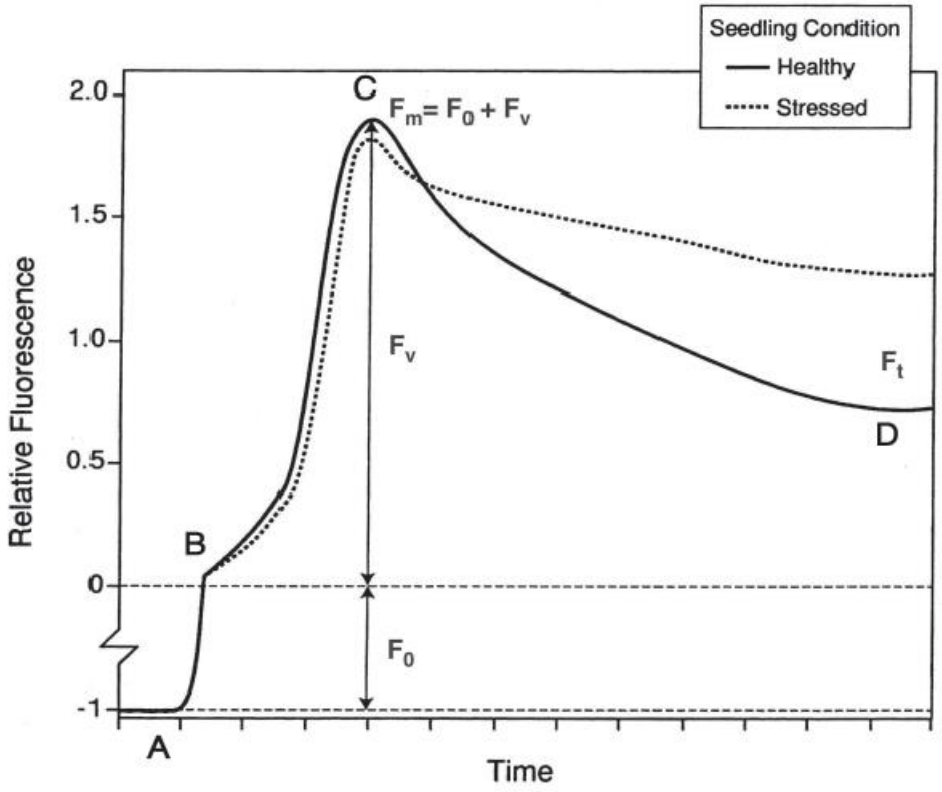

Figure 2. A typical chlorophyll emission curve for a leaf made with a "Kautsky" fluorometer. A is at the point of the actinic light pulse; B is the chlorophyll emission when all reaction centers are open; C is the emission peak; and D is the emission approaching steady state. F_o is the fluorescence emanating from the light harvesting complex. F_m is maximum fluorescence. F_v,variable fluorescence = $F_m - F_o$. Ft is steady state fluorescence. If the leaf is under significant stress, say from cold damage, the emission curve may resemble the upper dotted line. Source: Ritchie, 2006.

Before the generation of AOS under high energy conditions, there is always a decrease in the efficiency of photosynthetic energy conversion. This is defined as photoinhibition (Demmig-Adams and Adams, 1992). Photoinhibition is an indication of either an increase in the thermal dissipation of energy excessive of that which can be utilised in photosynthesis or damage of the photosynthetic system. Measurements of F_v/F_m can detect photoinhibition. However, these measurements cannot determine the extent to which the two incidents are contributing to the photoinhibition (Demmig-Adams and Adams, 1992).

Water stress and photoinhibition

Environmental stresses are known to decrease photosynthesis and plant growth as they usually alter carbon and nitrogen metabolic processes (Yordanov et al., 2003; Cornic and Massacci, 1996). Water stress occurs either when available soil water becomes depleted or when the rate of transpiration increases considerably (Reddy et al., 2004). Plants that strive to maintain stable water status at low soil moisture are termed isohydric, while those that have a responsive water potential to available water are anisohydric (Franks et al., 2007). To our knowledge, Japanese plums have not previously been categorised as isohydric or anisohydric.

Water stress conditions often occur in arid and semi-arid conditions typical of the summer conditions of the Western Cape of South Africa. With a Mediterranean-type climate, the summers are characterised by clear skies with typically high solar radiant energy. This results in an overload of energy on leaves and fruit, associated with insufficient dissipation capacities. In cases of high water potential, overheating can be avoided by transpirational cooling (Larcher, 1995). Therefore water stress has a significant impact on photosynthesis and quantum yield (Yordanov et al., 2003). Björkman and Powles (1984) demonstrated the effects of water stress on photochemistry. Stomatal conductance, transpiration, carbon uptake and electron transport decreased in a water stressed oleander shrub (*Nerium oleander* L.) growing under full natural sunlight.

Similar effects were observed in plants growing under shade conditions when suddenly subjected to full sunlight. The authors attributed this to an inactivation of the PSII system as a result of photoinhibition. This is an indication of light energy absorbed in excess of that which can be utilized in carbon assimilation (Demmig-Adams et al., 1995). It increases as adverse environmental factors limit photosynthesis (Manuel et al., 2001). Even at low irradiance, Düring (1999) reported that the quantum yield of water-stressed grape vines decreased compared to well-watered ones.

Influence of light on temperature

The main energy input into plant leaves and other organs such as fruit is solar radiation (Lambers et al., 1998). Apart from being utilised in photochemistry, incident solar radiation can be reflected or transmitted. If not dissipated, energy in excess of that of the plant photochemical requirements would heat up the plant organ to 100 °C in a few seconds (Jones, 1985). However, there are several processes responsible for plant heat loss and ensuring steady state temperature regimes for

productivity. Temperature response varies from different plant species and different plant habitats offer different air microclimates.

When a leaf absorbs short wave solar radiation, one of the processes of heat loss involves emitting long wave infrared radiation (Lambers et al., 1998). However, it concurrently absorbs long wave radiation emitted by the sky and other nearby objects. Therefore, depending on the magnitude of emission and absorption, the net energy balance might be positive or negative. This brings about differences in air and plant temperatures. In such a scenario, convective heat transfer proceeds along the temperature gradient.

Another process involved in heat loss is that of cooling by transpiration (Lambers et al., 1998). The rate of transpiration can be affected by leaf diffusion of water vapour (g_w). This in turn is a function of the leaf stomatal conductance (g), boundary layer conductance (g_a) and the leaf and air vapour pressure gradient (e_i - e_a). Vapour pressure gradient is regulated by leaf temperature and relative humidity (RH). Apart from environmental factors, in plant organs such as the fruit, convectional heat loss can be affected by fruit peel permeability to water, the extent of fruit peel radiation reflectance and fruit size (Nordey et al., 2014).

Thermal stress

Temperature is an important factor in nearly all plant processes. It plays a significant role in biochemical processes such as enzyme catalysed reactions, membrane transport, and compound volatisation (Tiaz and Zeiger, 2002). It is equally important in physical plant processes such as transpiration. Therefore high temperature or thermal stress is a serious impediment to crop productivity (Hall, 2001). At the lower range of increasing temperatures, proteins are degraded in the organelles, enzymes inactivated, and membrane integrity lost. Extremely high temperatures denature and aggregate important cellular proteins and enzymes in the cell while increasing fluidity of lipids (Wahid et al., 2007) eventually resulting in death of the plant (Schöffl et al., 1999).

To avoid or minimise damage to cellular components by thermal stress, plants and other organisms make use of a response known as the heat shock response (Feder and Hoffman, 1999; Hochachka and Somero, 2002). This involves rapid synthesis and accumulation of a specific set of proteins, the heat shock proteins (hsps) (Iba 2002). Synthesis of the hsps is regulated by heat stress transcription factors which in turn are controlled by HSF encoding genes (Kotak et al., 2007). In addition to thermal damage evasion, hsps can facilitate repair of subsequent cellular damage.

Hsps are mostly categorised according to their molecular weight. They have three distinct classes, namely Hsp90, Hsp70 and low molecular weight proteins (lmwp) of 15-30 kDa (Wahid et al., 2007). Although different plant species have varying proportions of these proteins, under conditions of thermal stress, hsp90 and hsp70 can increase tenfold while lmwp increase by up to 200 fold.

The actual mechanism by which hsps provide thermo-tolerance is yet to be understood. However, many studies have indicated that they assume a chaperone role by mimicking the form and function of proteins that might have been denatured by high temperatures. The hsps persist for a long time in the cells (Schlesinger, 1990). This ensures continued physiological functionality under stressful and otherwise detrimental conditions.

Membrane stability under heat stress conditions is important in maintaining physiological function. High temperatures increase lipid fluidity, modifying membrane structure and composition (Wahid et al., 2007). Membrane disruption can lead to ion leakage (Stanley, 1991). This affects processes such as photosynthesis and respiration which depend on membrane-based enzymes and electron transfer systems. In fact, the thylakoid membranes of the chloroplasts are so heat-sensitive that the effects of high temperature stress affect photosynthesis before most biochemical processes (Valladares and Pearcy, 1997).

Ferguson et al. (1998) tracked diurnal gene expression for heat shock treatments in apples. The response was consistent with changes in typical daily temperature cycles. Hsp gene expression was highest after the peak afternoon temperatures. However, this persisted well into the night, but declined by the following morning due to the then prevailing low temperatures. The cycle recurs with an increase in temperature.

Lurie and Klein (1990) reported an induction of hsps in pears at 38 °C. A similar response at the same temperature was reported in avocado by Woolf and Lay-Yee (1997) and was associated with subsequent thermo-tolerance of temperatures as extreme as 50 °C. Therefore, the induction of hsps at high, but sub-lethal temperatures is important as it protects the plant against hazardous heat levels.

Effects of light and temperature on fruit quality

Light

Fruit colour is one of the most important attributes influencing consumer perception and the ultimate appeal of a product (Singh and Khan, 2010). Consumers generally prefer well coloured

fruit with the red colour masking the green/yellow ground colour. Although consumers get their initial perception from the peel colour, flesh colour can also be an important driver of consumer appeal. In all fruits, the perceived colour is derived from the pigment groups anthocyanins, chlorophylls, carotenoids or betalains (Steyn, 2009).

In plums, the pigments responsible for both peel and flesh colour are anthocyanins, carotenoids and chlorophylls (Manganaris et al., 2008). The pigment composition in the peel or flesh varies depending on the cultivar and stage of maturity. In red cultivars, anthocyanins are mostly found in the fruit peel, giving the fruit the characteristic red colour. In red-fleshed plum cultivars, anthocyanins are also found abundantly in the fruit flesh. The predominant anthocyanins in plums are cyanidin-3-rutinoside, cyanidin 3-glucoside and peonidin 3-rutinoside (Tomás-Barberán et al., 2001; Kim et al., 2003).

Light exposure is one of the most important factors influencing the accumulation of anthocyanins in most temperate Rosaceous fruit such as apples, peaches and apricots (Steyn, 2009). However, some cultivars of plums and other fruits such as blackberries, strawberries and grapes are even capable of developing colour, although to a lesser extent, in the absence of light (Steyn, 2009). In fruit that require light, literature is replete with reports indicating that the sun exposed fruit accumulate more anthocyanins during development compared to shaded fruit. Campbell and Marini (1992) demonstrated that prolonged exposure of apples to 250 µmol m^{-2} s^{-1} PPF linearly increased red colour intensity of ‘Delicious’ apples. In peach, shading the fruit with a screen cloth resulted in less red colour development (Erez and Flore, 1986). Fruit peel accumulation of anthocyanins in response to light differs with cultivars (Steyn, 2009). Pale-red coloured cultivars are more sensitive than dark-red, purple and black ones.

Arakawa et al. (1985) reported the role of UV-B in anthocyanin synthesis, particularly in blushed apple cultivars. Fan and Mattheis (1998) added that the discriminant eliminating of UV-B light marred red colour development of ‘Fuji’ apples. Under clear skies, there is an increase in the UV proportion due to a reduction in its absorption by the atmosphere. Environments with clear skies such as Washington State, USA, reportedly produce redder fruit (Nobel, 1983).

Carotenoids are fat soluble compounds that are derived from isoprene (Manganaris et al., 2008). The major carotenoids found in plums are beta carotene and cryptoxanthin (Gil et al., 2002). They are responsible for the green/yellow ground colour of the peel and the flesh colour in yellow-fleshed cultivars. The ground colour indicates fruit maturity and readiness for harvest. With most stone fruit

and plum cultivars, the ground colour changes from green to yellow due to a decline in chlorophyll and an increase in carotenoids. Unlike red colour development, ground colour is not affected by light and is therefore an accurate indicator of fruit maturity (Crisosto, 1994).

Pre-harvest low light conditions within the canopy have been associated with delayed fruit maturity in many fruit. In plums, greener fruit with poorer red colour, firmer flesh, and lower total soluble solids (TSS) have always been observed at harvest from shaded canopy positions compared to the sun exposed ones. (Murray et al., 2005; Manganaris et al., 2008). Shading can delay maturity by up to 14 days (Manganaris et al., 2008).

For greater consumer approval, plums must be harvested when they have at least attained a certain threshold level of TSS and reduced acids to develop a sweet taste. The TSS are largely comprised of sugars, and these are in the form of fructose, glucose, sucrose and sorbitol (Meredith et al., 1992; Brady 1993). Acids, on the other hand consist mostly of malic and citric acid (Crisosto, 1994). These attain a diminished level by harvest largely due to the degradation of malic acid (Ryall and Pentzer, 1982). Another important quality attribute affected by light in stone fruit is fruit size. Fruit in outer sun exposed canopy positions have consistently been reported to be bigger than inner shaded ones (Murray et al., 2005).

Temperature

High temperatures, which are usually experienced as spates of heat waves in the Western Cape, South Africa, cause two forms of heat damage in plums - internal heat damage and gel breakdown. Although cultivars differ in the way they internally respond to heat damage (De Kock, 2012), fruit generally become more susceptible with advanced harvest maturity (Taylor et al., 1994). Symptoms may not be apparent in the field and may later be detected in cold storage (De Kock, 2012). Fruit quality at harvest of most fruit, including plums, is a function of conditions that prevailed in the orchard. Post-harvest treatments such as cold storage therefore strive to maintain quality attained during pre-harvest development (Manganaris et al., 2008).

Internal heat damage (Pitburn)

According to De Kock (2012), internal heat damage manifests when air temperatures rise above 38 °C. However, this is based on personal observation and is not statistically tested. Symptoms appear as a dark brown discolouration of the inner mesocarp around the stone and spreading out to the outer tissue with increasing severity (Amiot et al., 1997). High ambient temperatures initiate high rates of respiration in the fruit (Cheng et al., 1998). High respiration rates depress internal O_2

within the fruit tissue while elevating internal CO_2 levels, promoting anaerobic respiration and subsequent accumulation of ethanol (Lange and Kader, 1977). This results in softening of the tissue around the stone, with concurrent oxidation of phenolic compounds (Amiot et al., 1997) which appears as a brown discolouration in the fruit flesh. The accumulation of alcohol and toxic metabolites and depletion of energy for maintaining cell respiration result in cell and tissue breakdown, softening the fruit flesh of the affected area (Franck et al., 2007).

The oxidation of phenolic compounds in fruit is catalysed by a group of enzymes known as polyphenol oxidase (PPO) into dark coloured *o*-quinones (Tomás-Barberán et al., 1997). In the initial step, PPO catalyses the hydroxylation of monophenols into colourless *o*-diphenols which are further oxidised by the same enzyme to colour-bearing *o*-quinones. Polyphenols are mostly restricted to the vacuole of the cell (about 97 %), with the rest in free space and none in the cytoplasm (Yamaki, 1984). On the other hand, PPO enzymes are located in the thylakoids and therefore the enzyme and substrate are separated by cell membrane compartments, preventing phenolic oxidation to occur. However, in conditions such as heat stress which cause loss of membrane integrity and leakage (Wahid et al., 2007), PPO and the phenolic compounds coalesce, initiating the oxidation process (Veltman, 2002). In addition, high CO_2 conditions have been reported to promote the activity of PPO, enhancing phenolic oxidation (Veltman, 2002).

Paul and Pandey (2014) have indicated that in general, metabolic processes and factors that regulate the rate of respiration, significantly affect fruit quality and storage life. Apart from the availability of respiratory substrate, fruit respiratory activity is a function of its internal gaseous composition, particularly O_2 concentration. Conditions such as high respiration rate or lower permeability of gases that reduce fruit internal oxygen concentrations are associated with the development of anaerobic stress and physiological disorders. Exposing 'Murcott' Mandarins to high nitrogen atmosphere triggered an increase in respiration, decreasing internal O_2 levels (Shi et al., 2007). In addition, the mandarin peels have low gas permeability. The result was an accumulation of ethanol and acetaldehyde and subsequent off flavours (Shi et al., 2005; 2007). Internal browning of pears was attributed to low O_2 concentrations in the pear cortex (Franck et al., 2007).

At ambient temperature, the fruit internal composition consists of a mixture of gases and volatiles such as O_2, CO_2, alcohols, aldehydes, aromatic hydrocarbons and water vapour (Toivonen, 1997; Baldwin et al., 2000, Pesis, 2005). During ripening, concentrations of CO_2 and ethylene increase, while O_2 decreases (Paul and Pandey, 2014). In addition, high CO_2 concentrations inhibit synthesis and activities of aerobic respiratory enzymes, promoting further anaerobic conditions (Lange and

Kader, 1977). Fruit of advanced maturity would therefore accumulate higher quantities of ethanol. Consistently, advanced maturity at harvest was reported to increase the likelihood of fruit developing internal heat damage (Taylor et al., 1993a; Abdi et al., 1997).

It was speculated that low plant water status predisposes fruit to internal heat damage (De Kock, 2012). Under the high heat conditions and moisture stress, leaves close their stomata to conserve water by avoiding transpirational water loss (Colaizzi et al., 2012). This minimises the fruit convectional heat loss to the environment, increasing its surface and pulp temperature. When micro-climatic conditions limit heat loss to the environment, fruit surface temperature can be 10-15 °C higher than the ambient temperature (Smart and Sinclair, 1976).

Gel breakdown

Plums that experience heat waves on the tree may also develop gel breakdown. Like pitburn, oxygen depletion and subsequent anaerobic conditions initiated by the high temperature seem to play a significant role in the development of gel breakdown in plums (Maxie and Claypool, 1956). Initial symptoms appear as a gelatinous breakdown in the mesocarp flesh around the stone turning into dark discolouration with increasing severity (Candan et al., 2008). This gives rise to mealy, woolly or hard textured flesh (Singh and Khan, 2010). Although the actual mechanism is not known, this change in fruit texture is considered to be a result of changes in membrane permeability and the accumulation of water soluble-pectins (Taylor et al., 1993b). The electrolyte leakage facilitates formation of gel complexes with the pectins that bind with water. Subsequently, extractable juice within the fruit is reduced, resulting in hard, mealy or wolly textured fruit.

The incidence of gel breakdown in the orchards is usually very low. If it manifests in the orchard, it is usually observed in fruit of advanced maturity (Taylor et al, 1994). Gel breakdown is more prominent when fruit is moved to shelf life conditions after cold storage. For this reason, it is often classified as a cold storage chilling injury disorder (Kapp and Jooste, 2006). Low temperatures affect cells in two ways to result in the symptoms of chilling injury (Stanley, 1991). The first involves structural disturbances of the lipid bilayer to result in loss of membrane integrity. The second affects the activities of pectolytic enzymes that are responsible for fruit softening. The membranes are naturally comprised of fluid lipid bilayer of phospholipids with imbedded proteins and sterols. This functional form is known as the liquid crystalline form (Stanley, 1991). Under chilling conditions, lipid domains undergo a phase transition from the crystalline state to the gel state (Marangoni et al., 1996). The gel state has packing imperfections that cause electrolyte leakages across the membranes (Stanley, 1991).

To avoid the prolonged storage of plums at chilling injury inducing temperature of -0.5 °C, fruit of susceptible South African plum cultivars are subjected to an intermittent warming storage protocol (dual remperature) (Taylor, 1996). The fruit are stored at -0.5 °C immediately after harvest for 8-10 days, depending on the cultivar. The temperature is then increased to 7.5 °C for 5-7 days before reverting to -0.5 °C for the remainder of the storage time. Although the physiology behind the reduction of chilling by intermittent warming is yet to be clarified, some hypotheses are suggested. Among these, it has been suggested that the variation in temperature promotes synthesis of polyunsaturated fatty acids which enable membranes to stay fluid at chilling temperatures (Wang, 2010; Jooste, 2012). Jooste (2012) found that the intermittent warming regime aids in maintaining the fruit's antioxidant levels, and thereby the fruit's antioxidant scavenging potential compared to a single temperature regime at -0.5 °C. In addition, he dual storage regime, in combination with optimal harvest maturity, reduce incidences of chilling injury and increases storage potential (Jooste, 2012). The reasons for this are that less mature fruit have less permeable, but more fluid cell membranes, and higher levels of antioxidants that can scavenge for free radicals. Optimally mature fruit should therefore be picked without delay.

Photo-thermal effects on fruit quality

Sunburn

High irradiance and coinciding high temperatures cause physiological discolouration on the fruit surface known as sunburn (Schrader et al., 2001). Although sunburn is a cause for major concern in Japanese plum production, previous research efforts on sunburn mostly focused on apple (Glenn et al., 2002; Racskó et al., 2005; Schrader et al., 2009; Racskó and Schrader, 2012). To our knowledge, there is no tangible literature describing the symptoms and threshold environmental conditions for the manifestation of sunburn in plums. As in other fruits such as apple, sunburn of plums under the Western Cape Province conditions appears as a brown to yellow discolouration on the fruit surface. Severe cases result in necrotic patches and cracking of the fruit peel.

Three types of sunburn have been identified and described in apple. The symptoms are related to the extent and timing of light and heat exposure. Sunburn browning occurs when fruit surface temperature reaches a certain minimum threshold in the presence of sunlight (Schrader et al., 2003). The threshold temperature varies across apple cultivars but often ranges between 46-49 ºC. Symptoms appear as brown to golden bronze discolourations on the sun exposed fruit side. Sunburn browning is the most prevalent form accounting for the greatest fruit cullage (Racskó and Schrader, 2012).

Sunburn necrosis occurs under more severe temperatures even in the absence of light (Schrader et al., 2001). When the apple fruit surface attains a temperature of 52 ± 1 ºC for more than 10 mins, dark necrotic patches appear as a result of thermal death of epidermal cells. Sunburn necrosis is therefore more visually prominent than sunburn browning. The third type, photo-oxidative sunburn has been described as a white spot that appears on previously shaded fruit surface that is suddenly exposed to the sun (Felicetti and Schrader, 2008). This can manifest at temperatures as low as just under 31ºC and can be detected within 24 hours after initiation.

Physiological mechanisms against photo-thermal stress

The photo-protective mechanisms in plants against injurious UV light include the synthesis of flavonoids and phenolic UV absorbing compounds (Caldwell et al., 1983). It has been reported that the synthesis of these photo-protective compounds can be affected by UV radiation exposure history (Singh et al., 1999), plant developmental stage or water and nutrient deficit (Wand, 1995). At higher altitudes, tropical regions have a smaller solar zenith angle compared to temperate regions and would therefore experience more UV-B radiation (Madronich et al., 1998). The Western Cape Province of South Africa has a Mediterranean-type climate. It is characterised by abundant visible light during the growing season and therefore plants are subjected to elevated UV-B level. These plants are likely to have a higher concentration of photo-protective phenolics. In addition, water stress and nutrient deficiency, particularly lack of phosphates (Murali and Teramura, 1985), increase the synthesis and concentration of UV-B attenuating phenolics in plant cells (Wand, 1995).

Although leaves are the chief photosynthetic organs on plants, fruit peel is also involved with carbon fixing, contributing about 1% in mango (Chauhan and Pandey, 1984), 3% in lychee (Hieke et al., 2002) and up to 10% in peach (Pavel and De Jong, 1993) compared to leaves. However, as the developing fruit matures, the fruit peel experiences colour transformation (Manganaris et al., 2008). Anthocyanins that accumulate in fruit play a photoprotective role by screening light from photo-sensitive fruit tissue under adverse conditions such as cold temperatures (Steyn et al., 2009). The anthocyanin levels in immature pear peels fluctuated in response to changes in temperature, disappearing under warmer temperature conditions. Steyn et al. (2009) suggested that anthocyanin levels in leaves are less transient because of lower photoinhibition compared to fruit.

Xanthophyll cycle

One of the most effective ways of dissipating hazardous excess energy that cannot be utilised by the plant in the photochemical process involves the xanthophyll cycle (Demmig-Adams and Adams,

1992). The cycle is made up of the reversible interconversion of three forms of carotenoid xanthophylls, which are violaxanthin, antheroxanthin and zeaxanthin. The forms of the xanthophylls are changed by the addition or subtraction of an epoxide group. The total three forms of the xanthophylls make up the xanthophyll pool size (Ma and Cheng, 2004). The xanthophyll pool size increases with an increase in the need for thermal dissipation (Demmig-Adams, 1990). However, ultimately photoprotection comes from the de-epoxidised form, zeaxanthin which is formed under high light by prior de-epoxidation of violaxanthin via antheroxanthin (Thiele et al., 1996). In addition to thermal dissipation, zeaxanthin plays a key role in quelling the singlet oxygen free radical formed by excess excitation energy (Havaux and Niyogi, 1999).

Anti-oxidants

AOS caused by excessive radiant energy and high temperatures are highly reactive and they are involved in a series of radical reactions that damage cellular components (Noctor and Foyer, 1998). Damage can be aggravated by other concomitant adverse environmental conditions such as water stress. Therefore, to avoid cellular damage, the plants must activate a protective mechanism to quell the destructive free radicals.

Scavenging enzymes and antioxidants are capable of quenching the AOS before they cause detrimental effects (Noctor and Foyer, 1998). Under more ideal growing and environmental conditions, a delicate balance exists between AOS formation and their degeneration by antioxidants (Gill and Tujeta, 2010). When adverse conditions prevail, the rate of AOS formation exceeds that of quenching by protective compounds. However, the plant up-regulates the production of these scavengers. Superoxide dismutase is the commonest enzymatic scavenger of AOS (Gill and Tujeta, 2010). It quenches superoxide anion (O^{-}_{2}), by catalysing its dismutase reducing it to H_2O_2 and oxidising it to O_2. Other important enzyme scavengers are ascorbate peroxidase, glutathione reductase and dehydroascorbate reductase (Noctor and Foyer, 1998).

The most effective and abundant non enzymatic water soluble AOS scavenger is ascorbic acid (Gill and Tujeta, 2010). It exists in reduced and oxidised forms (Foyer, 1993). Under less adverse environmental conditions, it predominantly exists in the reduced form (Gill and Tujeta, 2010). The oxidised form of ascorbic acid has a short half-life, and therefore, does not persist longer, unless it is regenerated in the reduced form (Foyer, 1993). The quenching ability of ascorbic acid comes from its ability to donate electrons in both enzymatic and non-enzymatic reactions (Smirnoff, 2000). Glutathione is another important non enzymatic scavenger and it targets free radicals like

$^1O_2$, OH·, H_2O_2. (Dixon et al., 1998). In addition, it plays a fundamental role in regenerating ascorbic acid in the ascorbate-glutathione cycle.

Manipulation of orchard climatic environment to improve fruit quality

Summer pruning and canopy light distribution

Tree canopy micro climate conditions play a significant role in fruit development and ultimate fruit quality at harvest (Garriz et al., 1997). Of these conditions, light availability and light level in the fruiting zone have major effects on fruit yield and quality (Crisosto et al., 1997). In most deciduous fruit, including plums, current season's flower buds were initiated and formed during the previous summer (LaRue, 1989). Therefore in summer, optimal light distribution in the canopy is critical for flower bud formation and fruit set.

Canopy light penetration and availability, are in turn, affected by row orientation, canopy size, and canopy form (Stadler and Stassen, 1985). Row orientation affects the manner in which light and/or shade is distributed within the canopy. A north-south (N-S) row orientation evenly spreads out light on either side of the row, with latitude having negligible effect. Growers in South Africa therefore strive for N-S oriented orchards (Stassen, 2014).

An ideal tree structure supports a spatial branch architecture that supports good leaf area for optimal light interception for photosynthesis. Therefore, tree pruning and training are conducted to obtain the ideal form and size that maximises on light distribution within the canopy (Stassen, 2014). Dormant pruning, which is carried out in winter, can promote vigorous growth in spring. This is a response to removal of a large portion of the tree while its energy reserve in the root system remains constant. This excessive vegetative growth can result in poor light penetration into the canopy and therefore undesirable shading. Summer pruning is therefore carried out during the growing season as this enables discriminatory removal of shoots and branches to give the desired canopy shape (Saure, 1987) with improved light distribution.

Improved light penetration into the tree canopy can enhance red colour development in stone fruit (Crisosto et al., 1997). However, the timing of summer pruning is important. Day et al. (1995) observed that summer pruning performed towards harvest resulted in a reduction in TSS and fruit size in peaches and nectarines. The effect increases with increased pruning severity. In addition, if not well manipulated, excessive light might be detrimental in that it would cause sunburn and downgrade the fruit.

Responses to summer pruning can be affected by timing of pruning, severity of pruning, type of cultivar, tree maturity (vigour) and geographic (climatic) location (Stadler and Stassen, 1985). However, it is of utmost importance to bear in mind where flowers and fruit will develop on the tree. Japanese plums bear fruit mostly on spurs and lateral branches that are two years old or older and, to a lesser extent, on one year old wood (LaRue, 1989).

Shade netting

Shade nets have become a widespread measure of mitigating incoming radiation on the fruit surface, particularly in apple production (Middleton and McWaters, 2002; Smit, 2007). By reducing the intensity of radiation reaching the fruit, shade nets subsequently lower the fruit surface temperature (Gindaba and Wand, 2005). However, the extent of radiation attenuation is dependent on the colour and density of the net.

Apart from regulating irradiance reaching the fruit surface, net density and colour also affects fruit quality attributes such as fruit size, red colour, rate of starch conversion, and total soluble solids (TSS) (Shahak et al., 2004). In 'Fuji' apples, Solomakhin and Blanke (2009) reported improved fruit quality (firmer fruit, higher TSS and redder fruit) under red/white and white shade nets compared to red/black and green/black nets. However, in general the effects of shade nets on fruit quality resembles the low light environment such as the inner canopy
(Génard and Bruchou, 1992). Seeley et al. (1980) reported a reduction in TSS and fruit weight in apples as a result of shade nets. Although they have been extensively used in other fruit crops, we have no knowledge of literature on the use of shade nets in plum production.

Conclusion

The ultimate quality of many fruits, including Japanese plums, is determined by a nexus of pre-harvest and post-harvest factors. Available literature on apples has undoubtedly indicated that an understanding of the pre-harvest components of tree canopy micro-climatic conditions and factors related to light distribution and utilisation is key in controlling sunburn. There is scarce published data on plums in this regard. Environmental conditions leading up to the manifestation of internal heat damage in plums during and after cold storage are not well understood. Reports on the little available information are based on personal observation and deductions. Literature on approaches such as the use of shade nets over plum orchards, which have been reported to successfully mitigate sunburn in apples, is not available. Research and publication around these areas can immensely contribute to the plum literature body.

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RESEARCH CHAPTER 1

THE EFFECT OF CLIMATIC FACTORS ON THE EXTERNAL AND INTERNAL QUALITY OF JAPANESE PLUMS (*PRUNUS SALICINA* LINDL.).

Abstract

High irradiance and high temperatures decrease Japanese plum quality by causing sunburn and two types of internal heat damage, namely pitburn and gel breakdown. We manipulated orchard conditions to determine how resultant light and temperature affected fruit quality and sunburn. In the 2013/14 season, early summer pruning (8 Dec. 2013), late pruning (7 Jan. 2014) and a no pruning control were applied in an 'African Delight' orchard at Môrelig farm, Wemmershoek in the Western Cape Province of South Africa. In 2014/15, 20% shade net was incorporated during the hottest part of the season. Photosynthetic photon flux (PPF), fruit surface temperature (FST) and sunburn were progressively assessed on typically hot days on upper, mid and lower canopy positions at Môrelig farm. Fruit quality and internal disorders were assessed at harvest. Upon realising that it could be difficult to increase FST if mild temperatures prevailed in the season, heat absorbing black stickers were used on 'Laetitia' in a supplementary trial conducted a week before harvest at Welgevallen Research farm in Stellenbosch. Treatments consisted of 1) applying a cluster of 13 mm black stickers on sun exposed fruit free of sunburn; 2) randomly choosing fruit that had developed sunburn naturally and; 3) choosing and maintaining fruit that did not develop sunburn. FST and flesh temperature were measured at midday, a day after trial establishment. In all trials, internal disorders and fruit quality were assessed at harvest and again after cold storage. At Môrelig farm, upper canopy positions received higher PPF, had bigger fruit which were redder, softer and with higher total soluble solids and sunburn incidence. Fruit that developed sunburn received >50% PPF of full sun on average while average FST exceeded 35 °C. Early summer pruning improved early light penetration and enabled vegetative regrowth for filtered light during the hottest part of the season, reducing sunburn and enhancing fruit quality. Late summer pruning increased sunburn while the control delayed fruit maturity and reduced fruit size. The shade net attenuated PPF, reduced sunburn severity and improved fruit red colour. No internal heat damage was observed in any treatment. 'Laetitia' fruit with black stickers developed sunburn necrosis and had the highest FST and flesh temperature. Except for the brown discolouration of flesh underneath the necrotic peel, no clear internal disorders were observed in this treatment. However, fruit with no sunburn symptoms had higher gel breakdown and internal browning incidence after cold storage compared to those which

naturally developed sunburn. This could be an indication of heat curing against cold storage injury. In conclusion, the timing of orchard light manipulation is essential and delaying summer pruning would predispose fruit to sunburn. Shade nets have potential to control sunburn in sensitive cultivars. However, they can potentially affect tree reproductive physiology negatively if used over the entire growing season and this warrants further investigation. We confirmed that 'African Delight' plums are tolerant to internal heat damage. Whether shade nets can prevent internal heat damage would require further trials on sensitive cultivars. Although no pitburn was detected in Laetitia, the cultivar seems to be more sensitive to gel breakdown and internal browning. Heat-related internal disorders appearing after cold storage in plums could respond to pre-harvest heat curing.

INTRODUCTION

Solar radiation is fundamental to plant productivity. Photosynthetically active radiation (PAR) within 400-700 nm of the electromagnetic spectrum is utilised by plants to assimilate carbon dioxide in the process of photosynthesis (Majnooni-Heris, 2014). This is quantified as photosynthetic photon flux (PPF). PAR in excess of that which can be utilised in photosynthesis results in the inhibition of the process and in severe cases, damages the photosynthetic apparatus (Baker and Bowyer, 1994). In addition, plants are exposed to other even more energetic and therefore detrimental components of solar radiation such as ultraviolet (UV) radiation. Persistent exposure to UV radiation between 280-320 nm (UV-B) can result in chlorophyll degradation and impairment of the plant photosynthetic system (Förschler et al., 2003; Glenn et al., 2008). Damage is aggravated when combined with adverse conditions such as high temperatures (Woolf and Ferguson, 2000).

Compared to leaves, fruit peel has reduced photosynthetic capability (Aschan and Pfanz, 2003). Fruit peel subjected to high PPF density (PPFD) and high temperatures easily exhibit symptoms of photo-thermal damage or adaptations to such conditions known as sunburn. In apples, temperatures between 46 to 49 ˚C with concurrent high PPFD result in sunburn browning, which is a golden-bronze discoloration of fruit peel (Schrader et al., 2003a). Higher temperatures exceeding 52 ˚C damage epidermal and sub-epidermal tissue resulting in sunken necrotic spots. Due to high summer temperatures and high irradiation, sunburn is a major impediment to premium quality fruit production in the Western Cape province of South Africa.

Unlike in pome fruit, stone fruit such as Japanese plums that may not exhibit external damage might still develop internal heat related disorders (De Kock, 2012). Internal heat damage or pitburn is thought to be a result of increased respiration rates initiated by high temperature (Cheng et al., 1998). High respiration rates depress internal O_2 while elevating internal CO_2, which subsequently promote anaerobic respiration (Lange and Kader, 1977), and hence, disorder development in the fruit (Franck et al., 2007). Threshold irradiation and temperature conditions for manifestation of the external and internal disorders in Japanese plums have not yet been reported although up to 20% of the crop in sensitive cultivars like Laetitia and Fortune can be affected (Jooste, personal communication).

Interception of irradiation by the fruit is a function of the canopy size, training or trellising system and row orientation with respect to the position of the sun (Jackson, 1980). In 'Granny Smith' apple, outer canopy fruit were exposed to PPFD as high as 2000 µmol m^{-2} s^{-1}, which steeply decreased with canopy depth and was lower than 20 µmol m^{-2} s^{-1} for shaded, innermost fruit (Fouché et al., 2010). As a result, temperature disparities greater than 20 °C between exposed and shaded apple fruit have been reported (Corelli-Grappadelli, 2003). Due to filtering by leaves, light distribution within the canopy may not be uniform. While fruit quality and consumer appeal in apple have been shown to be directly related to the light and ensuing temperature regime under which the fruit develop in the tree canopy (Hamadziripi et al., 2014), little is known about Japanese plums in this regard.

Canopy manipulation such as summer pruning can be used to improve light penetration within the canopy in order to improve and increase the uniformity of fruit quality (Rom, 1991). Summer pruning is a standard practice in commercial Japanese plum production in South Africa. Research in apple indicated that fruit are more susceptible to sunburn from three months prior to harvest (Schrader et al., 2003b). Although the most critical developmental stage at which plums become susceptible is yet to be confirmed, it is apparent that the timing of summer pruning is crucial as it would have a bearing on shoot regrowth and subsequent light and shading dynamics.

In addition to canopy manipulation, incoming radiation incident on exposed apple fruit can be reduced by the use of shade nets (Middleton and McWaters 2002). In South Africa, shade nets are also increasingly being adopted to decrease sunburn in apple. Smit (2007) reported a reduction in sunburn on 'Fuji' and 'Braeburn' apples using a 20% black shade net. However, the reduction in sunburn was associated with poor red blush development. We have no

knowledge of any literature pertaining to the use of nets on Japanese plums to decrease light and temperature-induced disorders. Murray et al. (2005) enclosed branches of red 'Laetitia' and yellow 'Songold' Japanese plums in nets of various transmittance levels in order to induce differences in fruit maturity and to study the effect of mixed maturity at harvest on fruit quality after storage. Although they did not assess the incidence of sunburn while incidence of internal disorders was negligible, the authors found that shading decreased red colour development prior to harvest in 'Laetitia'. For shading of less than 30%, the deficit in red colour could be recovered through further red colour development during postharvest storage.

The objective of this study was to investigate the effects of orchard manipulation on micro-climatic canopy conditions viz. light and temperature in relation to fruit quality and the manifestation of internal and external damage in Japanese plums. Knowledge in this regard as it pertains to plums is scarce and could contribute to improved profitability of plum production.

MATERIALS AND METHODS

Trials were conducted during the 2013/14 and 2014/15 growing seasons at Môrelig farm (33° 51' S, 19° 02' E) and Welgevallen farm (33º 55' S, 18º 53'E) near Paarl and Stellenbosch, respectively, in the Western Cape Province of South Africa. The regions have a Mediterranean-type climate with warm, dry growing seasons (November to March) and mild, wet winters (April to October) (Tyson and Preston-Whyte, 2000). Peak summer temperatures are experienced in February with a maximum average around 27 °C (Tadross and Johnston, 2012). Daily maximum temperature of up to 42°C can be recorded in summer.

2013/14 season

Two separate trials were established adjacent to each other in an 'African Delight' plum orchard at Môrelig farm. At 7% of total plantings, 'African Delight' is the fifth most planted and third most exported plum cultivar in South Africa (HORTGRO, 2016). The cultivar ripens late and the first pick normally takes place the last week of February. Since February is the warmest month of the year in the production region, 'African Delight' is very susceptible to sunburn and heat damage while red colour development may also be poor (Von Mollendorf et al., 2008). The orchard used was established in 2008 with trees on the vigorous rootstock SAPO 778. The trees were planted at a spacing of 3.5 m x 1.25 in a NW-SE row direction, giving a compass bearing of 330º NW. They were trained on a staggered V-trellising system. The vertical poles of 2.5 m height were 0.5 m apart and inclined 15° off the perpendicular line.

One cross-pollinator after every three trees was planted between the V rows using any one of 'Harry Pickstone', 'Pioneer' or 'Ruby Nel' plums. Full bloom occurred on 23 Aug. 2013.

In the first trial, twenty trees on either side of the V-double row were tagged. On both the NE and SW row sides, three fruit on the lower, intermediate (hereinafter mid) and upper canopy position were tagged to progressively assess PPF, fruit surface temperature (FST) and sunburn. Lower canopy fruit were within 0.75 m from the ground, while mid canopy fruit were between 0.75 -1.50 m from the ground. Upper canopy fruit were higher than 1.50 m aboveground.

PPF was measured tree by tree using a quantum sensor attached to a light meter (LI 250 LI-COR, Lincholin, NE) which was placed on the fruit and directed towards the sun. FST was measured on the same position of the fruit using a hand held infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA). Additional whole season weather data was obtained from an automatic weather station at Lamotte Wine farm (33° 53' S, 19° 04' E), 4 km away from Môrelig farm.

Sunburn assessment was based on a modified Schrader and McFerson sunburn severity chart (Schrader et al., 2003a). In the chart, a score of 0 represents no sunburn while 5 represents the severest form. In our study, sunburn classes 1 and 2 could pass for export and shall hereinafter be referred to as low severity. Classes 3 and 4 could be marketed at the local market for a lower price and shall be referred to as high severity. Sunburn class 5 has undesirable necrotic patches and cracked fruit peel and is therefore not marketable. This class shall hereinafter be referred to as unmarketable. All measurements were assessed on four typically sunny days during fruit development around 120, 135, 150 and 180 DAFB (17 Dec. 2013, 31 Dec 2013, 14 Jan. 2014 and 14 Feb. 2014) hourly between 08h00 and 17h00.

At harvest (17 Feb. 2014), 20 representative fruit from each of the tagged positions were assessed for fruit quality in the Department of Horticultural Science, Stellenbosch University. Another sample of 10 fruit from the same positions was stored for 42 days at -0.5 °C and approximately 90% relative humidity. The fruit were assessed for post-harvest cold storage internal disorders such as pitburn, gel breakdown and internal breakdown after cold storage.

Total soluble solids concentration (TSS) and titratable acidity (TA) were measured by crushing and extracting juice from pooled plum pieces in a blender. A hand held refractometer (Model N1, Atago, Tokyo, Japan) was used to measure TSS from the juice. 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) and was expressed as percentage of malic acid (g 100 g^{-1} juice). Fruit colour was assessed using a 1-12 colour chart (Casselman PL 23, Deciduous Fruit Board, South Africa) where a value of 1 denoted the least coloured fruit (greenest) and 12 the reddest. Cold storage internal disorders were subjectively categorised according to severity as slight, moderate or severe.

In the second trial, early pruning (8 Dec. 2013), late pruning (07 Jan 2014), and a no pruning control were applied in a randomised complete block design (RCBD) with eight replications. In all pruning treatments, undesirable and densely spaced interior vertical shoots were cut back. Water shoots were completely removed. Sunburn, PPF, FST and fruit quality at harvest were assessed as described in the first trial.

2014/15 season

The same 'African Delight' orchard at Môrelig farm was used, with trees reaching full bloom on 25 Aug. 2014. The first trial of the previous season was not repeated and therefore only the second trial was conducted. The same pruning treatments as the previous season were applied with 20 percent black and white shade net (20 BLK/WHT, Knittex, Randfontein SA) in a split plot design comprising of two main plot levels and three subplot treatments. Shade net and no shade net control formed the main plots while early pruning, late pruning and no pruning control were subplot treatments. The main plots were replicated four times. The early and late pruning treatments were applied on 04 Dec. 2014 and 05 Jan. 2015, respectively while the shade net was installed on 22 Jan. 2015 (20-30 days before harvest). This coincided with the hottest part of the growing season in the region, the months of January and February.

Shading the trees involved directly draping the net over two rows along the length of the plot as shown in Figure 1. The net was kept in place by anchoring it to the ground with metal rods and huge stones on outermost row sides. Fruit were tagged on the same canopy positions as described for Experiment 1 in the previous season but PPF, FST and sunburn measurements were taken on innermost row sides that were not in direct contact with the net (Figure 1).

A supplementary trial was laid out at Welgevallen farm upon realising that assessment of heat tolerance of plums under field conditions at Môrelig farm could be difficult if mild temperatures prevailed in the season. The objective therefore was to increase FST in the field with the aid of heat absorbing black stickers.

In a RCBD replicated five times, the first treatment was a cluster of four 13 mm black stickers (DIA 13 mm, Tower Muizenberg, SA) applied on 14 Jan. 2015 on randomly selected sun exposed 'Laetitia' plums without prior sunburn. Laetitia was chosen as it is a susceptible cultivar, whose maturity coincides with the onset of heatwaves in the Western Cape Province (De Kock, 2012). Each replication consisted of 10 fruit, to give a total of 50 fruit per treatment.

The second treatment consisted of selecting an equal number of fruit at harvest (20 Jan. 2015) that developed sunburn naturally. The last treatment, a control, had fruit free of sunburn selected at harvest. FST and pulp temperature were measured the following day between 12h00 and 14h00 midday. Pulp temperature was measured by means of thermocouples (C22, Comark Instruments, Beaverton, USA).

The 'Laetitia' orchard used was planted in 1998 on 'Mariana' rootstock. Trees were trained to a palmette system. The planting distance was 4 m between the rows and 1.25 m within the row in a NE to SW row direction. Full bloom was attained on 15 Sept. 2014.

Fruit pulp concentration of the antioxidants glutathione and ascorbic acid were assayed using a high performance liquid chromatography (HPLC) autosampler (Series 1100, Agilent Technologies, Inc., Waldbronn, Germany) according to Davey et al. (2003), and adjustments by Jooste (2012). Glutathione and ascorbic acid exist in reduced and oxidised forms (Foyer, 1993). However, only the reduced antioxidants (RA) can be directly determined by the HPLC. To determine oxidised forms of the antioxidants (OA), they have to first undergo reduction. The resultant analysis would therefore give total reduced and oxidised antioxidant concentration (TAC). A sub sample directly determining RA allows computation of the quantity of OA by the following equation:

$$OA = TAC - RA \quad \text{(Equation 1).}$$

To directly determine RA, a milled liquid-nitrogen preserved and frozen (-80 °C) 5.0 g fruit pulp sample from each tree plot was added to 10 ml of extraction buffer (3% metaphosphoric acid, 1mM ethylenediaminetetracetic acid, 2% polyvinylpolypyrolidone, MQ water) kept at 4 °C, and stirred well. The mixture was left to stand for 20 mins at 4 °C before extracting 1.8 mL into a microtube and centrifuging at 20 000 rpm at 4 °C in a centrifuge (Eppendorf 5417R,

Hamburg, Germany). A supernatant of 1.0 mL was transferred to a 1.5 mL microtube before further centrifuging under the same conditions to get a clearer supernatant (CS). From the CS, 0.6 mL aliquot was transferred to the autosampler for analysis. TAC was determined by adding 40 µL of CS to 20 µL of 400 mM DL-dithiothreitol in 400 mM Trisma base. This was stirred well and left to stand for 20 mins at room temperature. The reduction reaction was then terminated by adding 20 µL of 8.5% ortho-phosphoric acid. This was transferred to the autosampler for analysis.

A twenty fruit sample from each tree plot was subjected to cold storage and assessed for post harvest internal disorders as described for 'African Delight'. Glutathione and ascorbic acid were assessed as previously described for the samples at harvest.

RESULTS

Trial 1

Average hourly ambient temperature and irradiance over the whole growing season of 2013/14 and 2014/15 are shown in Figure 2 and 3, respectively. Figure 4 (a and b) show the progression of fruit surface temperature (sun exposed positions) and canopy weather conditions within a typical field measurement period, averaged over four measurement days. Figure 5 and 6 further depict the typical irradiance incident on fruit and the subsequent FST respectively within specific canopy positions (lower, mid and upper canopy) and row sides (NE and SW).

The NE row side received more light than the SW side early in the day, with the mid and lower canopy positions only becoming shaded just before midday (Figure 5). Full sunlight, still persisted on the upper NE canopy for two to three hours after midday. At this point, more light would have shifted to the SW side with the upper canopy receiving full light from midday, lasting more than five hours. Consequently, high FST prevailed early in the day on the NE row side while on the SW side peak temperatures were only observed after midday (Figure 6). In general, PPF decreased from upper to lower canopy (Figure 5).

Fruit that developed sunburn received an average PPF greater than 50% of the full sun while average FST within the field measurement period exceeded 35 °C (Figure 7). However, sunburn was predominantly of the low severity class. High severity sunburn was almost negligible with no observed incidences of unmarketable fruit (Table 1). Sunburn incidence in

the upper and mid canopy did not differ significantly on the NE row side but was significantly higher than that observed in the lower canopy. Contrary, on the SW side sunburn incidence in the lower and mid canopy did not significantly differ from each other, but was significantly lower than that in the upper canopy. Regardless of row side, an increase in canopy height resulted in an increase in fruit diameter, fruit weight, fruit red colour and TSS, albeit not always significant (Table 2). Flesh firmness and TA remained unchanged except on the SW side where fruit on lower canopy height were firmer than on the mid canopy position (Table 2). After cold storage, no post-harvest internal disorders were observed.

Trial 2

Early summer pruning improved early light penetration and enabled adequate vegetative regrowth for filtered light during the months of January and February, the hottest part of the season. Fruit in the upper NE canopy received about 60-100% of full sunlight before midday. About two hours after midday, when the shade was cast on the NE row side, PPF reaching the fruit in the upper canopy dropped significantly and fluctuated between 2-5% of full sunlight. In the mid and lower canopy, PPF reaching the fruit on average increased from 7% in the morning to about 85% by midday before sharply dropping to less that 5% of full sunlight for the remainder of the day. On the SW row side, PPF varied between less than 1% (lower canopy) and 20% (upper canopy) of full sunlight before midday. After midday, the upper canopy fruit received 70-100% full sunlight while the mid and bottom canopy fruit received between 40-50%.

Fruit in the late pruning treatment were exposed to PPF between 80-100% full sunlight in the upper canopy during the hottest part of the season. Mid and lower canopy fruit experienced about four hours of around 50% full sunlight before and after midday on the NE and SW row side, respectively.

Control trees were characterised by excessive vegetative growth and fruit were mostly shaded. The shade was intensified by water shoots, which were predominantly located in the upper canopy. PPF reaching the fruit generally stayed below 30% full sunlight by the hottest part of the season. However, depending on angle of the sun, occasional sun fleck discrepancies were noted where 50-60% of full sunlight would be received within the canopy.

The no pruning control had lower sunburn compared to the summer pruning treatments (Table 3). Delayed summer pruning resulted in more sunburn than pruning early in the season.

Although the statistical difference was barely non-significant (p=0.0560), the proportion of unmarketable sunburn in 2013/14 was higher for the late pruning treatment than that of early pruning and no pruning control (Table 3). In 2014/15, late pruning had resulted in higher sunburn than the no pruning control in the upper and mid canopies but there we no significant differences under the shade net (Table 4). Low severity sunburn was the most predominant while unmarketable sunburn was least prevalent. The unmarketable sunburn was significantly lower under the 20% shade net compared to the unshaded treatment in the upper and mid canopies.

The no pruning control had smaller fruit with poor red colour and lower TSS compared to the summer pruning treatments in both seasons (Tables 5, 6 and 7). There were no significant differences in these fruit quality attributes between the summer pruning treatments in 2013/14 (Table 5). In 2014/15, early summer pruning resulted in better red colour development and higher TSS but significant differences for red colour development were only observed in the upper canopy (Tables 6 and 7). No significant differences in fruit diameter and fruit weight were observed (Tables 6). There were no significant differences in TA between the control and summer pruning treatments in both seasons (Table 6). There were no internal cold storage disorders observed after cold storage in both seasons.

The maximum PPF under the shade net was approximately 83.5% of full sunlight. On average, this decreased FST between 1-2 °C. Fruit under the shade net therefore received lower PPF and generally had lower FST compared to the no shade control. A comparison of FST and PFF for the no net control and shade net for the different treatments and canopy positions is shown in Table 8.

At Welgevallen farm, the heat absorbing black stickers increased FST and fruit pulp temperature significantly while fruit showing no sunburn symptoms had significantly lower temperatures compared to other treatments (Table 9). The fruit used were of comparable size as there were no significant differences in fruit diameter and fruit weight (Table 10). The heat absorbing stickers and natural sunburn treatments were associated with low firmness, TA and high TSS (Table 10). The fruit peel around the heat absorbing black stickers showed sunburn necrosis (Figure 8).

The total pitburn observed in all treatments was negligible. Normal fruit had a significantly higher incidence of gel breakdown or internal browning compared to the sunburnt fruit

although these were predominantly low in severity (Table 9). As for the fruit treated with heat absorbing stickers, no clear flesh breakdown was observed apart from the dark brown flesh discolouration due to thermal damage below the stickers.

At harvest, fruit that had the heat absorbing black stickers had significantly higher ascorbic acid concentrations (Table 11). Sunburnt fruit had slightly higher total ascorbic acid concentrations than fruit that did not develop sunburn although they did not differ significantly. No significant difference was observed in the concentration of the reduced form of the ascorbic acid. However, for the oxidised form of ascorbic acid, fruit from the heat absorbing stickers had significantly higher concentration while the unburnt, control fruit had the lowest. There were no significant differences observed in all forms of glutathione (Table 11).

After cold storage the concentrations of both ascorbic acid and glutathione were notably lower than at harvest. There were no significant differences in total and reduced ascorbic acid concentrations (Table 11). The oxidised ascorbic acid concentration of the fruit covered with heat absorbing stickers was still significantly higher than the control but did differ significantly from that of sunburnt fruit. As was the case at harvest, there were no significant differences in the concentrations of all forms of glutathione (Table 11).

DISCUSSION

Fruit surface temperature and the subsequent development of sunburn and red colour was a function of PPF reaching the fruit. In addition, fruit size, and TSS also seemed to be influenced by canopy position and hence light availability. Although not always statistically different, an increase in canopy height, and therefor light exposure, resulted in an increase in fruit diameter, fruit weight and TSS, regardless of row side. However, significant differences for these parameters were almost always observed between the upper and lower canopies. With the well documented consistent phenomenon of a decrease in light with a decrease in canopy height, the observed response of the fruit quality parameters to canopy height can therefore be attributed to light availability. TA, however, seemed less responsive to any of the light changing variables.

Pre-harvest red colour development in some Japanese plums is due to the presence of the light dependent pigment anthocyanin (Jackson, 1980). However, in other cultivars, red colour can still develop in the absence of light, particularly during post-harvest cold storage (Allen, 1932).

Murray et al. (2005) reported the importance of canopy position and light exposure for red colour development in 'Laetitia' plums. In our study on 'African Delight', PPF generally decreased from upper to lower canopy position as described by Barritt et al. (1987), Warrington et al. (1996) and Fouché et al. (2010). Therefore fruit in the exposed upper canopy were redder. The same was observed for summer pruning treatments which resulted in redder fruit compared to the no pruning treatment due to increased light penetration into the tree canopy.

However, sunburn may develop if exposure to light exceeds a certain threshold and is accompanied by high temperature. In our study, fruit that developed sunburn received an average PPF greater than 50% of full sunlight while FST within the measurement period exceeded 35 °C. These fruit were more prevalent in late summer pruned trees and upper tree canopies. The fruit was larger, with lower firmness and higher TSS. It was previously reported that an increase in light reaching the fruit increased TSS and fruit size in stone fruit (Marini et al., 1991; Muleo et al., 1994; Murray et al., 2005). This could be in response to enhanced carbon assimilation and partitioning to the fruit (Murray et al., 2005). Similar effects of light exposure on fruit quality have been reported in pome fruit (Hamadziripi et al., 2014) although sunburn was consistently associated with increased flesh firmness in apples (Makeredza et al., 2013).

While sunburn reduces fruit market value (Bergh et al., 1980), the concomitant low flesh firmness observed in our study further reduces fruit quality by shortening shipping and storability windows. Murray et al. (2005) indicated that light advances maturity in stone fruit and fruit that grow in more exposed canopy positions have less firm flesh compared to the shaded ones at harvest. According to Manganaris et al. (2008), shading can delay maturity in plums by 10-14 days. Therefore, to avoid shipments of variable maturity and quality, growers must always maintain selective early picking of upper canopy or more exposed fruit.

Fruit on the NE row side attained the threshold temperature more gradually early in the day. The temperature persisted for about three hours at most. Conversely, on the SW row side FST only steeply increased beyond 35 °C just after midday, but continuing until sundown. In the 2013/14 season, sunburn incidence was higher on the NE row side compared to the SW side while in 2014/15 there was no significant difference. However, in both seasons, fruit on the upper SW side consistently had more severe sunburn. The observed high sunburn severity on the SW row side, particularly the upper canopy fruit, can therefore be attributed to more

prolonged PPF exposure and high FST during the hottest part of the day. Proper light manipulation strategies to ameliorate the sunburn-inducing environmental conditions while maintaining red colour, fruit size and TSS are therefore important.

Different pruning strategies were adopted to manipulate vegetative growth, and subsequent light reaching the fruit. In addition, shade net was incorporated during the hottest part of the season in 2014/15. Although summer pruning improved fruit red colour and increased TSS, delaying it increased sunburn by suddenly exposing more mature and therefore more sunburn-susceptible fruit to high irradiance. Sudden exposure of fruit to irradiance in apples has been associated with a form of sunburn known as photo-oxidative sunburn, which involves bleaching of fruit peel pigments (Felicetti and Schrader, 2008). We did not observe this form of sunburn in plums as all fruit either had sunburn browning or necrosis.

Unpruned trees had smaller fruit compared to the summer pruning treatments. There was no significant difference in fruit size between pruning treatments although we expected late summer pruning to reduce fruit size. Day et al. (1995) observed that summer pruning performed towards harvest resulted in a reduction of fruit size in peaches and nectarines. As the rapid increase in fruit size of stone fruit occurs 4-6 weeks before harvest (soon after pit hardening). The exocarp (peel) and mesocarp (flesh) cells previously formed during the rapid cell division elongate at this stage, thereby dramatically increasing fruit size. The onset of this period coincides with slowing down of shoot growth. Therefore delaying summer pruning, results in insufficient time to regrow adequate vegetative cover.

Early summer pruning permitted adequate vegetative regrowth that filtered excess irradiance during the hottest part of the season. Although we did not measure the vegetative regrowth, Ferree et al. (1984) postulated that the earlier summer pruning is carried out, the more vegetative regrowth occurs. Similar finding were reported by Rom, (1982) and Myers and Ferree (1983). The vegetative regrowth brought about by early summer pruning resulted in lower sunburn and bigger fruit than late summer pruning in 2014/15 season. Awad et al. (2001) reported that light scattering by foliage within the canopy can reduce UV-B radiation by between 48-50% and thus reducing solar injury. In addition, the observed low sunburn could be a result of fruit being able to acclimatise to high irradiance and temperature by being exposed early in the season (Huner et al., 1998; Racskó and Schrader, 2012). The increased fruit size could be the effect of a good balance of leaf to fruit ratio that channels sufficient photosynthates towards fruit growth (Ashraf and Ashraf, 2014).

Control trees were characterised by excessive vegetative growth and fruit were mostly shaded. The shade was intensified by water shoots, which were predominantly located in the upper canopy. Although the disproportionate shading significantly reduced sunburn incidence, it decreased red colour development and reduced fruit size and TSS. This was in agreement with Marini et al. (1991) and Murray et al. (2005) who reported that prolonged shading before harvest drastically reduced fruit size of stone fruit. The growing water shoots can act as strong sinks that compete with the fruit for assimilates and thus reduce fruit size (Stassen, 2014).

The maximum PPF under the 20% shade net installed in the 2014/15 season was approximately 83.5% of full sunlight. This attenuation of PPF reduced sunburn, albeit not significantly, of the later summer pruning treatment, particularly in the upper canopy. Although there were no clear differences in the proportion of low and high severity sunburn classes between treatments, shade net to significantly reduced the unmarketable sunburn in the top canopies.

Murray et al. (2005) showed that light has to be less than 70% to negatively affect fruit quality in ‘Laetitia’ plum. Therefore in future, studies such as this should incorporate a wider range of shading levels. In addition, the shade net was installed towards the end of the season (18 days before harvest). Several stone fruit studies (Kappel and Flore, 1983; DeJong and Doyle, 1985; Marini et al., 1991) suggest that light is most important during the final stages of rapid fruit growth, when pit hardening ends. In our study, shade nets were installed when the rapid fruit growth phase was already underway.

Although most internal physiological disorders in plums manifest after cold storage, internal browning and pitburn originate before harvest and are both related to heat stress during fruit maturation and or a delay in harvest (De Kock and Taylor, 2010). The physiology of these cold storage disorders is based on loss of membrane integrity as a result of oxidative stress and disruptions in lipid constitutions (Sevillano et al., 2009). There were no internal disorders observed in ‘African Delight’ plums at harvest and after cold storage. De Kock (2012) indicated that for internal disorders such as pitburn to occur, air temperatures have to rise above 38 °C. Although the 2013/15 and 2014/15 growing seasons seemed to be fairly mild (hottest temperatures falling under this threshold), ‘African Delight’ seems to be tolerant to internal disorders.

It has been reported that late maturing cultivars are more resistant to gel breakdown and internal browning compared to early cultivars (Abdi, et al., 1997). These disorders were observed in 'Laetitia' plums, while 'African Delight' had negligible incidences. Normal fruit that did not develop sunburn and matured predominantly under shade conditions had a significantly higher incidence of gel breakdown than sunburnt fruit. Fruit subjected to exceedingly high temperatures by the heat absorbing black stickers only had burnt epidermis and discoloured pulp immediately below the epidermis, but no other internal disorders were observed at harvest and after cold storage. It therefore appears that for susceptible cultivars, the higher the temperatures fruit get subjected to during pre-harvest development, the more tolerant they become to post harvest chilling injury disorders. Vlachonasios et al. (2001) controlled chilling injury in tomatoes by heat curing at 42 °C for 36 or 48 minutes before storing the tomatoes at 2 °C for two weeks. This can be attributed to accumulation of heat shock proteins (hsps). The hsps play a chaperone role by mimicking the form and function of proteins that may be denatured by high temperatures (Wang et al., 2004). This maintains membrane integrity and reduces ion leakage, the chief cause of high and low temperature physiological disorders due to oxidative stress.

Apart from hsps profile modification, fruit that is tolerant to chilling injury has been reported to adopt certain antioxidant capacities and lipid compositions (Lurie, 2003). In plums, chilling injury tolerant cultivars have been found to be capable of adjusting their membrane and antioxidants compositions (Jooste, 2012). In our study, we were not able to assess lipid composition. We, however, evaluated fruit pulp glutathione and ascorbic acid levels at harvest and immediately after cold storage. There were no significant differences in the total glutathione concentrations. However, total ascorbic acid increased due to an increase in oxidized ascorbic acid with an increase in pre-harvest temperature. This is consistent with the work of Jooste (2012) who observed an increase in the levels of the oxidised form of ascorbic acid in plums with an increase in temperature when comparing two growing seasons

We therefore suggest that ascorbic acid plays a more important role in thermo-tolerance than glutathionine in plums. Ascorbic acid is the chief water soluble antioxidant in plants and its concentration in plant tissue can be regulated by numerous pathways apart from the major ascorbate-glutathione pathway (Noctor and Foyer, 1998). It has been reported that under adverse conditions such as light or water stress, ascorbic acid scavenge the destructive active oxygen species (AOS) (Noctor and Foyer, 1998), which in this case, cause heat stress symptoms to manifest. An increase in AOS triggers an increase in the concentration of the

antioxidant (Potters et al., 2002), and our results were consistent with this assertion. Also consistent with our findings, Lurie and Crisosto (2005) reported that peaches and nectarines that developed in shaded inner canopy positions had higher incidences of post-harvest chilling injury and internal browning compared to outer canopy fruit exposed to higher irradiance and warmer temperatures. It is therefore possible that an increase in pre-harvest antioxidants and changes in hsps profiles triggered by high pre-harvest orchard temperatures would protectively precondition the fruit against manifestation of the internal cold storage disorders such as chilling injury and gel breakdown. Heat pre-conditioning for cold storage disorders can be as short as 10 minutes at 45 °C (Serrano et al., 2004). However, as was the case with 'African Delight', it was apparent that high pre-harvest temperatures advance maturity as evidenced by significantly lower flesh firmness in the high temperature treatments. TSS was expectedly higher, while TA was lower.

CONCLUSION

Fruit from exposed, upper canopy positions were larger, advanced in maturity but more susceptible to sunburn. Contrary, inadequate light exposure, mostly in the lower canopy resulted in smaller fruit with delayed maturity and less red colour. This indicates the importance of regularising light exposure and temperature for optimum fruit quality. Delaying summer pruning until 40 days before harvest predisposed fruit to sunburn and did not enhance fruit quality. Earlier summer pruning of 70 days before harvest decreased sunburn, increased fruit size and TSS and improved fruit red colour. Therefore there is need for producers to shift to early pruning practices. Abstaining from pruning reduced fruit quality although it reduced sunburn. Shade net during the hottest part of the season reduced PPF and FST and reduced sunburn severity caused by delayed summer pruning particularly in the upper canopy. While it is apparent that the timing of orchard light manipulation is essential, the use of shade net still has to be evaluated over the entire growing season to ascertain its full value over a wide range of cultivars.

'African Delight' plums seem to be tolerant to internal defects such as pitburn and post-harvest chilling injury disorders. Although no pitburn was detected in 'Laetitia' plums, the cultivar seems to be more sensitive to gel breakdown and internal browning. High pre-harvest temperatures triggered production of ascorbic acid which in turn protected the fruit against cold storage oxidative stress that results in chilling injury. However, high pre-harvest temperatures concomitantly caused external defects such as sunburn.

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Table 1. Categorised average sunburn incidence and severity in 'African Delight' plums at Môrelig farm during the 2013/14 season at harvest (17 Feb. 2014).

	Sunburn incidence (%)			
	Low severity	High severity	Unmarketable	Total
NE row side				
Lower canopy	4 ± 2.2	1 ± 1	0	5 ± 2.7
Mid canopy	12 ± 3.3	2 ± 2	0	14 ± 3.7
Upper canopy	13 ± 4.2	0	0	13 ± 4.2
SW row side				
Lower	4 ± 2.2	1 ± 1	0	5 ± 2.2
Mid	5 ± 2.2	0	0	5 ± 2.2
Upper	14 ± 5.2	1 ± 1	0	15 ± 5.0

Table 2. Effect of canopy position and row side on fruit quality of 'African Delight plums' at Môrelig Farm during the 2013/14 season at harvest.

Position	Flesh firmness (kg cm^{-2})	Fruit Diameter (mm)	Fruit Weight (g)	Fruit Peel Colour[z]	Titratable Acidity (%)	Total Soluble Solids (º BRIX)
NE row side						
Lower	7.99 ± 0.24	57.4 ± 0.23	98.5 ± 1.29	8.7 ± 1.30	0.89 ± 0.02	13.7 ± 0.29
Mid	7.70 ± 0.27	59.6 ± 0.54	108.5 ± 2.66	9.2 ± 0.11	0.83 ±0.02	13.6 ± 0.19
Upper	7.52 ± 0.33	60.1 ± 0.19	114.0 ± 1.27	10.3 ± 0.81	0.84 ±0.02	14.6 ± 0.16
SW row side						
Lower	7.70 ± 0.37	58.0 ± 0.67	101.2 ± 3.18	8.3 ± 0.43	0.90 ± 0.03	13.6 ± 0.31
Mid	6.52 ± 0.59	59.9 ± 0.99	110.1 ± 5.08	10.0 ± 0.10	0.88 ±0.04	14.08 ± 0.30
Upper	7.07 ± 0.22	60.5 ± 0.68	115.5 ± 3.54	10.9 ± 0.12	0.86 ±0.04	14.9 ± 0.30

Table 3. The effect of time of summer pruning on sunburn incidence and severity of 'African Delight' plums at Môrelig farm during the 2013/14 season at harvest (17 Feb. 2014). Early pruning and late pruning treatments were carried out on 08 Dec. 2013 and 7 Jan. 2014 respectively.

	Sunburn incidence (%)			
	Low severity	High severity	Unmarketable	Total
Treatments				
Early pruning	8.1 a	2.70 ns	0 ns	10.8 b
Late pruning	11.0 a	6.04	1.25	18.1 a
Control	4.4 b	6.67	0.63	11.7 b
F test	*0.0062*	*0.0614*	*0.0560*	*0.0246*

Table 4. The effect of summer pruning and 20% black and white shade net on sunburn incidence and severity of 'African Delight' plums at Môrelig farm during the 2014/15 growing season at harvest (10 February 2015). Early pruning and late pruning treatments were carried out on 08 Dec. 2014 and 7 Jan. 2015 respectively.

	No net Control				20% black/white shade net			
	Sunburn incidence (%)				Sunburn incidence (%)			
	Low severity	High severity	Unmarketable	Total	Low severity	High severity	Unmarketable	Total
Upper canopy								
Early pruning	8.5 ± 2.4	1.9 ± 1.4	0.7 ± 0.7	10.7 ± 2.6	7.5 ± 2.8	1.9 ± 0.7	0	9.4 ± 3.2
Late pruning	6.9 ± 1.2	5.0 ± 2.1	3.2 ± 1.9	15.1 ± 3.7	8.8 ± 3.1	1.3 ± 0.7	0	10.0 ± 3.2
Control	3.8 ± 1.8	2.6 ± 1.3	0.7 ± 0.7	6.9 ± 1.9	6.9 ± 1.4	1.3 ± 1.3	0	8.2 ± 2.5
Mid canopy								
Early pruning	4.4 ± 3.0	4.4 ± 2.3	0.7 ± 0.7	9.4 ± 5.5	5.7 ± 1.7	1.3 ± 1.3	0	6.9 ± 2.1
Late pruning	4.4 ± 1.7	4.4 ± 0.7	1.9 ± 1.4	10.7 ± 3.6	10.1 ± 3.1	0.7 ± 0.7	0	10.7 ± 3.4
Control	1.3 ± 1.3	1.9 ± 1.4	0.7 ± 0.7	3.8 ± 3.2	5.7 ± 2.8	0.7 ± 0.7	0	6.3 ± 2.4
Lower canopy								
Early pruning	3.2 ± 1.9	3.2 ± 1.9	0.7 ± 0.7	6.9 ± 3.0	6.3 ± 4.2	0	0.7 ± 0.7	6.9 ± 4.3
Late pruning	3.8 ± 1.7	1.9 ± 1.4	1.3 ± 0.7	6.9 ± 2.3	3.2 ± 1.7	1.3 ± 0.7	0.7 ± 0.7	5.0 ±1.0
Control	4.9 ± 2.3	0.7 ± 0.7	0	5.7 ± 2.8	2.6 ± 1.9	0.7 ± 0.7	0.7 ± 0.7	3.8 ± 1.9

Table 5. The effect of time of summer pruning on fruit quality of 'African Delight' plums at Môrelig farm during the 2013/14 season at harvest. Early pruning and late pruning treatments were carried out on 08 Dec. 2013 and 7 Jan. 2014 respectively.

Treatment	Flesh firmness ($kg\ cm^{-2}$)	Fruit Diameter (mm)	Fruit Weight (g)	Fruit Peel Colour[z]	Titratable Acidity (%)	Total Soluble Solids (º BRIX)
Early pruning	7.71 b	60.6 a	109.6 a	10.8 a	0.91	15.9 a
Late pruning	7.51 b	59.4 a	108.6 a	10.6 a	0.92	15.2 a
Control	8.28 a	57.2 b	101.4 b	9.1 b	0.86	13.4 b
F test	*0.0001*	*0.0002*	*<0.0001*	*<0.0001*	*0.2946*	*0.0055*

[z] Fruit colour was assessed using a 1-12 colour chart where a value of 1 denoted the least coloured fruit (greenest) and 12 the best (reddest).

Table 6. The effect of summer pruning and shade net on fruit size and colour of 'African Delight' plums at Môrelig farm during the 2014/15 season at harvest (10 Feb. 2015). Early pruning and late pruning treatments were carried out on 08 Dec. 2014 and 7 Jan. 2015 respectively.

	No net Control			20% black/white shade net		
	Fruit Diameter (mm)	Fruit Weight (g)	[z]Fruit Colour	Fruit Diameter (mm)	Fruit Weight (g)	Fruit Colour
Upper canopy						
Early pruning	54.6 ± 0.41	105.7 ± 2.50	10.5 ± 0.11	55.1 ± 0.51	102.8 ± 1.19	11.2 ± 0.27
Late pruning	53.9 ± 0.27	102.3 ± 1.79	9.9 ± 0.17	54.0 ± 0.30	96.7 ± 3.42	10.8 ± 0.07
Control	52.8 ± 0.41	98.5 ± 1.01	8.6 ± 0.27	52.1 ± 0.48	94.3 ± 3.14	9.1 ± 0.15
Mid canopy						
Early pruning	53.7 ± 0.19	97.3 ±1.26	9.8 ± 0.17	53.9 ± 0.16	101.1 ± 1.14	10.1 ± 0.11
Late pruning	53.1 ± 0.44	94.6 ± 3.20	10.7 ± 0.33	53.5 ± 0.11	95.4 ± 2.38	10.2 ± 0.26
Control	52.9 ± 0.26	92.3 ± 1.25	7.9 ± 0.22	52.5 ± 0.27	93.7 ± 2.24	9.4 ± 0.23
Lower canopy						
Early pruning	53.2 ± 0.22	95.2 ± 2.72	9.9 ± 0.18	52.4 ± 0.18	97.2 ± 3.41	10.2 ± 0.26
Late pruning	52.6 ±0.49	94.4 ± 4.11	9.4 ± 0.15	52.9 ± 0.48	96.3 ± 2.82	9.8. ± 0.18
control	52.0 ± 0.30	91.5 ± 2.34	7.6 ± 0.23	51.2 ± 0.13	93.5 ± 1.55	8.7 ± 0.23

z Fruit colour was assessed using a 1-12 colour chart (Casselman PL 23, Deciduous Fruit Board, South Africa) where a value of 1 denoted the least coloured fruit (greenest) and 12 the reddest.

Table 7. The effect of summer pruning and shade net on fruit quality of 'African Delight' plums on the South western row side at Môrelig farm during the 2014/15 season at harvest (10 Feb. 2015). Early pruning and late pruning treatments were carried out on 08 Dec. 2014 and 7 Jan. 2015 respectively.

	No net Control			20% black/white shade net		
	Flesh firmness ($kg\ cm^{-2}$)	TSS (º Brix)	TA (%)	Flesh firmness ($kg\ cm^{-2}$)	TSS (º Brix)	TA (%)
Upper canopy						
Early pruning	7.00 ± 0.18	16.9 ± 0.38	1.12 ± 0.01	7.14 ± 0.07	15.8 ± 0.51	1.12 ± 0.03
Late pruning	7.02 ± 0.22	15.2 ± 0.51	1.14 ± 0.03	7.13 ± 0.27	15.9 ± 0.48	1.10 ± 0.03
Control	7.99 ± 0.19	14.1 ± 0.22	1.14 ± 0.02	7.83 ± 0.31	14.5 ± 0.36	1.10 ± 0.02
Mid canopy						
Early pruning	7.18 ± 0.21	15.4 ± 0.28	1.10 ± 0.03	7.31 ± 0.33	15.2 ± 0.11	1.09 ± 0.01
Late pruning	7.52 ± 0.23	14.7 ± 0.17	1.09 ± 0.05	7.30 ± 0.25	14.3 ± 0.10	1.10 ± 0.02
Control	7.91 ± 0.42	13.7 ± 0.33	1.11 ± 0.04	7.87 ± 0.23	13.6 ± 0.40	1.11 ± 0.03
Lower canopy						
Early pruning	7.86 ± 0.40	13.2 ± 0.34	1.13 ± 0.03	7.81 ± 0.34	12.9 ± 0.37	1.10 ± 0.02
Late pruning	7.70 ± 0.31	14.6 ± 0.27	1.13 ± 0.03	7.00 ± 0.18	13.9 ± 0.33	1.09 ± 0.04
control	8.03 ± 0.26	13.7 ± 0.25	1.11 ± 0.02	7.93 ± 0.20	13.1 ± 0.56	1.10 ± 0.03

Table 8. The effect of summer pruning and shade net on fruit surface temperature and photosynthetic photon flux reaching 'African Delight' plums at Môrelig farm during the 2014/15 growing season (07 Feb. 2015). Early pruning and late pruning treatments were carried out on 08 Dec. 2014 and 7 Jan. 2015 respectively. Values are means (n=5) ± SE.

	No net Control		20% black/white shade net	
	FST (°C)	PPF (µmol.m^{-2}s^{-1})	FST (°C)	PPF (µmol.m^{-2}s^{-1})
Upper canopy				
Early pruning	32.1 ± 0.76	1075.6 ± 53.6	31.6 ± 0.65	988.0 ± 51.18
Late pruning	36.2 ± 0.78	1215.1 ± 61.8	34.1 ± 1.07	1049.7 ± 28.4
Control	29.9 ± 0.63	592.4 ± 143.1	29.0 ± 0.56	587.0 ± 66.7
Mid canopy				
Early pruning	29.9 ± 0.43	998.6 ± 55.4	29.5 ± 0.60	942.1 ± 162.3
Late pruning	32.2 ± 0.44	1016.0 ± 74.8	31.2 ± 0.76	985.3 ± 98.6
Control	30.2 ± 0.70	688.2 ± 217.9	29.5 ± 0.39	806.0 ± 66.6
Lower canopy				
Early pruning	28.9 ± 0.42	261.3 ± 66.1	28.6 ± 0.50	265.2 ± 86.0
Late pruning	30.7 ± 0.41	621.7 ± 85.2	30.0 ± 0.35	468.4 ± 161.2
Control	30.2 ± 0.29	407.5 ±145.3	29.6 ± 0.61	398.4 ± 46.3

Table 9. The effect of pre-harvest heat stress on gel breakdown of 'Laetitia' plums from Welgevallen Research farm after cold storage during the 2014/15 growing season.

Treatment	FST	Pulp temperature	Gel breakdown/Internal browning			
			None	Slight	Moderate	Severe
Heat absorbing stickers	47.2 a	42.1 a	100 a	0.0 b	0.0	0.0
Natural sunburn	41.3 b	37.2 b	96.0 a	3.0 b	0.0	1.0
No sunburn (control)	34.1 c	31.5 c	78.0 b	13 a	7.0	2.0
F test	*<0.0001*	*<0.0001*	*<0.0170*	*0.0213*	*0.1647*	*<0.4096*

Table 10. Fruit quality of 'Laetitia' plums subjected to different light and heat exposures at Welgevallen Research farm during the 2014/15 growing season.

	Fruit diameter (mm)	Fruit weight (g)	Firmness (kg cm^{-2})	TSS (º Brix)	TA (%)
Heat absorbing stickers	50.0	84.3	3.94 b	12.9 a	1.606 b
Natural sunburn	50.5	84.0	4.08 b	12.8 a	1.584 b
No sunburn (control)	50.6	84.7	6.47 a	10.9 b	1.892 a
P value	*0.2592*	*0.8356*	*0.0006*	*0.0016*	*0.0012*

Table 11. Effect of heat stress on glutathione and ascorbic acid concentration of 'Laetitia' plums at Welgevallen Research farm during the 2014/15 growing season.

	Total ascorbic acid (µg g^{-1} FW)	Reduced ascorbic acid (µg g^{-1} FW)	Oxidised ascorbic acid (µg g^{-1} FW)	Total glutathione (µg g^{-1} FW)	Reduced glutathione (µg g^{-1} FW)	Oxidised glutathione (µg g^{-1} FW)
At harvest						
Heat absorbing stickers	42.5 a	22.2	20.3 a	22.9	20.0	2.85
Natural sunburn	32.5 b	21.8	12.4 b	22.4	21.1	1.33
No sunburn (control)	30.0 b	20.1	6.15 c	23.7	20.2	3.42
F test	*0.0005*	*0.4551*	*0.0009*	*0.7497*	*0.8241*	*0.2194*
After cold storage						
Heat absorbing stickers	27.9	19.7	8.3 a	15.8	14.0	1.86
Natural sunburn	26.4	19.8	6.2 ab	16.3	14.7	1.61
No sunburn (control)	26.0	21.7	4.6 b	15.2	13.5	1.72
F test	*0.4804*	*0.2383*	*0.0152*	*0.7986*	*0.7252*	*0.8992*

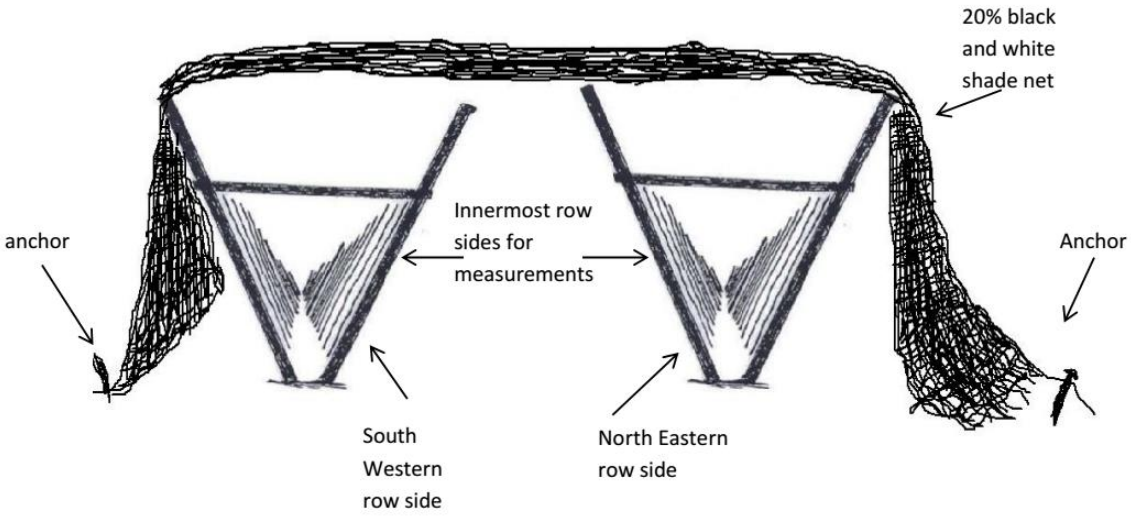


Figure 1. An illustration of how 20% black and white shade net was hung over two staggered V trellised 'African Delight' plum rows at Môrelig farm during the 2014/15 growing season.

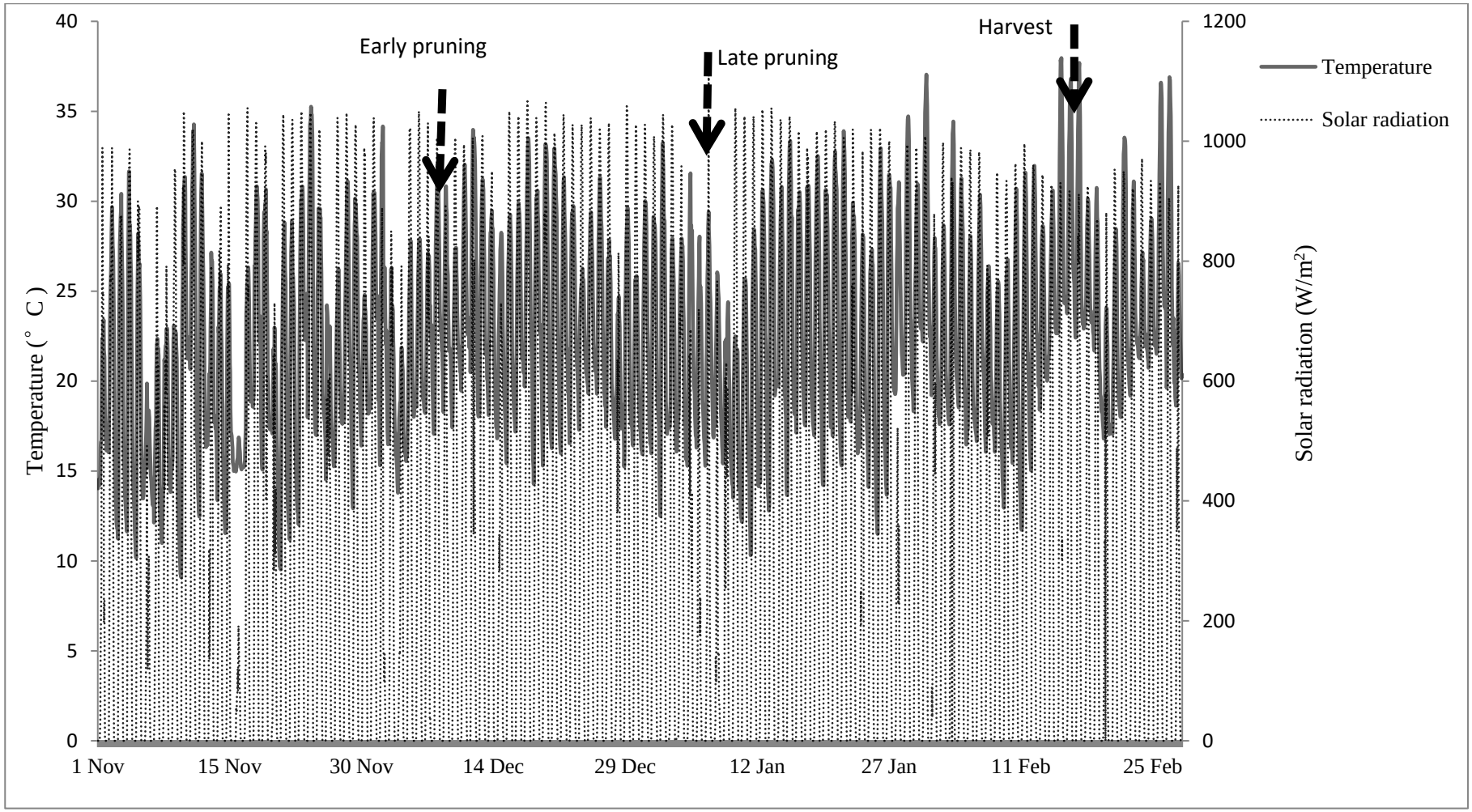


Figure 2. Average hourly temperature and solar radiation data at Môrelig farm in the 2013/14 growing season. Early pruning and late pruning treatments were carried out on 08 Dec. 2013 and 7 Jan. 2014 respectively. Fruit were harvested on 17 Feb. 2014

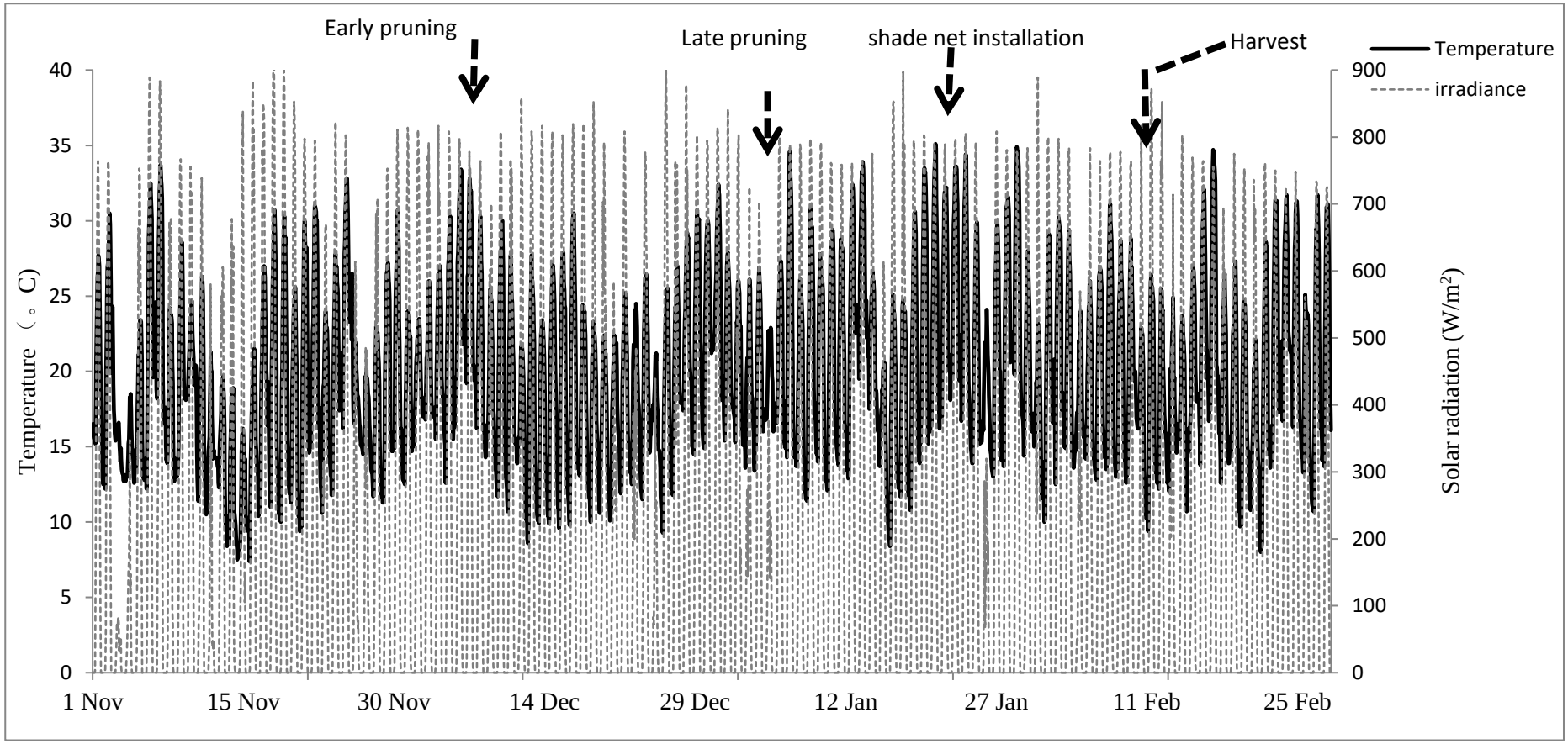
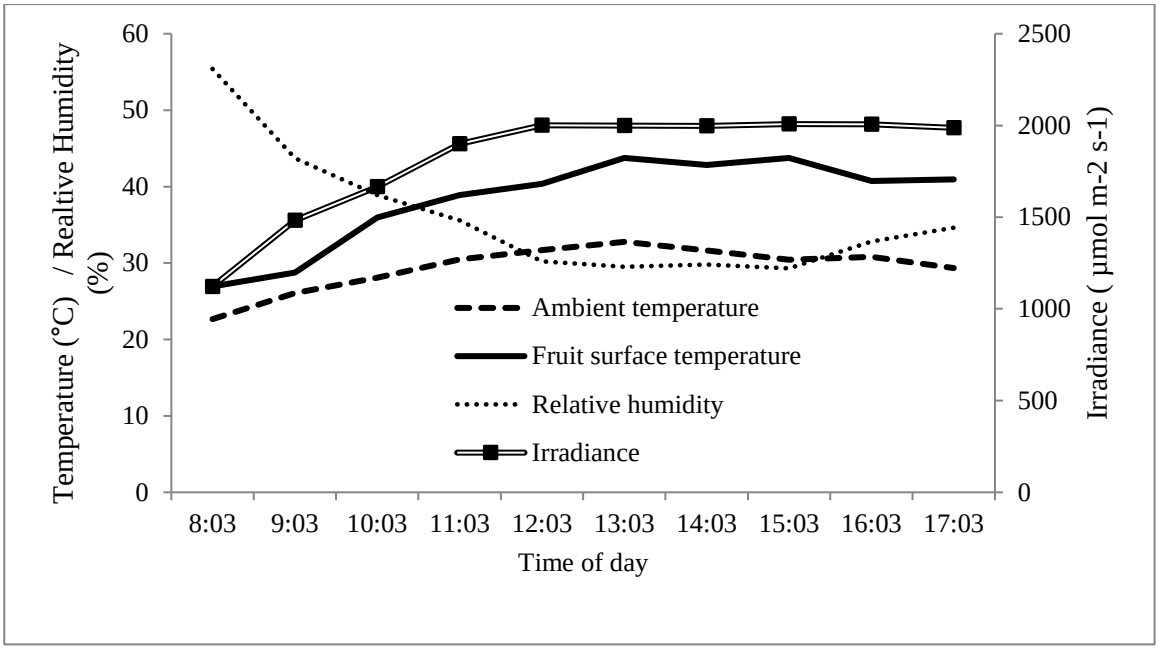
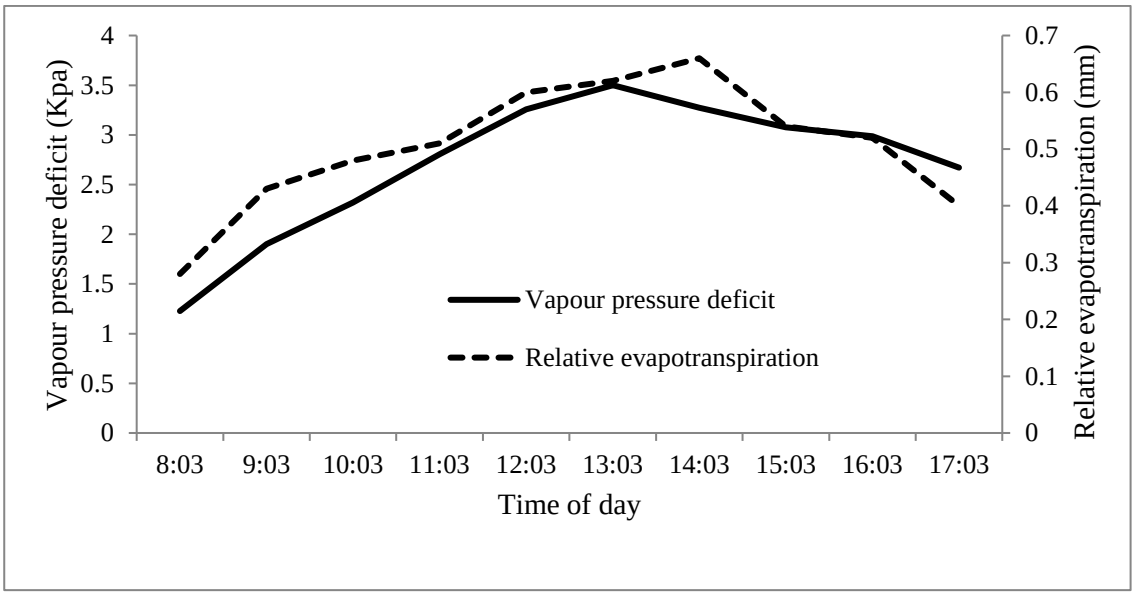


Figure 3. Average hourly temperature and solar radiation data at Môrelig farm in the 2014/15 growing season. Early pruning and late pruning treatments were carried out on 08 Dec. 2014 and 7 Jan. 2015 respectively. Fruit were harvested on 10 Feb. 2015.



(4a)



(4b)

Figure 4. Progression of ambient temperature, fruit surface temperature, relative humidity and irradiance (4a) and vapour pressure deficit and relative evaporation (4b) in the canopy of 'African Delight' plums at Môrelig farm within a typical field measurement period (14 Feb. 2014). Fruit surface temperature measurements are based on sun exposed fruit positions.

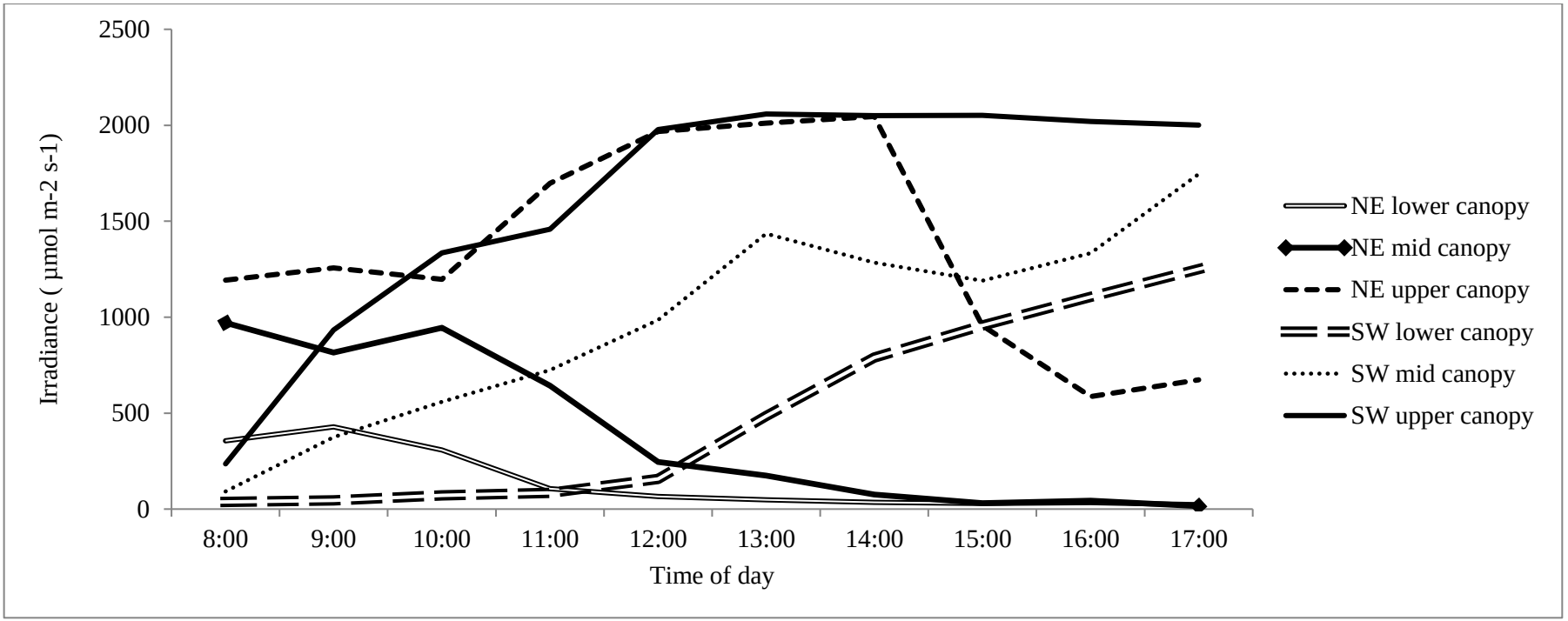


Figure 5. Changes of irradiance incident on the fruit surface of 'African Delight' plums in specific canopy positions and row sides at Môrelig farm within a typical field measurement period during the 2013/14 growing season. NE and SW = North Eastern and South Western row sides respectively. Values are averages of four measurement days (17 Dec., 31 Dec., 14 Jan. and 14 Feb).

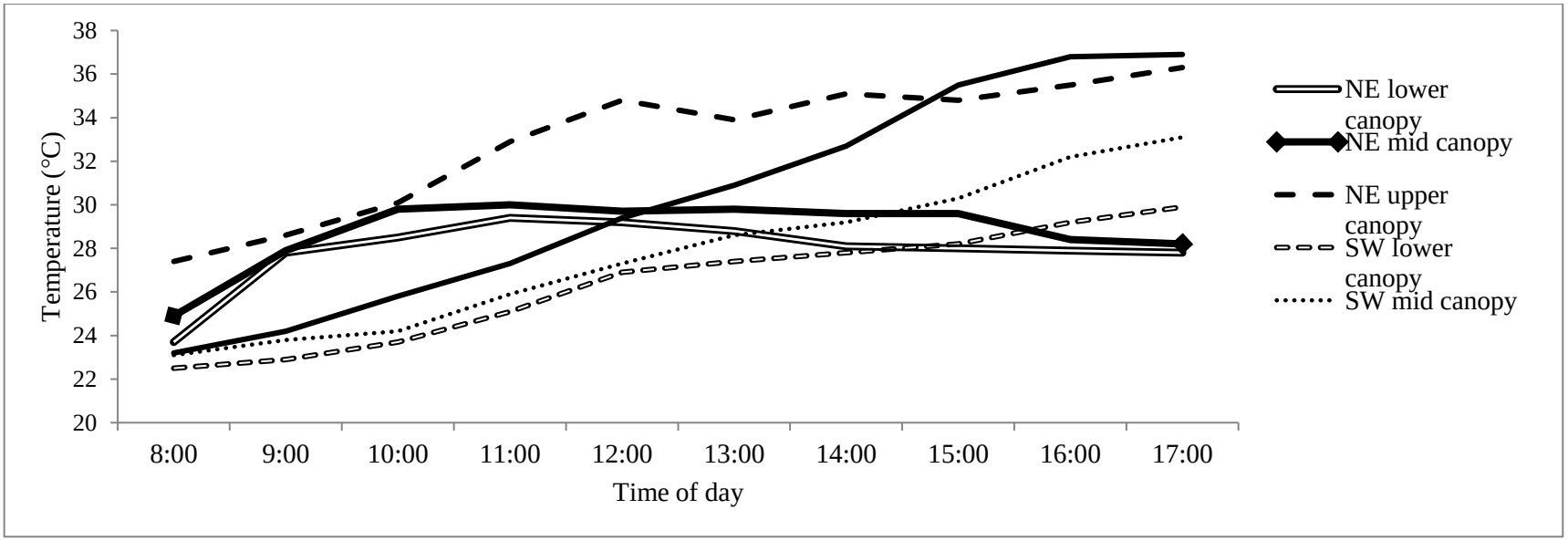


Figure 6. Changes of fruit surface temperature of 'African Delight' plums in specific canopy positions and row sides at Môrelig farm within a typical field measurement period during the 2013/14 growing season. NE and SW = North Eastern and South Western row sides respectively. Values are averages of four measurement days (17 Dec., 31 Dec., 14 Jan. and 14 Feb).

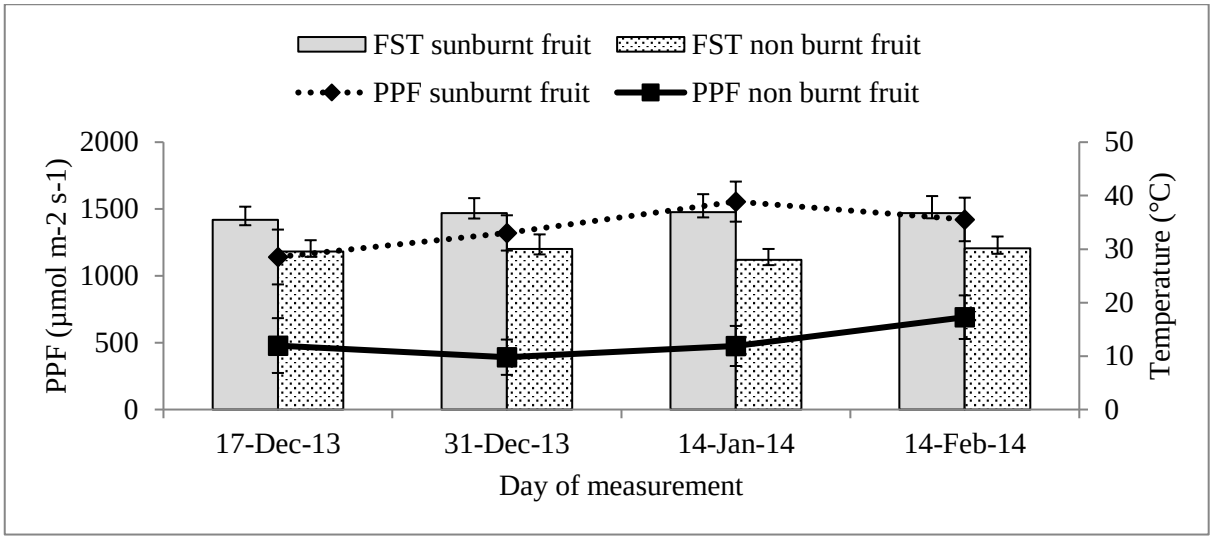


Figure 7 Average photosynthetic photon flux (PPF) and fruit surface temperature (FST) of 'African Delight' plums that did or did not develop sunburn at Môrelig farm during the 2013/14 season. The average full light was 2040.5 µmol m^{-2} s^{-1}. Values are means (n=4) ± standard error of means.

Figure 8. An illustration of the effect of heat absorbing black stickers on ‘Laetitia’ plums from Welgevallen Research farm during the 2014/15 season.

RESEARCH CHAPTER 2

PLANT WATER STATUS AND SUNBURN IN JAPANESE PLUMS (*PRUNUS SALICINA* LINDL.).

Abstract

A study was carried out to investigate the effect of plant water status on fruit surface temperature (FST), sunburn development in Japanese plum. To establish the link between tree water status and sunburn development, irrigation was manipulated at two sites in the Western Cape Province of South Africa, Sandrivier Estate and Welgevallen Research farm in Wellington and Stellenbosch, respectively. Half and double irrigation treatments and a farmer practice control were used on 'African Delight' plums at Sandrivier Estate during the 2012/13 and 2013/2014 seasons. At Welgevallen Eperimental farm, full irrigation (control), half irrigation and no irrigation treatments were used 14 days in the early season (3 to 18 Dec 2013) and again 14 days late in the season (3 to 18 Jan. 2014) on different trees of 'Laetitia' plums in the 2013/14 season. In the 2014/15 season, the same experiment was conducted once late in the season (30 Dec 2014 to 13 Jan 2015) on previously untreated 'Laetitia' trees. Soil moisture, stem water potential (SWP), photochemistry, canopy temperature, fruit quality, FST and sunburn were assessed at all sites. Chlorophyll fluorescence measurements consisting of fruit and leaf potential quantum yield of photosystem II (F_v/F_m)„ actual yield of PSII photochemistry ($^{\varphi}$PSII) and photochemical and non-photochemical quenching were carried out at Welgevallen farm. SWP, flesh firmness and total soluble solids decreased while canopy temperature, FST and sunburn increased linearly with a decrease in irrigation. This was more prominent in the late season. Higher irrigation levels did not result in significantly lower FST and sunburn than the control. Titratable acidity seemed to be insensitive to irrigation levels. There was a general decrease in gas exchange with a decrease in irrigation although there were notable inconsistences between sites and seasons. A significant reduction in (F_v/F_m), with a reduction in irrigation was only observed in leaves after 14 days. A similar and consistent trend was observed in fruit but was not statistically significant. No differences were observed for non-photochemical quenching. There was no evidence for leaf and fruit tissue damage as values for F_v/F_m ranged within 0.7 and 0.8, above the critical threshold of 0.6, below which indicates cellular damage. We ascertained that low water potential reduces photochemical light utilisation but there was no evidence of increased non-photochemical quenching with low irrigation. We concluded that low plant water potential increases FST and sunburn possibly due to canopy heating and loss of convectional cooling and excessive irrigation does not reduce sunburn.

INTRODUCTION

Apart from Chile, South Africa is the main exporter of stone fruit in the southern hemisphere. Of these stone fruit, Japanese plums (*Prunus salicina* Lindl.) represent a considerable share of production and export. Although plums are second to peaches in terms of total planted area and production volume, they are the largest income contributor among stone fruit in the Province, with R1.244 billion per annum (HORTGRO, 2017).

The Western Cape in South Africa has a Mediterranean-type climate characterised by warm, dry summers that can attain daily maximum temperatures of up to 42°C (Tadross and Johnston, 2012). The exposure of plums to these climatic conditions results in discolouration of the fruit peel, a condition known as sunburn. Sunburn renders the fruit less appealing to the lucrative export market, thereby reducing profitability by decreasing export volumes.

Despite plums having a big export share, most of previous research on sunburn focussed on apples. Fruit culling of up to 50% due to sunburn have been reported in apple orchards in the Province (Bergh et al., 1980) while little is known about sunburn incidence in plums. In the apple research, sunburn has been categorised into three types (Felicetti and Schrader, 2008; Schrader et al., 2001). Fruit surface temperatures (FST) between 46 to 49 °C in the presence of light result in superficial sunburn browning. Higher FST up to 52 °C kill epidermal and sub-epidermal tissue leaving dark necrotic spots of sunburn necrosis. At lower FST of about 30 °C, previously shaded fruit suddenly exposed to light suffer photo-bleaching which may become necrotic.

In Japanese plums, damage manifests as a pale yellow discolouration on the fruit peel (sunburn browning). Severe symptoms include dark blotches on the fruit peel which usually crack open (sunburn necrosis). To our knowledge, photo-bleaching has never been observed in plums.

Woolf and Ferguson (2000) considered sunburn as the detrimental effect attained when plants surpass the benefits of exposure to irradiation. Although leaves are the chief photosynthetic organs on plants, fruit peel is also involved in carbon fixing, contributing about 1% in mango (Chauhan and Pandey, 1984), 3% in lychee (Hieke et al., 2002) and up to 10% in peach (Pavel and De Jong, 1993) compared to leaves. The leaves and fruit peel possess light harvesting pigment complexes (LHCs) to absorb photosynthetically active radiation (PAR) that drives photochemistry. Compared to leaves, fruit peel has a lower chloroplast density (Aschan and Pfanz, 2003) and is therefore more sensitive to light stress (Blanke and Lenz, 1989; Hetherington, 1997).

When LHCs in the photosynthetic organs absorb PAR, it excites chlorophyll molecule *a* into a highly energetic singlet state (Müller et al., 2001). This molecule can revert to ground state when the excitation energy is channelled to a photosynthetic reaction centre from where it is used for carbon assimilation. The energy can also be emitted as heat, or re-emitted as light of longer wavelength, in what is known as chlorophyll fluorescence (Maxwell and Johnson, 2000). All these processes occur competitively and a reduction in one would increase the flux of the others.

When photosynthetic organs absorb more PAR than it can utilise in photochemistry, the singlet chlorophyll *a* can dissipate excitation energy by transforming to a triplet state (Müller et al., 2001; Gill and Tujeta, 2010). However, the triplet molecule passes on energy to oxygen-containing molecules, to form highly reactive and hazardous active oxygen species (AOS). These highly reactive AOS degrade cellular components and macromolecules, including photosynthetic pigments and apparatus (Gill and Tujeta, 2010). The major AOS initiating photo-oxidative damage are the superoxide anion O^{-}_{2} and its derivatives and singlet oxygen $^{1}O_{2}$ (Gill and Tujeta 2010; Şen, 2012).

High temperatures impact negatively on photosynthesis because its main components for electron transfer, namely Photosystem I (PSI), Photosystem II (PSII), cytochrome complex and ATP synthase are protein in nature (Mathur et al., 2011). The proteins of PSII have been reported to be very sensitive to heat denaturation (Cheng and Cheng, 2009; Zhang and Starkey, 2009). Rubisco is also inactivated by heat following denaturation of rubisco activase, a protein responsible for release of sugar phosphates from rubisco. In the PSII reaction centre, thermal stress initiates the dissociation of the important manganese-stabilising 33kDa protein, with subsequent release of manganese atoms (Enami et al., 1994). This inhibition of the PSII can further affect the LHC in this reaction centre, aggravating the effects of light stress (Li et al., 2009).

To survive the hazardous effects of high light and temperature, it is vital that plants have efficient photo-thermal protective measures. Thermo-tolerance can be achieved by rapid synthesis and accumulation of a specific set of proteins, the heat shock proteins (hsps) (Iba, 2002). The synthesis of the hsps is regulated by heat stress transcription factors which in turn are controlled by heat shock factor encoding genes (Kotak et al., 2007). The actual mechanism by which hsps effect thermo-tolerance is yet to be understood. However, many studies have indicated that they assume a chaperone role by mimicking the form and function of proteins that might have been denatured by high temperatures (Pitcon and Grierson, 1988; Wang et al., 2004).

Scavenging enzymes and antioxidants such as ascorbic acid and glutathione have the ability to quell the AOS before they cause detrimental effects (Foyer and Mullineaux, 1994). Under more ideal growing and environmental conditions, a delicate balance exists between AOS formation and their degeneration by antioxidants (Gill and Tujeta, 2010). When adverse conditions prevail, the rate of AOS formation exceeds that of quenching by protective compounds (Moran et al., 1994). However, the plant up-regulates the production of these scavengers (Gill and Tujeta, 2010) and measurement of their concentration can give an indication of the magnitude of oxidative stress.

An unequivocal way that plants deal with excessive energy before it initiates the formation of AOS is by thermal dissipation in the xanthophyll cycle located in the thylakoid membrane (Demmig-Adams et al., 1995). The cycle is light dependent and it involves reversible inter-conversion of oxidised carotenoids from one form to the other. At high PFD, violaxanthin, which is a xanthophyll carotenoid, is de-epoxidized to anteroxantin and then to zeaxanthin. Failure of the xanthophyll cycle to dissipate the excess energy might result in degradation and breakdown of chlorophyll causing discolouration of leaves and fruit parts (Lambers et al., 1998) and thus the manifestation of sunburn.

Environmental stresses have been reported to influence the xanthophyll cycle (Demmig-Adams and Adams, 1992). These stresses reduce the ability of photosynthetic apparatus to utilise light in carbon fixing, causing photoinhibition (Manuel et al., 2001). Subsequently, the need to dissipate the excessive energy increases. Water stress affects photosynthesis by impairing the Calvin cycle (Tezara et al., 1999). Under high irradiance and water stress, carbon assimilation therefore would not provide an adequate sink to deal with electrons generated in the electron transport chain (Edreva, 2005). Consequently, the formation of AOS will increase under water stress conditions (Moran et al., 1994). In apples, sunburn incidence was found to increase when withholding irrigation for 14 days (Makeredza et al., 2013). Fruit surface temperatures is to a great extent controlled by irradiation, air temperature and air movement (Smart and Sinclair, 1967; Morandi et al., 2010). In addition, the rate of evapotranspiration determines heat loss from the fruit surface, ultimately affecting its temperature. A decrease in plant water potential is associated with decreased rate of transpiration (Álvarez et al., 2011; Verma et al., 2014). Therefore in addition to inducing photoinhibition, water stress may increase fruit surface temperature due to diminished transpirational cooling. While the photoinhibitory effect of water stress is well acknowledged, the detailed mechanism by which water status affects sunburn has been little studied.

The objective of this study was to investigate the effect of plant water status on photo-thermal tolerance of Japanese plums and how this would affect fruit surface temperature and sunburn development. Our hypothesis was that low plant water status renders Japanese plums more susceptible to photo-thermal damage by increasing photoinhibition in the photosynthetic system and reducing transpirational cooling under adverse summer conditions.

We therefore assessed photochemistry and closely monitored photosynthetic efficiency by evaluating chlorophyll fluorescence at different plant water statuses. In addition, the photoprotective role of antioxidants was investigated.

MATERIALS AND METHODS

The trial was conducted at two different locations, namely Sandrivier Estate (33° 35' S, 18° 55' E) and Welgevallen Research farm (33º 55' S, 18º 53'E) in the Western Cape Province of South Africa. The locations are situated near the towns of Wellington and Stellenbosch, respectively.

Sites and plant material

At Sandrivier Estate, the trial was conducted during the 2012/13 and 2013/14 growing seasons using 'African Delight' plum on Marianna rootstock. The orchard was established in 2008 in North-South row orientation and was trained on a V trellising system inclining 18° off the vertical line. The inclined planes were planted from the same horizontal line with opposite trees staggered at 0.5 m, but the whole orchard plant spacing was 3.5 x 1 m. Blocks used in the trial were on fairly flat terrain although rows were established on ridges about 0.45 m high. The soil in the orchard is shallow clayey albic luvisols of the Kroonstad class (Soil Classification Working Group, 1991). The tree height was maintained at 2.4 m. At the time of trial establishment, the orchard was self-pollinating although 'Pioneer' was later incorporated as cross pollinator at one in every ten trees in the row. Full bloom was attained on 29 Aug. 2012 and 27 Aug 2013 during the 2012/13 and 2013/14 growing seasons, respectively. Fruit were harvested on 15 Feb. 2013 and 07 Feb. 2014.

At Welgevallen Research farm, the experiment was conducted in the 2013/14 and 2014/15 growing seasons using 'Laetitia' plums. The trees on 'Marianna' rootstock were planted in 1998. The soil in the orchard is an Othric Planosol in the Kroonstad class (Soil Classification Working Group, 1991) and has a high clay-content. The trees were trained to a palmette system in a NE to SW row orientation with a planting distance of 4 m between the rows and 1.25 m within the row. The pollinator was 'Songold', which was planted as every tenth tree in the row. Full bloom was attained

on 9 Sept. in 2013/14 and 15 Sept. 2014/15. Harvest dates were 28 and 29 January for the 2013/14 and 2014/15 seasons, respectively.

Treatments and experimental design

Three irrigation treatments, namely low irrigation, high irrigation and control were effected in a randomised complete block design (RCBD) at Sandrivier Estate. The treatment consisted of four tree plots separated by two buffer trees. The treatments were replicated ten times.

The low irrigation treatment had micro sprinklers (Gulf MSCR2002-4002, Agriplas, SA) that delivered water at 20 L h^{-1} (Gulf MSCR 2002) while the high irrigation micro sprinklers delivered 40 L h^{-1} (Gulf MSCR4002). The control was the producer irrigation practice of irrigating at 30 L h^{-1} (Gulf MSCR 3002). All the micro sprinklers had a wetting radius of 1.5 m. The micro sprinklers were placed 1 m apart within the row, with an inter-row spacing of 3.5 m to give a precipitation rate of 5.7 mm, 8.6 mm and 11.4 mm for the low irrigation treatment, control and high irrigation treatments, respectively. The micro sprinklers were approximately 0.5 m on either side of the tree trunk. Irrigation was scheduled whenever necessary using continuous capacitance probe logging data, but was within 10-14 h per week in peak summer season (December-February).

The experiment at Welgevallen Research farm was conducted twice to give early and late season observations during the 2013/14 season. In the early season, single tree plots separated by two buffer trees were subjected to three different irrigation regimes randomised in 8 blocks over a 14 day period running from 3 to 18 Dec 2013. The experimental blocks were restricted to non-sloping terrain to avoid the effect of irrigation water run-off. The control treatment employed the normal irrigation spray nozzle (6 mm h^{-1}). To attain half irrigation, the normal spray nozzles were substituted with ones that delivered 3 mm h^{-1} while stoppers were put in place of nozzles to completely cut off irrigation in the no irrigation treatment (0 mm h^{-1}). A microjet irrigation system was used with microjects placed 0.5 m on either side of the tree. Irrigation was delivered every Tuesday and Friday for 2.5 h.

During the late season period, the experiment was conducted on a different set of trees from 3 to 18 Jan. 2014. In the 2014/15 growing season, the experiment was conducted once on previously untreated trees in the 2014/15 season from 30 Dec 2014 to 13 Jan.

Data were subjected to analysis of variance (ANOVA) by General Linear Methods using SAS version 9.1.3 (SAS Institute Inc. 2003, Cary, USA). Means were separated by Least Significant Difference (LSD) where significant differences occurred at 0.05 level. Single degree of freedom orthogonal linear and quadratic contrasts were fitted for irrigation level.

Measurements

Weather data

Maximum daily temperature, relative humidity and rainfall data for Sandrivier Estate and Welgevallen Research farm were obtained from automatic weather stations at Abendruhe and Helderfontein, respectively. Both weather stations were approximately 5 km away from their respective trial sites.

Vapour pressure deficit (VPD) was computed using the equation

VPD (Pascals) = {1-(RH/100)}*SVP (Equation 1)

Where RH is the relative humidity and SVP is the saturated vapour pressure.

$SVP = 610.7*10^{7.5T/(237.3+T)}$ (Equation 2)

Where T is the ambient temperature.

Hourly canopy temperature at Welgevallen Research farm was logged using thermocron buttons (DS 1922L, Maxim Integrated, California, USA). A total of three buttons were used repeatedly in every experiment, one on each tree of a different treatment. The thermocron buttons, protected from direct sunlight by white wooden lanterns were installed at shoulder height at the centre of the tree canopy.

Soil moisture content

At both sites, soil moisture content of each treatment was assessed using a single soil capacitance moisture probe (DFM Software Solutions, SA). Each soil moisture probe, placed 0.3 m from the tree, was well within the spray radius of the micro sprinkler. To correspond with stem water potential measurements, which were recorded at noon, only midday (12h00) percentage soil moisture content was reported at a soil depth of 40 cm, representative of the core of the rooting zone. Due to limited availability, soil moisture probes were only installed on 03 Dec. in 2012, a week after trial establishment at Sandrivier Estate.

Stem water potential and photochemistry

Midday stem water potential was assessed between 12h00 and 14h00 at both Sandrivier Estate and Welgevallen Research farm. At Sandrivier Estate, three sun-exposed healthy leaves from the middle two trees of each four-tree plot on the western row side were sampled. At Welgevallen Research farm, the leaves were sampled from the NW side. All sampled leaves were from mid canopy (about shoulder height) from fruit bearing wood closest to the tree trunk. Still attached to the branch, each leaf was enclosed in opaque silver bag for an hour before determining its leaf water potential in a pressured chamber (Model 600, PMS Instrument Co, USA).

An equal number of leaves from similar tree positions were used for net carbon assimilation, stomatal conductance and transpiration assessment in an infrared gas analyser (LI-6400, Lincolin, Nedbraska, USA) chamber. Environmental conditions were controlled in the chamber to give a CO_2 concentration of 380 µmol·mol^{-1}, irradiance of 1500 µmol·m^{-2}· s^{-1} and leaf temperature of 25 ^{o}C. Measurements were taken between 07h00 and 10h00 to avoid midday photochemical depression. In the 2012/13 season, the stem water potential and photochemistry measurements at Sandrivier Estate were conducted on 20 and 27 Dec. 2012 and 03, 08 and 10 Jan. 2013. In the following season, measurements were taken on 04, 18, and 30 Dec. 2013, and 19 and 24 Jan. 2014.

At Welgevallen Research farm, measurements were taken for Day 0, Day 7 and Day 14 of the trial period. In the 2013/14 season these days were on 03, 10, and 17 Dec. 2013 respectively for early season observations. For the late season, they were on 03, 10, and 17 Jan. 2014. In the 2014/15 season, measurements were taken on 30 Dec. 2013, 06 Jan. and 13 Jan. 2015, representing Day 0, Day 7 and Day 14, respectively. However, all Day 0 measurements were baseline measurements before treatment to ascertain a uniform starting point. As no significant differences were found in all Day 0 measurements, they are not reported.

Fruit surface temperature

Fruit surface temperature was determined by pointing a hand held infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA) directly onto the fruit surface facing the sun at noon (between 12h00 and 14h00). Ten fruit in sun-exposed positions from each plot were selected on either side of the row at all sites. Assessments were done on the same days as stem water potential and photochemistry.

Sunburn

Sunburn severity was evaluated using an arbitrary 0-5 scale adapted from Schrader and McFerson scale (Schrader et al., 2003) where 0 represented no sunburn and 5 the severest form (Figure 1). We modified this as low sunburn (classes 1 and 2), high sunburn (classes 3 and 4) and unmarketable sunburn (class 5).The low sunburn fruit can still pass for the lucrative export market. High sunburn fruit cannot be sold on the export market but can still be marketed on the local market, albeit for considerably lower prices. The class 5 fruit is rendered unmarketable because of necrotic patches and cracks on the skin surface.

At Sandrivier Estate, sunburn incidence was expressed as the proportion of fruit showing sunburn symptoms out of 50 fruit randomly sampled per tree at harvest during the 2012/13 season. In the 2013/14 season, sunburn incidence was progressively assessed during the season on twenty randomly tagged fruit per treatment plot, ten from each row side. Assessments were made on 04, 18, and 30 Dec. 2013, and 19 and 24 Jan. 2014. At harvest (07 Feb. 2014), all the tagged fruit were included in the 50 fruit sample per treatment plot for assessment. At Welgevallen Research farm sunburn was assessed at harvest as described for Sandrivier Estate 2012/13 season at harvest.

Fruit quality

All fruit quality assessments were conducted on a 20 fruit sample from each plot, 10 on either side of the row. Flesh firmness was measured on one peeled cheek per fruit using a flesh texture analyser (Guss electronic model GS 20, Strand, South Africa) fitted with an 11.1 mm tip. Total soluble solids concentration (TSS) and titratable acidity (TA) were assessed by crushing plum flesh pieces, pooled per treatment replicate, in a blender to extract juice. A hand held refractometer (Model N1, Atago, Tokyo, Japan) was used to measure TSS from the juice. 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). This was expressed as percentage of malic acid (g 100 g^{-1} juice). Fruit colour was assessed using a 1-12 colour chart (Casselman PL 23, Deciduous Fruit Board, South Africa) where a value of 1 denoted the least coloured fruit (greenest) and 12 the reddest.

Antioxidants (Ascorbic acid and glutathione concentration)

Fruit pulp (Sandrivier Estate) and fruit peel (Welgevallen Research farm) concentration of the antioxidants glutathione and ascorbic acid were assayed using a high performance liquid chromatography (HPLC) autosampler (Series 1100, Agilent Technologies, Inc., Waldbronn,

Germany) according to Davey et al. (2003) with adjustments by Jooste (2012) as described in Research Chapter 1.

Chlorophyll fluorescence

Chlorophyll fluorescence measurements were only conducted at Welgevallen Research farm on control and no irrigation treatments due to time limitations. All leaf and fruit samples were measured using a pulse amplitude modulated fluorimeter (FSM 2, Hansatech Instruments, Kings Lynn, UK). Healthy sun exposed samples were taken from the same positions as those sampled for photochemistry and stem water potential around 17h00. Three leaf and fruit samples per plot were taken for measurement of potential quantum yield of photosystem II (F_v/F_m), while only two were sampled for quenching analysis as this was time consuming. Upon sampling, all leaf and fruit samples were immediately put in opaque dark brown bags and further dark adapted overnight at room temperature before taking measurements the following morning at 08h00.

Potential quantum yield of photosynthesis was measured as F_v/F_m, where F_v is variable fluorescence and F_m, maximal flurorescence. Variable fluorescence was calculated at room temperature by subtracting fluorescence yield in absence of PAR (F_0) at a saturating light pulse of 10, 800 µmol m^{-2} s^{-1} for 0.7 s. Actual yield of PSII photochemistry (φPSII) was determined as $(F'_m - F)/F'_m$ by gradually increasing actinic light, but not exceeding 1400 µmol m^{-2} s^{-1} PFD. Transitory alternation of actinic light with far red radiation for 5 s was used to obtain F'o. Photochemical quenching (q_p) was thus calculated as $(F'_m - F)/(F'_m - F'_o)$, with non-photochemical quenching (q_{np}) being $1-(F'_m - F'_o)/(F_m - F_o)$. Measurements were taken on day 8 and day 15.

RESULTS

Weather data

Maximum daily temperature and average irradiance data at Sandrivier Estate for the 2012/13 and 2013/14 seasons are shown in Figures 2 and 3, respectively. The highest temperature recorded in 2012/13 before harvest was 35.5 °C, while in 2013/14 it peaked at 37.8 °C. Vapour pressure data for the two seasons is shown in Figures 4 and 5. Comparing the seasons, irradiance and vapour pressure deficit were similar. Rainfall during peak summer season was negligible and is therefore not reported.

Figure 6 and 7 illustrate maximum daily temperature and average irradiance data at Welgevallen Research farm for the 2013/14 and 2014/15 seasons, respectively. Fruit were exposed to the highest

temperature of 36.1°C before harvest in 2013/14 with a high of 33.7 °C being experienced in the following season. Vapour pressure deficit for both seasons at this site tended to increase towards harvest and is shown in Figures 8 and 9.

Canopy temperatures for the different treatments in the 2013/14 season at Welgevallen Research farm are shown in Figures 10 and 11. The temperatures were invariably similar in this season. However, in the 2014/15 season, the no irrigation treatment occasionally had higher canopy temperatures. These were observed on Days 10, 13, 14 and 15 as illustrated in Figure 12.

Soil moisture content

Percentage soil moisture content data for Sandrivier Estate are illustrated in Figure 13 for the 2012/13 season. The high irrigation treatment with the highest soil moisture content fluctuated around a maximum of 55% and a 45% minimum during the December and January peak summer irrigation period. This was followed by the control within 41-54% during the same period. The low irrigation treatment attained maximum around 40% and a minimum of 29%. After the peak irrigation period, soil moisture content for all treatments gradually declined with the high irrigation treatment still maintaining the highest moisture content, followed by the control and then the low moisture content. However, due to a technical fault on one of the loggers in the 2013/14 season, soil moisture content data for this site is not presented.

As expected, the control consistently had the highest soil moisture content, with the no irrigation treatment having the lowest at Welgevallen Research farm over all the 14-day period experiments. Figures 14 and 15 show early and late season soil moisture content respectively during the 2013/14 season. In 2014/15, the experiment was only conducted late in the season and the percentage soil moisture content is shown in Figure 16. In all cases, peak soil moisture content for the control was around 40%. The half irrigation generally peaked around 35% in all the experiments, but where it was occasionally higher it did not exceed 38%. Soil moisture content for the no irrigation treatment gradually declined reaching minimum levels of around 26% in the early season experiment and 22 % for the later season ones.

Stem water potential

There were no significant trends and differences in stem water potential observed until 10 Jan. 2013 at Sandrivier Estate in the 2012/13 season (Figure 17). However, by 08 Jan. 2013, there was an almost significant linear trend ($p=0.0857$) in which a reduction in irrigation resulted in a reduction

in stem water potential. This trend became significant (p=0.0027) by 10 Jan. 2013. The high irrigation treatment had significantly higher stem water potential than the control and low irrigation treatments. However, although the low irrigation treatment had a considerably lower stem water potential than the control, they did not differ significantly.

In the 2013/14 season, a significant linear trend in stem water potential in response to irrigation was not observed until 30 Dec. 2013 (Figure 18). At this point, a clear linear increase in stem water potential was observed from the high irrigation to the low irrigation treatment.

At Welgevallen Research farm, a reduction in early and late season irrigation resulted in a linear reduction in stem water potential at day 14 in the 2013/14 season (Figure 19). However, the stem water potential of the half irrigation treatment did not differ significantly from that of the control although it was consistently lower. In the 2014/15 season, irrigation was only manipulated during the late season and a similar linear trend of reduction of stem water potential with decreased irrigation was observed as early as day 7 with the control having a higher stem water potential than the two deficit irrigation treatments (Figure 20). The linear trend continued up to day 14, but the control and the half irrigation treatments by then had significantly higher stem water potentials than the no irrigation treatment.

Photochemistry

In the 2012/13 season, there were no significant differences in photochemistry at Sandrivier Estate (Table 1). However, towards the end of the season, net carbon assimilation tended to be low in the low irrigation treatment. Clear linear trends in stomatal conductance and transpiration were observed on 20 Dec. 2012 and 03 Jan. respectively whereby both parameters increased from low to high irrigation treatments. In the 2013/14 season, the effect of irrigation on net carbon assimilation was only apparent by 19. Jan 2014 (Table 2). Net carbon assimilation, stomatal conductance and transpiration all followed a significant linear trend of increasing with an increase in irrigation. Comparing the treatments, the low irrigation treatment had significantly lower net carbon assimilation than the control and high irrigation treatments. The high irrigation treatment had a higher rate of transpiration than the low irrigation treatment. The control did not differ significantly with either treatment.

In the 2013/14 season, there was a linear reduction in photosynthesis with a decrease in irrigation in both the early and late season at Welgevallen Research farm (Tables 3 and 4). There were no

significant differences observed in transpiration and stomatal conductance in this season (Tables 3 and 4).

There was a linear reduction in photosynthesis, stomatal conductance and transpiration with a decrease in irrigation by Day 14 in the 2014/15 season (Table 5). This trend was observed as early as Day 7 for photosynthesis.

Chlorophyll fluorescence

Due to time constraints, all chlorophyll fluorescence measurements were done only on the no irrigation and control treatments. The leaves of control treatment consistently had a higher maximum quantum yield of Photosystem II (F_v/F_m) by Day 14 in all seasons (Tables 6, 7 and 8). However, in 2013/14 season, this was observed by Day 7 in early season measurements (Table 6). Although the F_v/F_m of the control was always slightly higher than the no irrigation treatment, except on Day 14 in early season (Table 6) no significant differences were observed for fruit at any point. Significant differences between treatments for photochemical and non-photochemical quenching of fluorescence were not observed either (Tables 6, 7 and 8).

Fruit surface temperature

A significant linear increase in FST with a reduction in irrigation was observed by 27 Dec. 2012 at Sandrivier Estate (Figure 21) in the 2012/13 season. This trend discontinued as there was neither a significant linear fit nor significant treatment differences by 03-08 Jan. 2013. However, the previously observed linear trend resumed on 10 Jan 2014. FST for the control and high irrigation treatment did not significantly differ, but were significantly lower than that of the low irrigation treatment at this point

In the 2013/14 season, a significant linear increase in FST with a reduction in irrigation was observed by 30 Dec. 2013 and it persisted until 24 Jan. 2014 (Figure 22). However, significant treatment differences only first became apparent on 19 Jan. 2014. Fruit on the low irrigation trees had significantly higher FST than those on the control and high irrigation trees. The same observation was seen on 24 Jan. 2014.

At Welgevallen Experimantal Farm, early season irrigation manipulation in 2013/14 did not result in differences in fruit surface temperature (Table 9). Late in the season, there was a linear increase in fruit surface temperature with a reduction in irrigation although on day 7 individual treatments

did not differ significantly (Table 9). At day 14 the no irrigation treatment had significantly higher fruit surface temperature than the control. The half irrigation treatment was intermediary and it did not significantly differ with either treatment. In the following season, the same linear trend as the previous season was observed on day 14 (Table 10). There was no significant difference between fruit surface temperature for the control and half irrigation treatments, however, these treatments had significantly lower fruit surface temperature than the no irrigation treatment.

Sunburn

The low sunburn category was the most predominant, while the unmarketable sunburn had the lowest incidence at Sandrivier Estate in the 2012/13 season. All sunburn classes exhibited a linear trend whereby a reduction in irrigation increased sunburn incidence (Table 11). The low irrigation treatment resulted in higher sunburn incidence of the low sunburn class compared to the high irrigation treatment. The control did not significantly differ with either treatment. There were no significant individual treatment effects observed for the high sunburn class. The low irrigation treatment resulted in significantly higher unmarketable sunburn than the control and high irrigation treatment. Ultimately, the low irrigation treatment had significantly higher total sunburn incidence compared to the control and high irrigation treatments whereas the latter two did not differ significantly.

In the following season, sunburn was progressively assessed on the same tagged fruit throughout the season at Sandrivier Estate. Treatment effects were first observed on 30 Dec. 2013 (Table 12). From this point until 24 Jan. 2014, sunburn increased linearly with a reduction in irrigation. The low irrigation treatment consistently had higher sunburn incidence than the control and high irrigation treatments which never differed significantly. During this period, sunburn incidence increased for all treatments. However, sunburn incidence for the control and high irrigation treatment increased at a faster rate than that of the low irrigation treatment, but both remained lower than it. As was the case in the previous season, the low sunburn class was the most predominant, followed by high sunburn class and then unmarketable sunburn.

Almost similar observations were noted at harvest (Table 13). Although there were no significant treatment differences and significant trends that could be fitted for the different sunburn categories, a reduction in irrigation quadratically increased total sunburn incidence. Once more, although the high irrigation treatment had lower sunburn than the control, it did not significantly differ from it.

At Welgevallen Research farm, there was no effect on sunburn due to early season irrigation manipulation in the 2013/14 season (Table 14). On the other hand, late season water stress linearly increased total sunburn (Table 14). The no irrigation treatment resulted in significantly higher total sunburn incidence than the control. The sunburn incidence for the half irrigation treatment was in between the control and no irrigation treatments, but did not statistically differ from either. The low sunburn class was more predominant in all treatments when irrigation was manipulated in the early season and there were no discernible trends between the sunburn classes. Later in the season, unmarketable sunburn linearly increased with a reduction in irrigation (Table 14).

A similar linear increase in total sunburn with a reduction in irrigation was observed in the 2014/15 season. In this season, there were no significant differences observed in all sunburn classes. However, sunburn was predominantly in the low and high classes (Table 15).

Ascorbic acid and glutathione concentration

In the 2012/13 season, there was a significant quadratic trend among the treatments at Sandrivier Esate where reducing or increasing irrigation beyond the control resulted in an increase in total ascorbic acid concentration (Table 16). Therefore the low irrigation treatment had a significantly higher total ascorbic acid concentration than the control, but did not significantly differ from the high irrigation treatment. The same trend was observed for the reduced form of ascorbic acid although treatments were barely significant ($p=0.0527$) from each other. As for the oxidised ascorbic acid, the concentration increased linearly with a reduction in irrigation. The low irrigation treatment had a significantly higher concentration of oxidised ascorbic acid. No significant trends and treatment effects were observed for all forms of glutathione.

In the 2013/14 season, there were no significant differences observed in all forms of the antioxidant glutathione. Total ascorbic acid in fruit from the low irrigation treatment was significantly higher than that of the control and high irrigation treatments (Table 17). The concentration of the reduced and oxidised ascorbic acid did not differ significantly between the treatments although the concentration of the oxidised form seemed to increase linearly with a reduction in irrigation ($p=0.0624$) (Table 17).

The concentration of glutathione and ascorbic acid in fruit peel at harvest was not affected by early season irrigation manipulation in the 2013/14 season at Welgevallen Research farm except for reduced glutathione (Table 18). The reduced glutathione concentration increased with a reduction in

irrigation. In the late season, the concentration of peel total glutathione and reduced glutathione increased quadratically with a decrease in irrigation (Tables 19). The concentration of the total and reduced form of glutathione for the no irrigation treatment was higher than the control and half irrigation treatments. In both forms of these antioxidants, the control and half irrigation treatment did not significantly differ from each other. There were no significant differences for oxidised glutathione. The control had a significantly higher concentration of total ascorbic acid than the no irrigation treatment. The concentration of total ascorbic acid in the half irrigation treatment did not significantly differ from the latter treatments. There was a quadratic increase in reduced and oxidized ascorbic acid with a reduction in irrigation (Table 19). In 2014/15 season the total and reduced forms of both glutathione and ascorbic acid increased quadratically with a reduction in irrigation (Table 20). No significant differences were observed for oxidized glutathione and ascorbic acid.

Fruit quality

There were no significant trends and significant treatment effects in fruit size, peel colour, fruit flesh firmness, and titratable acidity in 2012/13 season at Sandrivier Estate (Table 21). A significant linear trend and treatment effects were only observed for TSS. A reduction in irrigation linearly increased the concentration of TSS. The low irrigation treatment had significantly higher TSS than the control and high irrigation treatment.

In the following season, there were significant linear trends observed for fruit size, TSS and flesh firmness. Fruit size increased with an increase in irrigation (Table 22). The low irrigation fruit had significantly smaller fruit diameter than the high irrigation treatment while the control was intermediate and did not significantly differ from either treatment. The low irrigation treatment had significantly lower fruit weight than the control and high irrigation treatment. Flesh firmness and TSS increased with a reduction in irrigation. The low irrigation treatment had significantly firmer fruit than the high irrigation treatments. TSS for the low irrigation treatment and control were significantly higher than the high irrigation treatment while titratable acidity remained insensitive to irrigation level.

At Welgevallen Research farm, no early season irrigation effects were observed for fruit size, fruit colour and flesh firmness in the 2013/14 season (Table 23). However, TSS and TA increased linearly with a reduction in irrigation. Late in the season, fruit weight and diameter linearly decreased while flesh firmness, TA and TSS increased with a reduction in irrigation (Table 23). In

2014/15, fruit colour and TA were not affected by irrigation while a reduction in irrigation linearly increased TSS nad flesh firmness while decreasing fruit diameter and fruit weight (Table 24).

DISCUSSION

Syvertsen and Lloyd (1994) explained fluctuations in the plant water status as the primary response to environmental changes and stress. As expected in our study, a reduction in irrigation was reflected by low soil moisture content and subsequently, lower stem water potential. Plants that strive to maintain stable water status even at low soil moisture levels are termed isohydric, while those whose water potential is sensitive to environmental changes are anisohydric (Franks et al., 2007). To our knowledge, Japanese plums have neither been previously categorised as isohydric nor anisohydric. However, the reduction in stem water potential in response to reduced irrigation, particularly towards the end of the season, seem to suggest anisohydric tendencies in 'African Delight' and 'Laetitia' plums.

Although there were inconsistencies between sites and seasons in terms of the response of gas exchange to irrigation, there was a general decrease in gas exchange with a decrease in irrigation. In the 2013/14 season at Sandrivier Estate, there was a general decrease in photosynthesis with a reduction in irrigation, but a prominent and significant drop in photosynthesis was only observed late in the season where the low irrigation treatment had significantly lower photosynthesis than the control and high irrigation treatments. This coincided with an almost significant reduction in stomatal conductance ($p=0.0896$, Table 2) and significant reduction in transpiration rate. Similar relationships were also noted at Welgevallen Research farm.

We can therefore attribute the reduction in photosynthetic rate to stomatal closure triggered by the low plant water status, as documented for many plants (Centrito et al., 2002; Cornic, 2000; Rosati et al., 2006; Rahmati et al., 2015). However, at Welgevallen Research farm in 2014/15 season, we also noted a reduction in photosynthesis at day 7 before we observed a reduction in stomatal conductance and transpiration for the no irrigation treatment.

Vu and Yelenosky (1988) indicated that under moisture stress conditions, there is a reduction in the amount of the enzyme ribulose-1, 5-biphosphate (rubisco). It is well known that plant photosynthetic rates are dependent on the activity of rubisco (Crafts-Brandner and Salvucci, 2000; Vico and Porporato 2008). In addition to reducing quantities of the enzyme, moisture stress partially inactivates the available rubisco, further slowing down carbon assimilation (Vu and Yelenosky, 1988). Tezara et al. (1999) ascribed the reduction in rubisco to a reduction in adenosine triphosphate (ATP).

Our observations therefore confirm the assertion that under low plant water potential, the capacity to use light energy in carbon assimilation is reduced. This reduction in photosynthetic light use causes the prevalence of excess light in the system, a condition referred to as photoinhibition. During photoinhibition, absorbed light energy not used for photosynthesis accrues as heat and increase the levels of AOS (Foyer et al., 1994; Racskó and Schrader, 2012). If not properly dissipated, the energy may become detrimental to plant tissue. According to Pastenes et al. (2005), the extent of photoinhibition is dependent upon the photosynthetic capacity of the plant organ and prevailing irradiance. These authors reported photoinhibition in bean leaves under moisture stress and attributed it to stomatal closure.

Fruit peel has a lower chloroplast density (Carrara et al., 2001; Aschan and Pfanz, 2003) compared to leaves. As such, fruit have a small photosynthetic capacity compared to leaves (1% in mango, Chauhan and Pandey, 1984, and up to 10% in peach, Pavel and De Jong, 1993). The lower photosynthetic capacity of fruit renders them more sensitive to light stress than leaves (Hetherington, 1997; Steyn et al., 2009). On the other hand, due to the small photosynthetic contribution, induced photoinhibition in fruit may not be as important as it is in leaves.

In our study we observed significantly lower leaf maximum quantum efficiency of PSII for the no irrigation treatment by day 14 as indicated by F_v/F_m measurements. However, we did not observe any significant differences between treatments for fruit peel although the F_v/F_m values for the control were consistently higher than for the no irrigation treatment.

Our observations did not give evidence for cellular damage at both the leaf and fruit level. The F_v/F_m values for healthy photosynthesising plant parts range within 0.7 and 0.8 (Ritchie, 2006). Values around and below 0.6 would suggest damage to the photosynthetic system. Although leaves from the no irrigation treatment had significantly lower maximum quantum efficiency of PSII than the control, all observations were within the normal range. It would therefore seem that the ‘Laetitia’ plum trees did not show chronic photoinhibition under the water deficit conditions. Similar observations were reported in sweet orange (Ribeiro and Machado, 2007). This would imply that damage to the photosynthetic apparatus may only occur under extremely prolonged unfavourable conditions.

Yordanov et al. (2000) suggested that the reduction in PSII photosynthetic efficiency under deficit water conditions is a regulatory process aimed at protecting the system from photo-damage. This

triggers an increase in the non-photochemical chlorophyll fluorescence quenching (q_{np}) which dissipates the overloaded light energy as heat (Ruban and Horton, 1995; Schindler and Litchtender, 1996). We did not observe any significant differences in q_{np} between the control and the no irrigation treatment in both leaves and fruit treatments. This could indicate that physiological heat dissipation, particularly for the low water potential trees did not play a major photo-protective role. Numerous photo-protective measures exist for plants and the use of any of these or their combination under adverse light conditions is a complex process that depends on factors such as magnitude of stress, cultivar and ecological conditions (Demmig-Adams and Adams, 2006).

However, we were certain that plant water status, particularly late in the season towards harvest, played a role in influencing fruit surface temperature and eventually sunburn. This was consistent with findings by Makeredza et al. (2013) and Mupambi (2017) where withholding irrigation for two weeks increased fruit surface temperature in 'Cripps Pink' and 'Granny Smith' apples.

The photosynthetic heat overload on fruit increases as the fruit matures. This can be due to a decrease in the fruit peel photosynthetic rates with maturity (Aschan and Pfanz, 2003) and subsequently a decrease in light utilization. With anthocyanin red colour development which intensifies as fruit matures for cultivars such as 'Laetitia' and 'African Delight', light reflectance on fruit surface is decreased (albedo increased), causing an increase in radiant heat absorption. This increases fruit surface temperature, particularly on clear sunny days that mostly prevail during fruit maturation. Evans (2004) also attributed the increase in sunburn as fruit mature to an increase in thermal mass. With a substantially lower surface to volume ratio compared to leaves, fruit surface temperature can be 10-15 °C higher than ambient temperature (Smart and Sinclair, 1976). Micro-climatic conditions affecting heat transfer would therefore greatly influence the ultimate fruit surface temperature. Plant water status contributes to tree micro-climate by affecting canopy temperature.

A decrease in plant water potential is associated with a decreased rate of transpiration as we observed, due to an increase in leaf stomatal closure (Álvarez et al., 2011; Verma et al., 2014). This can result in an increase in canopy temperature (Colaizzi et al., 2012), with subsequent increase in fruit surface temperature due to diminished radiative heat loss to the environment. Using thermal remote sensing imagery, Sepulcre-Canto´ et al. (2006) detected higher canopy temperatures in olive trees under deficit irrigation compared to well-watered ones.

In our study, we did not observe any marked differences in canopy temperature between the irrigation treatments in the 2013/14 season. However in the 2014/15 season, we observed notable differences in canopy temperatures. On occasions, the no irrigation canopy temperature was higher than the control and half irrigation treatments. This was more prominent on Day 8, Day 11 and Day 12. These occasions also coincided with times of high ambient temperatures, irradiation and vapour pressure deficit. These conditions are conducive to induce increases in fruit surface temperature and initiation of sunburn. We therefore cannot rule out the effect of irrigation level on canopy temperature, fruit surface temperature and ultimately the manifestation of sunburn.

To our knowledge, no work has been done on the evapotranspiration from the plum fruit surface. Although stem water potential is important in xylem and phloem flow into fruit (Morandi et al., 2010), it appears it might not be critical in ultimately determining the rate of fruit transpiration in plums and most fruit. The rate of transpiration of most fruit is to a great extent determined by the environmental vapour pressure deficit (Morandi et al., 2010; Léuchadel et al., 2013). This is chiefly because fully developed fruit lack stomata, which degenerate early in fruit development and become lenticels (Burton, 1982; Dietz et al., 1988).

Lenticels have no guard cells that regulate opening and closing in response to changes in plant water status. However, with small cells beneath the epidermis and large intercellular spaces, they are responsible for gaseous exchange and moisture loss between the fruit and the environment (Tamjinda et al., 1992). As the fruit transpiration increases at high vapour pressure deficit, the fruit is capable of drawing more water from the vascular stream to maintain high rates of transpiration. High fruit transpiration is usually maintained at high vapour pressure deficits with the result that fruit shrink if they cannot maintain constant water potential (Morandi et al., 2007).

It is important to note that we did not observe significant differences in FST at harvest after different early season irrigation manipulation treatments at Welgevallen Research farm in 2013/14. Therefore, the early treatments did not predispose trees to sunburn later in the season. However, prolonging deficit irrigation throughout the season, as was the case at Sandrivier Estate, rendered the low irrigation treatment susceptible to sunburn sooner than the control and high irrigation treatments. This seems to suggest there must be a specific threshold period in the season where deficit irrigation begins to predispose fruit to sunburn. This requires further investigation.

At all sites, the low sunburn class was the most predominant, followed by the high sunburn class. Unmarketable sunburn was low, but in cases where it was prevalent it was mostly in the low and no irrigation treatments. Unmarketable sunburn was observed as early as more than a month before harvest at Sandrivier Estate in the low irrigation treatment. In the control and high irrigation treatments, unmarketable sunburn was yet to be observed at this point. Therefore in addition to increasing sunburn incidence, low plant water potential seems to increase sunburn severity.

At Welgevallen Research farm there were no indications of increased oxidative stress for fruit whose trees were subjected to early season low moisture levels compared to the control at harvest. Similar results were reported by Sofo et al. (2005) who observed a decline in AOS and scavenging compounds activity upon re-watering of water stressed *Prunus* hybrids especially under high irradiance.

At all sites, fruit from the low irrigation treatment trees, which eventually had a lower plant water potential and higher fruit surface temperatures, had a significantly higher total ascorbic acid pool at all sites. Several studies have indicated that high antioxidant levels are stress indicators (Lester, 2003; Lurie, 2003; Jooste, 2012). The reduced forms of the antioxidants are the more predominant and active forms in the plant cell (Foyer, 1993). However, in our study we observed an increase in the oxidized form of ascorbic acid with a reduction in irrigation level. An accumulation of this could be a result the antioxidant quenching the AOS, further confirming that these fruit were undergoing stress (Jooste, 2012).

There were no significant differences observed between treatments at Sandrivier Estate for reduced forms of anti-oxidants. However, the significantly higher concentration of the oxidised form of ascorbic acid in the low irrigation treatment observed in 2012/13 season could have been the oxidation of the reduced form under stressful conditions (Léchaudel et al., 2013). At Welgevallen Research farm, the concentration of the reduced form of glutathione for the no irrigation treatment was higher than the control and half irrigation treatments, while that of the ascorbic acid was lower than these treatments. The observed high concentration of reduced glutathione regulates the ascorbate/glutathione cycle by reconverting the oxidised ascorbic acid back to the active reduced form (Gill and Tuteja, 2010).

Studies on apples have indicated that apart from rendering fruit less appealing to consumers, sunburn is associated with other changes in fruit textural and chemical qualities (Klein et al., 2001; Schrader et al., 2009; Makeredza, 2013). At Sandrivier Estate, the low irrigation treatment, which

had higher sunburn than the control and high irrigation, had significantly higher TSS. The same was observed at Welgevallen Research farm in the late season experiments. This was also consistent with findings in apples by Klein et al. (2001) and Makeredza (2013) who reported high TSS in fruit with sunburn. However, high fruit TSS have also been reported under deficit irrigation in various fruit. Rahmati et al. (2015) attributed this to an accumulation of the soluble solids as a result of reduced water movement into the fruit.

The high TSS associated with low irrigation levels could be a result of fruit osmotic adjustment. Léchaudal et al. (2013) indicated that high transpirational water loss at high vapour pressure deficit and low plant water potential concentrates carbon compounds in the fruit. This also supports findings by Bertin et al. (2000) who observed high sugar content in tomatoes exposed to high vapour pressure deficit at low plant water potential. Guichard et al. (2005) further confirmed that such tomatoes lost more water by transpiration than they received into the fruit by the xylem.

Observations for TA were inconsistent. At Sandrivier Estate, TA levels were not associated with sunburn or sensitive to plant water status as we did not observe any significant differences between treatments. However, at Welgevallen Research farm, this was the case only in the early season experiment of 2013/14. In the late season experiments TA decreased with a decrease in water potential.

We expected sunburn to be associated with firmer fruit as reported by Racskó et al. (2005), Schrader et al. (2009) and Makeredza (2013) in apples. Although this was consistent with our expectation in 2013/14 at Sandrivier Estate, we observed lower firmness in sun-exposed fruit being associated with sunburn (Research Chapter 1). It appears in plums an exposure to light advances fruit maturity. Fruit firmness decreases rapidly with an increase in maturity in plums and this could explain the reduced firmness in sunburnt fruit. However, when irrigation was completely withheld for 14 days late in the season at Welgevallen Research farm, fruit that developed sunburn were firmer than fruit from the control and half irrigation treatments. Naor et al. (2004) also reported higher fruit firmness in plums under deficit irrigation. The seemingly high firmness of sunburnt fruit observed in 2013/14 at Sandrivier Estate could also be a direct effect of low fruit water content rather than sunburn. In addition, the increase in firmness with a reduction in irrigation could be related to the correasponding reduction in fruit size. A reduction in fruit size was reported to correlate with an increase of fruit flesh firmness in apples (De Salvador et al., 2006).

CONCLUSION

We conclude that lower irrigation levels increase sunburn in 'African Delight' and 'Laetitia' plums. However, there was no conclusive evidence that water in excess of good normal agronomic practice would decrease sunburn. Proper irrigation management should therefore not be employed as the sole strategy for sunburn reduction. This has to be augmented with management practices such as light manipulation, which are directly aimed at ameliorating the sunburn-inducing environmental conditions. However, our study ascertained the importance of adequate irrigation in maintaining fruit quality attributes such as fruit size.

Physiologically, it appears plums are resilient to the effects of low plant water status due to deficit irrigation early in the season. Notable reductions in stem water potential and photochemistry were only observed towards the end of the season although photochemistry did not seem highly responsive. Thererefore, plums could be semi-anisohydric. However, the magnitude of response to deficit irrigation late in the season was adequate to elevate the fruit heat load, with subsequent development of significantly higher sunburn. Further studies that investigate the effect of irrigation restoration on sunburn after early season deficit irrigation would therefore be worthwhile. Sunburn could have been aggravated by increases in canopy temperature, due to diminished evaporative cooling as a result of reduced transpiration due to low plant water potential.

Although we ascertained that low water potential reduces photochemical light utilisation, there was no evidence of increased non-photochemical quenching in deficit irrigation treatments. However, an increase in the total glutathione and ascorbic acid concentrations in fruit peels from trees of low irrigation levels was an indication of adapting to low water potential and subsequently high light and temperature stress.

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Table 1. Effect of irrigation on photosynthesis, stomatal conductance and transpiration of 'African Delight' plum trees at Sandrivier Estate during the 2012/13 growing season.

	Net CO_2 assimilation rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal conductance ($mol \cdot m^{-2} \cdot s^{-1}$)	Transpiration ($mol \cdot m^{-2} \cdot s^{-1}$)
20 Dec. 2012			
Low irrigation	10.9	0.081	1.23
Control	11.2	0.085	1.48
High irrigation	10.9	0102	1.42
F test	*0.8824*	*0.0811*	*0.1006*
Contrasts			
Linear	*0.9918*	*0.0379*	*0.1147*
Quadratic	*0.6218*	*0.3903*	*0.1325*
27 Dec. 2012			
Low irrigation	10.8	0.084	1.59
Control	11.1	0.082	1.45
High irrigation	11.4	0.094	1.58
F test	*0.8366*	*0.3492*	*0.3367*
Contrasts			
Linear	*0.5587*	*0.2710*	*0.9328*
Quadratic	*0.9418*	*0.3445*	*0.1462*
03 Jan. 2013			
Low irrigation	9.59	0.079	1.35
Control	9.61	0.083	1.42
High irrigation	9.26	0.088	1.60
F test	*0.6852*	*0.6910*	*0.0585*
Contrasts			
Linear	*0.4761*	*0.3994*	*0.0218*
Quadratic	*0.6284*	*0.9229*	*0.5506*
10 Jan. 2013			
Low irrigation	9.50	0.072	1.41
Control	10.51	0.088	1.45
High irrigation	10.23	0.087	1.61
F test	*0.0880*	*0.1836*	*0.1365*
Contrasts			
Linear	*0.1165*	*0.1227*	*0.0613*
Quadratic	*0.1080*	*0.3067*	*0.5161*

Table 2. Effect of irrigation on photosynthesis, stomatal conductance and transpiration of 'African Delight' plum trees at Sandrivier Estate during the 2013/14 season.

	Net CO_2 assimilation rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal conductance ($mol \cdot m^{-2} \cdot s^{-1}$)	Transpiration ($mol \cdot m^{-2} \cdot s^{-1}$)
18 Dec. 2013			
Low irrigation	10.9	0.10	1.17
Control	11.6	0.12	1.39
High irrigation	12.7	0.14	1.51
F test	*0.2163*	*0.1318*	*0.2312*
Contrasts			
Linear	*0.0867*	*0.0474*	*0.0958*
Quadratic	*0.8393*	*0.9700*	*0.7772*
30 Dec. 2013			
Low irrigation	12.7	0.099	1.00
Control	12.2	0.086	1.01
High irrigation	13.3	0.094	1.14
F test	*0.4428*	*0.5742*	*0.3555*
Contrasts			
Linear	*0.4859*	*0.6604*	*0.2132*
Quadratic	*0.2883*	*0.3446*	*0.4782*
19 Jan. 2014			
Low irrigation	9.30 b	0.073	0.666 b
Control	11.10 a	0.082	0.937 ab
High irrigation	11.25 a	0.095	1.246 a
F test	*0.0119*	*0.0896*	*0.0089*
Contrasts			
Linear	*0.010*	*0.0312*	*0.0025*
Quadratic	*0.0768*	*0.8144*	*0.8906*

Table 3. Effect of early season irrigation manipulation on photosynthesis, stomatal conductance and transpiration of 'Laetitia' plum trees at Welgevallen Research farm during the 2013/14 season. The irrigation treatments were effected from 03 to 18 Dec 2013 using 6 $mm.h^{-1}$ spray nozzles (control), 3 $mm.h^{-1}$ (half irrigation) and 0.00 $mm.h^{-1}$ stoppers (no irrigation).

	Net CO_2 assimilation rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal conductance ($mol \cdot m^{-2} \cdot s^{-1}$)	Transpiration ($mol \cdot m^{-2} \cdot s^{-1}$)
Day 7			
Control	18.5	0.048	1.87
Half irrigation	18.2	0.048	1.72
No irrigation	17.9	0.047	1.82
F test	*0.7287*	*0.9540*	*0.7446*
Contrasts			
Linear	*0.6578*	*0.7765*	*0.8734*
Quadratic	*0.4532*	*0.6233*	*0.3419*
Day 14			
Control	17.7	0.039	1.63
Half irrigation	17.4	0.041	1.61
No irrigation	17.0	0.044	1.57
F test	*0.9335*	*0.7160*	*0.9008*
Contrasts			
Linear	*0.8776*	*0.2722*	*0.3106*
Quadratic	*0.7604*	*0.4311*	*0.5974*

Table 4. Effect of late season irrigation manipulation on photosynthesis, stomatal conductance and transpiration of 'Laetitia' plum trees at Welgevallen Research farm during the 2013/14 season. The irrigation treatments were effected from 03 to 18 Jan. 2014 using 6 $mm.h^{-1}$ spray nozzles (control), 3 $mm.h^{-1}$ (half irrigation) and 0.00 $mm.h^{-1}$ stoppers (no irrigation).

	Net CO_2 assimilation rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal conductance ($mol \cdot m^{-2} \cdot s^{-1}$)	Transpiration ($mol \cdot m^{-2} \cdot s^{-1}$)
Day 7			
Control	17.3	0.033	1.21
Half irrigation	17.2	0.038	1.10
No irrigation	16.8	0.040	1.10
F test	*0.4987*	*0.2134*	*0.3567*
Contrasts			
Linear	*0.1004*	*0.2564*	*0.3838*
Quadratic	*0.5120*	*0.7644*	*0.4829*
Day 14			
Control	17.2 a^{z}	0.045	1.43
Half irrigation	16.3 ab	0.041	1.45
No irrigation	15.5 b	0.043	1.52
F test	*0.0331*	*0.9248*	*0.8985*
Contrast			
Linear	*0.0319*	*0.7655*	*0.7211*
Quadratic	*0.1543*	*0.8547*	*0.6755*

Table 5. Effect of irrigation on photosynthesis, stomatal conductance and transpiration of 'Laetitia' plum trees at Welgevallen Research farm during the 2014/15 season. Treatments were effected from 30 Dec 2014 to 13 Jan 2015 using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation).

	Net CO_2 assimilation rate (µmol·m^{-2}·s^{-1})	Stomatal conductance (mol·m^{-2}·s^{-1})	Transpiration (mol·m^{-2}·s^{-1})
Day 7			
Control	17.8 a[z]	0.046	1.35
Half irrigation	17.2 ab	0.042	1.28
No irrigation	16.8 b	0.039	1.24
F test	*0.0215*	*0.2153*	*0.2150*
Contrast			
Linear	*0.0069*	*0.0880*	*0.0875*
Quadratic	*0.6668*	*0.7687*	*0.8075*
Day 14			
Control	18.3 a	0.0497 a	1.672 a
Half irrigation	17.1 b	0.0441 ab	1.538 ab
No irrigation	15.9 c	0.0405 b	1.456 b
F test	*0.0006*	*0.0147*	*0.0209*
Contrast			
Linear	*0.0001*	*0.0045*	*0.0067*
Quadratic	*0.9160*	*0.6946*	*0.6551*

[z] Means in the same column with different letters are significantly different at 0.05 level.

Table 6. The effect of early season two-week moisture stress on leaf and fruit chlorophyll fluorescence of 'Laetitia' plums at Welgevallen Research farm during the 2013/14 growing season. Treatments were effected from 03 to 18 Dec 2013 using 6mm.h^{-1} spray nozzles (control) and 0.00mm.h^{-1} stoppers.

	Leaves				Fruit			
	F_v/F_m	$_{\varphi}$PSII	q_p	q_{np}	F_v/F_m	$_{\varphi}$PSII	q_p	q_{np}
Day 7								
Control	0.83 a[z]	0.159	0.389	0.904	0.824	0.157	0.325	0.833
No irrigatiom	0.80 b	0.153	0.387	0.900	0.818	0.160	0.319	0.826
F test	*0.0141*	*0.7573*	*0.9416*	*0.5600*	*0.5446*	*0.9265*	*0.9361*	*0.7642*
Day 14								
Control	0.824 a	0.167	0.403	0.897	0.813	0.150	0.340	0.859
No irrigatiom	0.801 b	0.146	0.372	0.907	0.813	0.148	0.337	0.877
F test	*0.0155*	*0.1955*	*0.3467*	*0.0921*	*0.9472*	*0.9449*	*0.9743*	*0.5647*

[z] Means in the same column with different letters are significantly different at 0.05 level

F_v/F_m -maximum quantum yield of photosystem II (PSII)

$_{\varphi}$PSII -actual efficiency (photon yield) of PSII photochemistry

q_p -photochemical quenching of fluorescence

q_{np} -non-photochemical quenching of fluorescence

Table 7. The effect of late season two week moisture stress on leaf and fruit chlorophyll flourescence of 'Laetitia' plums at Welgevallen Research farm during the 2013/14 growing season. Treatments were effected from 03 to 18 Jan 2013 using 6mm.h^{-1} spray nozzles (control) and 0.00mm.h^{-1} stoppers.

	Leaves				Fruit			
	F_v/F_m	$_{\varphi}$PSII	q_p	q_{np}	F_v/F_m	$_{\varphi}$PSII	q_p	q_{np}
Day 7								
Control	0.811	0.152	0.398	0.907	0.801	0.211	0.456	0.848
No irrigation	0.804	0.151	0.388	0.910	0.800	0.217	0.475	0.860
F test	*0.4638*	*0.9677*	*0.7429*	*0.9134*	*0.9228*	*0.8142*	*0.7501*	*0.4439*
Day 14								
Control	0.815 a[z]	0.146	0.371	0.919	0.807	0.198	0.392	0.904
No irrigation	0.797 b	0.143	0.354	0.913	0.802	0.203	0.371	0.897
F test	*0.0297*	*0.9110*	*0.2389*	*0.9021*	*0.4319*	*0.7654*	*0.7220*	*0.6786*

[z] Means in the same column with different letters are significantly different at 0.05 level

F_v/F_m -maximum quantum yield of photosystem II (PSII)

$_{\varphi}$PSII -actual efficiency (photon yield) of PSII photochemistry

q_p -photochemical quenching of fluorescence

q_{np} -non-photochemical quenching of fluorescence

Table 8. The effect of two week moisture stress on leaf and fruit photoinhibition of 'Laetitia' plums at Welgevallen Research farm during the 2014/15 growing season. Treatments were effected from 30 Dec 2014 to 13 Jan 2015 using 6mm.h^{-1} spray nozzles (control) and 0.00mm.h^{-1} stoppers.

	Leaves				Fruit			
	F_v/F_m	$_{\varphi}$PSII	q_p	q_{np}	F_v/F_m	$_{\varphi}$PSII	q_p	q_{np}
Day 7								
Control	0.821	0.170	0.412	0.878	0.802	0.143	0.378	0.900
No irrigation	0.818	0.169	0.396	0.891	0.782	0.157	0.389	0.903
F test	*0.9350*	*0.5713*	*0.1347*	*0.3284*	*0.5854*	*0.1603*	*0.5396*	*0.7913*
Day 14								
Control	0.803 a[z]	0.164	0.349	0.896	0.743	0.156	0.417	0.913
No irrigation	0.768 b	0.163	0.360	0.894	0.729	0.155	0.407	0.904
F test	*0.0035*	*0.7004*	*0.5254*	*0.5705*	*0.1551*	*0.9351*	*0.3235*	*0.0929*

[z] Means in the same column with different letters are significantly different at 0.05 level

F_v/F_m -maximum quantum yield of photosystem II (PSII)

$_{\varphi}$PSII -actual efficiency (photon yield) of PSII photochemistry

q_p -photochemical quenching of fluorescence

q_{np} -non-photochemical quenching of fluorescence

Table 9. Effect of irrigation on fruit surface temperature of 'Laetitia' plum at Welgevallen Research farm during the 2013/14 season. Early season treatments were effected from 03 to 18 Dec 2013 using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation). Late season treatments were manipulated from 03 to 18 Jan 2014.

	Fruit surface temperature (°C)	
	Early season	Late season
Day 7		
Control	36.9	34.9
Half irrigation	37.1	35.8
No irrigation	35.4	36.3
F test	*0.2634*	*0.0778*
Contrasts		
Linear	*0.7888*	*0.0286*
Quadratic	*0.1127*	*0.6573*
Day 14		
Control	36.8	34.5 b[z]
Half irrigation	36.6	35.1 ab
No irrigation	37.0	36.3 a
F test	*0.9208*	*0.0201*
Contrasts		
Linear	*0.7936*	*0.0067*
Quadratic	*0.7626*	*0.5508*

[z] Means in the same column with different letters are significantly different at 0.05 level

Table 10. Effect of irrigation on fruit surface temperature of 'Laetitia' plum at Welgevallen Research farm during the 2014/15 season. Treatments were effected from 30 Dec 2014 to 13 Jan 2015 using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation).

[z] Means in the same column with different letters are significantly different at 0.05 level

	Fruit surface temperature (°C)	
	Day 7	Day 14
Control	36.0	37.2 b[z]
Half irrigation	36.9	38.0 b
No irrigation	37.0	39.0 a
F test	*0.1565*	*0.0073*
Contrasts		
Linear	*0.0831*	*0.0021*
Quadratic	*0.3970*	*0.7264*

Table 11. Categorised sunburn incidence of 'African Delight' plums at Sandrivier Estate during the 2012/13 season.

	Sunburn incidence (%)			
	Low sunburn	High sunburn	Unmarketable	Total
Irrigation levels				
Low irrigation	30.5 a	9.75	5.25 a	45.5 a
Control	21.3 ab	8.50	2.75 b	32.5 b
High irrigation	17.8 b	5.25	1.75 b	24.8 b
	0.0467	0.0938	0.0101	0.0032
F test				
Contrasts				
Linear	*0.0176*	*0.0369*	*0.0035*	*0.0009*
Quadratic	*0.5047*	*0.5702*	*0.4163*	*0.5692*

Table 12. Effect of irrigation on sunburn progression of African Delight plums at Sandrivier Estate during the 2013/14 growing season.

	Sunburn incidence (%)			
	Low sunburn	High sunburn	Unmarketable	Total
30 Dec 2013				
Irrigation levels				
Low irrigation	13.1 a	7.7	1.6 a	22.4 a
Control	6.6 b	4.4	0 b	11.0 b
High irrigation	5.8 b	4.8	0 b	10.7 b
F test	*0.0292*	*0.3063*	*0.0410*	*0.0047*
Contrasts				
Linear	*0.0155*	*0.2167*	*0.0276*	*0.0034*
Quadratic	*0.2360*	*0.3580*	*0.1832*	*0.0842*
19 Jan 2014				
Irrigation levels				
Low irrigation	15.6 a	9.9	1.7	27.2 a
Control	9.7 b	6.9	1.1	17.1 b
High irrigation	8.3 b	6.3	0.6	15.8 b
F test	*0.0203*	*0.2309*	*0.6287*	*0.0205*
Contrasts				
Linear	*0.0088*	*0.1780*	*0.3426*	*0.0105*
Quadratic	*0.3035*	*0.2842*	*0.9621*	*0.2240*
24 Jan 2014				
Irrigation levels				
Low irrigation	16.3	10.1	1.8	29.9 a
Control	12.1	8.8	1.7	22.0 b
High irrigation	11.4	8.2	1.1	21.4 b
F test	*0.1329*	*0.6553*	*0.8851*	*0.0326*
Contrasts				
Linear	*0.0662*	*0.5579*	*0.6549*	*0.0188*
Quadratic	*0.4140*	*0.4848*	*0.8454*	*0.2131*

Table 13. Categorised sunburn incidence and severity in 'African Delight' plums at Sandrivier Estate during the 2013/14 season.

	Sunburn incidence (%)			
	Low sunburn	High sunburn	Unmarketable	Total
Irrigation levels				
Low irrigation	21.3	7.92	1.25	30.4 b
Control	17.1	5.83	1.33	23.2 a
High irrigation	16.7	3.75	0.83	21.3 a
F test	*0.3644*	*0.4038*	*0.9128*	*0.0106*
Contrasts				
Linear	*0.2618*	*0.5190*	*0.9524*	*0.0225*
Quadratic	*0.4218*	*0.2564*	*0.6762*	*0.0328*

Table 14. Effect of early and late season irrigation manipulation on categorised sunburn incidence in 'Laetitia' plums at Welgevallen Research farm during the 2013/14 season. Early season treatments were effected from 03 to 18 Dec 2013 using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation). Late season treatments were manipulated from 03 to 18 Jan 2014.

[z] Means in the same column with different letters are significantly different at 0.05 level

	Sunburn incidence (%)			
	Low sunburn	High sunburn	Unmarketable	Total
Early Season				
Irrigation levels				
Control	8.8	3.8	1.3	13.8
Half irrigation	10.0	5.0	0	15.0
No irrigation	10.0	2.5	2.5	15.0
F test	*0.9626*	*0.6802*	*0.2338*	*0.9626*
Contrasts				
Linear	*1.000*	*0.3884*	*0.0939*	*1.000*
Quadratic	*0.7861*	*1.000*	*1.000*	*0.7861*
Late season				
Irrigation levels				
Control	1.3	7.5	2.5 b[z]	11.3 b
Half irrigation	0	6.3	10.0 ab	16.3 ab
No irrigation	3.8	5.0	16.3 a	25.0 a
F test	*0.1776*	*0.8269*	*0.0306*	*0.0355*
Contrasts				
Linear	*0.2159*	*0.5447*	*0.0095*	*0.0185*
Quadratic	*0.1567*	*1.000*	*0.8771*	*0.6812*

Table 15. The effect of irrigation manipulation on categorised sunburn incidence and severity in 'Laetitia' plums at Welgevallen Research farm during the 2014/15 season. Treatments were effected from 30 Dec 2014 to 13 Jan 2015 using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation).

	Sunburn incidence (%)			
	classes 1 & 2	classes 3 & 4	class 5	Total
Irrigation levels				
Control	5.75	3.00	0.50	9.25 b
Half irrigation	7.75	4.75	0.75	13.25 ab
No irrigation	11.25	6.50	1.75	19.5 a
F test	*0.1335*	*0.2638*	*0.4747*	*0.0373*
Contrasts				
Linear	*0.0510*	*0.1087*	*0.2558*	*0.0122*
Quadratic	*0.7418*	*1.000*	*0.6817*	*0.7210*

Table 16. Effect of irrigation level on the glutathione and ascorbic acid concentrations for 'African Delight' plums of Sandrivier Estate assessed at harvest (15 February 2013) during the 2012/13 growing season

	Total glutathione ($\mu g\ g^{-1}$ FW)	Reduced glutathione ($\mu g\ g^{-1}$ FW)	Oxidised glutathione ($\mu g\ g^{-1}$ FW)	Total ascorbic acid ($\mu g\ g^{-1}$ FW)	Reduced ascorbic acid ($\mu g\ g^{-1}$ FW)	Oxidised ascorbic acid ($\mu g\ g^{-1}$ FW)
At harvest						
Low irrigation	21.9	20.7	1.2	63.4 a	48.7	14.7 a
Control	21.9	20.7	2.0	48.4 b	40.8	7.6 b
High irrigation	21.5	19.9	0.8	62.0 a	58.0	4.0 b
F test	*0.9297*	*0.7006*	*0.2840*	*0.0448*	*0.0527*	*0.0031*
Contrasts						
Linear	*0.7610*	*0.9944*	*0.6249*	*0.8163*	*0.1700*	*0.0009*
Quadratic	*0.8240*	*0.4054*	*0.1346*	*0.0143*	*0.0396*	*0.4600*

Table 17. Effect of irrigation level on the glutathione and ascorbic acid concentrations for 'African Delight' plums of Sandrivier Estate assessed at harvest (07 February 2014) during the 2013/14 season.

	Total glutathione ($\mu g\ g^{-1}$ FW)	Reduced glutathione ($\mu g\ g^{-1}$ FW)	Oxidised glutathione ($\mu g\ g^{-1}$ FW)	Total ascorbic acid ($\mu g\ g^{-1}$ FW)	Reduced ascorbic acid ($\mu g\ g^{-1}$ FW)	Oxidised ascorbic acid ($\mu g\ g^{-1}$ FW)
At harvest						
Low irrigation	20.3	18.7	1.58	60.1 a	39.5	20.6
Control	22.2	20.4	1.81	43.3 b	26.8	16.6
High irrigation	21.4	20.5	0.88	45.1 b	33.6	11.4
F test	*0.2497*	*0.2275*	*0.2316*	*0.0106*	*0.1049*	*0.1665*
Contrasts						
Linear	*0.3465*	*0.1299*	*0.2157*	*0.0116*	*0.3121*	*0.0624*
Quadratic	*0.1678*	*0.4136*	*0.2320*	*0.0624*	*0.0596*	*0.8850*

Table 18. Effect of irrigation level on the glutathione and ascorbic acid concentration in 'Laetitia' plum fruit peel from Welgevallen Research farm during the 2013/14 season. Early season treatments were effected from 03 to 18 Dec 2013 using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation).

	Total glutathione (µg g^{-1} FW)	Reduced glutathione (µg g^{-1} FW)	Oxidised glutathione (µg g^{-1} FW)	Total ascorbic acid (µg g^{-1} FW)	Reduced ascorbic acid (µg g^{-1} FW)	Oxidised ascorbic acid (µg g^{-1} FW)
Control	23.4	20.6	2.77	140.3	69.5	70.8
Half irrigation	22.5	19.9	2.58	129.43	76.0	53.4
No irrigation	24.3	22.1	2.21	137.1	72.7	64.4
F test	*0.2348*	*0.1099*	*0.4286*	*0.7343*	*0.7100*	*0.5148*
Contrasts						
Linear	*0.0945*	*0.0432*	*0.3943*	*0.5950*	*0.6750*	*0.4736*
Quadratic	*0.9670*	*0.6242*	*0.3277*	*0.5716*	*0.4835*	*0.3721*

Table 19. Effect of late season irrigation manipulation on the glutathione and ascorbic acid concentration in 'Laetitia' fruit peel from Welgevallen Research farm. The late season treatments were effected from 03 to 18 Jan 2013 using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation).

	Total glutathione (µg g^{-1} FW)	Reduced glutathione (µg g^{-1} FW)	Oxidised glutathione (µg g^{-1} FW)	Total ascorbic acid (µg g^{-1} FW)	Reduced ascorbic acid (µg g^{-1} FW)	Oxidised ascorbic acid (µg g^{-1} FW)
Control	22.5 b	19.9 b	2.61	149.7 b	80.0 a	69.7 b
Half irrigation	23.6 b	20.5 b	3.08	169.5 ab	88.8 a	80.7 b
No irrigation	31.5 a	29.1 a	2.45	213.a	29.2 b	183.9 a
F test	*0.0009*	*<0.0001*	*0.5312*	*0.0260*	*<0.0001*	*<0.0001*
Contrasts						
Linear	*0.0014*	*0.0001*	*0.2882*	*0.0576*	*<0.0001*	*0.0001*
Quadratic	*0.0113*	*0.0040*	*0.7506*	*0.3890*	*0.0270*	*0.0029*

Table 20. Effect of irrigation manipulation on the glutathione and ascorbic acid concentration in 'Laetitia' fruit peel from Welgevallen Research farm. Treatments were effected from 30 Dec 2014 to 13 Jan 2015 using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation).

	Total glutathione (µg g^{-1} FW)	Reduced glutathione (µg g^{-1} FW)	Oxidised glutathione (µg g^{-1} FW)	Total ascorbic acid (µg g^{-1} FW)	Reduced ascorbic acid (µg g^{-1} FW)	Oxidised ascorbic acid (µg g^{-1} FW)
Control	24.3 b	21.6 b	2.71	144.3 b	108.8 b	35.5
Half irrigation	24.6 b	22.0 b	2.61	152.8 b	117.1 b	35.7
No irrigation	28.3 a	25.6 a	2.73	187.8 a	162.0 a	25.9
F test	*0.0003*	*0.0002*	*0.9841*	*0.0014*	*0.0003*	*0.4411*
Contrasts						
Linear	*0.0002*	*0.0002*	*0.9856*	*0.0006*	*0.0001*	*0.2786*
Quadratic	*0.0273*	*0.0316*	*0.8609*	*0.1453*	*0.0571*	*0.5061*

Table 21. Effect of irrigation level on the fruit quality of 'African Delight' plums of Sandrivier Estate assessed at harvest (15 February 2013) during the 2012/13 season.

	Fruit Diameter (mm)	Fruit weight (g)	Peel colour	Flesh firmness (kg cm^{-2})	Total titratable acidity (%)	Total soluble solids (º BRIX)
Irrigation level						
Low irrigation	51.5	91.6	8.7	9.7	0.70	19.8 a
Control	51.9	92.6	9.0	9.7	0.73	18.8 b
High irrigation	52.4	93.5	8.7	9.8	0.71	18.4 b
F test	*0.5400*	*0.9383*	*0.6984*	*0.9513*	*0.3034*	*0.0150*
Contrast						
Linear	*0.2700*	*0.7254*	*0.9291*	*0.7770*	*0.5413*	*0.0049*
Quadratic	*0.9315*	*0.9829*	*0.4059*	*0.8966*	*0.1586*	*0.5281*

Table 22. Effect of irrigation level on the fruit quality of 'African Delight' plums of Sandrivier Estate assessed at harvest (07 February 2014) during the 2013/14 season.

	Fruit Diameter (mm)	Fruit weight (g)	Peel colour	Flesh firmness (kg cm^{-2})	Total titratable acidity (%)	Total soluble solids (º BRIX)
Irrigation level						
Low irrigation	51.6 b	92.0.0 b	9.59	9.16 a	0.668	18.6 a
Control	52.8 ab	98.1 b	9.59	8.58 ab	0.683	17.3 b
High irrigation	53.9 a	100.3 a	9.47	8.30 b	0.667	17.2 b
F test	*0.0085*	*0.0143*	*0.8409*	*0.0360*	*0.4656*	*0.0001*
Contrast						
Linear	*0.0023*	*0.0052*	*0.6237*	*0.0124*	*0.9446*	*<0.0001*
Quadratic	*0.9301*	*0.4017*	*0.7546*	*0.5792*	*0.2234*	*0.0299*

Table 23. Effect of irrigation level on the fruit quality of 'Laetitia' plums at Walgevallen farm in the 2013/14 season. Early season treatments were effected from 03 to 18 Dec 2013 using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation). Late season treatments were manipulated from from 03 to 18 Jan 2014.

[z] Peel colour was assessed using an arbitrary 1-12 scale where 1 denoted the least coloured (green) fruit and 12 the most (reddest).

	Fruit diameter (mm)	Fruit weight (g)	Peel colour[z]	Flesh firmness (kg cm^{-2})	Total titratable acidity (%)	Total soluble solids (º BRIX)
Early season						
Control	53.6	73.7	7.01	7.31	1.40 b	11.6 b
Half irrigation	55.2	76.3	7.28	7.48	1.50 ab	12.1 ab
No irrigation	53.0	73.7	7.61	7.70	1.52 a	13.0 a
F test	*0.1757*	*0.1996*	*0.3368*	*0.6220*	*0.0480*	*0.0448*
Contrasts						
Linear	*0.6156*	*0.9859*	*0.1477*	*0.3396*	*0.0208*	*0.0152*
Quadratic	*0.0756*	*0.0777*	*0.9375*	*0.9435*	*0.3793*	*0.6853*
Late season						
Control	52.8 a[y]	76.0 a	6.93	7.14 a	1.47 b	11.1 b
Half irrigation	52.5 a	75.4 a	6.49	7.24 a	1.52 ab	11.1b
No irrigation	48.7 b	62.1 b	7.40	6.00 b	1.60 a	12.3 a
F test	*0.0012*	*0.0028*	*0.1516*	*0.0037*	*0.0281*	*0.0088*
Contrasts						
Linear	*0.0080*	*0.0019*	*0.3032*	*0.0040*	*0.0094*	*0.0066*
Quadratic	*0.0522*	*0.0665*	*0.0959*	*0.0357*	*0.6083*	*0.0874*

Table 24. Effect of irrigation level on the fruit quality of 'Laetitia' plums at Walgevallen farm in the 2014/15 season. Treatments were effected from 30 Dec 2014 to 13 Jan 2015 using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation).

[z] Peel colour was assessed using an arbitrary 1-12 scale where 1 denoted the least coloured (green) fruit and 12 the most (reddest).

	Fruit diameter (mm)	Fruit weight (g)	Peel colour[z]	Flesh firmness (kg cm^{-2})	Total titratable acidity (%)	Total soluble solids (^{o} BRIX)
Control	54.5 a	76.5 a	8.08	6.93 b	1.52	11.2 b
Half irrigation	52.2 b	73.4 ab	7.89	7.59 a	1.50	12.0 a
No irrigation	51.2 b	71.9 b	7.78	8.01 a	1.50	12.2 a
F test	*0.0006*	*0.0218*	*0.5738*	*0.0029*	*0.9026*	*0.0106*
Contrasts						
Linear	*0.0002*	*0.0075*	*0.3078*	*0.0008*	*0.6756*	*0.0043*
Quadratic	*0.2662*	*0.5246*	*0.8570*	*0.6778*	*0.8796*	*0.2866*

[y] Means in the same column with different letters are significantly different at 0.05 level.

Figure 1. Illustration of sunburn severity assessment guide for ‘African Delight’ plums adapted from the chart of Schrader et al. (2003) where 0 represented no sunburn and 5 the severest form. Classes 0, 1 and 2 can be sold on the export market. Classes 3 and 4 can be sold only on the local market. Class 5 is unmarketable.

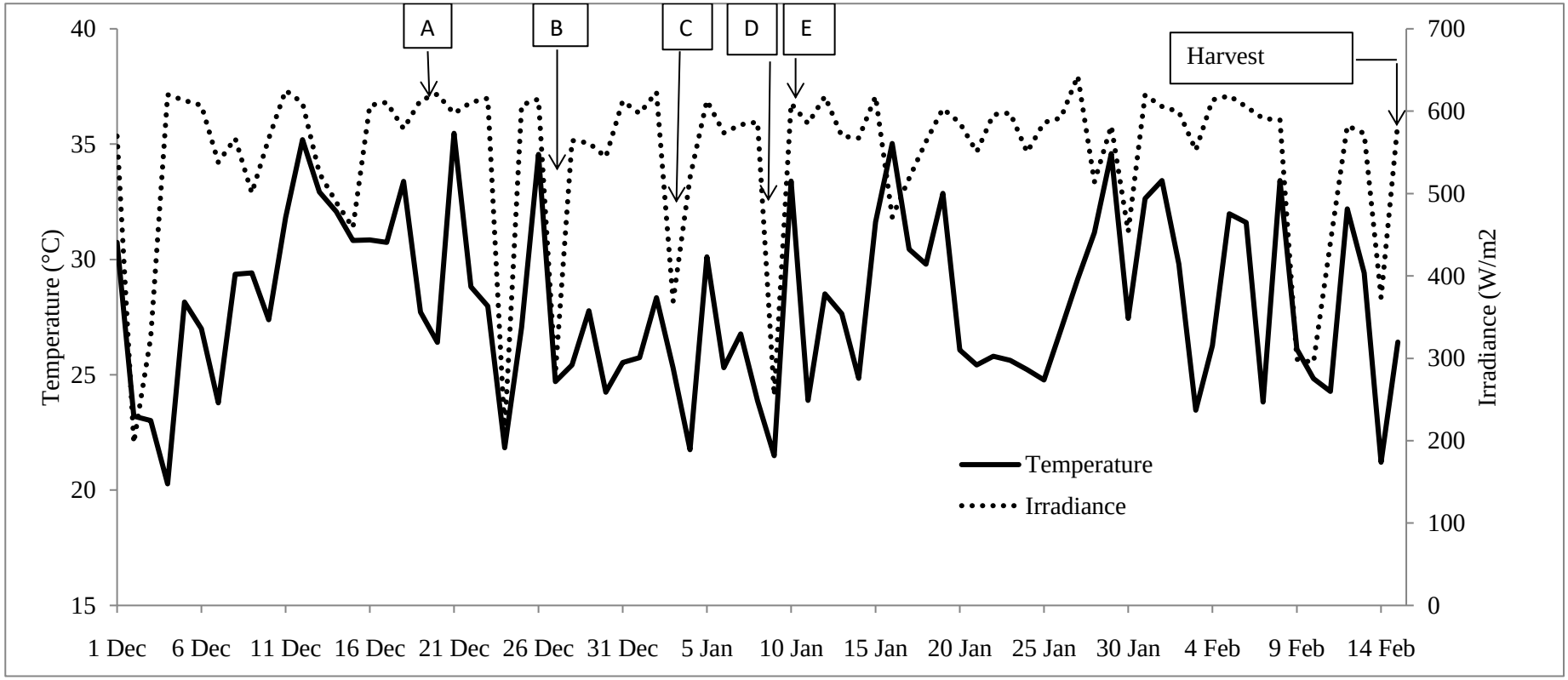


Figure 2. Daily maximum air temperature and average irradiance for the Wellington area from 1 December to 15 February during the 2012/13 growing season. Irradiance data was averaged from hourly values between 07h00 and 19h00. Data was obtained from Abendruhe weather station, approximately 5km from Sandrivier Estate. Letters A, B, C, D and E point to 20 Dec., 27 Dec. (2012), 03 Jan., 08 Jan. and 10 Jan. (2013), respectively. These are the dates when photochemistry, stem water potential and fruit surface temperature measurements were conducted. Fruit were harvested on 15 Feb. 2014.

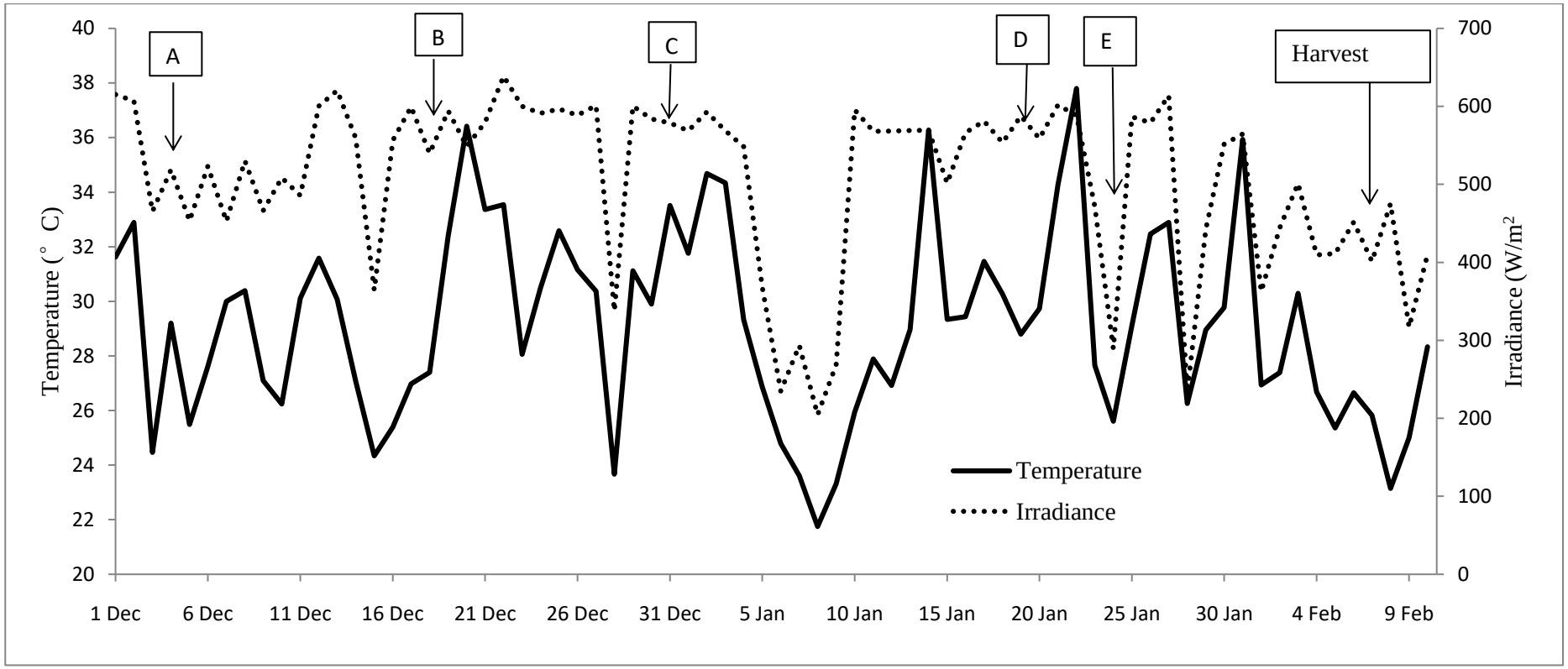


Figure 3. Daily maximum air temperature and average irradiance for the Wellington area from 1 December to 10 February during the 2013/14 growing season. Irradiance data was averaged from hourly values between 07h00 and 19h00. Data was obtained from Abendruhe weather station, approximately 5km from Sandrivier Estate. Letters A, B, C, D and E point to 04 Dec., 18 Dec., 30 Dec. (2013), 19 Jan. and 24 Jan. (2014), respectively. These are the dates when photochemistry, stem water potential and fruit surface temperature measurements were conducted. Fruit were harvested on 07 Feb. 2014.

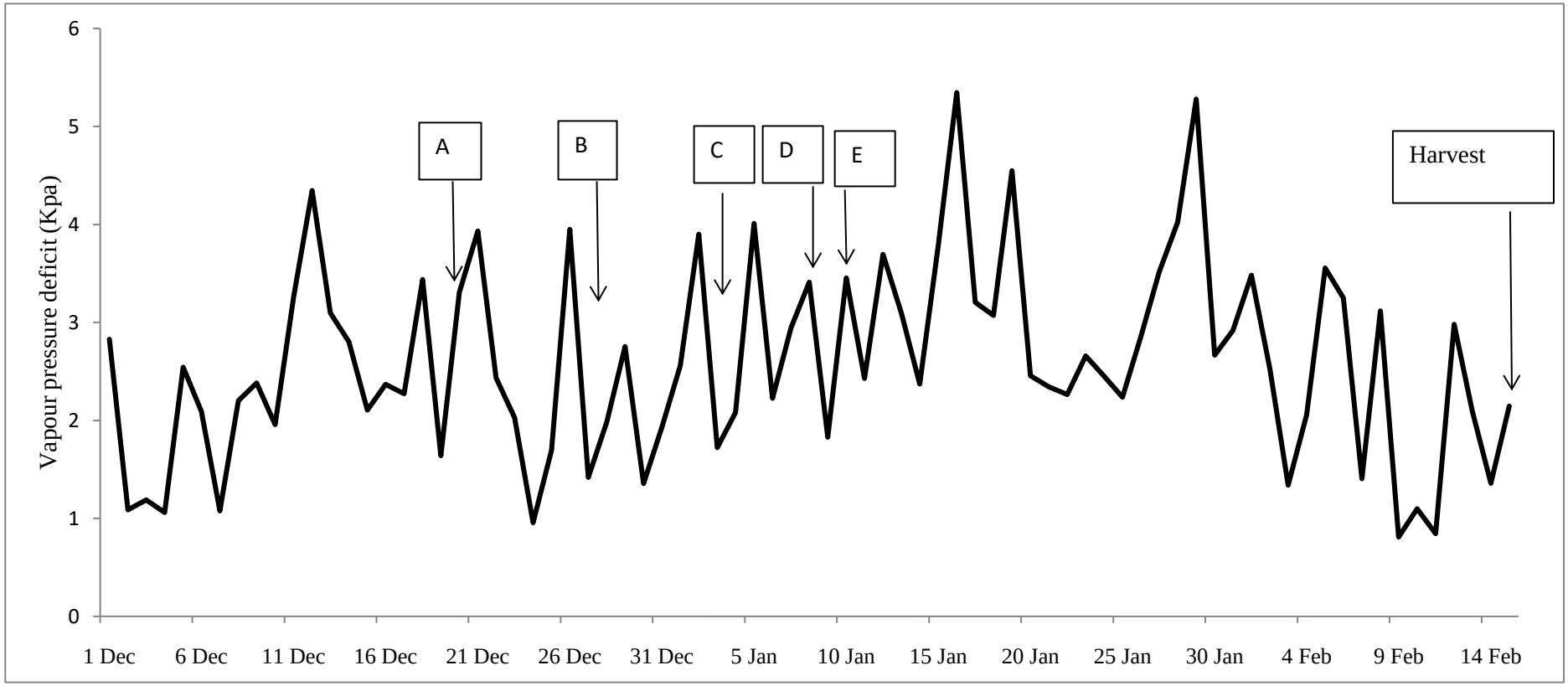


Figure 4. Daily maximum vapour pressure deficit for the Wellington area from 1 December to 15 February during the 2012/13 growing season. Data was obtained from Abendruhe weather station, approximately 5km from Sandrivier Estate. Letters A, B, C, D and E point to 20 Dec., 27 Dec. (2012), 03 Jan., 08 Jan. and 10 Jan. (2013), respectively. These are the dates when photochemistry, stem water potential and fruit surface temperature measurements were conducted. Fruit were harvested on 15 Feb. 2014.

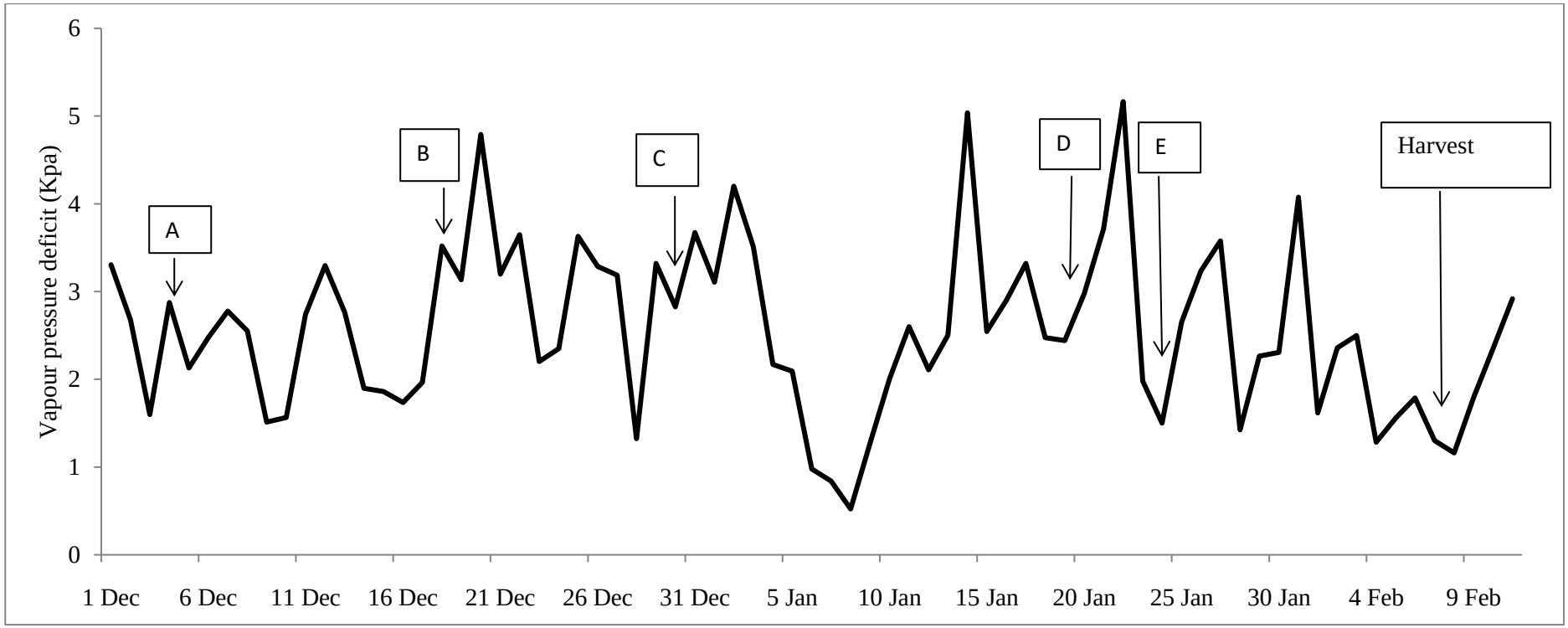


Figure 5. Daily maximum vapour pressure deficit for the Wellington area from 1 December to 15 February during the 2012/13 growing season. Data was obtained from Abendruhe weather station, approximately 5km from Sandrivier Estate. Letters A, B, C, D and E point to 04 Dec., 18 Dec., 30 Dec. (2013), 19 Jan. and 24 Jan. (2014), respectively. These are the dates when photochemistry, stem water potential and fruit surface temperature measurements were conducted. Fruit were harvested on 07 Feb. 2014.

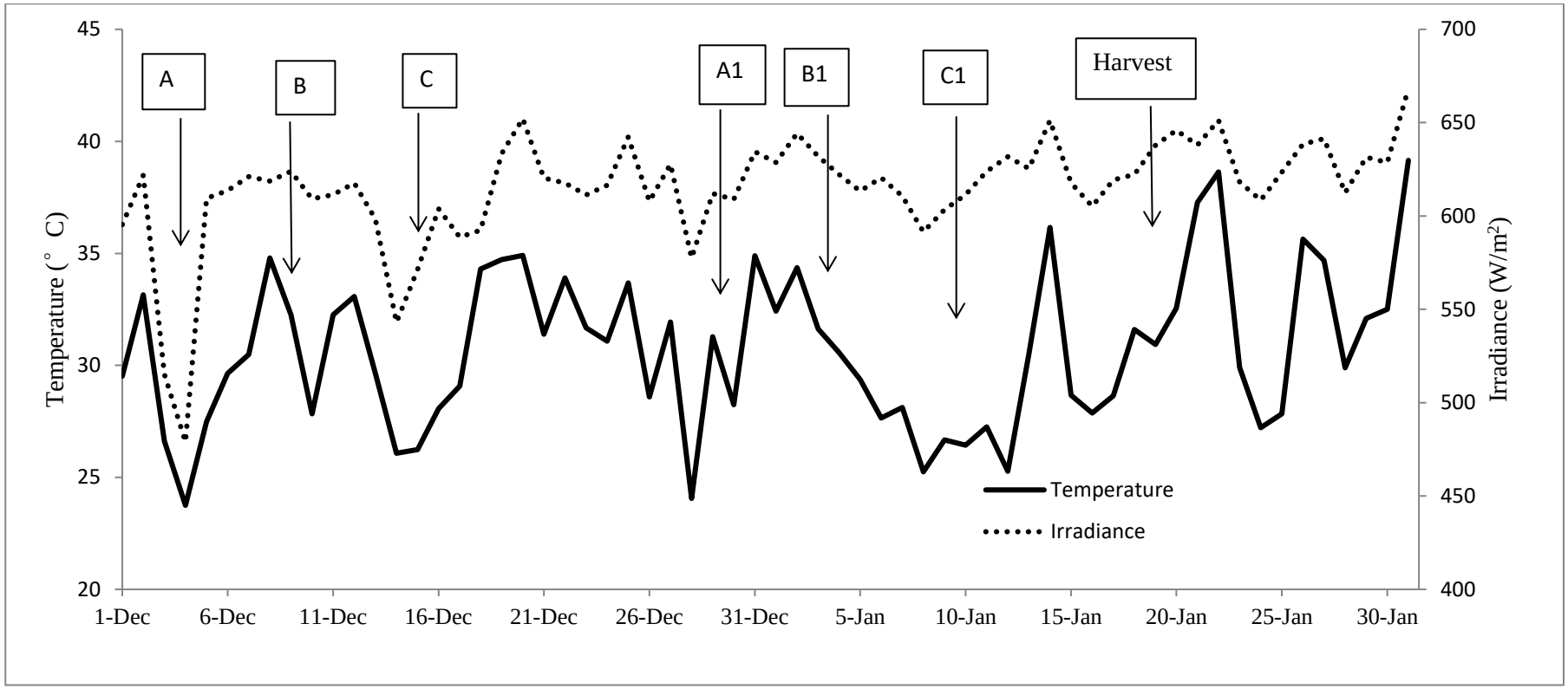


Figure 6. Daily maximum temperature and average irradiance for the Stellenbosch area from 1 December to 30 January during the 2013/14 growing season. Data was obtained from Helderfontein weather station, located within 5km from Welgevallen Research farm. Letters A, B, C, indicate the dates 03 Dec., 10 Dec and 17 Dec. 2013 representing the onset of early season 2013/14 irrigation manipulation and measurements at Day 7 and Day 14 respectively. The start of late season dates is shown by A1 (03 Jan), with B1 and C1 showing Day 7 and Day 14 measurement dates (10 & 17 Jan. 2014).

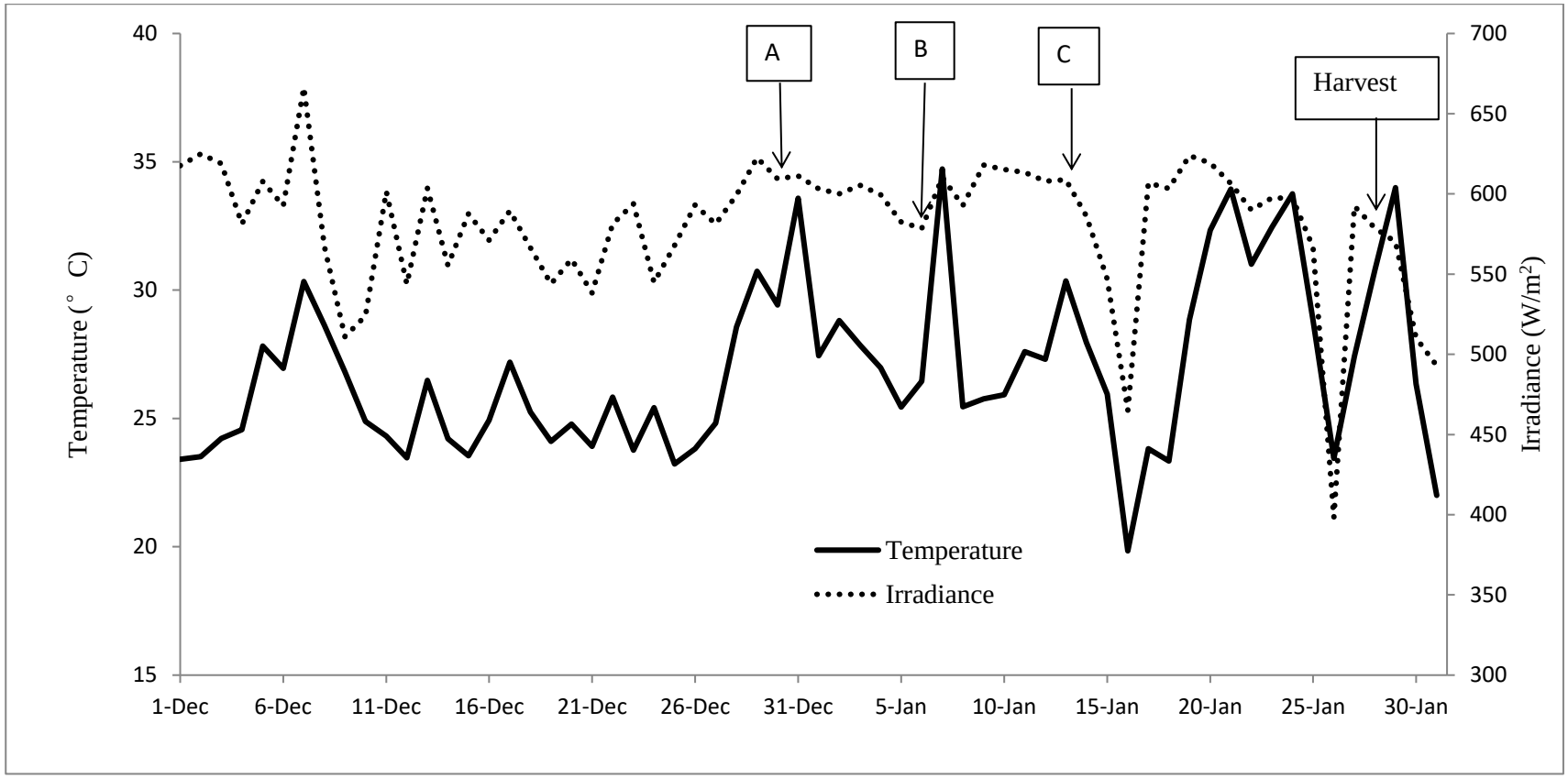


Figure 7. Daily maximum temperature and average irradiance for the Stellenbosch area from 1 December to 30 January during the 2014/15 growing season. Data was obtained from Helderfontein weather station, located within 5km from Welgevallen Research farm. Letters A, B, C, indicate the dates 30 Dec. 2014 and 6 &13 Jan. 2015, representing the onset of 2014/15 irrigation manipulation and measurements at Day 7 and Day 14 respectively.

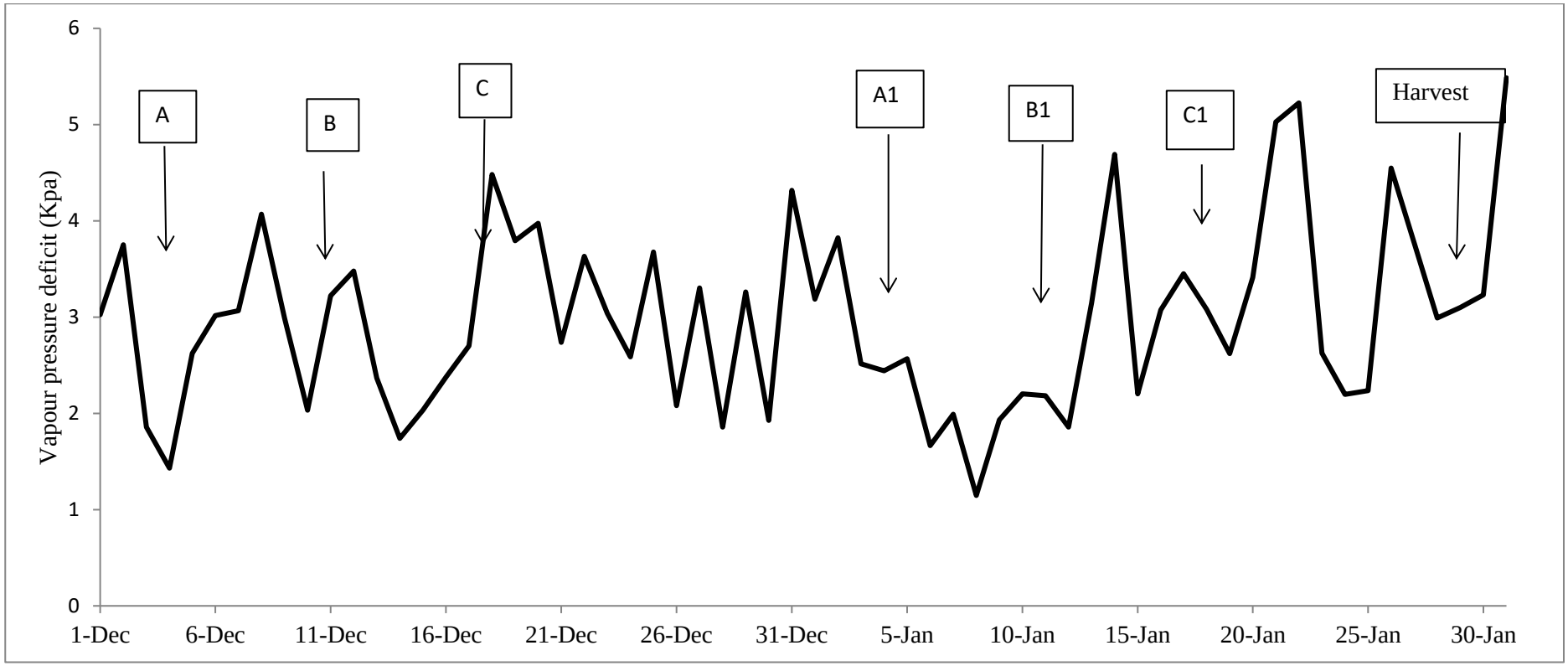


Figure 8. Daily maximum vapour pressure deficit for the Stellenbosch area from 1 December to 30 January during the 2013/14 growing season. Data was obtained from Helderfontein weather station, located within 5km from Welgevallen Research farm. Letters A, B, C, indicate the dates 03 Dec., 10 Dec and 17 Dec. 2013 representing the onset of early season 2013/14 irrigation manipulation and measurements at Day 7 and Day 14 respectively. The start of late season dates is shown by A1 (03 Jan), with B1 and C1 showing Day 7 and Day 14 measurement dates (10 & 17 Jan. 2014).

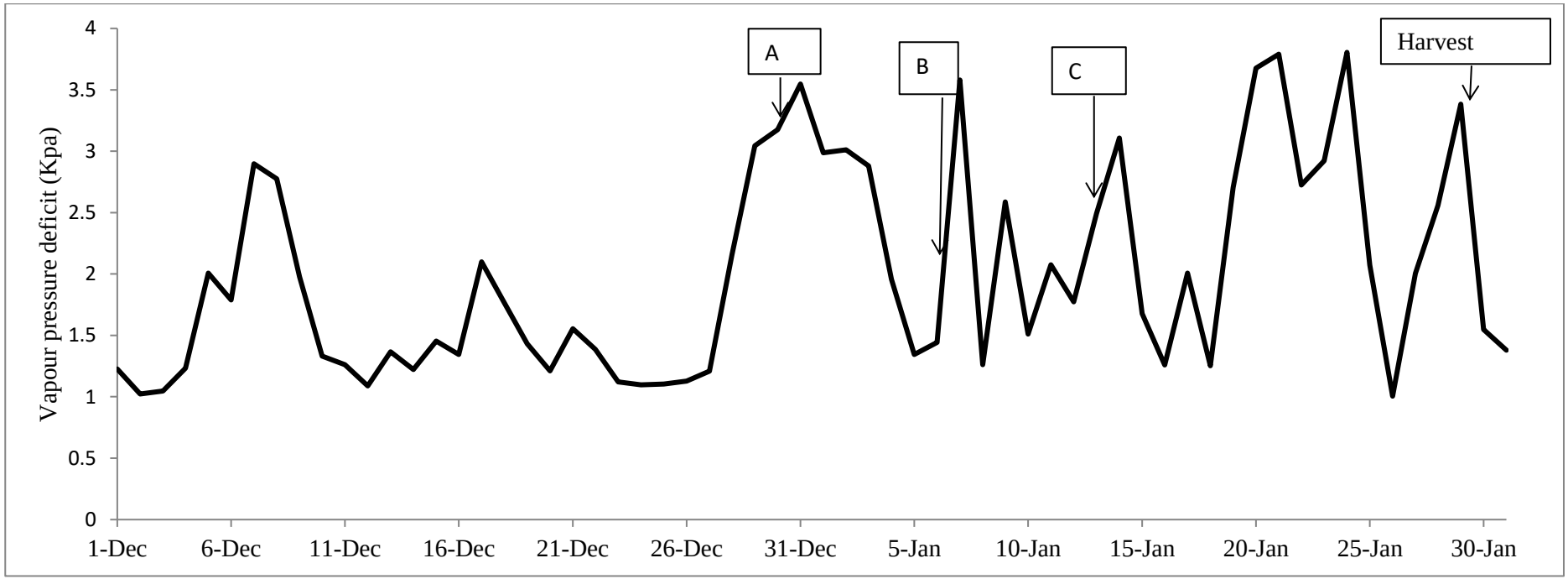


Figure 9. Daily maximum temperature and average irradiance for the Stellenbosch area from 1 December to 30 January during the 2014/15 growing season. Data was obtained from Helderfontein weather station, located within 5km from Welgevallen Research farm. Letters A, B, C, indicate the dates 30 Dec. 2014 and 6 &13 Jan. 2015, representing the onset of 2014/15 irrigation manipulation, measurements at Day 7 and Day 14 respectively.

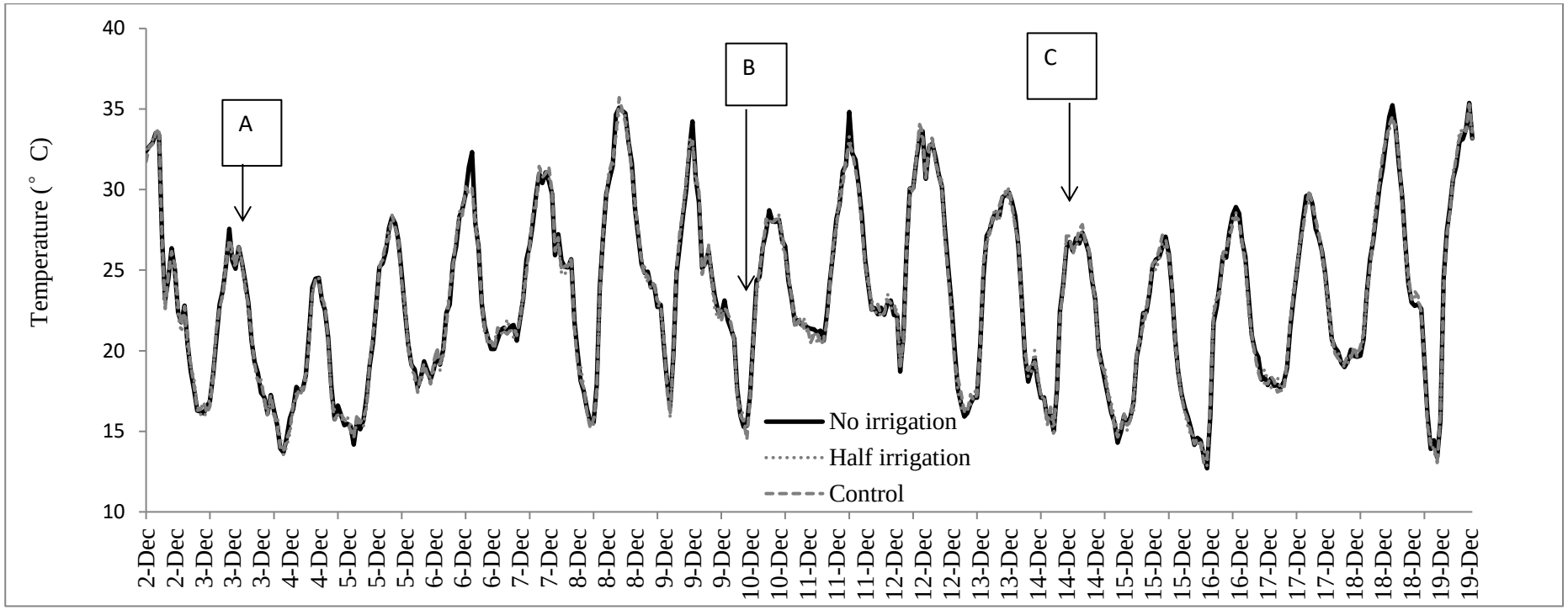


Figure 10. Effect of early season irrigation level on canopy temperature of 'Laetitia' plums during the 2013/14 season at Welgevallen Research farm. Letters A, B, C, indicate the dates 03 Dec., 10 Dec and 17 Dec. 2013 representing the onset of early season 2013/14 irrigation manipulation, measurements at Day 7 and Day 14 respectively.

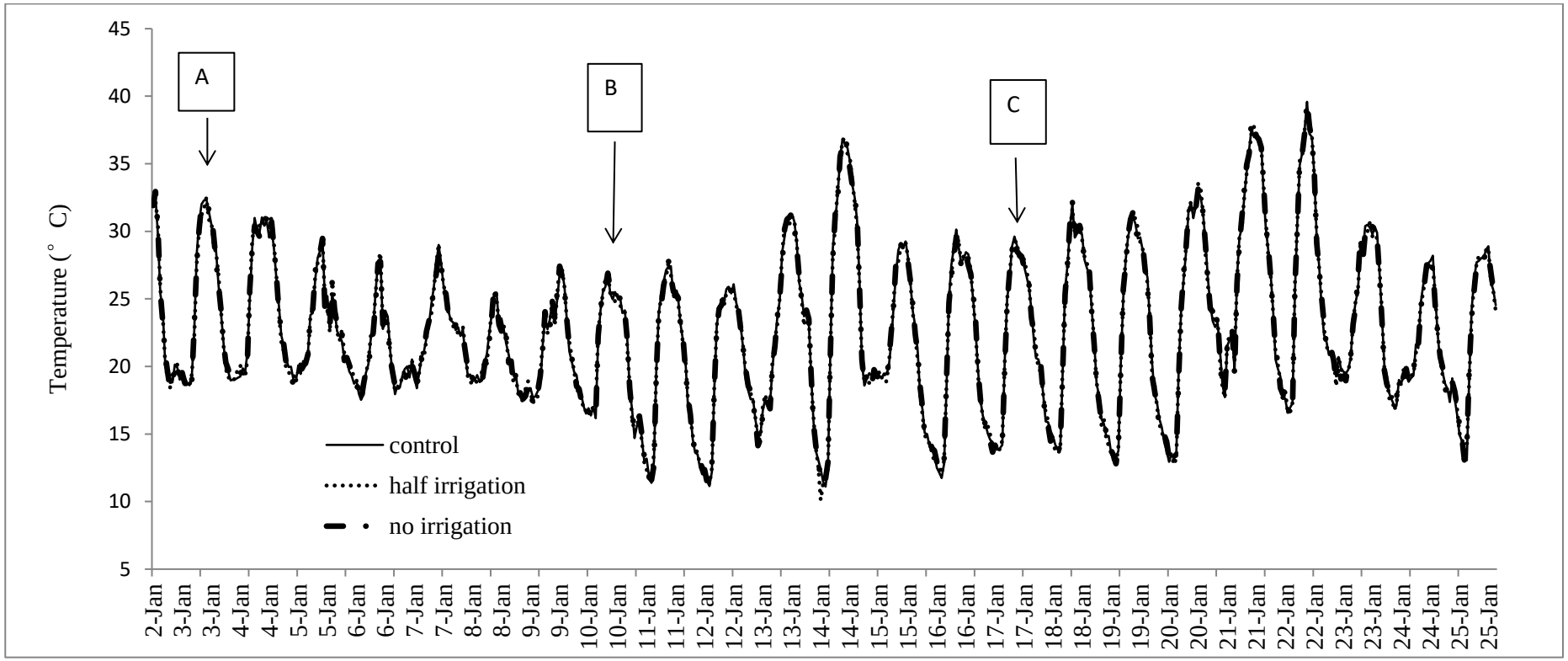


Figure 11. Effect of late season irrigation level on canopy temperature of 'Laetitia' plums during the 2013/14 season at Welgevallen Research farm. The start of late season dates is shown by A (03 Jan), with B and C showing Day 7 and Day 14 measurement dates (10 & 17 Jan. 2014).

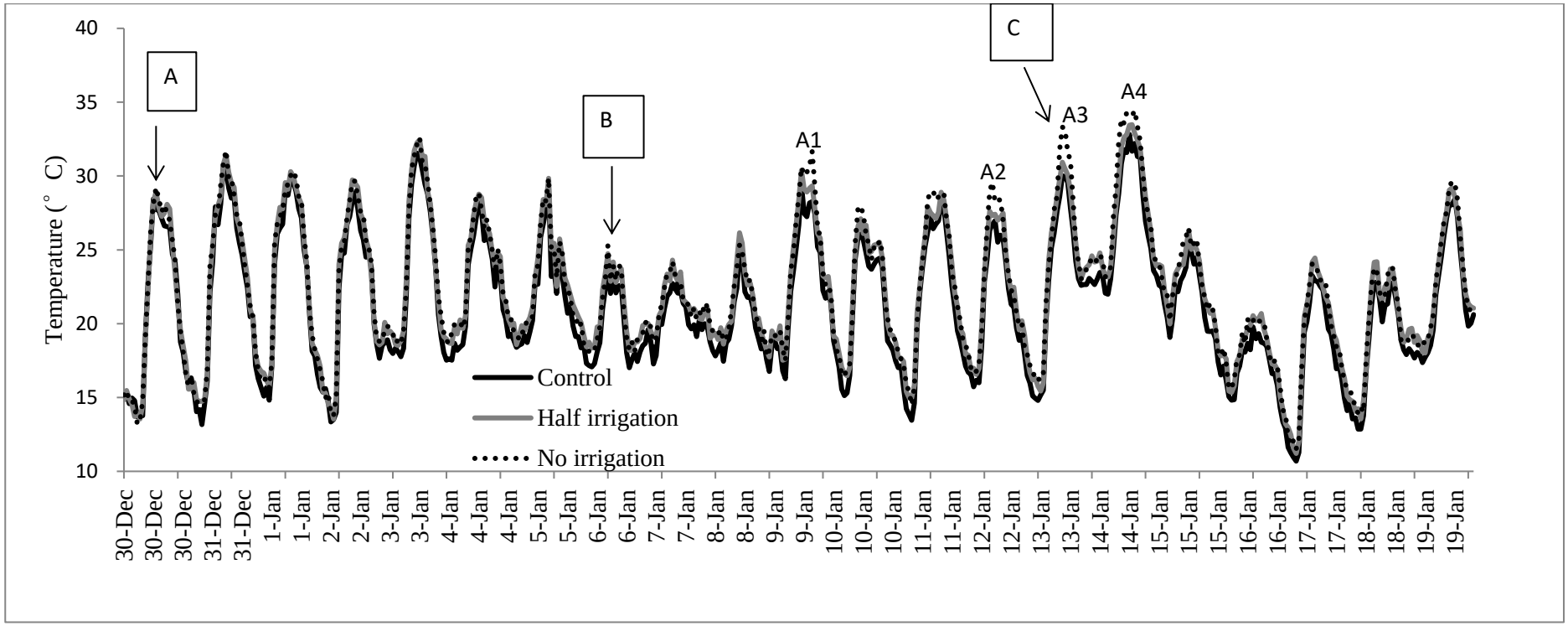


Figure 12. Effect of irrigation level on canopy temperature of 'Laetitia' plums during the 2014/15 season at Welgevallen Research farm. Letters A, B, C, indicate the dates 30 Dec. 2014 and 6 &13 Jan. 2015, representing the onset of 2014/15 irrigation manipulation and measurements at Day 7 and Day 14 respectively. A1, A2, A3 and A4 show peaks where the no irrigation treatement had higher canopy temperature and these were on days 10, 13, 14 and 15 respectively.

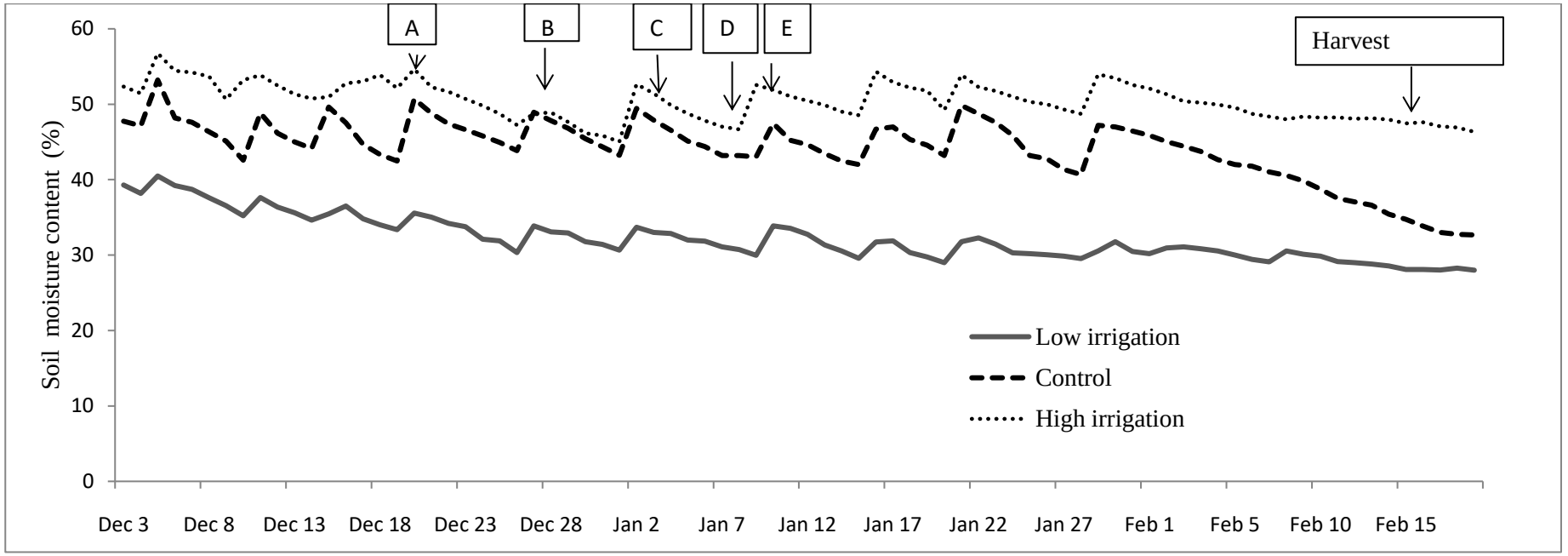


Figure 13. The effect of irrigation level on daily midday (12h00) percentage soil moisture content of 'African Delight' plums orchard at Sandrivier Estate during the 2012/13 growing season. The precipitation rates were 5.7 mm, 8.6 mm and 11.4 mm for the low irrigation treatment, control and high irrigation treatment, respectively. Letters A, B, C, D and E point to 20 Dec., 27 Dec. (2012), 03 Jan., 08 Jan. and 10 Jan. (2013), respectively. These are the dates when photochemistry, stem water potential and fruit surface temperature measurements were conducted. Fruit were harvested on 15 Feb. 2014.

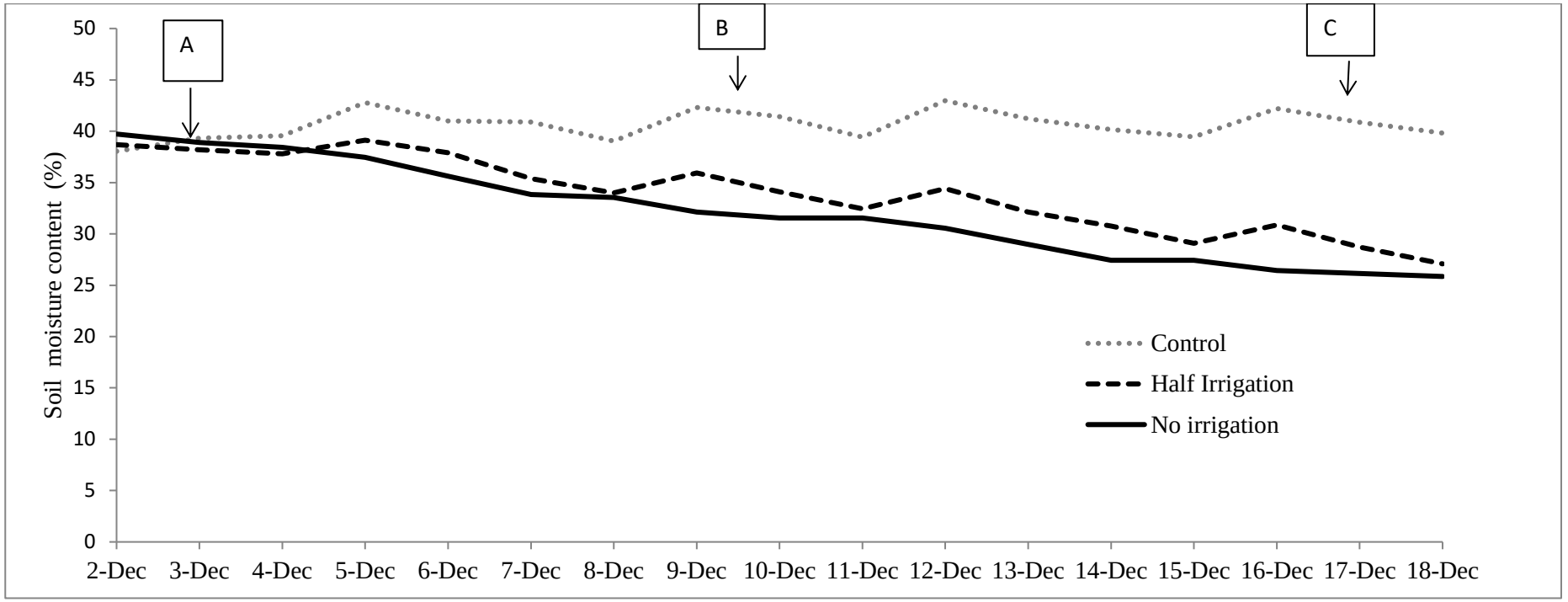


Figure 14. The effect of early season irrigation level on daily midday (12h00) percentage soil moisture content of 'Laetitia' plums orchard at Welgevallen Research farm during the 2013/14 season. Letters A, B, C, indicate the dates 03 Dec., 10 Dec and 17 Dec. 2013 representing the onset of early season 2013/14 irrigation manipulation and field measurements at Day 7 and Day 14 respectively.

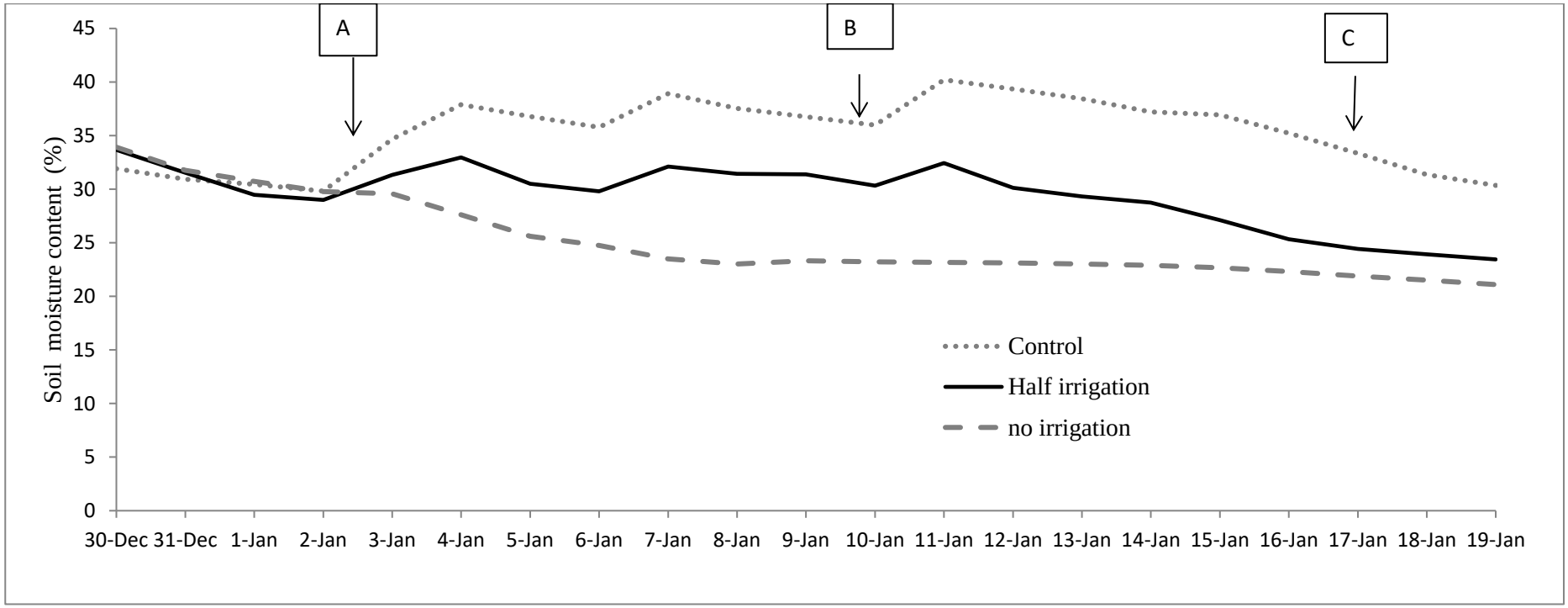


Figure 15. The effect of late season irrigation level on daily midday (12h00) percentage soil moisture content of 'Laetitia' plums orchard at Welgevallen Research farm during the 2013/14 season. The start of late season dates is shown by A (03 Jan), with B and C showing Day 7 and Day 14 field measurement dates (10 & 17 Jan. 2014).

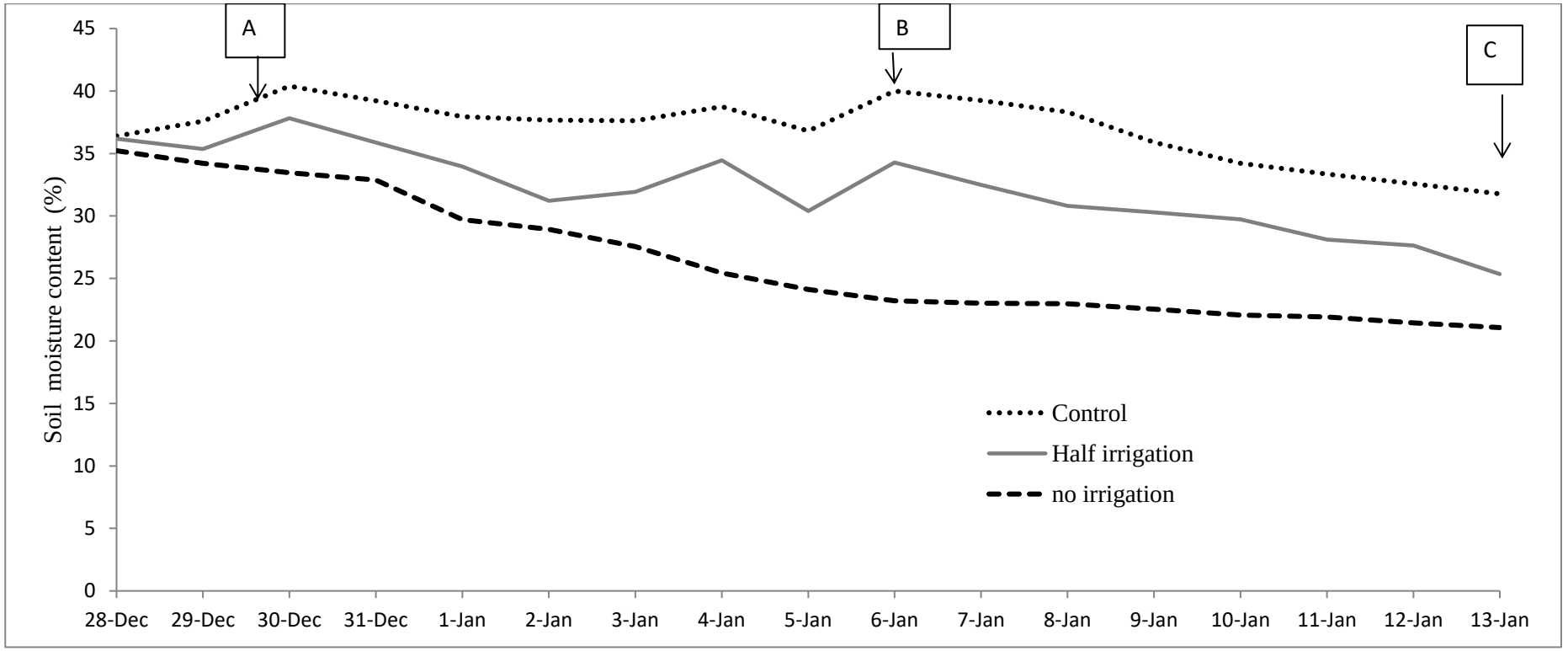
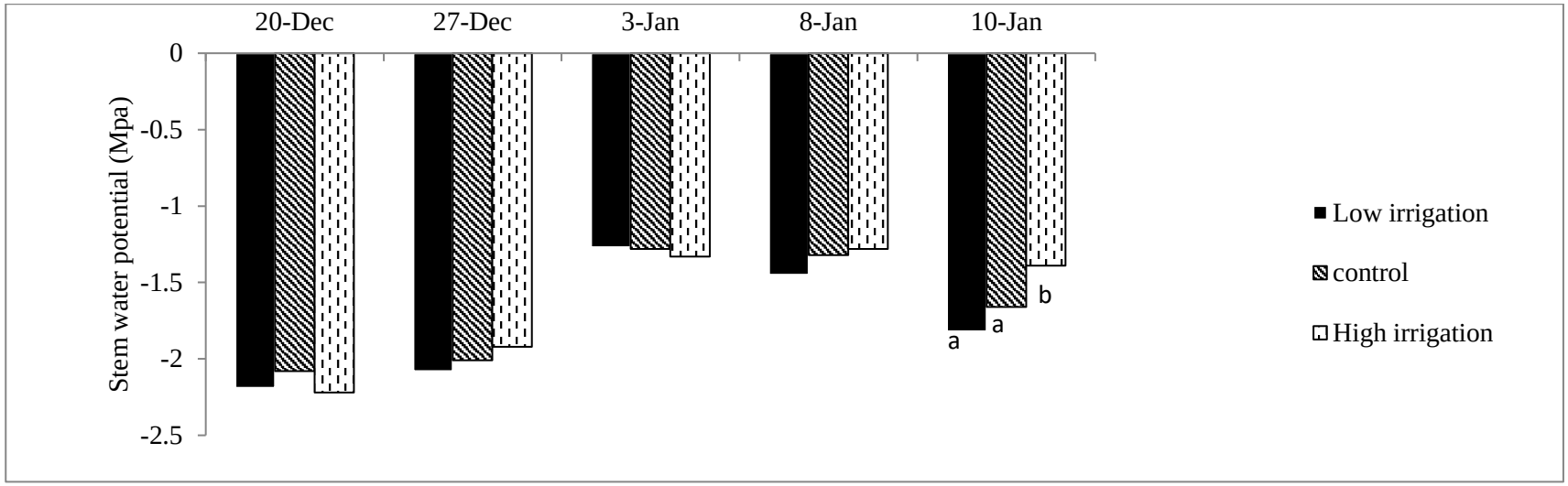
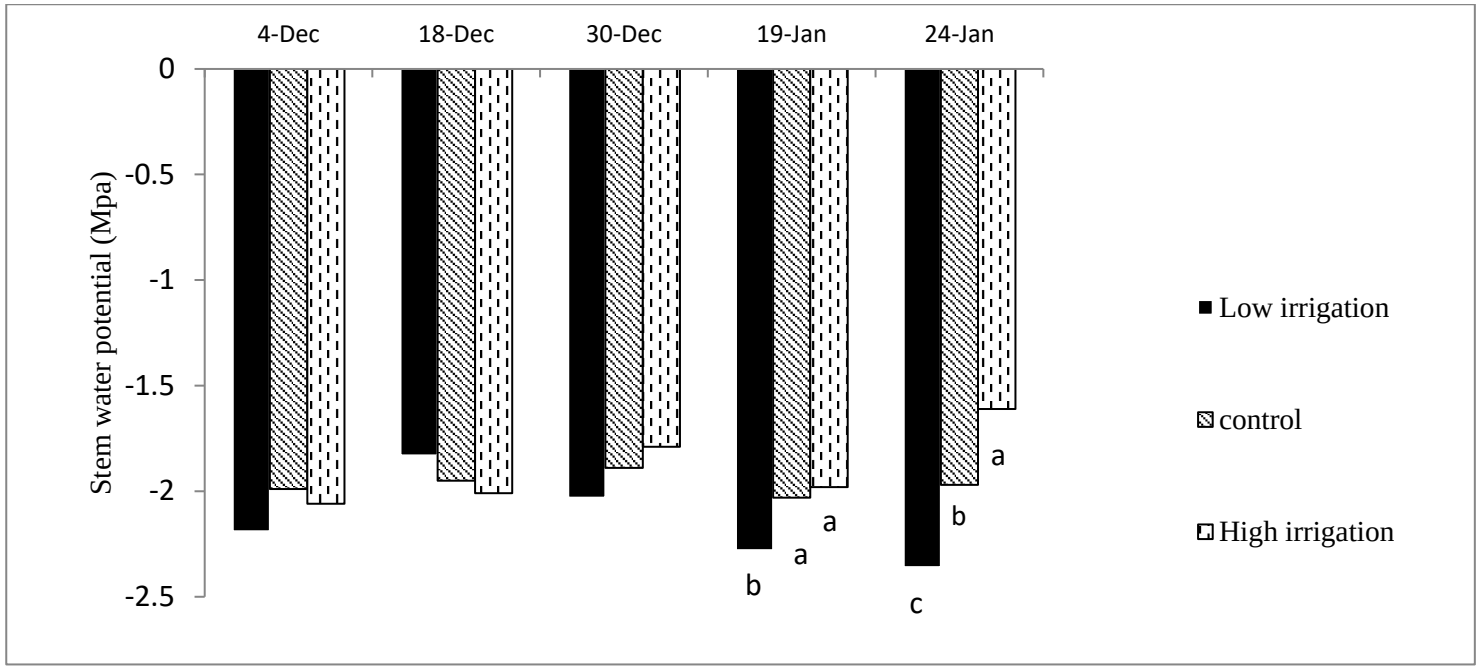


Figure 16. The effect of irrigation level on daily midday (12h00) percentage soil moisture content of 'Laetitia' plums orchard at Welgevallen Research farm during the 2014/15 season. Letters A, B, C, indicate the dates 30 Dec. 2014 and 6 &13 Jan. 2015, representing the onset of 2014/15 irrigation manipulation and measurements at Day 7 and Day 14 respectively.



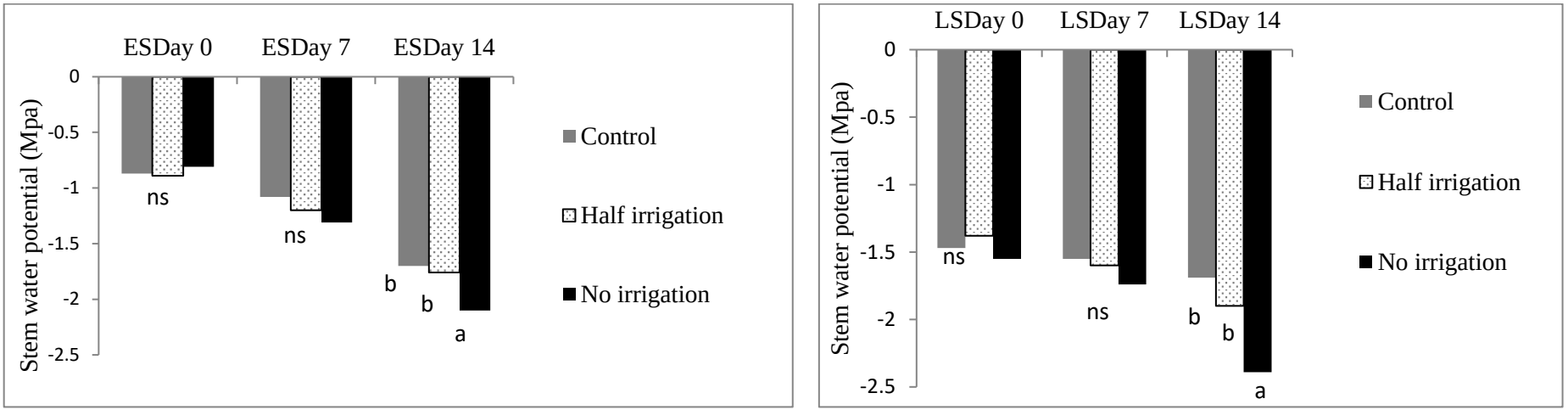
	20 Dec 2012	27 Dec 2012	03 Jan 2013	08 Jan 2014	10 Jan 2014
F test	*0.7669*	*0.5175*	*0.4843*	*0.0821*	*0.0090*
Linear contrast	*0.8680*	*0.2588*	*0.2671*	*0.0857*	*0.0027*
Quadratic contrast	*0.4849*	*0.9621*	*0.6640*	*0.1323*	*0.6073*

Figure 17. Effect of irrigation on stem water potential of 'African Delight' plums at Sandrivier Estate during the 2012/13 growing season



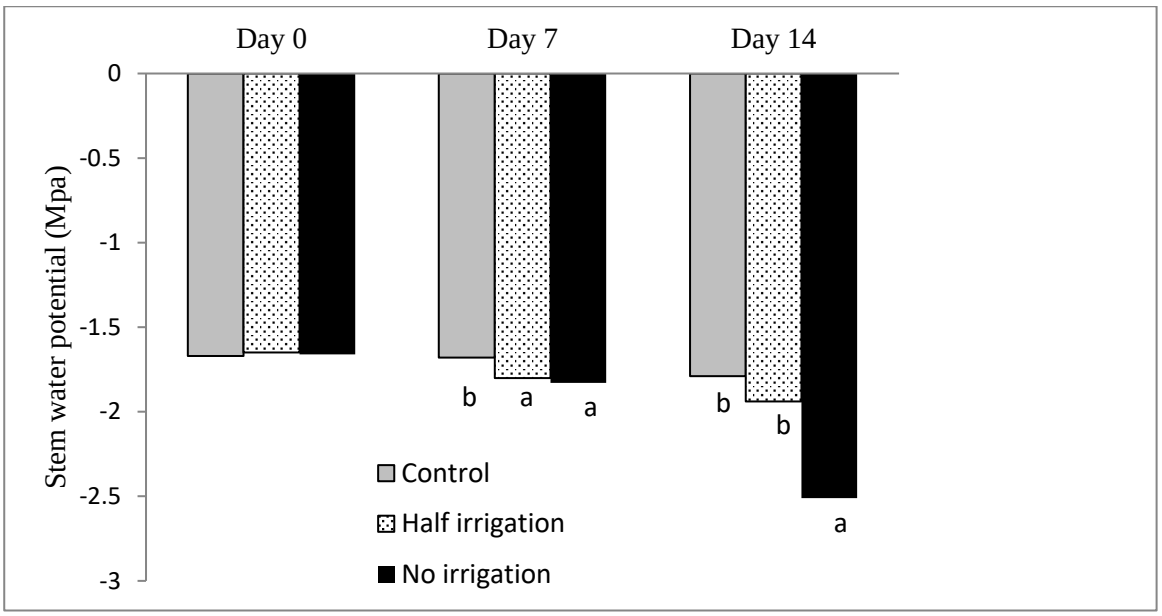
	04 Dec	18 Dec	30 Dec	19 Jan	24 Jan
F test	*0.1800*	*0.1439*	*0.1226*	*0.0198*	*0.0020*
Linear contrast	*0.2216*	*0.0615*	*0.0477*	*0.0093*	*0.0006*
Quadratic contrast	*0.1507*	*0.6194*	*0.8106*	*0.2269*	*0.8855*

Figure 18. Effect of irrigation on stem water potential of 'African Delight' plums at Sandrivier Estate during the 2013/14 growing season.



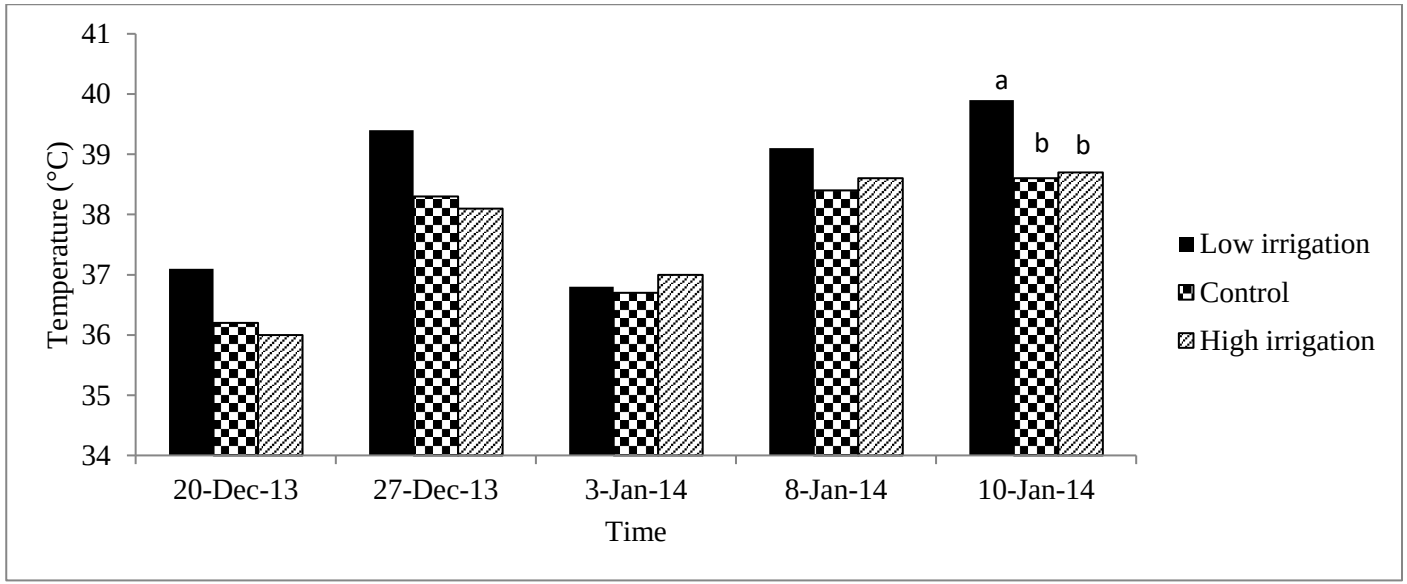
	ES Day0	ES Day7	ES Day14	LS Day0	LS Day 7	LS Day14
F test	*0.6272*	*0.1704*	*0.0041*	*0.3340*	*0.3363*	*0.0009*
Linear contrast	*0.5138*	*0.0679*	*0.0019*	*0.4764*	*0.1626*	*0.0003*
Quadratic contrast	*0.4902*	*0.9752*	*0.1136*	*0.1986*	*0.7182*	*0.2012*

Figure 19. The effect of early season (ES) and late season (LS) irrigation manipulation on stem water potential of 'Laetitia' plums at Welgevallen Research farm during the 2013/14 growing season. Early season treatments were effected from 03 to 18 Dec 2013 using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation). Late season treatments were manipulated from 03 to 18 Jan 2014.



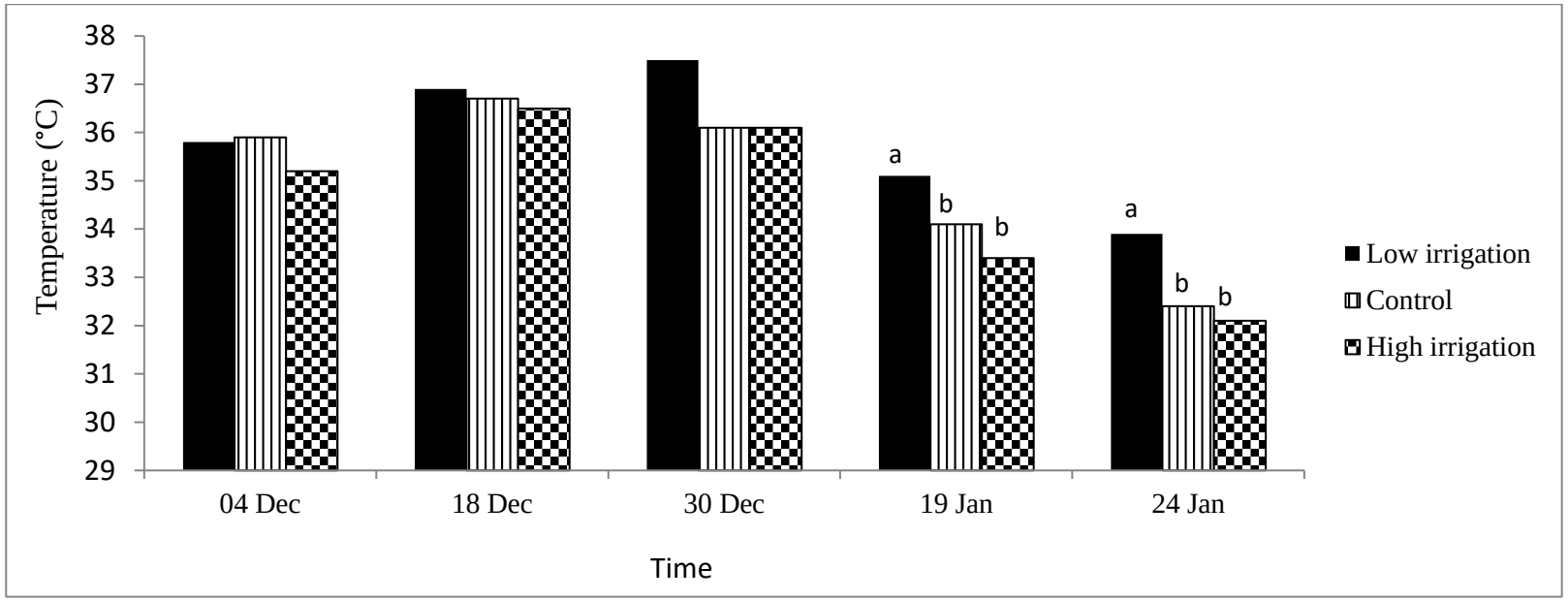
	Day 0	Day 7	Day 14
F test	*0.9603*	*0.0340*	*<0.0001*
Linear contrast	*0.8900*	*0.0151*	*<0.0001*
Quadratic contrast	*0.8110*	*0.3170*	*0.3162*

Figure 20. Effect of two-week irrigation manipulation on stem water potential of 'Laetitia' plums at Welgevallen Research farm during the 2014/15 growing season. Irrigation was manipulated using 6mm.h^{-1} spray nozzles (control), 3mm.h^{-1} (half irrigation) and 0.00mm.h^{-1} stoppers (no irrigation)



	20-Dec-13	27-Dec-13	03-Jan-14	08-Jan-14	10-Jan-14
F test	*0.2459*	*0.0515*	*0.7089*	*0.3621*	*0.0399*
Linear contrast	*0.1194*	*0.0228*	*0.5749*	*0.2822*	*0.0351*
Quadratic contrast	*0.5545*	*0.3745*	*0.5479*	*0.3497*	*0.1276*

Figure 21. The effect of irrigation level on fruit surface temperature of ‘African Delight’ plums at Sandrivier Estate during the 2012/13 growing season.



	04 Dec	18 Dec	30 Dec	19 Jan	24 Jan
F test	*0.7705*	*0.9548*	*0.0796*	*0.0096*	*0.0003*
Linear contrast	*0.5500*	*0.7656*	*0.0457*	*0.0026*	*0.0001*
Quadratic contrast	*0.6986*	*0.9847*	*0.2750*	*0.6617*	*0.0688*

Figure 22. The effect of irrigation level on fruit surface temperature of 'African Delight' plums at Sandrivier Estate during the 2013/14 growing season

RESEARCH CHAPTER 3

AN INVESTIGATION INTO THE EFFECT OF SIMULATED PRE-STORAGE THERMAL STRESS ON POSTHARVEST QUALITY OF JAPANESE PLUMS

Abstract

Heat damage has become a major problem in recent seasons when heat waves occur close to or during the harvesting window of Japanese plums in the Western Cape Province of South Africa. Damage can manifest externally as sunburn or internally as pitburn or gel breakdown, mostly after cold storage. Sensitivity differs among cultivars and maturity levels. The physiology of internal damage is not known but it is speculated that a rise in fruit internal ethanol concentration plays a significant role. High temperatures speed up respiration, depleting internal fruit oxygen and promoting anaerobic respiration and ethanol accumulation. We realised that internal heat damage symptoms do not easily manifest in fairly mild seasons. We therefore simulated conditions in the laboratory and subjected a sensitive cultivar, Laetitia, at early and late harvest maturities and tolerant 'African Delight' at one maturity to 30 °C, 40 °C and 45 °C for 1, 2 and 3 hours in the 2013/14 season. In 2014/15, the simulation was refined by comparing the response of the sensitive cultivar, Fortune, to heat wave and mild summer temperature conditions in a controlled atmosphere temperature treatment system (CATTS). Respiration, rates of ethanol evolution, fruit quality and heat damage were assessed at harvest and again after cold storage and shelf life in both seasons. No symptoms of internal heat damage were observed in 'African Delight', although an increase in temperature and exposure duration increased external peel damage. Tolerance to heat damage was possibly due to this cultivar's high skin permeability that prevented accumulation of threshold ethanol levels. 'Laetitia' and 'Fortune' were more sensitive to gel breakdown than pitburn, with symptoms predominantly manifesting after cold storage. In 'Laetitia', the incidence was higher in more mature fruit that experienced between 30 °C and 40 °C compared to 45 °C. Consistently in 'Fortune', fruit that had been treated to heat wave conditions had lower internal defects than those that experienced mild summer day conditions after cold-storage and shelf-life.

The lower incidence of internal heat damage at high temperatures could be attributed to heat curing. Manifestation of internal heat damage was related to respiration and ethanol evolution rates observations at harvest and there were no significant differences in respiration rates observed in all cultivars after cold storage. After shelf life, there were no clear trends in respiration and ethanol evolution rates. These observations did not relate to heat damage symptoms either. Flesh firmness notably increased under high temperature treatments, exhibiting the potential of extending fruit shelf life, particularly in 'Fortune' and 'Laetitia' that seemed to withstand peel damage better.

Although there seemed to be a reduced rate of loss of TA, there was a general increase in TSS with heat treatments. This can be correlated positively with increased consumer perception of fruit. In conclusion, we confirmed tolerance of 'African Delight' to internal heat damage. 'Laetitia' and 'Fortune' were, however, more tolerant to peel damage. In these cultivars, gel breakdown occurred more predominantly than pitburn and susceptibility increased with advance in maturity. With further research, high temperature treatments can potentially be used for curing against cold storage in cultivars more tolerant to peel damage.

INTRODUCTION

Maximum temperatures of 35 °C and higher persisting for about three days is deemed a heat wave in terms of potential effect on fruit quality in the Western Cape Province of South Africa (De Kock, 2015). Heat waves occurring just prior to, or in the harvesting window of various Japanese plum (*Prunus salicina* Lindl.) cultivars in the province, are thought to be a major causative factor for external and internal defects with subsequent modifications of fruit quality. This downgrades export-bound first grade fruit destined for highly paying lucrative markets.

High temperatures of up to 40 °C is considered to induce heat damage on plums in as little as one day (De Kock, 2015). When associated with high irradiance, the high temperatures cause external peel damage known as sunburn (Kossuth and Biggs, 1978; Wade et al., 1993; Schrader et al., 2001). Although not widely documented in plums, sunburn appears as a brown to yellow discolouration on the fruit surface (Research Chapter 2). Severe cases result in necrotic patches and cracking of the fruit peel. Although there are no formal data for plums, sunburn losses of up to 50% have been reported for apples in the Western Cape Province (Bergh et al., 1980).

In the absence of high irradiance, damage due to a heat wave is not always apparent at harvest in plums, but can manifest progressively during cold storage as two forms of internal damage, namely pitburn and gel breakdown. Pitburn (Figure 1A) manifests as a dark brown discolouration of the fruit flesh, starting out from the inner mesocarp around the pit and spreading out to the outer tissue with increasing severity (Amiot et al., 1997). High pre-harvest temperatures initiate high rates of respiration (Cheng et al., 1998) with concurrent oxidation of phenolic compounds (Amiot et al., 1997). The high fruit respiration rates depress internal O_2 while elevating internal CO_2 which subsequently promote anaerobic respiration. This results in softening of the tissue around the pit, with phenolic oxidation appearing as a brown discolouration or pitburn.

Gel breakdown (GB) (Figure 1B) first appears as a gelatinous breakdown in the mesocarp flesh around the pit which develops a dark discolouration over time (Candan et al., 2008). The symptoms

often appear when fruit is moved to shelf life conditions after cold storage. For this reason, it is often classified as a cold storage chilling injury disorder (Kapp and Jooste, 2006). However, fruit that experiences heat waves on the tree may also develop GB. The actual mechanism of GB is not known, but is considered to be a result of changes in membrane permeability and the accumulation of water soluble-pectins (Taylor et al., 1993). Like pitburn, anaerobic conditions initiated by high temperature oxygen depletion also seem to play a significant role in the development of GB in plums (Maxie and Claypool, 1956).

Studies have indicated that a high plant water status alleviates heat damage in fruit. Kotzé and Bothma (1989) demonstrated that withholding irrigation towards harvesting in peaches resulted in heat damage of 84%, but only 24% where irrigation was applied. We tested this in Research Chapter 2 and confirmed that low plant water status aggravates photo-thermal damage. However, irrigating beyond normal agronomic practices had no remedial effects on photo-thermal damage. The problem of heat damage on the tree, or progressively during cold-storage, therefore, appears inevitable as fruit ripen during heat waves. In order to prevent losses there is an urgent need to develop protocols for optimal post-harvest handling of fruit that experienced heat wave conditions just prior to or during harvest.

Stepwise forced air cooling is a protocol that works well for ‘Laetitia’ to minimise the incidence of internal heat damage during forced air cooling and cold storage (HORTGRO, 2016). Rapid cooling of fruit picked during a heat wave increases the incidence of heat damage (Jooste, personal communication). Fruit should be cooled to -0.5 °C within 48 to 72 h after harvest as opposed to the shorter periods of 24 h considered optimal for cultivars less sensitive to heat damage.

In general, the susceptibility of the fruit to heat damage increases with an increase in fruit maturity (Taylor et al., 1994). However, cultivars differ in the way they internally respond to heat damage. Heat waves occurring early in the season normally affect ‘Sapphire’ and ‘Fortune’ (De Kock, 2015), which are harvested early to mid-December. ‘Laetitia’ and ‘Songold’, which are harvested mid to late January, are affected later in the season. All these cultivars are prone to internal and external heat damage. De Kock (2015) indicated that cultivars that mature during the hottest part of the season, which is January and February, have a greater likelihood of being affected by heat waves as this is the period when most heat waves occur in the Western Cape summer months. We, therefore, expected African Delight, a late maturing cultivar, to be more susceptible to both internal and external heat damage since it is exposed to several heat waves during the season before it is

harvested. However, according to observations in the Western Cape Province, 'African Delight' only shows symptoms of external damage with little to no internal damage.

The objective of this study was to obtain an understanding of how respiration rate in plums is affected by heat, and how this subsequently affects fruit quality if the fruit is harvested immediately after a heat wave event. We selected the cultivars African Delight, Laetitia and Fortune because of their varied tolerance levels to heat damage. Laetitia and Fortune are mid-season cultivars that are sensitive to both external and internal fruit damage due to heat waves while African Delight usually only exhibits external damage and no internal damage during heat wave conditions. The findings would enable us to relate respiration rate to heat damage sensitivity. The different responses between the cultivars could also be a function of differences in their peel permeabilities to gas diffusion (Theron, 2015), which would ultimately affect respiration.

Our initial assessment of respiration was under field conditions in the 2012/13 season. However, fairly mild weather conditions prevailed in the season with no notable heat waves. Subsequently, no internal heat damage was observed. Findings in the 2012/13 season, therefore, constituted preliminary work upon which subsequent seasons were based on. Maxie and Claypool (1956) were able to induce internal heat damage symptoms under controlled laboratory conditions. However, they did not take into consideration the changes in temperature at night. To ascertain the prevalence of internal heat damage we modified the work of Maxie and Claypool (1956) by manipulating temperature in growth chambers to also include night time temperatures during heat waves. In the final season, the more accurate controlled atmosphere temperature treatment system (CATTS) was used. This enabled the comparison of refined treatments that simulated day and night temperature conditions of heat waves and mild summer days.

MATERIALS AND METHODS

'Laetitia', 'African Delight' and 'Fortune' plums were sampled at harvest to evaluate their heat tolerance under simulated heat wave conditions. 'Laetitia' and 'African Delight' were evaluated in the 2013/14 growing season and were obtained from Môrelig Farm (33° 51' S, 19° 02' E) near Wemmershoek in the Western Cape Province of South Africa. In 2014/15, 'Fortune' plums were sampled from Sandrivier Estate (33° 35' S, 18° 55' E), in Wellington, within the same province.

In 2013/14, 'Laetitia' fruit were sampled at two maturities; at the upper end (approx. 8.5 kg cm^{-2}), and at the lower end (approx. 6.0 kg cm^{-2}) of the picking window, hereinafter referred to as H1 and

H2, respectively. H1 fruit were sampled on 6 Feb. 2014, and H2 on 18 Feb. 2014. 'African Delight' fruit were sampled once on 04 Mar. 2014. In the 2014/15 growing season, 'Fortune' was sampled twice on 13 and 18 Dec. 2014 at approx. 8.0 kg cm^{-2} (H1) and 6.5 kg cm^{-2} (H2), respectively.

In 2013/14 all sampled fruit were treated and assessed at the Department of Horticultural Science, Stellenbosch University. For 'Laetitia' a three-factor complete randomised design (CRD), replicated three times, was used whereby fruit of each harvest maturity were concurrently subjected to 30, 35, 40 or 45 °C (heat exposure) for 1, 2, or 3h (heat exposure duration) in growth chambers (Model ECD01E, Snijders Scientific, Tilburg, Holland). However, the 35 °C chamber broke down before completion of the trial and that treatment will, therefore, not be reported.

General fruit quality, ethanol evolution as an indicator of anaerobic respiration (Maxie and Claypool, 1956; Paul and Pandey, 2014), antioxidant concentration as an indicator of stress (Jooste, 2012) and internal and external defects were assessed after treatments and again after cold storage and shelf life. Total soluble solids concentration (TSS) and titratable acidity (TA) were measured by pooling 10 fruit per replicate of each treatment and crushing them in a blender to extract juice. A hand-held refractometer (Model N1, Atago, Tokyo, Japan) was used to measure TSS from the juice. TA was determined by titrating 0.1 M NaOH to a pH of 8.2 with an automated titrator (Model 719 S, Metrohm AG, Hersiau, Switzerland) and was expressed as percentage of malic acid (g 100 g^{-1} juice). Flesh firmness was measured on one peeled cheek per fruit of all 10 fruit in each replicate using a flesh texture analyser (Guss electronic model GS 20, Strand, South Africa) fitted with an 11.1 mm tip.

Respiration rate and ethylene evolution were determined by placing a two-fruit sample of pre-determined mass and volume in a 5 L airtight glass jar at ambient conditions. After 30 min, three replicates of 10 mL were drawn from the headspace of each jar into gastight syringes. Gas from the syringes were injected into a gas chromatograph (Model N6980, Agilent technologies, Wilmington, USA) fitted with flame ionization and thermal conductivity detectors.

The two-fruit samples were taken out of the glass jars, pitted, cut up and crushed in a blender to give pooled juice extract for ethanol concentration determination. Three 4 mL aliquots of the juice/pulp mixture from each or the three two-fruit replicates were transferred into separate 50 mL glass vials with 1 g of NaCl added. The vials were crimped closed with gastight caps before placing them on an electric mixer until all the NaCl had dissolved. The vials were placed in an oven at 50 °C. After 15 min they were removed from the oven and 10 mL of the headspace gas was drawn

from each vial into separate gastight syringes. Gas from the syringes was injected into a gas chromatograph (Model 3300, Varian Instrument Group, California, USA) fitted with a flame ionisation detector to determine ethanol concentration. Ethanol concentration in the fruit was determined by extrapolations from a standard curve.

Fruit flesh concentration of the antioxidants glutathione and ascorbic acid were assayed using a high performance liquid chromatograph (HPLC) fitted with an autosampler (Series 1100, Agilent Technologies, Inc., Waldbronn, Germany) according to Davey et al. (2003), and adjustments by Jooste (2012) as described in preceding chapters. Internal and external defects were expressed as a percentage of the proportion that developed defects in three 15 fruit replicates per treatment. External defects were unmarketable dark discolorations on the fruit peel. Internal defects included the internal heat damage symptoms of pitburn and GB. Pitburn symptoms were dark brown discolouration of the fruit flesh, mostly on the inner mesocarp around the pit. GB manifests as a gelatinous or translucent appearance of tissue around the pit.

In 2014/15, 'Fortune' fruit were subjected to a three factor CRD replicated three times. The factors consisted of H1 or H2 fruit as described in the previous season (harvest maturity), being exposed to heat wave or mild summer day temperature conditions (temperature regime) for 1 (Day 1), 2 (Day 2) or 3 (Day 3) consecutive days (number of exposure days). Heat wave and mild temperature regimes were determined using hourly temperature data logged from a stone fruit orchard during the 2013/14 season on a typically hot or mild summer's day, respectively.

For each harvest maturity, three replicates of 44 fruit each were used per treatment per day. General fruit quality, respiration rate, ethanol evolution, antioxidant concentration and internal and external heat damage were assessed after treatments and again after cold storage and shelf life as described for the 2013/14 season.

All treatments commenced at 08h00 in the Department of Conservation Ecology (DCE) (to simulate either mild summer day or heat wave temperatures), Stellenbosch University. After treatment the fruit was taken to the Department of Horticultural Science (DHS), Stellenbosch University, and kept at 25 °C until 20h00 in a controlled environment chamber (Model ECD01E, Snijders Scientific, Tilburg, Holland). The temperature was then decreased to 18 °C to simulate average summer night temperatures.

For the mild summer day temperature regime, the fruit pulp temperature was increased from 18 °C to 30 °C at a ramp rate of 0.024 °C min^{-1} in a controlled atmosphere temperature treatment system (CATTS) (Techni-System L.L.C, Washington, USA). The fruit remained in the CATTS for 3 h

upon attaining the 30 °C pulp temperature before being removed and transferred to DHS. To effect the heat wave temperature regime fruit pulp temperature was initially ramped to 30 °C at a rate of 0.04 °C min^{-1}. Upon the fruit attaining 30 °C core temperature, the ramp rate was increased at 0.165 °C min^{-1} until the core temperature was 40 °C. This temperature was maintained for 3 h before transfer to DHS. At DHS, fruit that had completed exposure day/days was either immediately assessed for fruit quality, respiration, ethylene evolution, ethanol and antioxidant concentration or cold-stored for further assessment of these parameters after cold storage and shelf life. Fruit with on-going exposure day/days were stored at 25 °C before 20h00 and reduced to 18 °C thereafter before being transferred to DCE at 08h00.

In cold storage, 'African Delight' was stored at single temperature while 'Laetitia' and 'Fortune' were stored at dual temperature regimes. Fruit stored at single temperature were maintained at -0.5 °C and 92-95% relative humidity (RH) for 42 days. The dual temperature regime had fruit stored at -0.5 °C for 10 days followed by 8 days at 7.5 °C and back to -0.5 °C for 14 days. After cold storage, the batch of fruit designated for assessments after shelf life simulation were transferred to storage conditions of 10 °C and 92-95% RH for 7 days.

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, means were separated by Least Significant Difference (LSD) with a 95% confidence interval. In addition, baseline measurements of 10 fruit per harvest maturity for all cultivars were conducted immediately after harvest for all the parameters before treatments were effected. The measurements were presented as averages with standard error of means.

RESULTS

2013/14 season (Laetitia)

Baseline measurements before treatments at harvest are shown in Table 1. The less mature fruit (H1) were firmer, had lower TSS and higher TA than the more mature fruit (H2). Ethanol evolution rates were similar for the two maturities. The respiration rate of the H2 fruit was higher compared to the H1 fruit. Ethylene was not detected at this stage of assessment.

Internal and external defects and fruit quality

No internal defects were detected in the fruit at the time of effecting treatments at harvest. After cold storage there was a very low incidence of pitburn with H2 fruit showing significantly higher levels of the defect compared to H1 fruit (Table 2). Although there was no significant differences due to heat exposure and exposure duration for pitburn after cold-storage, pitburn incidence tended

to increase with an increase in exposure duration. The majority of internal heat damage symptoms after cold storage were due to GB. There was a significant interaction between harvest maturity and heat exposure for GB incidence after cold-storage (Figure 2). H2 fruit had significantly higher GB levels compared to H1 for fruit previously treated at 30 °C and 40 °C, but no differences were observed between maturities for the 45 °C treatment. In both fruit maturities GB levels observed at 45 °C were also significantly lower compared to 30 °C and 40 °C.

After shelf life simulation there was an increase in the levels of pitburn compared to the levels observed after cold-storage, but GB was still the predominant defect observed in the fruit (Table 2). There were no significant differences in pitburn incidence due to harvest maturity or heat exposure duration. However, fruit that had previously been treated at 30 °C and 40 °C had higher pitburn levels than the 45 °C treatment. H2 fruit had higher GB levels than H1 fruit. Consistent with the findings after cold-storage, GB levels were significantly higher in fruit previously treated at 30 and 40 °C compared to 45 °C, with fruit treated at 40 °C having significantly the highest GB levels. Heat exposure duration did not have a statistically significant effect on GB incidence, but there was a tendency for GB levels to increase with an increase in exposure time.

External defects were only observed after shelf life (Figure 1C). Peel damage displayed a significant interaction between exposure temperature and exposure duration (Figure 3). Changes in percentage peel damage with an increase in exposure duration were insignificant for the high exposure temperatures of 40 °C and 45 °C. However, for the low exposure temperature of 30°C, significantly lower levels of peel damage were measured after 2 h of heat exposure, with a non-significant increase in peel damage after 3 h of exposure.

There was significant interaction between harvest maturity and heat exposure duration immediately after treatment at harvest for titratable acidity (TA) (Figure 4). TA did not differ significantly between the two harvest maturities for the first 2 h of treatment exposure. However, after 3 h H1 fruit had significantly higher TA levels compared to H2 fruit. After cold storage, H1 fruit had significantly higher TA levels compared to H2 fruit. TA levels were also significantly higher in fruit previously treated at 45 °C than that treated at 30 and 40°C after cold storage (Table 3). After shelf life some fruit samples were prematurely discarded before total soluble solids (TSS) and TA were measured and, therefore, results for these are not available.

At harvest, immediately after treatment, there was a significant three-way interaction for TSS between harvest maturity, heat exposure and heat exposure duration (Figure 5). The influence of

these factors on TSS was complex and did not exhibit a definite trend. Generally, the TSS of H1 fruit was lower than that of H2 fruit at this stage. However, after cold storage TSS levels tended to increase with an increase in heat exposure temperature, however the 40 °C and 45 °C treatments did not significantly differ from each other (Table 3). In addition, H1 fruit had lower TSS than H2 fruit at this point of assessment.

There was significant interaction between heat exposure and heat exposure duration for fruit firmness immediately after treatment at harvest (Figure 6). An increase in heat exposure duration for fruit subjected to 30 °C resulted in a decrease in flesh firmness with an increase in exposure time, although there were no significant differences between the second and third hour. No significant changes in flesh firmness were noted for fruit subjected to 40 and 45 °C with an increase in exposure time. However, flesh firmness was significantly higher in fruit exposed to 40 and 45 °C compared to 30 °C after 2 and 3 h of treatment. A somewhat similar trend was noted after cold storage for fruit previously subjected to 30 °C as there was a decline in firmness with an increase in exposure time (Figure 7). The decline was, however, only statistically significant after 3 h. Flesh firmness was still higher after cold-storage in fruit exposed to 40 and 45 °C for 2 and 3 h compared to the fruit exposed to 30 °C, however, the differences were only significant for fruit exposed to 40 °C for 2 h and for the 3 h exposure time. After shelf life, fruit previously subjected to 30 and 45 °C had significantly lower flesh firmness than fruit previously treated at 40 °C (Table 3). H1 fruit was significantly firmer compared to H2 fruit after shelf-life, irrespective of the treatment received.

Respiration rate, ethylene and ethanol evolution

After treatment at harvest there was a significant interaction between heat exposure and heat exposure duration for the fruit respiration rate (Figure 8A). The respiration rate of fruit treated at 30 °C decreased with an increase in exposure time with a significant reduction after 3 h of exposure. The respiration rate of the fruit treated at 40 °C did not change over the treatment time while fruit treated at 45 °C showed a significant increase in respiration rate after 2 h of treatment where after it decreased slightly, but not significantly after 3 h of exposure.

There was also a significant interaction between harvest maturity and heat exposure duration in influencing fruit respiration rate after the heat exposure treatments at harvest (Figure 8B). During the first 2 h of heat exposure H1 fruit respired significantly faster than the H2 fruit. However, after 3 h of exposure to heat, the respiration rate of the H1 fruit declined and was significantly lower than that of H2 fruit. Although the H1 fruit showed a decrease in respiration rate after 3 h of exposure,

the respiration rate of H2 fruit did not differ significantly between exposure durations. There were no significant differences in respiration rate after cold storage (data not shown).

After shelf-life simulation there was a significant interaction between harvest maturity and heat exposure duration for fruit respiration rate (Figure 9). During the first 2 h of heat treatment the respiration rate of the fruit did not change significantly per harvest maturity, but after the third hour of heat treatment the respiration rate of the H1 fruit decreased and that of the H2 fruit increased significantly. After the first and third hour of heat treatment the respiration rate of the H2 fruit was significantly higher compared to that of the H1 fruit.

After the heat treatments at harvest, H2 fruit had significantly higher ethanol concentrations compared to H1 fruit (Table 3). After cold storage there was a significant interaction between harvest maturity and heat exposure for ethanol evolution (Figure 10). The internal ethanol concentration was significantly lower in H1 than in H2 fruit at treated at 30 °C, but the two fruit maturities had similar concentrations at 40 and 45 °C. Ethanol concentrations did not differ significantly between H1 fruit treated at different temperatures, however H2 fruit showed a significant decrease in internal ethanol levels with an increase in treatment temperature. After shelf-life simulation internal ethanol concentration was, irrespective of harvest maturity and heat exposure duration, significantly the highest in fruit previously exposed to 30 °C (Table 3).

Ethylene evolution was only observed after shelf life simulation. There was a significant three-way interaction between harvest maturity, heat exposure, and heat exposure duration for ethylene evolution after shelf-life. (Figure 11). Ethylene evolution for H1 fruit that had been treated at 30 °C and 40 °C increased slightly, but not statistically significant, after 3 h of heat treatment. However, for fruit treated at 45° C, ethylene evolution increased significantly and peaked after 2 h of treatment before significantly decreasing again after 3 h of treatment. Ethylene evolution rates for H2 fruit were generally, but not always significantly, higher compared to the H1 fruit treated at the same temperature and exposure duration. Although there was variation between the H2 fruit, the differences were generally not significant. Only the H2 fruit treated at 30 °C showed a significant decrease after 3 h of treatment to a level that was also significantly lower than the H2 fruit treated at 45 °C for 3h.

Anti-oxidants

There was a significant interaction between harvest maturity and heat exposure for the total glutathione (Figure 12 A) and total ascorbic acid (Figure 12B) concentrations immediately after

treatment at harvest. Concentrations of both antioxidants remained unchanged in H1 fruit exposed to 30, 40 and 45 °C. However, H2 fruit had significantly lower concentrations of both antioxidants after treatment at 40 and 45 °C compared to H2 fruit treated at 30 °C, and compared to the H1 fruit treated at 40 °C and 45 °C.

H1 fruit had a significantly higher concentration of reduced glutathione and oxidised ascorbic acid immediately after treatment at harvest compared to H2 fruit (Table 4). Fruit treated at 30 °C had significantly lower levels of oxidised ascorbic levels, irrespective of harvest maturity, immediately after treatment at harvest. Reduced ascorbic acid and oxidised glutathione were not influenced by harvest maturity, heat exposure or heat exposure duration (data not shown).

After cold storage the concentration of total (Figure 13) and reduced (Figure 14) glutathione as well as total ascorbic acid (Figure 15) were influenced by a significant interaction of harvest maturity and heat exposure duration. The total and reduced glutathione concentrations for H1 fruit remained unchanged with an increase in heat exposure duration while that of H2 fruit decreased significantly after 2 h of heat exposure where after it remained unchanged (Figures 13 and 14). The total ascorbic acid levels of the H1 fruit were not influenced by an increase in exposure temperature, however at 40 and 45 °C there was a significant decrease in total ascorbic acid levels in H2 fruit after cold-storage (Figure 14). The concentration of oxidised glutathione was significantly higher in H1 fruit after cold-storage (Table 4). There were no treatment differences for reduced and oxidised ascorbic acid after cold-storage (data not shown).

After shelf life H1 fruit had significantly higher concentrations of oxidised glutathione, total ascorbic acid and oxidised ascorbic acid than H2 fruit (Table 5). Total ascorbic acid levels were highest in fruit treated at 40 °C.

2013/14 season (African Delight)

The maturity of ‘African Delight’ at harvest, before treatment, is illustrated in Table 6. The cultivar was strip picked towards the end of the season and therefore there is only one level of maturity.

Internal and external defects and fruit quality

No internal defects were observed in ‘African Delight’ at any of the evaluation stages. However, peel damage observed immediately after treatment at harvest was significantly higher in fruit treated at 40 °C and 45 °C than at 30 °C, and increased with an increase in exposure duration (Table 7).

Flesh firmness after treatment at harvest was lowest in fruit exposed to 40 °C and 45 °C, and in fruit treated for 2 h and 3 h (Table 7). No significant differences in firmness were observed after cold storage and shelf life (Tables 8 and 9). Significant differences in TA were only observed after cold storage whereby the shortest heat exposure duration of 1 h resulted in a lower TA compared to the 2 and 3 h durations (Table 8). There were no significant differences in TSS at all stages of assessment (data not shown).

Respiration, ethylene and ethanol evolution

There was a significant interaction between heat exposure and heat exposure duration for respiration rate of the fruit immediately after treatment on the harvest date (Figure 16). The respiration rate of the fruit exposed to 30°C and 40 °C remained unchanged with an increase in heat exposure duration. Fruit treated at 45 °C had significantly the highest respiration rate after 1 h of exposure. Subsequently the respiration rate of the fruit treated at 45 °C decreased significantly after 2 h of treatment, but it was still significantly higher compared to the fruit treated at 30 and 40 °C. After 3 h of exposure the fruit treated at 40 °C and 45 °C had significantly higher respiration rates compared to that treated at 30 °C. No significant differences regarding respiration rate were observed after cold storage (Table 8). After shelf life the fruit treated for 1 h, irrespective of the treatment temperature, had significantly the highest respiration rate (Table 9). Ethylene was not detected at any of the assessment stages.

The highest exposure temperature (45 °C) and longest exposure duration (3 h) had the highest ethanol concentration after treatments at harvest (Table 7). After cold storage, the 3 h heat exposure duration resulted in significantly higher ethanol concentration than in the 1 and 2 h durations, with no differences between heat exposure temperatures (Table 8). After shelf life simulation, no significant differences in ethanol concentrations were observed (Table 9).

Anti-oxidants

The total and reduced ascorbic acid concentrations for fruit treated at 30 °C were significantly lower than that of fruit treated at 40 °C and 45 °C immediately after heat exposure (Table 10). However, the oxidised ascorbic acid concentration at 30 °C did not differ significantly from that of 40 °C, and oxidised ascorbic acid concentrations at 30 °C and 40 °C were significantly lower than at 45 °C. No significant differences were observed in all forms of glutathione.

After cold storage, significant differences were only observed for oxidised glutathione and reduced ascorbic acid (Table 11). Oxidised glutathione was significantly lower for the high exposure temperatures and after the longer exposure durations. Reduced ascorbic acid was significantly increased after the 3 h exposure duration.

Fruit previously exposed to heat for 3 h had significantly higher total and reduced glutathione concentrations after shelf-life (Table 12). Oxidised glutathione levels were significantly the highest after exposure to 40 ºC and significantly the lowest after treatment for 2 h. No differences were observed for any of the forms of ascorbic acid after shelf-life.

2014/15 season (Fortune)

Measurements done at harvest confirmed that H2 fruit were of more advanced maturity than H1 fruit with lower flesh firmness and TA (Table 13). TSS was slightly higher in H2 fruit although there were no significant differences. Ethanol content and rate of respiration were extremely low and did not significantly differ between the harvest maturities. Ethylene was not detected at either fruit maturity.

Internal and external defects and fruit quality

There was a significant interaction between harvest maturity and temperature regime in affecting fruit external damage after treatments at harvest (Figure 17). The mild summer day temperature regime did not result in significant differences in external fruit damage between the two harvest maturities and levels of external damage detected were very low. On the other hand, H2 fruit were significantly more susceptible to external damage under the heat wave temperature regimes compared to H1 fruit. Levels of external damage detected in H1 and H2 fruit were also significantly higher than under the mild summer day simulation. At this assessment stage there was also a significant interaction between number of exposure days and temperature regime (Figure 18). There were no significant increases in external damage and levels of external damage were also very low with an increase in number of exposure days when fruit were treated to the mild summer day temperature regime. However, under the heat wave temperature regime there was an increase in external heat damage with an increase in the number of exposure days with fruit exposed for 3 days having significantly the highest levels of external damage.

There was negligible pitburn throughout all assessment stages for ‘Fortune’ (Table 14). It was the sole internal defect observed immediately after treatment on the harvest date. Thereafter, it occurred simultaneously with gel breakdown after cold storage and shelf life simulation. On assessment of

the fruit immediately after treatment on the harvest day fruit exposed to the heat wave temperature regime had higher pitburn compared to those that experienced mild summer day temperatures. Exposing the fruit for 3 days resulted in significantly higher pitburn incidence than those exposed for 1 or 2 days.

After cold storage and after shelf-life H2 fruit had significantly higher levels of internal defects compared to H1 fruit (Table 14). Fruit that had been treated to heat wave conditions had lower internal defects than those that experienced mild summer day conditions after cold-storage and shelf-life.

H1 fruit were significantly firmer, with higher TA than H2 fruit after treatment at harvest (Table 15). TSS did not show significant differences for all factors. After cold storage the temperature regime and number of exposure days significantly interacted in affecting fruit firmness (Figure 19). Fruit firmness decreased with an increase in exposure duration from one to two days at mild temperature, where after it stabilised. Under the heat wave conditions flesh firmness remained unchanged during the first two days of exposure where after it increased slightly to be significantly firmer compared to fruit exposed to heat wave conditions for only one day.

After shelf life, harvest maturity interacted with number of exposure days to affect fruit firmness (Figure 20). H1 fruit that were exposed to mild or high temperatures for one or two days were firmer than H2 fruit. However, exposure for 3 days resulted in higher firmness of H2 than H1 fruit. Firmness decreased significantly with each additional day of exposure in H1 fruit, but only from one to two days exposure in H2 fruit.

Harvest maturity interacted with temperature regime in influencing TSS after cold storage (Figure 21). In H1 fruit there were no significant differences in TSS between mild and heat wave temperature regime fruit. However, for H2 fruit, exposure to the heat wave temperature regime significantly increased TSS after cold storage. The heat wave treatment resulted in higher TA compared to that of mild summer day after cold storage (Table 16). In addition, H2 fruit had significantly higher TA compared to H1 fruit. Similar trends were observed after shelf life simulation. After cold storage and shelf life simulation, TA increased linearly with exposure duration although there were no significant differences observed after cold storage.

Respiration, ethylene and ethanol evolution

Fruit subjected to heat wave conditions respired at a faster rate than those under the mild summer day conditions after treatment at harvest (Table 15). After cold storage, no significant differences in respiration rate was observed (data not shown). Harvest maturity significantly interacted with temperature regime after shelf life simulation in influencing the rate of respiration (Figure 22). H1 fruit exposed to mild summer day conditions respired faster after shelf life conditions than fruit of the same maturity exposed to the heat wave temperature regime and H2 fruit exposed to mild summer day and heat wave conditions. H2 fruit treated with heat wave conditions had the lowest respiration rate after shelf-life, albeit it did not differ significantly from H2 fruit exposed to mild summer day conditions.

There was a significant interaction between harvest maturity and temperature regime for ethanol evolution after treatment at harvest (Figure 23). Ethanol evolution of H1 fruit was insensitive to exposure temperature, but high temperature increased ethanol levels in H2 fruit. After cold storage there was no significant differences observed in ethanol evolution in any of the treatments (Table 16).

After shelf-life harvest maturity significantly interacted with the temperature regime (Figure 24). The H2 fruit subjected to the heat wave temperature regime had significantly the highest internal ethanol levels compared to the mild summer day temperatures regime at the same maturity as well as the H1 fruit exposed to both temperature regimes. H1 fruit did not show differences between the temperature regimes. There was also a significant interaction between harvest maturity and number of exposure days after shelf-life (Figure 25). H1 fruit had a significantly higher ethanol evolution rate in all 3 consecutive exposure days compared to the H2 fruit. It was also observed that the ethanol evolution rates increased with an increase in number of exposure days in the H1 fruit. H2 fruit only showed a significant increase in ethanol evolution after 2 days of treatment where after the ethanol evolution rate decreased, albeit not statistically significant.

Antioxidants

Immediately after treatment on the respective harvest dates the total glutathione concentration was higher for H2 fruit, in the fruit exposed to heat wave conditions, and after exposure for 2 and 3 days (Table 17). Reduced glutathione showed similar results, but harvest maturities did not differ significantly. Oxidised glutathione was higher in H2 fruit.

There was a significant interaction between harvest maturity and temperature regime for the total ascorbic acid concentration immediately after treatment at harvest (Figure 26). Mild temperature resulted in lower total ascorbic acid concentrations, but the difference was only significant in H1 fruit. Reduced ascorbic acid concentration was affected by a significant three way interaction of harvest maturity, temperature regime and number of days of exposure (Figure 27). For both the H1 and H2 fruit the heat wave temperature regime resulted in significantly higher reduced ascorbic acid levels, except in H1 fruit after 1 day of exposure. Although it was not always statistically significant, H2 fruit tended to have higher levels of reduced ascorbic acid compared to the H1 fruit for the mild summer day and heat wave simulations. H1 fruit also showed an increase in reduced ascorbic acid levels with an increase in exposure time, while levels in the H2 fruit remained fairly constant.

H1 fruit had a higher concentration of oxidised ascorbic acid immediately after treatment at harvest (Table 17). Fruit exposed to only one day of treatment had a higher oxidised ascorbic acid concentration compared to fruit exposed for 2 or 3 days.

After cold storage there were no significant differences in total glutathione (data not shown). However, H1 and high temperature exposure treatment had higher reduced glutathione (Table 18). Fruit that experienced 1 day of exposure had the lowest while 3 days of exposure gave the highest levels of reduced glutathione irrespective fruit maturity or temperature regime. Fruit previously treated for 2 days had an intermediate concentration of reduced glutathione and did not significantly differ with either of the other durations. There was a significant interaction between harvest maturity and temperature regime for oxidised glutathione (Figure 28). Under mild summer day temperatures, H2 fruit had significantly higher levels of oxidised glutathione but there were no significant differences between maturities under heatwave conditions. High temperature exposure resulted in higher total and reduced ascorbic acid concentrations, while H2 had higher reduced ascorbic acid concentration (Table 18). Exposure duration significantly interacted with temperature regime for oxidised ascorbic acid (Figure 29). Fruit exposed to heat wave conditions had significantly lower concentrations of oxidised ascorbic acid starting from 2 days of exposure.

After shelf life there were no significant differences were observed between treatments for all forms of glutathione (data not shown). The heat wave temperature regime resulted in higher total ascorbic acid after shelf life (Table 18). There was a significant interaction between harvest maturity and temperature regime for reduced ascorbic acid concentration (Figure 30). H1 fruit did not show any differences in the reduced acid concentration between temperature regimes. The heat wave

temperature regime resulted in higher reduced ascorbic acid concentration than the mild summer day temperature regime in H2 fruit. There were no significant differences observed for oxidised ascorbic acid after shelf life (data not shown).

DISCUSSION

'Laetitia' and 'Fortune' are normally harvested by selective picking during the harvest window, giving early and late harvests. Hence, assessment of these cultivars at two maturity levels in this study. Perez-Lopez et al. (2014) indicated the significance of tissue maturity in determining responses to factors affecting respiratory metabolism such as temperature (Kays and Paull, 2004). On the other hand, African Delight is a late season cultivar that is strip-picked towards the end of the growing season in the Western Cape. Therefore, we assessed 'African Delight' at one maturity level. Apart from fruit maturity, fruit internal gas composition and ultimately the O_2 to CO_2 ratio in tissue is affected by peel permeability, and intercellular air space (Argenta et al., 2002).

In the susceptible cultivars Laetita and Fortune, we confirmed a reduction in heat damage and ethanol accumulation at high temperatures after cold storage, particularly in fruit of advanced maturity. In 'Laetitia' we observed increases in ethanol and heat damage between 30°C and 40°C and a decline at 45°C. It has been widely reported that high temperatures increase respiration rate, depleting internal fruit O_2 concentration while increasing CO_2 (Mitcham and McDonald, 1993; Shellie and Mangan, 2000). The reduced O_2 to CO_2 ratio promotes anaerobic respiration, with the accumulation of ethanol (Paul and Pandey, 2014; Kader, 1987), subsequently inducing internal heat damage symptoms. In the tolerant cultivar, African Delight, exposure to temperatures of up to 45 °C did not result in threshold ethanol levels for internal heat damage to manifest.

Although the symptoms of internal heat damage in the sensitive cultivars were prominent after cold storage, their incidence was related to respiration and ethanol observations at harvest. After cold storage, there were no significant differences in respiration rates observed in all cultivars. Generally, high respiration rates revert to normal respiration when fruit move from a high temperature environment to ambient or lower temperature conditions (Lurie and Klein, 1991). After shelf life simulation there were no definite trends in respiration and ethanol between treatments. These observations did not relate to internal heat damage symptoms either.

Laetitia is a mid-season maturing plum cultivar in the Western Cape Province. In this cultivar, all the internal heat symptoms were observed after cold storage (Table 2 and Figure 2). Gel breakdown

symptoms were more predominant than pitburn. However, both disorders were generally consistent with respiration and ethanol observations at harvest. Upon assessment after treatment at harvest, we generally noted that an increase in temperature or heat exposure duration increased the rate of respiration up to a point (45 °C and 3 h of exposure) where further increases become inhibitory (Figure 8). Consistent with this observation, ethanol concentration and internal heat damage increased in fruit previously exposed to 30 °C and 40 °C after cold-storage, but decreased at 45°C and this was more prominent in H2 fruit (Table 2 and Figure 10).

As fruit advance in maturity towards ripening, there is a general decrease in the internal O_2 to CO_2 ratio (Bufler and Bangerth, 1982; Paul and Pandey, 2014). The intercellular spaces collapse with increased maturity, decreasing the diffusivity of gases (Rajapakse et al., 1989; Argenta et al., 2002). The breakdown of cell walls due to pectin degradation at ripening results in the accumulation of cell fluids in the intercellular space. This decreases O_2 diffusivity, causing anaerobic respiration (Ho et al., 2006). Rajapakse et al. (1989) confirmed a decrease in O_2 concentration due to ripening associated decrease in intercellular spaces of nectarines. In addition, an increase in soluble sugars has been reported to reduce the diffusivity of O_2. Therefore the observed higher ethanol concentration in more mature fruit at high temperature could have been enhanced by the ripening-related structural cell changes, with further increases in anaerobic conditions being contributed by the increased levels of TSS in the ripening process.

The predominance of gel breakdown, particularly in H2 fruit, could be related to membrane permeability and the ability of water soluble pectins to bind to fluids (Kapp and Jooste, 2006). Leaky membranes and reduced ability of pectins to bind with water results in the formation of the gelatinous symptoms of gel breakdown (Taylor et al., 1994). Water soluble pectin increases with an increase in fruit maturity (Luza et al., 1992). Taylor et al. (1995) implied that fruit of advanced maturity lose cell membrane early in storage. These fruit have a higher sugar content and as membranes leak, cell fluids bind with pectins to form sugar-gel pectins. Therefore, modifications of pectin functions and cell membrane integrity by high temperature are likely to have more tangible effects in more mature than less mature fruit.

The membranes of less mature fruit contain a higher concentration of mono and poly unsaturated fatty acids compared to saturated fatty acids (Jooste, 2013). The structural chains of these unsaturated fatty acids are flexible and therefore promote fluidity and permeability of the membrane (Somerville et al., 2002; Upchurch 2008). Therefore, when stored at low temperature, the mono and poly unsaturated acids remain in their fluid state, while saturated fatty acids harden (Upchurch,

2008).

At 45 °C we observed a reduction in ethanol concentration and internal heat damage in 'Laetitia'. Most biochemical processes have been observed to proceed two to threefold faster for every 10 °C increase in temperature before reaching the inhibitory phase (Zagory and Kader, 1988). We, therefore, suggest that at high temperatures such as 45 °C, the function of the enzyme alcohol dehydrogenase, which is responsible for converting acetaldehyde into ethanol, being protein in nature, is reduced due to denaturation (Kim et al., 2000). In addition, an accumulation of ethanol within fruit tissue can inhibit its further synthesis (Ritenour et al., 1997).

Numerous reports have indicated the importance of pre-harvest heat treatments in inducing tolerance to cold storage disorders. In most cases, the cold storage disorders are a result of oxidative stress due to active oxygen species (AOS) increasing and exceeding the antioxidant quelling capacity (Schirra and Cohen, 1999). High temperatures promote an increase in the anti-oxidant pool of ascorbic acid and glutathione (Almeselami et al., 2006; Hasanuzzaman et al., 2013). These are the most important non-enzyme scavengers of AOS in plants (Foyer, 1993).

Heat treatments up regulate the antioxidant levels, preventing the accumulation of the hazardous AOS when fruit are exposed to subsequent temperature extremes (Vincete et al., 2006). In addition, moderate heat treatment enhances the maintenance of membrane integrity by preventing ion leakage during cold storage (Vicente et al., 2006). Reduced ion leakage across membranes and increased tolerance to cold storage disorders due to heat treatments has been reported in many fruits such as tomatoes (Salveit, 2005) and strawberries (Vicente et al., 2006).

Woolf and Ferguson (2000) reported heat curing of cold storage disorders in avocado exposed to pre-storage temperatures of up to 50 °C. Vicente et al. (2006) exposed strawberries to 45°C for 3 hours with no tissue damage. This fruit subsequently showed resistance to internal cold storage disorders. Similarly, pre-storage heat treatments were reported to reduce chilling injury of different peach cultivars (Cao et al., 2010). In this study, the much lower heat damage levels in 'Laetitia' exposed to 45 °C could, therefore, also be due to a curing effect the high temperatures had on the fruit.

High temperatures can also initiate changes in protein synthesis and gene expression (Lurie, 1998). The messenger ribonucleic acid (mRNA) proteins of genes such as those responsible for fruit ripening are disunited and reassembled as heat shock proteins (HSP) under high temperature

conditions (Picton and Grierson, 1988; Ferguson et al., 1994). The genetic expression of these proteins include folding so as to protect cell contents from being denatured by heat, thereby acting as chaperons (Wang et al., 2004). When proteins are denatured by high temperatures, the polypeptide chains aggregate into dysfunctional clusters. However, when present during heat stress, chaperons oversee the correct folding and assembly of protein subunits to avoid damage and maintain cell integrity. As plants have the ability to perceive the heat stimulus, transcriptional signals are relayed for the re-activation of the HSPs (Sharkey and Schrader 2006). Therefore, the HSPs offer thermo-tolerance to recurring heat damage.

One form of stress in plants not only pre-conditions the tissue against the same recurring stress, but also against a different form of stress (Lurie et al., 1994). Studies later revealed that HSPs can also pre-condition fruit against cold storage related disorders (Lurie and Klein, 1991; Salveit, 1991; Woolf et al., 1995; Lurie, 1998). Sabahat et al. (1996) compared the HSP profile of tomatoes stored at 2 °C and 20 °C degrees after heat stress. The HSP persisted in tomatoes kept at 2 °C but not in those at 20 °C. Therefore the heat stress initiated thermo-tolerance can be carried over to protect against low temperature stress in cold storage. It can, therefore, also be suggested that the lower levels of heat damage observed in 'Laetitia' exposed to 45 °C could also have been due to the production of heat shock proteins at these high temperatures.

In 'Laetitia' we observed that the less susceptible H1 fruit generally had higher levels of total glutathione and ascorbic acid soon after treatment at harvest compared to H2 fruit (Figure 12A and B). While total glutathione and ascorbic acid levels remained relatively constant in H1 fruit at the temperatures tested, levels were the highest at 30 °C (the coolest temperature), and decreased significantly at 40 and 45 °C in H2 fruit (which were more susceptible to heat damage than H1 fruit). Thermo tolerance, which seemed to occur at 45 °C, could have interfered with the antioxidant protective system. We suggest that antioxidant enzymes could have been denatured or reconstituted to HSPs at high temperatures. Immediately after treatment, H1 fruit also had higher levels of the reduced form of glutathione than H2 fruit (Table 4). Several studies have indicated that the existence of more reduced forms of antioxidants and less of the oxidised forms better adapts tissues to abiotic stress of high and low temperature (Noctor and Foyer, 1998; Szalai et al., 2009; Ummarat et al., 2011). In this study we observed that H1 fruit had significant higher levels of oxidised ascorbic acid levels soon after treatment at harvest (Table 4). Although similar observations in almost all levels of antioxidants were observed after cold storage, a comparison of H1 and H2 indicated less oxidised glutathione in H2. This could have been an indication of a weak or inefficient free radical quelling system in H2 fruit, hence more susceptible to oxidative cold

storage damage than H1 fruit. It has been noted in effective systems when plants undergo even moderate oxidative stress, the reduced glutathione to oxidised glutathione ratio can decrease twenty fold in a short period (Mhamdi, et al., 2010). Increases in antioxidant concentrations in H1 fruit were only observed after cold storage and shelf life and we suggest that this was a response to low temperature oxidative stress (Purvis, 2004; Ummarat et al., 2011).

Fortune is a heat-sensitive, mid-season cultivar often harvested at two maturities. From our findings it appears this cultivar is more sensitive than Laetitia as the first pitburn symptoms manifested immediately after treatment before cold storage (Table 14 and Figures 17 and 18). No gel breakdown symptoms were observed at harvest. However, after cold storage, pitburn symptoms almost always appeared simultaneously with gel breakdown. Consistent with observations in 'Laetitia', H2 had higher internal heat damage incidence at harvest. The predominance of internal heat damage in H2 fruit persisted after cold storage and shelf life simulation. In H1 fruit, ethanol evolution was insensitive to the different temperature regimes immediately after treatment on the harvest date, but in H2 fruit the heat wave temperature regime resulted in higher ethanol concentrations in the fruit (Figure 23).

We observed significantly lower internal defects in 'Fortune' fruit previously exposed to heat wave conditions compared to mild summer day temperatures after cold storage and after shelf-life (Table 14). This was also consistent with findings in 'Laetitia' after cold storage, which confirms our previous suggestion that pre-storage heat exposure-initiated tolerance to cold storage induced internal heat damage symptoms.

The antioxidant profiles of 'Fortune' after treatment at harvest were characterised by larger pools of the various forms of glutathione and ascorbic acid that were prominent in H2 and fruit undergoing heat stress. This was consistent with our expectations as higher levels of ascorbic acid have been observed in fruit experiencing high temperatures in sun exposed positions or green houses (Davey et al., 2000).

African Delight is a late maturing cultivar that is less sensitive to internal heat damage. In this study as well no internal heat damage symptoms was observed. However, there were clear indications that ethanol levels in 'African Delight' responded to temperature exposure and duration after treatments at harvest. Levels increased with increased temperature exposure or duration (Figure 16). Regardless of temperature, higher ethanol levels after cold storage were only observed in fruit that had received the 3 h exposure duration, with no differences after shelf life simulation. We suggest

that ethanol accumulation could be a function of the difference between the rate at which O_2 is consumed in respiration and the rate at which it diffuses into the fruit. This could be affected by peel permeability of the fruit.

Theron (2015) compared the peel permeability of different plum cultivars. 'African Delight' fruit peel was about 20% more permeable than that of 'Laetitia'. In addition, 'African Delight' peel is characterized by open hairline concentric rings which are prominent on the stem-end of the fruit. These rings enhance gaseous exchange between the internal fruit environment and the external atmosphere. Theron (2015) did not test peel permeability of 'Fortune'. However, observations in South Africa indicate that it is not as susceptible to moisture stress as 'African Delight' and 'Laetitia', suggesting that its peel is less permeable than the former two cultivars. In 'African Delight' it is therefore possible that respiration could use O_2 slightly faster than it diffuses into the fruit. This would result in a net accumulation of ethanol over time, albeit not high enough to cause internal damage. Therefore according to our observations, 'African Delight' would require temperatures above 45 °C for durations longer than 3 hours for heat damage to manifest.

No ethylene evolution was detected in 'African Delight' and 'Fortune' plums, while for 'Laetitia', it was only observed after shelf life simulation (Figure 10). Plums are mostly climacteric fruit as they are characterised by peak respiration and ethylene production during ripening. However, Abdi et al. (1997) identified cultivars termed suppressed climacteric which produce limited amounts of ethylene towards the end of the ripening phase. 'Laetitia' plums display a climacteric ripening pattern (Argenta et al., 2003) while 'Fortune' has been reported to have a suppressed climacteric pattern (Kapp, 2008). To our knowledge, 'African Delight' has neither been classified as fully climacteric nor suppressed climacteric. Based on our findings, we suggest that it might have a suppressed climacteric behaviour.

Exposure of these cultivars to heat treatments in our study could have completely inhibited ethylene synthesis during ripening. In 'Friar' plums, peak ethylene production was reported between 5 and 15 °C and increasing temperature to 25 °C during ripening completely inhibited ethylene production (Wang et al., 2016). A few hours exposure to heat treatment inhibited the biosynthesis of ethylene in tomatoes and apples (Biggs et al., 1988; Klein, 1989). In tomatoes an increase in temperature beyond 25 °C resulted in a decrease in ethylene biosynthesis and respiration (Inaba and Chachin, 1989). Lee and Young (1984) reported suppressed ethylene biosynthesis at temperatures above 30 °C in avocado.

The influence of the heat treatment on suppression of ethylene biosynthesis in 'African Delight' and 'Fortune' seemed to have disrupted or inhibited the normal fruit ripening processes in our study as some maturity indices seemed to deviate from the expected after cold storage. In 'Fortune', loss of TA was slower in fruit treated to the heat wave temperature regime after cold storage (Table 16) and shelf life simulation. Loss of TA is chiefly a result of the use of acids, particularly malic acid, as respiratory substrates (Eskin and Hoehn, 2013). Fruit treated at the heat wave temperature regime started out respiring faster than those treated to mild summer temperature conditions at harvest. Possibly due to the heat initiated biochemical disruptions and ethylene suppression, respiration slowed down and there were no significant differences observed after cold storage. This could have subsequently slowed down the ripening process as evidenced by the retarded loss of TA.

In both 'African Delight' and 'Fortune', fruit that received the longest heat exposure time had the highest TA although there were no significant differences for 'African Delight' after shelf life simulation (Table 9). For 'Fortune', the high temperatures and their longer exposure period seemed to affect the reduction of TA more in H2. From cold storage onwards, H2 significantly had higher TA levels.

In 'Fortune', we observed that fruit exposed to the heat wave temperature regime for 3 days were firmer after cold storage (Figure 18). In peaches and nectarines, Malakou and Nanos (2005) also reported that the heat-treated fruit were firmer than the control after 2 weeks in cold storage. Woolf and Ferguson (2000) advised to expect a delay in ripening in fruits subjected to high temperatures prior to harvesting. In their previous work, sun exposed avocados that attained temperatures of about 35 °C were firmer and took 1.5 days longer to ripen compared to shaded fruit (Woolf et al., 1999).

The maintenance of firmness in heat treatments could be a result of denaturation or inhibition of the synthesis of proteins, particularly the hydrolytic cell wall degrading enzymes such as pectin lyase, β-galactosidase and polygalacturonases (Yoshida et al., 1984; Lurie, 1998; Vicente et al., 2005; Spadoni et al., 2014). These are responsible for the fruit softening process. Strawberries that were treated to a temperature of 45 °C for 3 h had reduced levels of β-galactosidase and polygalacturonases (Vicente et al., 2005). A similar observation in plums (Tsugi et al., 1984), peaches and nectarines (Malakou and Nanos, 2005; Bakshi and Masoodi, 2009), tomatoes (Biggs et al., 1988) and pears (Maxie et al., 1974) has been reported before. Retarded loss in fruit firmness occurs at temperatures up to 40 °C (Lurie, 1998).

The effects of heat treatments and heat exposure duration after treatments on fruit peel damage at harvest were clearer in ‘African Delight’ and ‘Fortune’. An increase in heat exposure temperature and duration increased peel damage immediately after heat wave simulation at harvest in ‘African Delight’ (Table 7). As for ‘Fortune’, there was a significant interaction between harvest maturity and temperature regime in affecting fruit external damage immediately after treatment on the harvest date (Figure 17). The effect of mild summer temperature conditions on external damage on ‘Fortune’ was the same on both H1 and H2 fruit. On the other hand, H2 fruit were more susceptible to external damage under the heat wave temperature regimes compared to H1 fruit. This indicates that ‘Fortune’ is more susceptible to external heat damage in the later part of the fruit harvesting windows. In addition, an increase in number of exposure days under heat wave conditions increased external heat damage of ‘Fortune’ irrespective of fruit maturity (Figure 18).

CONCLUSION

In the sensitive cultivars Laetitia and Fortune, internal heat damage symptoms of fruit manifested most prominently after cold storage than immediately after heat treatment at harvest or after shelf life simulation. Both cultivars seemed more sensitive to gel breakdown than pitburn. However, 'Fortune' appeared to be more susceptible to pitburn than 'Laetitia.' We confirmed that 'African Delight' is highly tolerant to internal heat damage as it did not exhibit any symptoms of damage. This could be a result of increased peel permeability of 'African Delight' which enhanced availability of oxygen for increased aerobic respiration. In the sensitive cultivars, an increase in ethanol at harvest, caused by increases in temperature and longer durations at high temperature was related to an increase in internal damage after cold storage. This was generally between 30 °C and 40 °C.

There were no significant differences in respiration rates observed in all cultivars after cold storage. After shelf life simulation there were no clear trends in respiration and ethanol between treatments. These observations did not relate to heat damage symptoms either. Increases in ethanol and internal damage after treatments at harvest were higher in more mature fruit treated at 30 °C and 40 °C but tended to decline at 45 °C in 'Laetitia'. In 'Fortune' more mature fruit were consistently more susceptible to internal heat damage by the heat wave temperature regime. This first became evident at harvest and increased after cold storage. We, therefore, concluded that 'Fortune' is more susceptible than 'Laetitia'. However, the internal defects increased after cold storage in both susceptible cultivars, but it appeared fruit that had been subjected to high temperatures treatments were more tolerant in cold storage. High temperature treatments can potentially be used for curing against cold storage enhanced heat damaged if used with methods that circumvent external peel damage. 'Laetitia' and 'Fortune' were more tolerant to peel damage compared to 'African Delight'. This is important as external fruit appearance considerably influences consumer appeal.

All the treatment temperatures were high enough to suppress ethylene synthesis in 'Fortune' and 'African Delight' plums. This affected the ripening process and subsequently fruit quality, albeit not always negatively. This was more apparent in fruit of advanced maturity that received high heat treatments for longer durations. Flesh firmness notably increased under high temperature treatments. This has potential in extending shelf life of fruit. With further research, this may be recommended in cultivars such as Fortune and Laetitia that seemed to withstand heat damage better than African Delight. Although there seemed to be a reduced rate of loss of TA, there was a general increase in TSS with heat treatments. TSS has often correlated positively with increased consumer

perception of fruit. It is therefore recommended that studies be carried out to verify the likelihood of an overall increase in consumer appeal.

At a large commercial scale, the energy costs involved in pre-storage heat treatments would have to be quantified and compared to the realisable monetary benefits. It is also important to consider other practical aspects such as holding facilities of fruit, equipment, time and labour involved to efficiently carry out such treatments before commercial recommendations can be made. Long term approaches such as crossing the susceptible cultivars with African Delight might be worthwhile in that the new cultivars would be resistant to internal heat damage while retaining the desirable previous characteristics.

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Table 1. Harvest maturity measurements for 'Laetitia' plums at harvest, before simulated heat exposure treatments. The fruit were sampled from Môrelig farm packhouse, Wemmershoek, at two harvest maturities.

Parameter	Mean and Standard error of mean	
	Harvest 1	Harvest 2
Flesh firmness (kg cm^{-2})	7.88 ± 0.11	6.11 ± 0.08
Total soluble solids (%)	11.2 ± 0.13	11.7 ± 0.08
Titratable acidity (%)	1.5 ± 0.09	1.3 ± 0.03
Ethanol (%)	0.022 ± 0.002	0.021 ± 0.003
CO_2 evolution (mg kg^{-1} h^{-1})	0.027 ± 0.005	0.048 ± 0.005

Table 2. Effect of harvest maturity, exposure temperature and exposure duration on pitburn of 'Laetitia' plums after cold storage and after shelf-life. Fruit were sampled from Môrelig farm, Wemmershoek at two harvest maturities.

Treatment	After cold-storage	After shelf-life	
	Pitburn (%)	Pitburn (%)	Gel breakdown (%)
Harvest maturity			
Harvest 1	0.78b	3.89	11.0b
Harvest 2	1.56a	5.00	15.6b
Heat exposure (°C)			
30	1.11	6.55a	16.5b
40	1.44	5.61a	20.3a
45	0.94	1.17b	2.88c
Heat exposure duration (h)			
1	0.89	4.22	12.0
2	1.28	5.17	13.6
3	1.33	3.94	14.2
F test			
Harvest maturity (HM)	*0.0022*	*0.2237*	*0.0004*
Heat exposure duration (HED)	*0.2550*	*0.5130*	*0.2806*
Heat exposure (HE)	*0.2222*	*<0.0001*	*<0.0001*
*HM*HED*	*0.1693*	*0.3219*	*0.1030*
*HM*HE*	*0.5967*	*0.1500*	*0.3800*
*HE*HED*	*0.8762*	*0.2574*	*0.1588*
*HM*HE*HED*	*0.8252*	*0.7567*	*0.1582*

Table 3. Effect of heat exposure and heat exposure duration on ethanol evolution of 'Laetitia' plums after simulated heat exposure at harvest and after shelf-life and on flesh firmness after shelf-life in the 2013/14 season. The fruit were sampled from Môrelig farm packhouse, Wemmershoek, at two harvest maturities.

Treatment	TA (%)	TSS (%)	Flesh firmness (kg cm^{-2})	Ethanol evolution (%)	
	After cold-storage	After cold-storage	After shelf life	At harvest	After shelf life
Harvest 1	1.17a	11.4b	3.16a	0.031 a	1.113
Harvest 2	0.928b	12.1a	2.42b	0.149 b	1.015
Heat exposure (°C)					
30	1.02b	11.5b	2.69b	0.110	2.054a
40	1.04b	11.9a	2.96a	0.038	0.695b
45	1.09a	11.9a	2.73b	0.118	0.442b
Heat exposure duration (h)					
1	1.02	11.9	2.85	0.0492	1.372
2	1.08	11.7	2.70	0.1497	0.930
3	1.06	11.7	2.82	0.0716	0.888
F test					
Harvest maturity (HM)	*<0.0001*	*<0.0001*	*<0.0001*	*0.0055*	*0.7533*
Heat exposure (HE)	*0.0218*	*0.0245*	*0.0122*	*0.2058*	*0.0003*
Heat exposure duration (HED)	*0.0648*	*0.2366*	*0.2668*	*0.1128*	*0.3719*
*HE*HED*	*0.1382*	*0.3440*	*0.8204*	*0.0863*	*0.7797*
*HM*HED*	*0.1501*	*0.1812*	*0.8278*	*0.1653*	*0.1279*
*HM*HE*	*0.0873*	*0.6198*	*0.9741*	*0.4476*	*0.2853*
*HM*HE*HED*	*0.0794*	*0.8324*	*0.1612*	*0.0696*	*0.6149*

Table 4. Effect of harvest maturity, exposure temperature and exposure duration on glutathione and ascorbic acid concentration of 'Laetitia' plums at harvest and after cold storage. Fruit were sampled at two harvest maturities from Môrelig Farm, Wemmershoek, during the 2013/14 season.

	After heatwave simulation at harvest		After cold storage
	Reduced glutathione (µg g^{-1} FW)	Oxidised ascorbic acid (µg g^{-1} FW)	Oxidised glutathione (µg g^{-1} FW)
Harvest maturity			
Harvest 1	20.3a	11.9a	3.93a
Harvest 2	18.4b	8.3b	0.61b
Heat exposure duration (h)			
1	20.2	9.6	2.13
2	20.2	9.9	2.54
3	17.7	10.8	2.13
Heat exposure (°C)			
30	20.4	7.6b	2.52
40	18.7	8.8a	2.26
45	18.9	8.1a	2.02
F test			
Harvest maturity (HM)	*0.0489*	*0.0374*	*<0.0001*
Heat exposure duration (HED)	*0.0581*	*0.8227*	*0.1656*
Heat exposure (HE)	*0.2719*	*0.0061*	*0.1315*
*HE*HED*	*0.2189*	*0.1755*	*0.0915*
*HM*HED*	*0.2466*	*0.9362*	*0.0820*
*HM*HE*	*0.1859*	*0.0577*	*0.5538*
*HM*HE*HED*	*0.6341*	*0.4718*	*0.5706*

Table 5. Effect of harvest maturity, exposure temperature and exposure duration on glutathione and ascorbic acid concentration of 'Laetitia' plums after shelf life. Fruit were sampled from Môrelig farm in Wemmershoek at two harvest maturities.

	Total glutathione (µg g^{-1} FW)	Reduced glutathione (µg g^{-1} FW)	Oxidised glutathione (µg g^{-1} FW)	Total ascorbic acid (µg g^{-1} FW)	Reduced ascorbic acid (µg g^{-1} FW)	Oxidised ascorbic acid (µg g^{-1} FW)
Harvest maturity						
Harvest 1	21.4	20.0	1.4a	20.7a	14.3	6.5a
Harvest 2	22.0	21.1	0.9b	15.4b	13.9	1.5b
Heat exposure (°C)						
30	21.8	20.1	1.7	18.3b	12.6	5.7
40	22.2	19.7	2.3	21.2a	14.5	6.7
45	22.8	20.8	2.0	18.7b	13.6	5.3
Heat exposure duration (h)						
1	20.7	19.4	1.4	19.2	13.5	5.5
2	21.6	20.2	1.4	19.4	13.8	5.4
3	22.1	20.8	1.3	18.9	13.9	5.1
F test						
Harvest maturity (HM)	*0.2311*	*0.4523*	*0.0250*	*0.0039*	*0.0989*	*0.0059*
Heat exposure duration (HED)	*0.5129*	*0.7545*	*0.7721*	*0.2345*	*0.7856*	*0.1076*
Heat exposure (HE)	*0.5455*	*0.6655*	*0.0725*	*0.0014*	*0.1366*	*0.2768*
*HM*HED*	*0.1209*	*0.3556*	*0.1232*	*0.3321*	*0.5646*	*0.1873*
*HM*HE*	*0.8376*	*0.7310*	*0.5668*	*0.1156*	*0.2767*	*0.5435*
*HE*HED*	*0.1943*	*0.4332*	*0.4775*	*0.4354*	*0.1244*	*0.5252*
*HM*HE*HED*	*0.7129*	*0.4291*	*0.1317*	*0.6876*	*0.3233*	*0.1369*

Table 6. Harvest maturity measurements for 'African Delight' plums sampled at Môrelig Farm, Wemmershoek, before heat exposure simulation at harvest.

Parameter	Mean and Standard error of mean
Firmness (kg)	8.19 ± 0.125
Total soluble solids (%)	13.0 ± 0.15
Titratable acidity (%)	0.97 ± 0.01
Ethanol evolution (%)	0.027 ± 0.002
CO_2 evolution (mg kg^{-1} h^{-1})	0.028 ± 0.004

Table 7. Effect of heat exposure and heat exposure duration on fruit quality, flesh firmness and ethanol evolution of 'African Delight' plums after simulated heat exposure at harvest. The fruit were sampled from Môrelig farm, Wemmershoek.

	Peel damage (%)	Flesh firmness (kg cm^{-2})	Ethanol evolution (%)
Heat exposure (°C)			
30	18.5b	8.36a	0.015b
40	40.0a	7.89b	0.038b
45	45.2a	7.75b	0.123a
Heat exposure duration (h)			
1	20.7c	8.40a	0.020b
2	35.6b	7.80b	0.000b
3	47.4a	7.79b	0.156a
F test			
Heat exposure (HE)	*<0.0001*	*0.0112*	*0.0268*
Heat exposure duration (HED)	*0.0001*	*0.0067*	*0.0012*
*HE*HED*	*0.3616*	*0.7215*	*0.0806*

Table 8. Effect of heat exposure and heat exposure duration on fruit quality, respiration rate and ethanol evolution and of 'African Delight' plums after cold storage in the 2013/14 season. The fruit were sampled from Môrelig farm packhouse, Wemmershoek.

	Flesh firmness (kg cm^{-2})	TA (%)	CO_2 evolution (mg $kg^{-1}h^{-1}$)	Ethanol evolution (%)
Heat exposure (°C)				
30	6.73	0.61	0.042	0.065
40	7.35	0.60	0.019	0.025
45	7.24	0.62	0.025	0.078
Heat exposure duration (h)				
1	7.29	0.59b	0.023	0.007b
2	6.81	0.63a	0.020	0.016b
3	7.20	0.63a	0.044	0.146a
F test				
Heat exposure	*0.0802*	*0.4355*	*0.3978*	*0.6337*
Heat exposure duration	*0.1993*	*0.0498*	*0.3321*	*0.0459*
*HE*HED*	*0.4169*	*0.2237*	*0.4899*	*0.8430*

Table 9. Effect of heat exposure and heat exposure duration on fruit quality, respiration rate and ethanol evolution of 'African Delight' plums after shelf life in the 2013/14 season. The fruit were sampled from Môrelig farm packhouse, Wemmershoek.

	Flesh firmness (kg cm^{-2})	Titratable acidity (%)	CO_2 evolution (mg $kg^{-1}h^{-1}$)	Ethanol evolution (%)
Heat Exposure (°C)				
30	5.44	0.60	0.019	0.029
40	5.80	0.57	0.019	0.026
45	6.16	0.58	0.020	0.090
Heat exposure duration (h)				
1	5.82	0.57	0.022a	0.134
2	5.53	0.58	0.018b	0.010
3	6.05	0.59	0.017b	0.003
F test				
Heat exposure (HE)	*0.1269*	*0.3078*	*0.7620*	*0.5212*
Heat exposure duration (HED)	*0.3206*	*0.8083*	*0.0347*	*0.0924*
*HE*HED*	*0.4408*	*0.1773*	*0.2452*	*0.4780*

Table 10. Effect of exposure temperature and exposure duration on glutathione and ascorbic acid concentration of 'African Delight' plums immediately after treatment at harvest. Fruit were sampled from Môrelig farm, Wemmershoek.

	Total glutathione (µg g^{-1} FW)	Reduced glutathione (µg g^{-1} FW)	Oxidised glutathione (µg g^{-1} FW)	Total ascorbic acid (µg g^{-1} FW)	Reduced ascorbic acid (µg g^{-1} FW)	Oxidised ascorbic acid (µg g^{-1} FW)
Temperature (°C)						
30	21.8	19.9	1.88	48.4b	40.9b	7.47b
40	19.6	18.6	1.00	64.6a	57.4a	7.26b
45	20.1	18.7	1.47	68.2a	53.4a	14.8a
Duration (h)						
1	19.3	18.4	0.97	58.0	45.6	12.4
2	22.7	20.2	2.45	62.3	53.7	8.60
3	19.5	18.5	0.93	60.9	52.5	8.47
F test						
Heat exposure (HE)	*0.4835*	*0.7616*	*0.6484*	*0.0002*	*0.0023*	*0.0060*
Heat exposure duration (HED)	*0.1558*	*0.5952*	*0.2072*	*0.5686*	*0.1351*	*0.1781*
*HE*HED*	*0.9725*	*0.9995*	*0.7196*	*0.8515*	*0.6043*	*0.1830*

Table 11. Effect of harvest maturity, exposure temperature and exposure duration on glutathione and ascorbic acid concentration of 'African Delight' plums after cold storage. Fruit were sampled from Môrelig farm in Wemmershoek and treated at harvest.

	Total glutathione (µg g^{-1} FW)	Reduced glutathione (µg g^{-1} FW)	Oxidised glutathione (µg g^{-1} FW)	Total ascorbic acid (µg g^{-1} FW)	Reduced ascorbic acid (µg g^{-1} FW)	Oxidised ascorbic acid (µg g^{-1} FW)
Heat exposure (°C)						
30	19.0	16.4	2.54a	40.3	31.2	9.8
40	16.7	15.6	1.09b	45.7	36.1	9.0
45	19.3	18.8	0.37b	49.3	35.4	11.1
Heat exposure duration (h)						
1	17.5	15.1	2.34a	42.0	30.8b	12.4
2	18.5	17.5	0.99b	40.5	30.8b	9.8
3	18.9	18.2	0.66b	52.9	41.3a	11.5
F test						
Heat exposure (HE)	*0.2331*	*0.1561*	*0.0002*	*0.2764*	*0.4890*	*0.1244*
Heat exposure duration (HED)	*0.6520*	*0.1754*	*0.0017*	*0.0702*	*0.0407*	*0.1001*
*HE*HED*	*0.3396*	*0.1740*	*0.1679*	*0.9954*	*0.8637*	*0.0645*

Table 12. Effect of exposure temperature and exposure duration on glutathione and ascorbic acid concentration of 'African Delight' plums after shelf life. Fruit were sampled from Môrelig farm.

	Total glutathione (µg g^{-1} FW)	Reduced glutathione (µg g^{-1} FW)	Oxidised glutathione (µg g^{-1} FW)	Total ascorbic acid (µg g^{-1} FW)	Reduced ascorbic acid (µg g^{-1} FW)	Oxidised ascorbic acid (µg g^{-1} FW)
Heat exposure (°C)						
30	13.8	11.4	2.4 b	16.5	12.5	4.0
40	15.6	12.2	3.5 a	16.9	13.5	3.4
45	15.4	13.3	2.1 b	16.2	13.2	3.0
Heat exposure duration (h)						
1	13.5 b	10.5 b	3.0 a	17.3	13.5	3.8
2	12.4 b	10.6 b	1.8 b	15.8	13.5	3.6
3	18.6 a	15.7 a	3.1 a	16.4	12.2	3.0
F test						
Heat exposure (HE)	*0.1541*	*0.1240*	*0.0352*	*0.7179*	*0.3612*	*0.3409*
Heat exposure duration (HED)	*<0.0001*	*<0.0001*	*0.0312*	*0.2611*	*0.1263*	*0.4420*
*HE*HED*	*0.2670*	*0.1214*	*0.8765*	*0.3082*	*0.4008*	*0.0643*

Table 13. A comparison of fruit quality parameters, respiration rate and internal ethanol levels between early (harvest 1) and late (harvest 2) maturities of 'Fortune' plums before treatment in a Controlled Atmosphere Temperature Treatment System (CATTS) chamber. Fruit were harvested from Sandrivier Estate, Wellington.

Parameter	Mean and Standard error of mean
Harvest 1	
Flesh firmness (kg cm^{-2})	8.75 ± 0.18
Total soluble solids (%)	13.4 ± 0.21
Titratable acidity (%)	1.82 ± 0.027
Ethanol (%)	0.00026 ±0.0003
CO_2 evolution (mg kg^{-1} h^{-1})	0.0062 ±0.002
Harvest 2	
Flesh firmness (kg cm^{-2})	6.94 ± 0.06
Total soluble solids (%)	13.5 ± 0.12
Titratable acidity (%)	1.72 ± 0.019
Ethanol (%)	0.00068±0.0002
CO_2 evolution (mg kg^{-1} h^{-1})	0.0051±0.001

Table 14. The effect of harvest maturity, temperature regime and number of exposure days on internal defects of 'Fortune' plums at harvest, after cold storage and shelf life simulation. Fruit were sampled from Sandrivier Estate, Wellington at two harvest maturities and treated to simulated mild summer day and heat wave temperature regime in a Controlled Atmosphere Temperature Treatment System (CATTS).

	Internal defects (pitburn) after treatment at harvest (%)	Internal defects (pitburn and gel breakdown) after cold storage (%)	Internal defects (pitburn and gel breakdown) after shelf life (%)
Harvest maturity			
Harvest 1	2.22	6.1 b	9.44b
Harvest 2	2.78	12.2a	18.3a
Temperature regime			
Mild summer day	0.55b	16.1a	23.8a
Heat wave	4.44a	2.2b	3.88b
Number of exposure days			
1	0.83b	8.33	13.3
2	1.67b	9.17	15.8
3	5.00a	10.0	12.5
F test			
Harvest maturity (HM)	*0.6647*	*0.0323*	*0.0012*
Temperature regime (TR)	*0.0055*	*0.0001*	*0.0001*
Number of exposure days (NED)	*0.0317*	*0.8793*	*0.5052*
*HM*NED*	*0.8259*	*0.5787*	*0.2095*
*HM*TR*	*0.6647*	*0.5396*	*0.5432*
*NED*TR*	*0.2797*	*0.9579*	*0.3386*
*HM*TR*NED*	*0.2797*	*0.8793*	*0.6887*

Table 15. The effect of harvest maturity and number of exposure days to a simulated mild summer day and heat wave temperature regime on fruit quality of 'Fortune' plums after treatment at harvest. Fruit were sampled from Sandrivier Estate, Wellington, and treated in a Controlled Atmosphere Temperature Treatment System (CATTS).

	Flesh firmness (kg cm^{-2})	TSS (%)	TA (%)	CO_2 evolution (mg $kg^{-1}h^{-1}$)
Harvest maturity				
Harvest 1	8.48a	13.6	1.89a	0.054
Harvest 2	7.72b	13.3	1.70b	0.049
Treatment regime				
Mild summer day	8.25	13.7	1.83	0.045b
Heat wave	7.95	13.3	1.76	0.057a
Number of exposure days				
1	8.22	13.3	1.76	0.053
2	8.07	13.5	1.79	0.054
3	8.01	13.7	1.83	0.047
F test				
Harvest (HM)	*0.0012*	*0.1946*	*<0.0001*	*0.1014*
Temperature regime (TR)	*0.1543*	*0.0677*	*0.0801*	*0.0006*
Number of exposure days (NED)	*0.6714*	*0.2918*	*0.2982*	*0.0969*
*TR*NED*	*0.1776*	*0.8469*	*0.7234*	*0.8438*
*HM*NED*	*0.3896*	*0.9053*	*0.1167*	*0.2019*
*HM*TR*	*0.9958*	*0.9425*	*0.0633*	*0.0673*
*HM*TR*NED*	*0.9742*	*0.6778*	*0.0996*	*0.6245*

Table 16. The effect of harvest maturity, temperature regime and number of exposure days to a simulated mild summer day and heat wave temperature regime on titratable acidity and ethanol evolution of 'Fortune' plums after cold storage. Fruit were sampled from Sandrivier Estate, Wellington and treated in a Controlled Atmosphere Temperature Treatment System (CATTS) chamber on the harvest date.

	Ethanol evolution (%) after cold storage	Titratable acidity (%) after cold storage	Titratable acidity (%) after shelf life
Harvest maturity			
Harvest 1	0.0016	1.60 b	1.74 a
Harvest 2	0.0017	1.77 a	1.59 b
Temperature regime			
Mild summer day	0.0017	1.65 b	1.58 b
Heat wave	0.0016	1.73 a	1.75 a
Number of exposure days			
1	0.0023	1.63	1.59 c
2	0.0016	1.68	1.66 b
3	0.0010	1.74	1.74 a
F test			
Harvest maturity (HM)	*0.8574*	*0.0001*	*<0.0001*
Temperature regime (TR)	*0.8005*	*0.0360*	*<0.0001*
Number of exposure days (NED)	*0.2231*	*0.0654*	*0.0009*
*TR*NED*	*0.1219*	*0.7779*	*0.3851*
*HM*NED*	*0.5238*	*0.6458*	*0.8090*
*HM*TR*	*0.8405*	*0.2696*	*0.1002*
*HM*TR*NED*	*0.3560*	*0.5847*	*0.0767*

Table 17. The effect of harvest maturity, temperature regime and number of exposure days on the concentration of glutathione of 'Fortune' plums on the harvest date immediately after treatment. Fruit were sampled from Sandrivier Estate, Wellington.

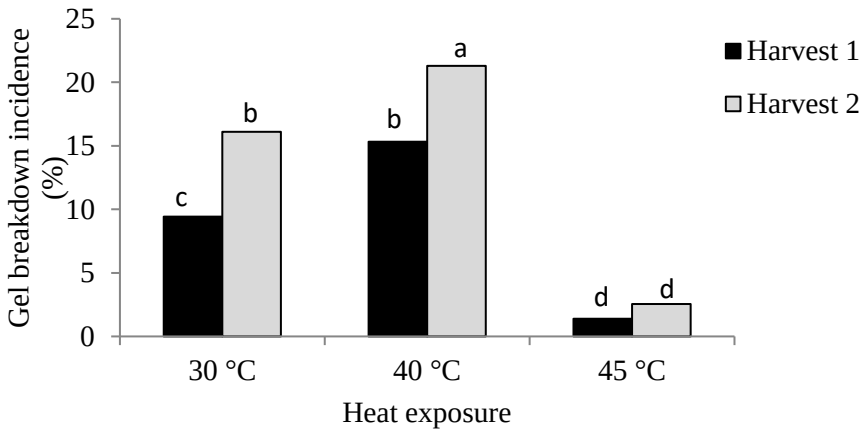
	Total glutathione (µg g^{-1} FW)	Reduced glutathione (µg g^{-1} FW)	Oxidised glutathione (µg g^{-1} FW)	Oxidised ascorbic acid (µg g^{-1} FW)
Harvest maturity				
Harvest 1	7.537b	6.524	1.012b	29.348a
Harvest 2	8.695a	7.155	1.539a	25.427b
Temperature regime				
Mild summer day	6.678b	5.470b	1.207	28.024
Heat wave	9.555a	8.210a	1.344	26.752
Number of exposure days				
1 Day	6.997b	5.814b	1.183	31.074a
2 Days	8.980a	7.524a	1.456	25.015b
3 Days	8.371a	7.181a	1.189	26.075b
F test				
Harvest maturity (HM)	*0.0054*	*0.1415*	*0.0307*	*0.0155*
Temperature regime (TR)	*<0.0001*	*<0.0001*	*0.5550*	*0.4039*
Number of exposure days (NED)	*0.0009*	*0.0066*	*0.5463*	*0.0071*
*HM*NED*	*0.9676*	*0.9054*	*0.9042*	*0.1372*
*HM*TR*	*0.3753*	*0.4316*	*0.9735*	*0.0765*
*NED*TR*	*0.8189*	*0.2385*	*0.1146*	*0.7381*
*HM*TR*NED*	*0.1223*	*0.0635*	*0.3365*	*0.0547*

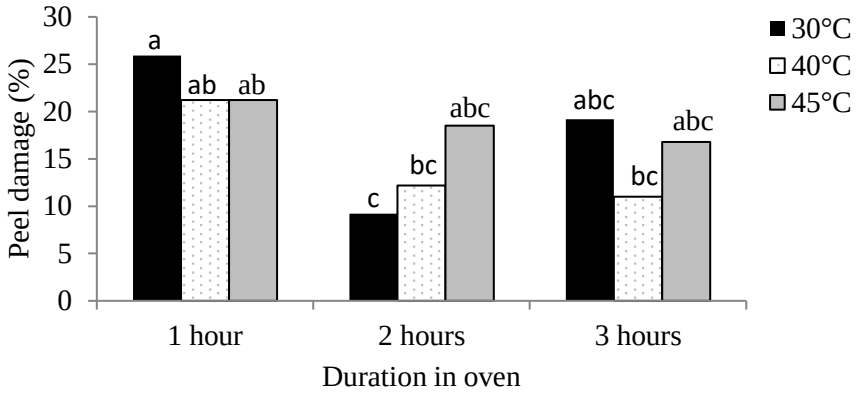
Table 18. The effect of harvest maturity, temperature regime, and number of heat exposure days on reduced glutathione concentration after cold storage and total ascorbic acid concentration after shelf life of 'Fortune' plums. Fruit were sampled from Sandrivier Estate, Wellington, and treated in a Controlled Atmosphere Temperature Treatment System (CATTS) chamber at harvest.

	After cold-storage			After shelf life
	Reduced glutathione (µg g^{-1} FW)	Total ascorbic acid (µg g^{-1} FW)	Reduced ascorbic acid (µg g^{-1} FW)	Total ascorbic acid (µg g^{-1} FW)
Harvest maturity				
Harvest 1	9.05a	23.2	10.3b	17.6
Harvest 2	8.32b	22.9	13.0a	17.7
Temperature regime				
Mild summer day	7.30b	21.3b	7.19b	17.4b
Heat wave	10.1a	24.9a	16.1a	18.0a
Number of exposure days				
1	8.18b	23.3	11.3	17.5
2	8.67ab	22.1	11.5	17.9
3	9.21a	23.8	12.2	17.6
F test				
Harvest maturity (HM)	*0.0323*	*0.6722*	*0.0050*	*0.6768*
Temperature regime (TR)	*<0.0001*	*<0.0001*	*<0.0001*	*0.0326*
Number of exposure days (NED)	*0.0495*	*0.0680*	*0.6853*	*0.5586*
*HM*NED*	*0.5753*	*0.8289*	*0.7885*	*0.5970*
*HM*TR*	*0.0611*	*0.3074*	*0.1792*	*0.4823*
*NED*TR*	*0.2255*	*0.6456*	*0.0533*	*0.8348*
*HM*TR*NED*	*0.5226*	*0.9402*	*0.3806*	*0.8886*

A **B** **C**

Figure 1 Typical symptoms of pitburn （A）, gel breakdown （B）and heat damage (C) of heat treated plums. The pitburn shown is on ‘Fortune’ plum, and the gel breakdown and heat damage are depicted on ‘Laetitia’.



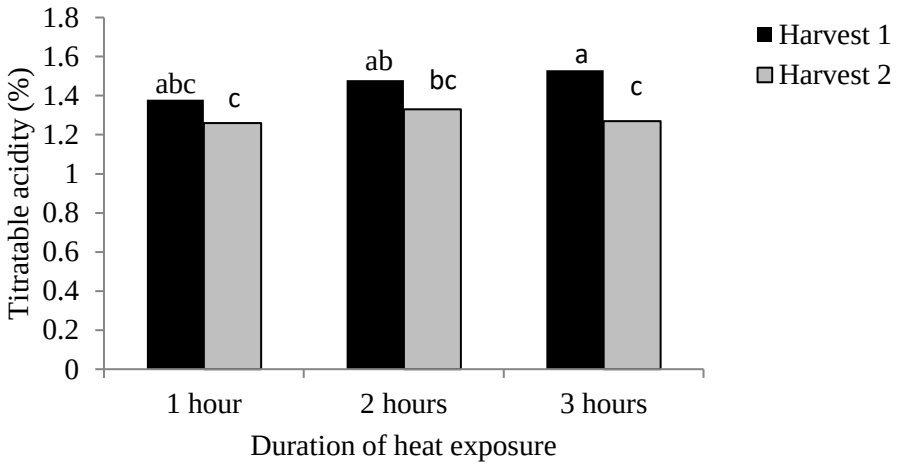


	F test
Harvest maturity (HM)	*<0.0001*
Heat exposure (HE)	*<0.0001*
Heat exposure duration (HED)	*0.0643*
*HE*HED*	*0.3698*
*HM*HED*	*0.0870*
*HM*HE*	*<0.0001*
*HM*HE*HED*	*0.2619*

Figure 2. Interaction between harvest maturity and heat exposure for 'Laetitia' internal heat damage (gel breakdown) after cold storage. Fruit were sampled from Môrelig farm in Wemmershoek and treated at harvest.

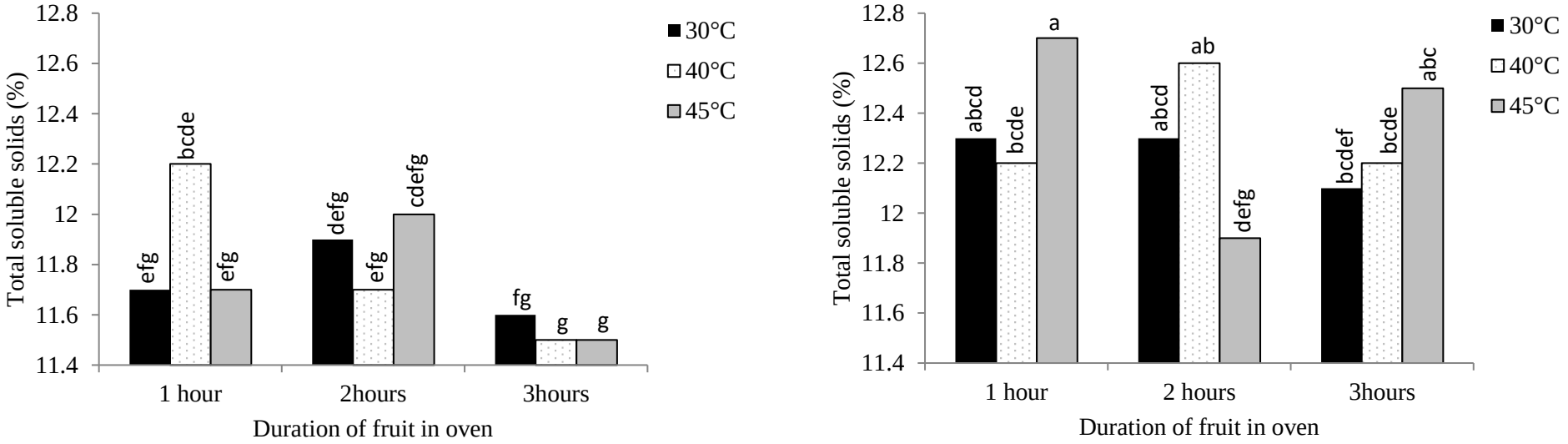
	F test
Harvest maturity (HM)	*<0.0001*
Heat exposure (HE)	*0.3661*
Heat exposure duration (HED)	*0.0653*
*HE*HED*	*0.0075*
*HM*HED*	*0.0620*
*HM*HE*	*0.4105*
*HM*HE*HED*	*0.4569*

Figure 3. Interaction between exposure temperature and duration of exposure for peel damage of 'Laetitia' plums during the 2013/14 season. Fruit were sampled from Môrelig farm in Wemmershoekon. Peel damage was assessed after shelf life.



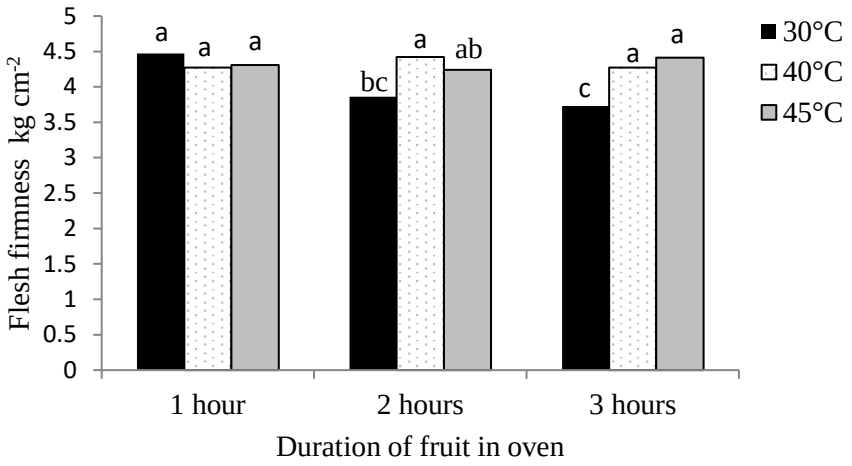
	F test
Harvest maturity (HM)	*<0.0001*
Heat exposure (HE)	*0.0002*
Heat exposure duration (HED)	*<0.0001*
*HE*HED*	*0.1023*
*HM*HED*	*0.0003*
*HM*HE*	*0.1500*
*HM*HE*HED*	*0.1519*

Figure 4. Interaction between harvest maturity and exposure temperature for titratable acidity of 'Laetitia' plums after treatment at harvest. Fruit were sampled from Môrelig Farm.



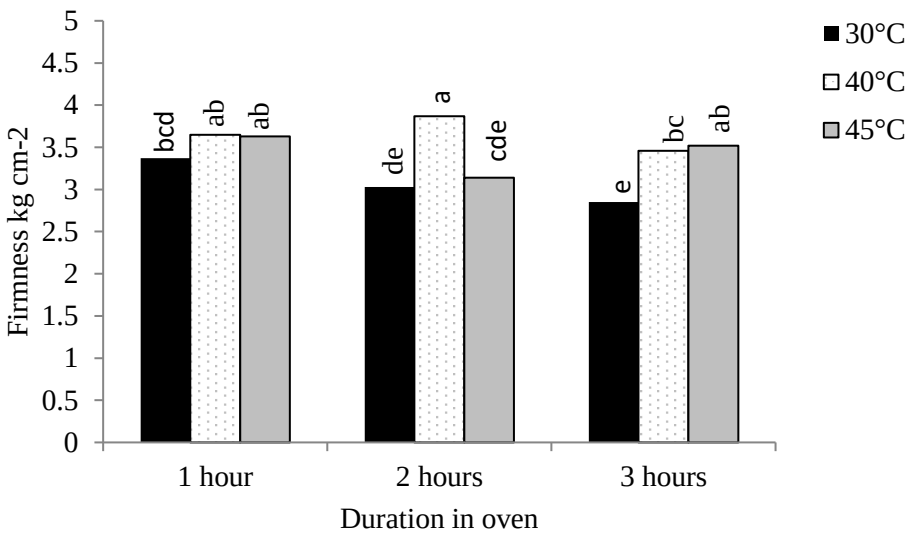
Harvest 1	Harvest 2
F test	*F test*
Harvest maturity (HM)	*<0.0001*
Heat exposure (HE)	*0.7204*
Heat exposure duration (HED)	*0.0999*
*HE*HED*	*0.6485*
*HM*HED*	*0.4366*
*HM*HE*	*0.7465*
*HM*HE*HED*	*0.0088*

Figure 5. Three-way interaction for harvest maturity, exposure temperature and exposure duration as it influenced total soluble solids of 'Laetitia' plums after treatment at harvest. Fruit were sampled from Môrelig farm at harvest during the 2013/14 season. The two graphs have been divided between harvest maturities for better clarity and perception of the three-way interaction and should therefore be considered as a single unit.



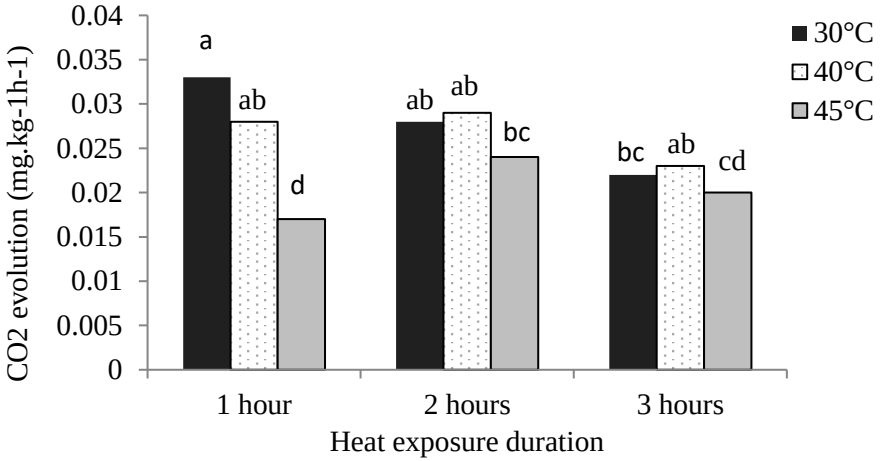
	F test
Harvest maturity (HM)	*<0.0001*
Heat exposure (HE)	*0.0150*
Heat exposure duration (HED)	*0.1412*
*HE*HED*	*0.0161*
*HM*HED*	*0.9573*
*HM*HE*	*0.4098*
*HM*HE*HED*	*0.2625*

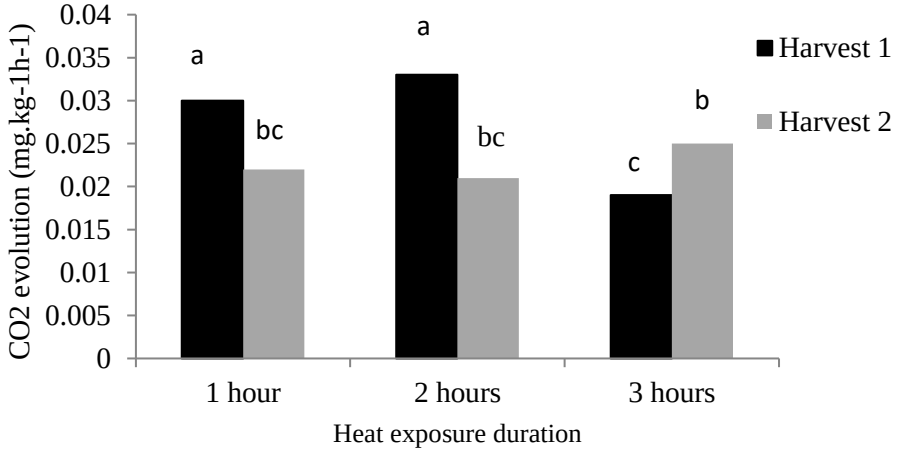
Figure 6. Interaction between exposure temperature and duration of exposure for flesh firmness of 'Laetitia' plums after heat wave simulation on the harvest date. Fruit were sampled from Môrelig Farm in Wemmershoek.



	F test
Harvest maturity (HM)	*<0.0001*
Heat exposure (HE)	*0.0150*
Heat exposure duration (HED)	*0.1412*
*HE*HED*	*0.0161*
*HM*HED*	*0.9573*
*HM*HE*	*0.4098*
*HM*HE*HED*	*0.2625*

Figure 7. Interaction between exposure temperature and duration of exposure on flesh firmness of 'Laetitia' plums after cold storage. Fruit were sampled from Môrelig Farm in Wemmershoek.





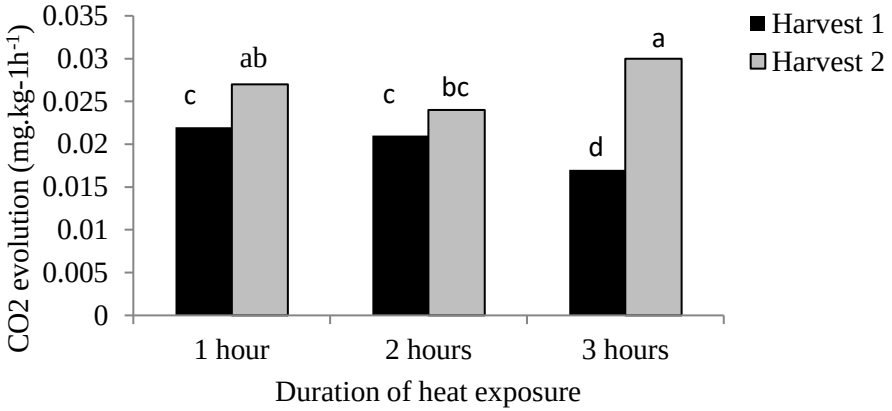
A

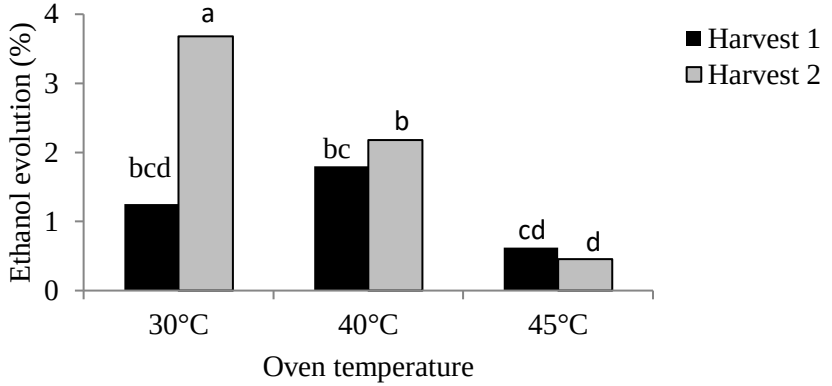
	F test
Harvest maturity (HM)	*0.0018*
Heat Exposure (HE)	*<0.0001*
Heat Exposure Duration (HED)	*0.0066*
*HE*HED*	*0.0187*
*HM*HED*	*<0.0001*
*HM*HE*	*0.2094*
*HM*HE*HED*	*0.9708*

B

	F test
Harvest maturity (HM)	*0.0018*
Heat Exposure (HE)	*<0.0001*
Heat Exposure Duration (HED)	*0.0066*
*HE*HED*	*0.0187*
*HM*HED*	*<0.0001*
*HM*HE*	*0.2094*
*HM*HE*HED*	*0.9708*

Figure 8. Interaction between heat exposure and heat exposure duration (A) and harvest maturity and heat exposure duration (B) for respiration of ‘Laetitia’ plums after simulated heat exposure at harvest. Fruit were sampled from Môrelig farm, Wemmershoek, at two harvest maturities.



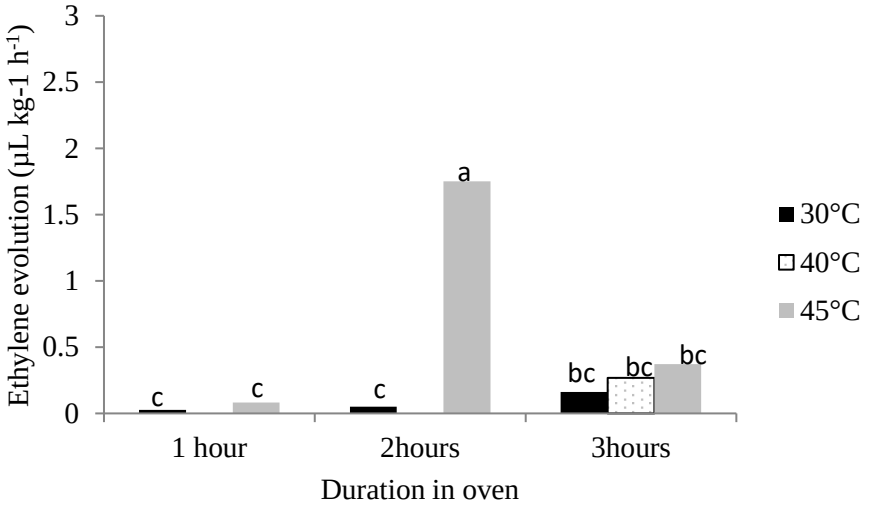


	F test
Harvest maturity (HM)	*<0.0001*
Heat exposure (HE)	*0.6588*
Heat exposure duration (HED)	*0.3262*
*HE*HED*	*0.0719*
*HM*HED*	*0.0051*
*HM*HE*	*0.7474*
*HM*HE*HED*	*0.1384*

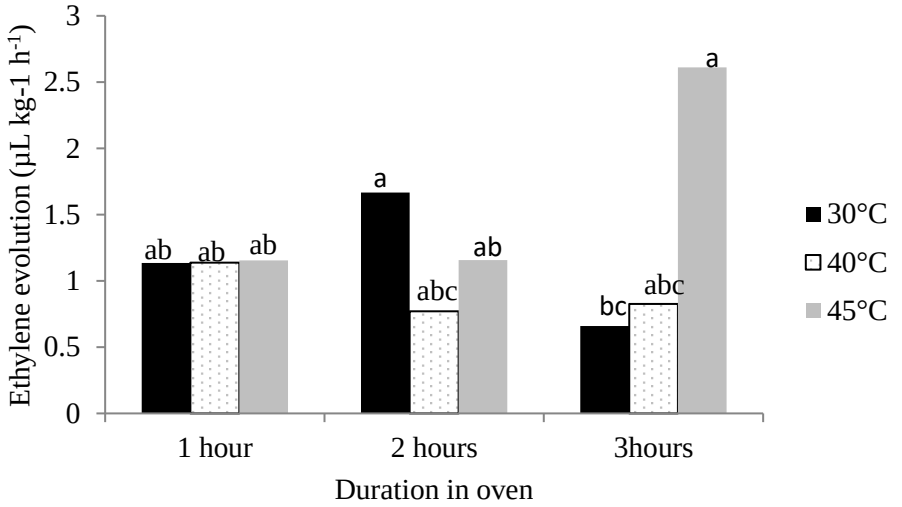
Figure 9. Interaction between harvest maturity and exposure duration for respiration rate of 'Laetitia' plums after shelf-life. The fruit were sampled from Môrelig farm in Wermmershoek.

	F test
Harvest maturity(HM)	*0.0248*
Heat exposure (HE)	*0.0005*
Heat exposure duration (HED)	*0.0725*
*HE*HED*	*0.6696*
*HM*HED*	*0.2271*
*HM*HE*	*0.0193*
*HM*HE*HED*	*0.2622*

Figure 10. Interaction between harvest maturity and heat exposure for internal ethanol concentration of 'Laetitia' plums after cold-storage. The fruit were sampled from Môrelig farm in Wermmershoek.



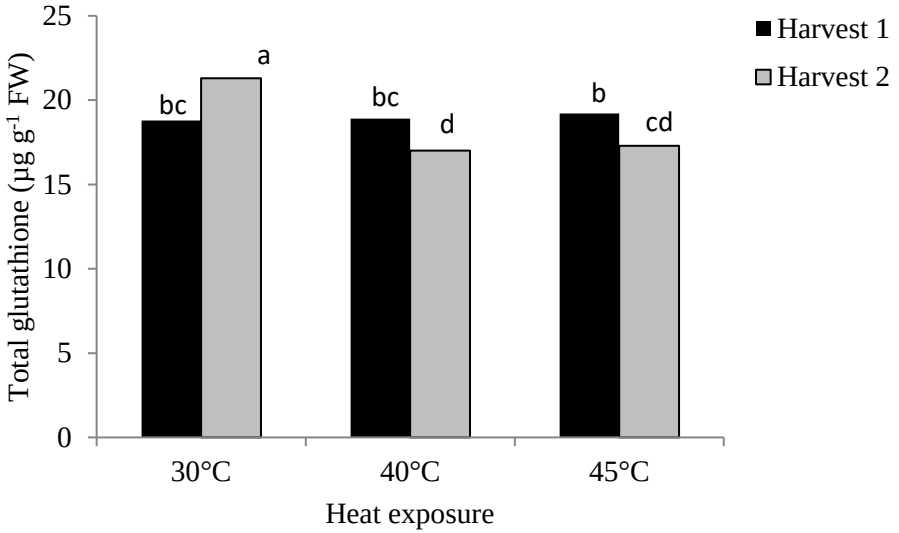
Harvest 1



Harvest 2

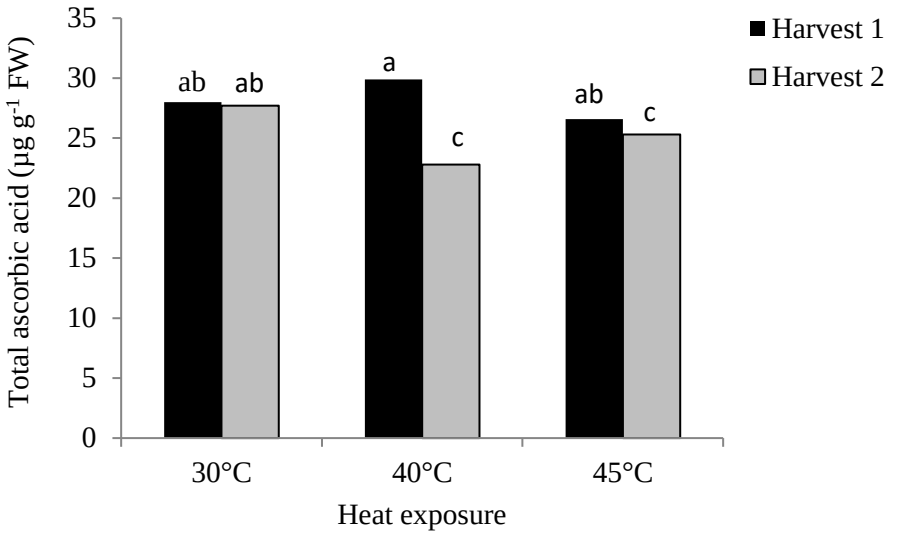
	F test
Harvest maturity(HM)	*<0.0001*
Heat exposure (HE)	*0.0046*
Heat exposure duration(HED)	*0.3760*
*HE*HED*	*0.1001*
*HM*HED*	*0.3075*
*HM*HE*	*0.8782*
*HM*HE*HED*	*0.0045*

Figure 11. Three-way interaction for harvest maturity, heat exposure and heat exposure duration as it influenced ethylene evolution after shelf life of 'Laetitia' plums sampled from Môrelig farm during the 2013/14 season. The two graphs have been divided between harvest maturities for better clarity and perception of the three-way interaction and should, therefore, be considered as a single unit.



A

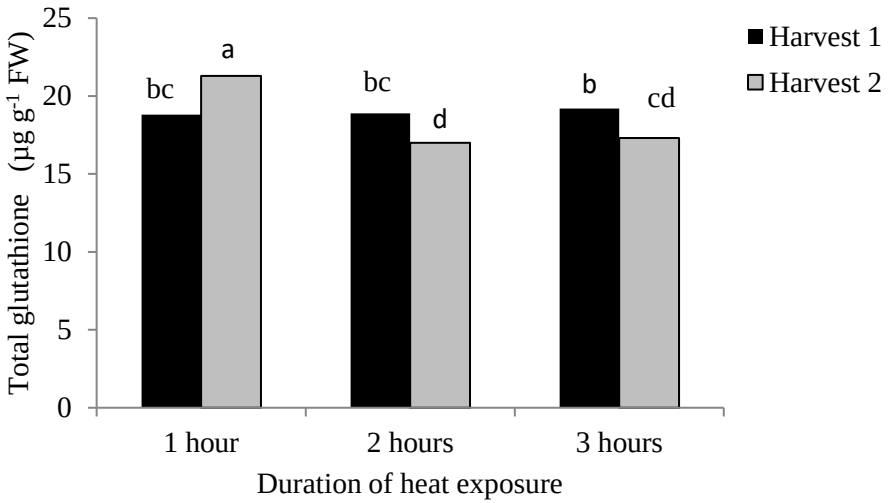
	F test
Harvest maturity (HM)	*0.6272*
Heat exposure (HE)	*0.2546*
Heat exposure duration (HED)	*0.0789*
*HE*HED*	*0.0793*
*HM*HED*	*0.9595*
*HM*HE*	*0.0205*
*HM*HE*HED*	*0.3361*



B

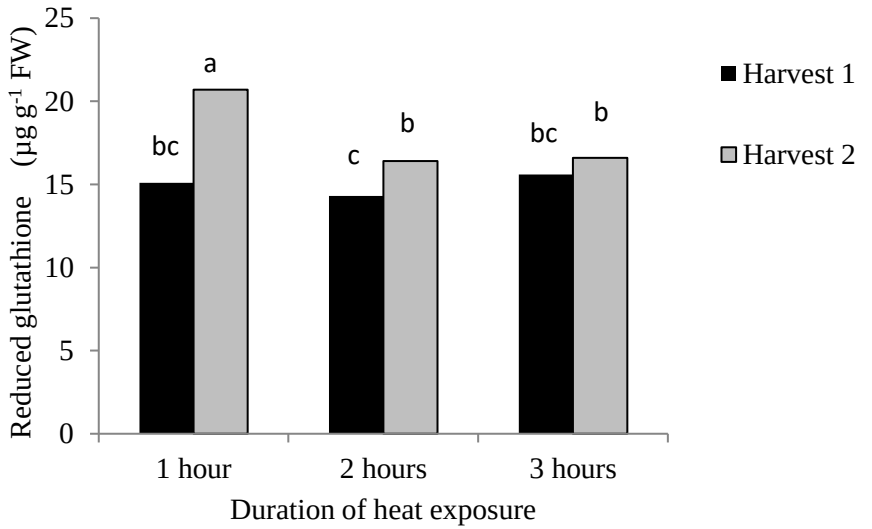
	F test
Harvest maturity (HM)	*0.0032*
Heat exposure (HE)	*0.0624*
Heat exposure duration (HED)	*0.7820*
*HE*HED*	*0.8350*
*HM*HED*	*0.7220*
*HM*HE*	*0.0367*
*HM*HE*HED*	*0.8212*

Figure 12. Interaction between harvest maturity and heat exposure on total glutathione concentration (A) and total ascorbic acid (B) of ‘Laetitia’ plums after treatment at harvest. Fruit were sampled from Môrelig Farm during the 2013/14 season.



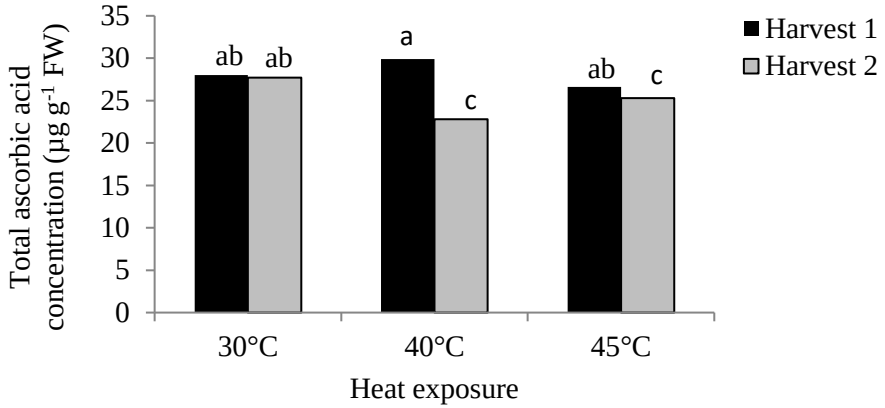
	F test
Harvest maturity (HM)	*0.3780*
Heat exposure (HE)	*0.5018*
Heat exposure duration (HED)	*0.0020*
*HE*HED*	*0.4365*
*HM*HED*	*0.0005*
*HM*HE*	*0.8578*
*HM*HE*HED*	*0.8263*

Figure 13. Interaction between harvest maturity and heat exposure duration for total glutathione concentration of 'Laetitia' plums after cold-storage. Fruit were sampled from Môrelig farm in Wemmershoek and treated at harvest.



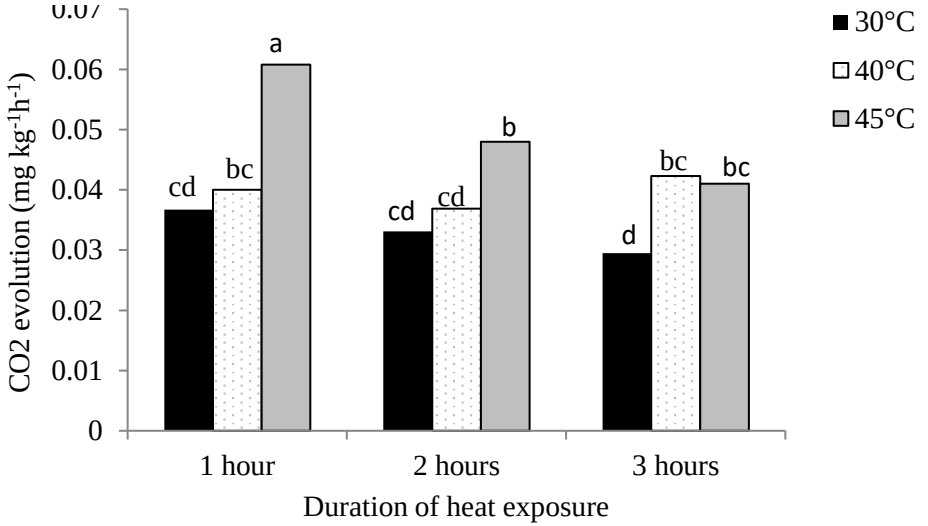
	F test
Harvest maturity (HM)	*0.0001*
Heat exposure (HE)	*0.5891*
Heat exposure duration (HED)	*0.0003*
*HE*HED*	*0.2663*
*HM*HED*	*0.0007*
*HM*HE*	*0.9212*
*HM*HE*HED*	*0.6601*

Figure 14. Interaction between harvest maturity and heat exposure duration for reduced glutathione concentration of 'Laetitia' plums after cold-storage. Fruit were sampled from Môrelig farm in Wemmershoek and treated at harvest.



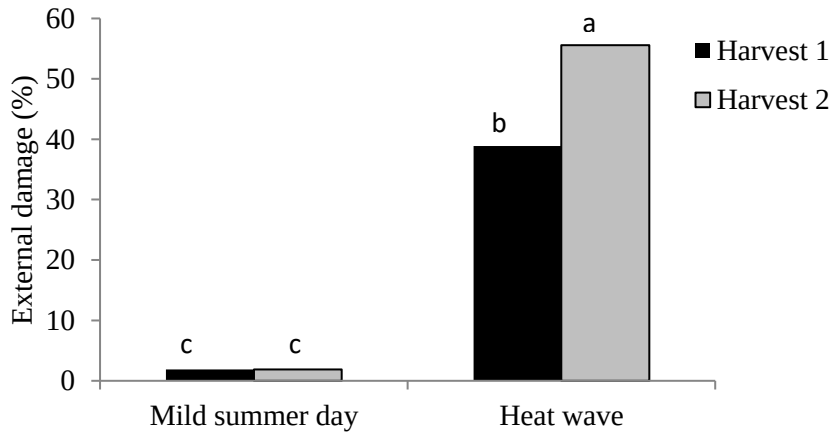
	F test
Harvest maturity (HM)	*0.0046*
Heat exposure (HE)	*0.2587*
Heat exposure duration (HED)	*0.2323*
*HE*HED*	*0.6048*
*HM*HED*	*0.5260*
*HM*HE*	*0.0145*
*HM*HE*HED*	*0.2064*

Figure 15. Interaction between harvest maturity and heat exposure temperature for total ascorbic acid concentration of 'Laetitia' plums after cold-storage. Fruit were sampled from Môrelig farm in Wemmershoek and treated at harvest.



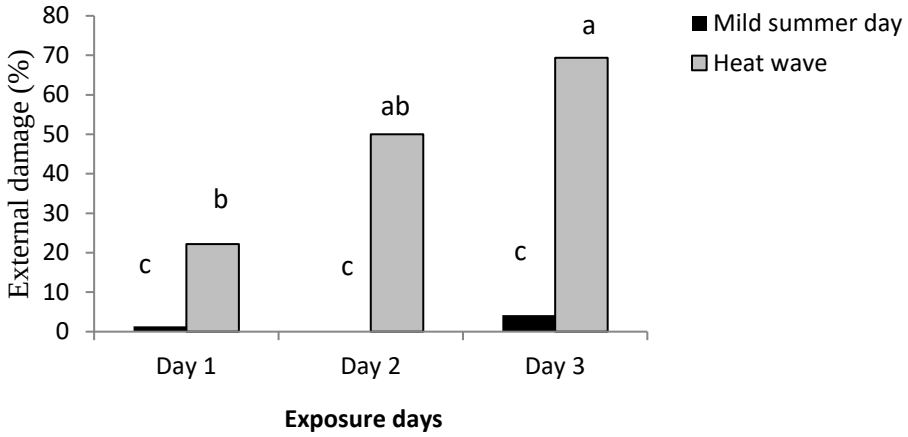
	F test
Heat exposure (HE)	*<0.0001*
Heat exposure duration (HED)	*0.0136*
*HE*HED*	*0.0411*

Figure 16. Interaction between heat exposure and heat exposure duration for respiration of 'African Delight' plums after simulated heat exposure at harvest. Fruit were sampled from Môrelig Farm, Wemmershoek.



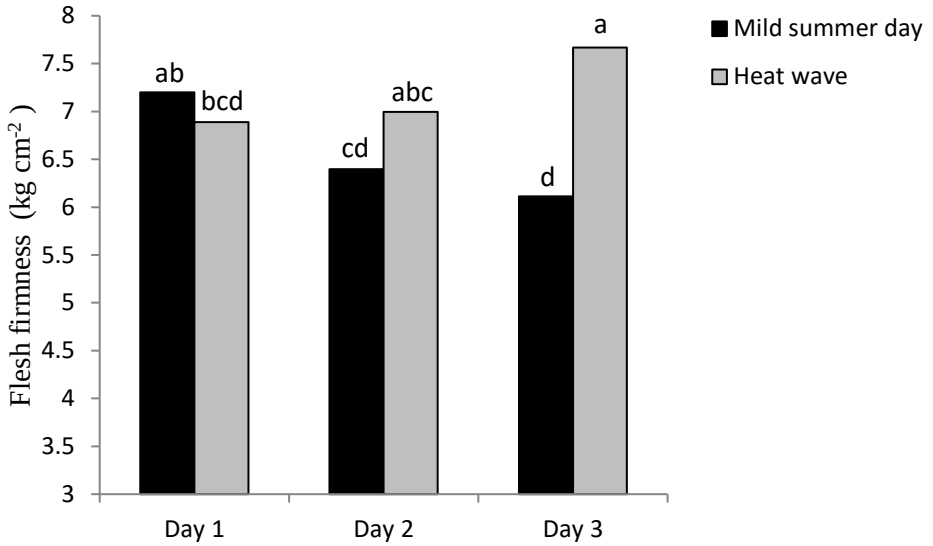
	F test
Harvest maturity (HM)	*0.0220*
Temperature regime (TR)	*<0.0001*
Number of exposure days (NED)	*<0.0001*
*TR*NED*	*<0.0001*
*HM*NED*	*0.0220*
*HM*TR*	*<0.0001*
*HM*TR*NED*	*0.3608*

Figure 17. Interaction between harvest maturity and temperature regime on external fruit peel damage of 'Fortune' plums after treatment at harvest. Fruit were sampled from Sandrivier, Wellington and treated to simulated mild summer day and heat wave temperature regime in a Controlled Atmosphere Temperature Treatment System (CATTS).



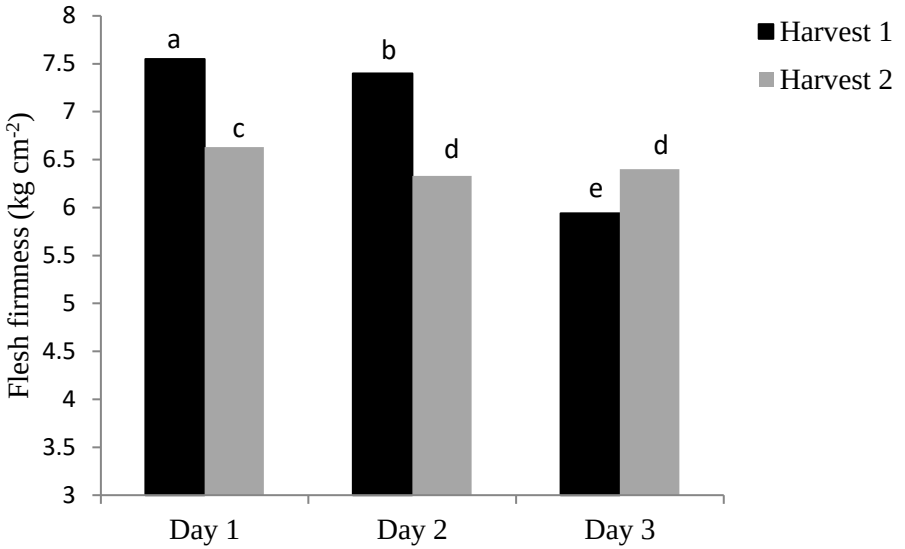
	F test
Harvest maturity (HM)	*0.0220*
Temperature regime (TR)	*<0.0001*
Number of exposure days (NED)	*<0.0001*
*TR*NED*	*<0.0001*
*HM*NED*	*0.0220*
*HM*TR*	*<0.0001*
*HM*TR*NED*	*0.3608*

Figure 18. Interaction between temperature regime and number of exposure days on external fruit peel damage of 'Fortune' plums after treatment at harvest. Fruit were sampled from Sandrivier Estate, Wellington and treated to simulated mild summer day or heat wave temperature regime in a Controlled Atmosphere Temperature Treatment System (CATTS).



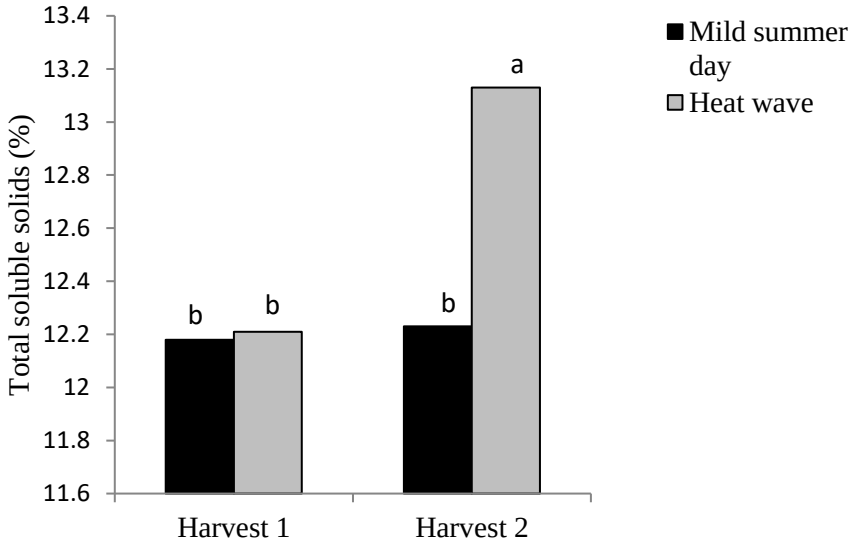
	F test
Harvest maturity (HM)	*<0.0001*
Temperature regime (TR)	*0.0095*
Number of exposure days (NED)	*0.4352*
*TR*NED*	*0.0071*
*HM*NED*	*0.3447*
*HM*TR*	*0.5462*
*HM*TR*NED*	*0.4769*

Figure 19. Interaction between temperature regimes and number of exposure days on flesh firmness of 'Fortune' plums after cold storage. Fruit were sampled from Sandrivier Estate, Wellington, at two harvest maturities and treated to heat wave or mild summer day temperature regimes in a Controlled Atmosphere Temperature Treatment System (CATTS) chamber at harvest



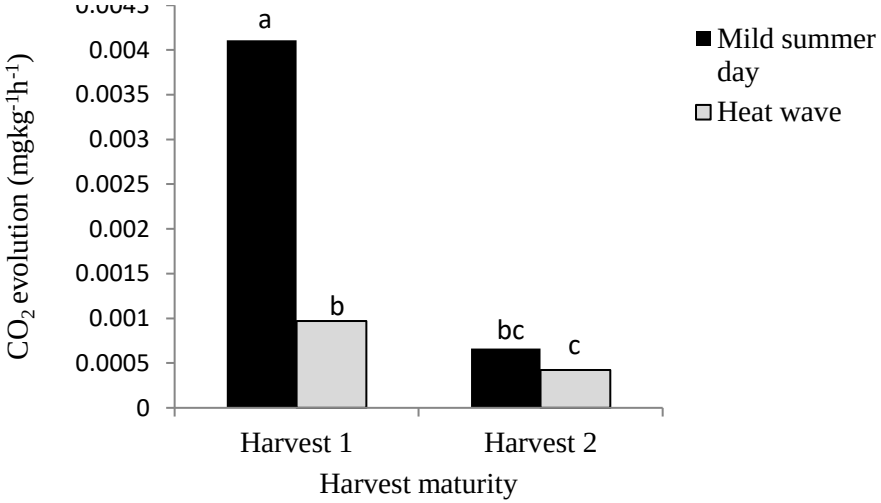
	F test
Harvest maturity (HM)	*0.0322*
Temperature regime (TR)	*0.0501*
Number of exposure days (NED)	*0.0067*
*TR*NED*	*0.0694*
*HM*NED*	*0.0170*
*HM*TR*	*0.3222*
*HM*TR*NED*	*0.0610*

Figure 20. The effect of interaction between harvest maturity and number of exposure days on flesh firmness of 'Fortune' plums after shelf life simulation. Fruit were sampled from Sandrivier Estate, Wellington, at two harvest maturities and treated at harvest to heat wave or mild summer day temperature regimes in a Controlled Atmosphere Temperature Treatment System (CATTS) chamber, for 1, 2 or 3 days.



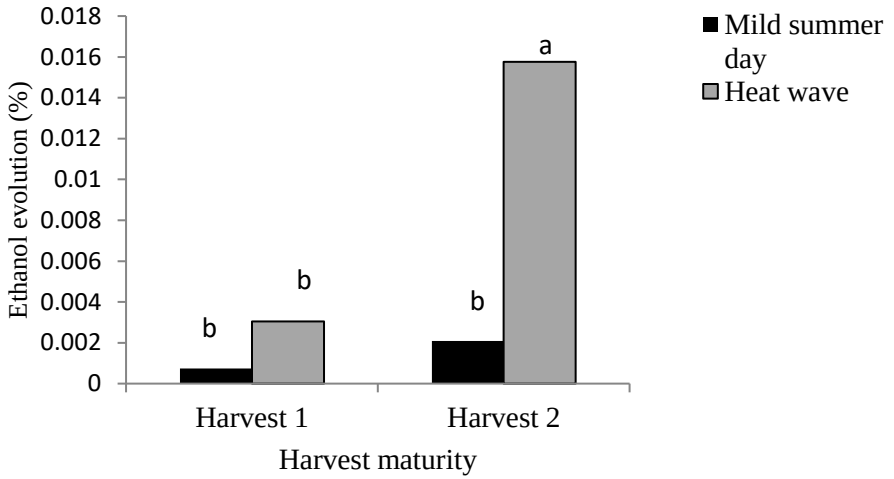
	F test
Harvest maturity (HM)	*0.0005*
Temperature regime (TR)	*0.0008*
Number of exposure days (NED)	*0.4870*
*TR*NED*	*0.4596*
*HM*NED*	*1.0000*
*HM*TR*	*0.0012*
*HM*TR*NED*	*0.2241*

Figure 21. The effect of interaction between harvest maturity and temperature regime on total soluble solids of 'Fortune' plums after cold storage. Fruit were sampled from Sandrivier Estate, Wellington, at two harvest maturities and treated to heat wave of mild summer day temperature regimes in a Controlled Atmosphere Temperature Treatment System (CATTS) chamber.



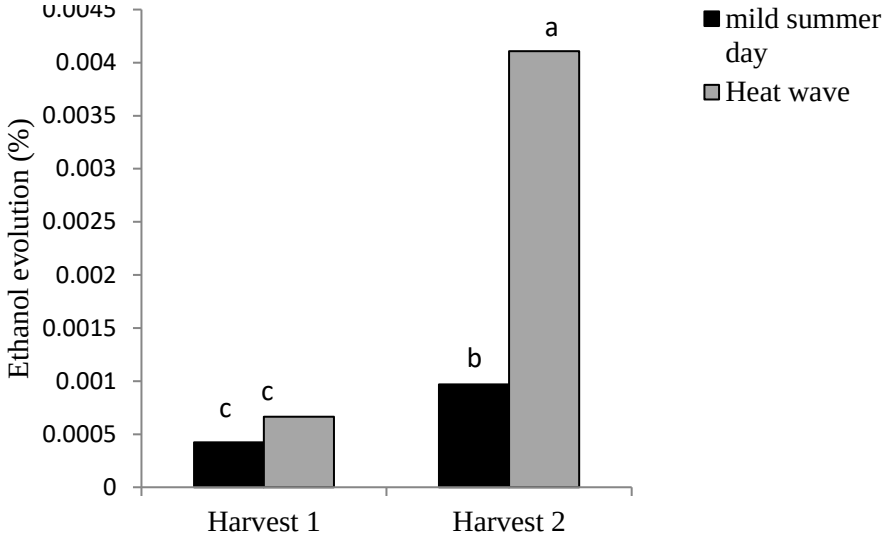
	F test
Harvest maturity (HM)	*0.0008*
Temperature regime (TR)	*0.8482*
Number of exposure days (NED)	*0.0635*
*TR*NED*	*0.2532*
*HM*NED*	*0.6819*
*HM*TR*	*0.0456*
*HM*TR*NED*	*0.4548*

Figure 22. Interaction between harvest maturity and temperature regime for respiration of 'Fortune' plums after shelf life simulation. Fruit were sampled from Sandrivier Estate, Wellington and treated to a simulated mild summer day and heat wave temperature regime in a Controlled Atmosphere Temperature Treatment System (CATTS).



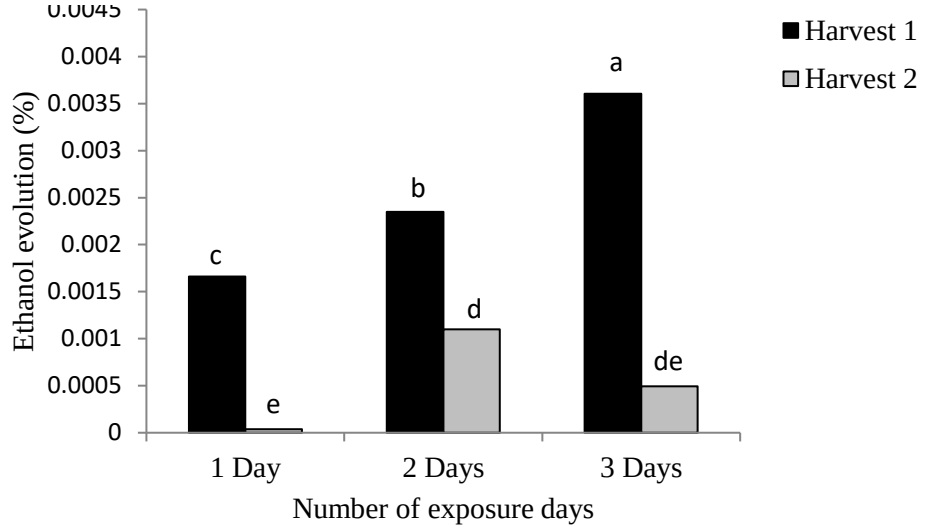
	F test
Harvest maturity (HM)	*0.0076*
Temperature regime (TR)	*0.0030*
Number of exposure days (NED)	*0.8978*
*TR*NED*	*0.8826*
*HM*NED*	*0.4512*
*HM*TR*	*0.0267*
*HM*TR*NED*	*0.4731*

Figure 23. Interaction between harvest maturity and temperature regime for ethanol evolution of 'Fortune' plums after treatment at harvest. Fruit were sampled from Sandrivier Estate, Wellington and treated to a simulated mild summer day and heat wave temperature regime in a Controlled Atmosphere Temperature Treatment System (CATTS).



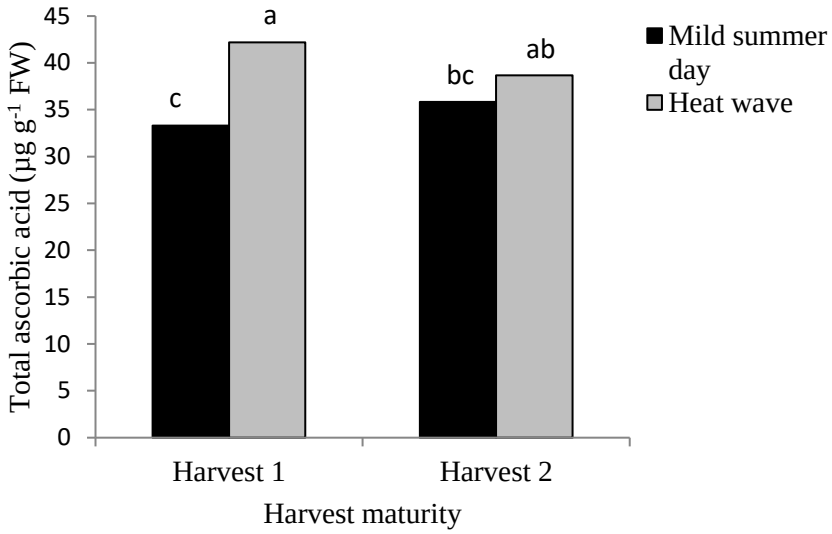
	F test
Harvest maturity (HM)	*0.0036*
Temperature regime (TR)	*0.0115*
Number of exposure days (NED)	*0.2758*
*HM*TR*	*0.0273*
*HM*NED*	*0.4366*
*TR*NED*	*0.0309*
*HM*TR*NED*	*0.0613*

Figure 24. Interaction between harvest maturity and temperature regime for ethanol evolution of 'Fortune' plums after shelf life. Fruit were sampled from Sandrivier Estate, Wellington and treated to simulated mild summer day and heat wave temperature regime in a Controlled Atmosphere Temperature Treatment System (CATTS) upon sampling.



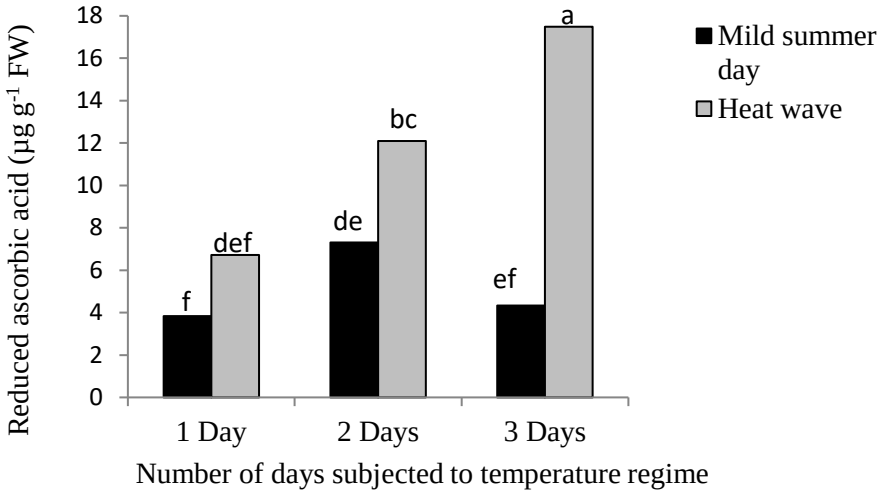
	F test
Harvest maturity (HM)	*0.0036*
Temperature regime (TR)	*0.0115*
Number of exposure days (NED)	*0.2758*
*HM*TR*	*0.0273*
*HM*NED*	*0.4366*
*TR*NED*	*0.0309*
*HM*TR*NED*	*0.0613*

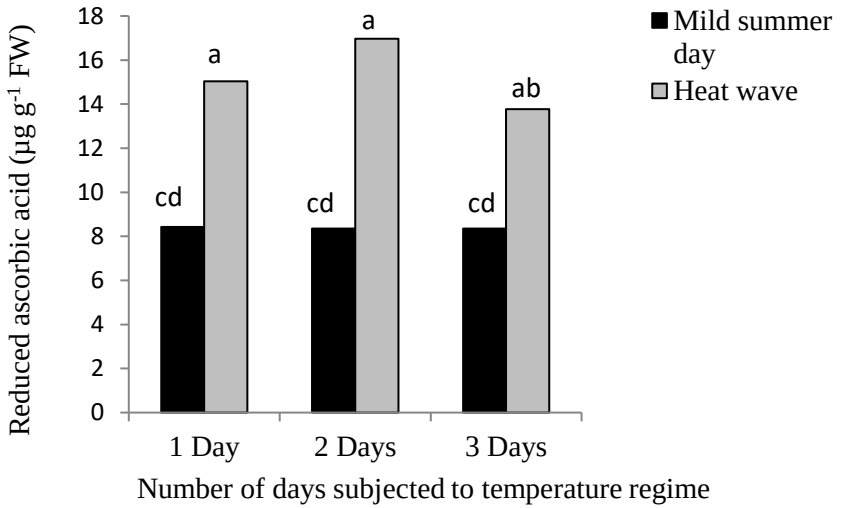
Figure 25. Interaction between harvest maturity and number of exposure days to simulated mild summer day and heat wave temperature regimes on ethanol evolution of 'Fortune' plums after shelf life. Fruit were sampled from Sandrivier Estate, Wellington, at two harvest maturities and treated in a Controlled Atmosphere Temperature Treatment System (CATTS) at harvest.



	F test
Harvest maturity	*0.7200*
Temperature regime	*0.0004*
Number of exposure days	*0.1948*
*HM*NED*	*0.7108*
*HM*TR*	*0.0436*
*NED*TR*	*0.1596*
*HM*TR*NED*	*0.4041*

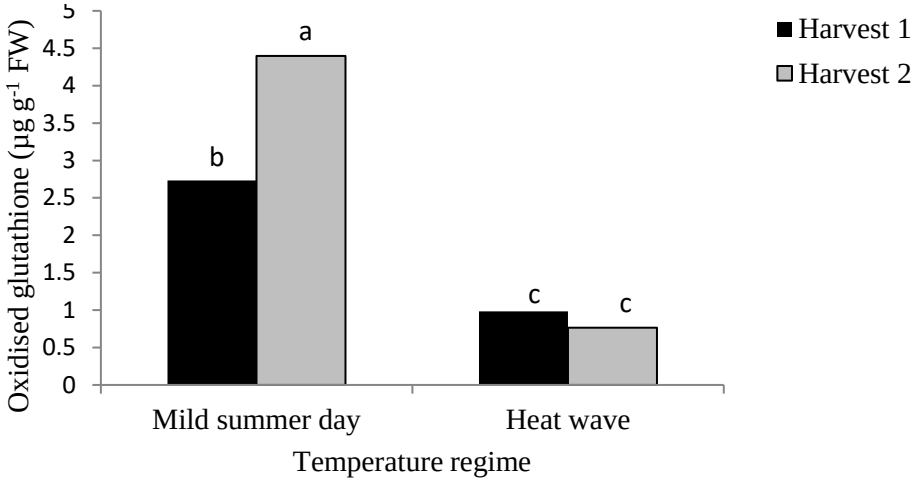
Figure 26. The effect of the interaction between harvest maturity and temperature regime on the concentration of total ascorbic acid of 'Fortune' plums at harvest. Fruit were sampled from Sandrivier Estate, Wellington and treated to simulated heat wave or mild summer day conditions in a Controlled Atmosphere Temperature Treatment System (CATTS) chamber





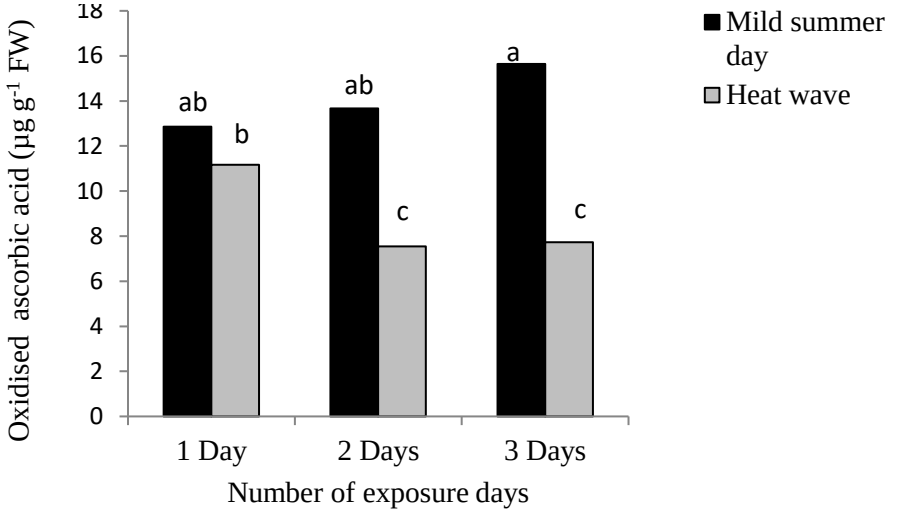
Harvest 1	Harvest 2
	F test
Harvest maturity (HM)	*0.0006*
Temperature regime (TR)	*<0.001*
Number of exposure days (NED)	*0.0193*
*TR*NED*	*0.1496*
*HM*NED*	*0.0155*
*HM*TR*	*0.7703*
*HM*TR*NED*	*0.0176*

Figure 27. Interaction for harvest maturity, temperature regime and number of exposure days on reduced ascorbic acid concentration of 'Fortune' plums immediately after treatment on the harvest date. Fruit were sampled from Sandrivier Estate, Wellington, and treated in a Controlled Atmosphere Temperature Treatment System (CATTS) for 1, 2 or 3 days. The two graphs have been divided between harvest maturities for better clarity should therefore be considered as a single unit.



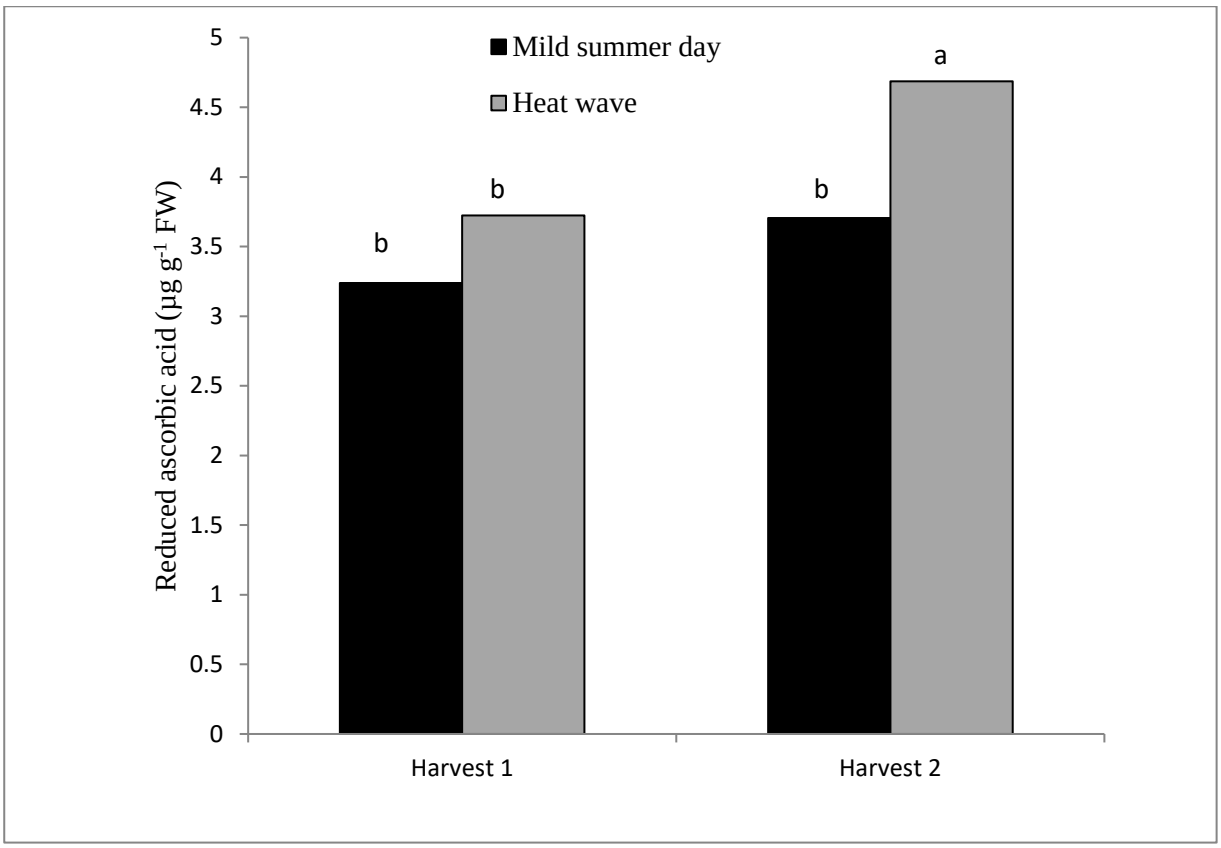
	F test
Harvest maturity(HM)	*0.0557*
Temperature regime (TR)	*<0.0001*
Number of exposure days (NED)	*0.5117*
*TR*NED*	*0.7033*
*HM*NED*	*0.2691*
*HM*TR*	*0.0150*
*HM*TR*NED*	*0.3491*

Figure 28. The effect of interaction between harvest maturity and temperature regime on oxidised glutathione concentration of 'Fortune' plums after cold storage. Fruit were sampled from Sandrivier Estate, Wellington and treated to heat wave or mild summer day temperature regime in a Controlled Atmosphere temperature Treatment System (CATTS) chamber at harvest.



	F test
Harvest maturity(HM)	*0.0008*
Temperature regime (TR)	*<0.0001*
Number of exposure days (NED)	*0.3038*
*TR*NED*	*0.0092*
*HM*NED*	*0.4577*
*HM*TR*	*0.4563*
*HM*TR*Days*	*0.2862*

Figure 29. The effect of interaction between temperature regime and number of exposure days on oxidised ascorbic acid concentration of 'Fortune' plums after cold storage. Fruit were sampled from Sandrivier Estate, Wellington and treated to heat wave or mild summer day temperature regime for 1, 2 or 3 days in a Controlled Atmosphere temperature Treatment System (CATTS) chamber at harvest.



	F test
Harvest maturity (HM)	*0.3842*
Temperature regime (TR)	*0.0158*
Number of exposure days (NED)	*0.6876*
*TR*NED*	*0.2765*
*HM*NED*	*0.7224*
*HM*TR*	*0.0182*
*HM*TR*NED*	*0.5143*

Figure 30. The effect of interaction between harvest maturity and temperature regime on reduced ascorbic acid concentration of 'Fortune' plums after simulated shelf life. Fruit were sampled from Sandrivier Estate, Wellington and treated to heat wave or mild summer day temperature regime in a Controlled Atmosphere temperature Treatment System (CATTS) chamber at harvest.

GENERAL DISCUSSION AND CONCLUSIONS

Introduction and background

High losses in Japanese plums have been reported due to both sunburn and internal heat damage as a result of heat waves experienced prior to (5-8 days) or during the harvesting window in the Western Cape Province of South Africa (Kapp and Jooste, 2006; De Kock, 2015). Maximum temperatures of 35°C or higher over three or more consecutive days are considered a heatwave (De Kock, 2015). A combination of high irradiance and high temperatures causes the externally appearing sunburn. In addition, the high temperature accelerates respiration, lowering O_2 levels within the fruit (Cheng et al., 1998), which leads to anaerobic respiration with the ultimate production of ethanol and purported manifestation of internal heat damage. Internal heat damage manifests in two possible forms, namely pitburn and gel breakdown. Internal heat damage that is not apparent at harvest in plums can manifest during or after cold storage (De Kock, 2012).

Previous research efforts on fruit radiant heat damage mostly focused on apple sunburn (Racskó and Schrader, 2012), where losses of up to 50% have been reported in the Western Cape Province (Bergh et al., 1980). Although the problem is also evident in plums, there is little information on sunburn, with no formal reporting of losses to sunburn . In addition, little information is available on how the environmental factors cause manifestation of internal heat damage. However, for profitable plum production, knowledge in this respect is required, particularly considering that the growing season climate will become more adverse to high quality deciduous fruit production due to climate change (Western Cape Government, 2018)

The main aim of this study was to fill the information void surrounding the apparent but not widely researched problem of sunburn, internal heat damage and subsequent fruit quality in plums, drawing from findings from apples. As climatic conditions and tree canopy factors are crucial pre-harvest factors affecting fruit quality, Chapter 1 of our study focused on the effects of light and temperature in different canopy positions on fruit quality and manifestation of sunburn and internal heat damage. Summer pruning, its timing and the use of light attenuating shade net were concurrently investigated as potential remedial practices to curb sunburn. To our knowledge, our study is the first that investigated the use of shade nets over Japanese plums for controlling sunburn.

In Chapter 2, the influence of plant water status on incidence of sunburn and fruit quality was investigated. This follows studies in apples that indicated that moisture stress rendered fruit more

susceptible to sunburn under conditions conducive for its development (Schrader et al., 2003; Yuri et al., 2004; Makeredza, 2013; Mupambi 2017).

In Chapter 3 of this study, we set out to establish a link between respiratory responses to high temperature of sensitive and tolerant cultivars and incidence of internal heat damage at different levels of fruit maturity. Laetitia and Fortune were the susceptible cultivars, at early and late harvest maturities while African Delight, the tolerant cultivar, had only one maturity level.

The initial assessment of respiration was under field conditions in the 2012/13 season where fairly mild weather conditions with no notable heat waves prevailed. No internal heat damage symptoms were observed. However, preliminary findings in this season became the basis upon which we manipulated conditions in growth chambers in an attempt to ascertain development of internal heat damage symptoms in the following seasons. Similar efforts were successfully reported by Maxie and Claypool (1956). In the final season, 2014/15, we modified the work of Maxie and Claypool (1956) by incorporating changes in temperature at night during heat waves in a controlled atmosphere temperature treatment system (CATTS). Simulated pre-harvest conditions of heat waves and mild summer days were therefore compared.

Canopy micro-climatic factors and sunburn

Canopy position and summer pruning

Consistent with many studies, irradiance decreased from the upper to lower canopy of the tree (Buler and Mika, 2009; Fouché et al., 2010; Ördög and Molnar, 2011). We generally observed a decrease in fruit quality with a decrease in irradiance. Upper canopy fruit were bigger, redder and were of advanced maturity compared to lower canopy fruit. Crisosto et al. (1997) observed for five consecutive seasons that fruit on upper canopy positions exposed to high light conditions had better storage potential than those that developed in shaded positions. This indicates the importance for growers to maintain fairly open canopies for easy light penetration and enhanced productivity, improved yield and better fruit quality in inner canopy positions. However, despite enhancing fruit quality in our study, fruit from the upper canopy positions showed higher sunburn incidence.

Light reaching the inner canopy fruit can be regulated by vegetative manipulation of the canopy through summer pruning (Rom, 1991). The practice should aim for good filtered light within the canopy, but its timing might coincide with the hottest part of the season and thereby increase the risk of sunburn. In our study, early summer pruning (08 Dec.) offered adequate time for vegetative regrowth before the hottest part of the season compared to late pruning (07 Jan.) Vegetative

regrowth was laterally branching, giving good filtered light. Early summer pruning therefore resulted in lower sunburn incidence. Knowledge of threshold light and temperature conditions for sunburn development is critical in canopy management. In our study, fruit that developed sunburn received an average photosynthetic photon flux (PPF) greater than 50% of the full sun while average fruit surface temperature (FST) exceeded 35 °C.

Shade nets

Shade nets have successfully reduced sunburn in sun exposed fruit, particularly in apple (Middleton and McWaters, 2002; Gindaba and Wand, 2005; Smit, 2007). Gindaba and Wand (2005) reported lower FST under shade net compared to evaporative cooling, a control strategy considered the most effective in reducing sunburn (Lal and Sahu, 2017). In this pioneering study in plums, we therefore tested the potential of 20% black and white shade net in reducing sunburn, specifically during the hottest part of the growing season.

The magnitude of PPF reaching the fruit in the upper canopy under the shade net was approximately 83.5% of full sunlight. On average, this decreased FST between 1-2 °C. Fruit under the shade net therefore received lower PPF and generally had lower FST and subsequently, slightly lower sunburn incidence compared to the no shade control. As the shade net was installed during the hottest part of the season (22 Jan.) some sunburn might have already manifested in some of the fruit before then. However, shade nets managed to significantly reduce sunburn severity, particularly in the top canopies. This indicates that shade nets have potential in the control of sunburn in plums. Further investigations can therefore be carried out over the entire growing season to ascertain and exploit the full value of the shade nets over a wider range of cultivars. In this study we tested shade nets on African Delight, a cultivar that is tolerant to internal heat damage. We could therefore not draw conclusions on the effects of shade net on internal heat damage. However, considering how shade nets modified the canopy environment and lowered FST in this study, they can equally have remedial effects on internal heat damage. Further studies in this area using sensitive cultivars such as Laetitia could be a worthwhile endeavour.

It is important to take note of a number of factors in choosing and installing the shade nets in plums over the entire season. Adequate levels of light are required for carbon assimilation into carbohydrates that must be partitioned into vegetative or fruit growth (Murray et al., 2005). Some studies in apples have revealed that shade net through greater partitioning of carbohydrates to vegetative growth at the expense of fruit growth, may result in smaller fruit in the current season (Mupambi et al., 2018). In the following season, the reduced allocation of assimilates to

reproductive sinks can reduce return bloom and fruit set (Solomakhin and Blanke, 2008; Mupambi et al., 2018). In addition, most plum cultivars in the Western Cape require a cross pollinator (De Kock, personal communication). Shade nets can hinder the movement of bees for effective cross pollination. No literature is available on how shade nets would affect flowering and fruit set in plums. Therefore, this is an area that needs further investigation before shade nets can be used as a control measure for sunburn.

In further studies, it is important to note that the weaving density and subsequent percentage shade of the chosen net should be custom-made to the grower's environment. For instance, increasing the shading percentage might result in a decrease in red colour development. Gindaba and Wand (2005) successfully reduced FST and sunburn in apples using 20% shade net but compromised red colour development in the process. In our study, shade net did not negatively affect fruit quality. It improved red colour and we suggest this is due to reduced loss of anthocyanins through bleaching as reported by Dussi et al. (1995) who partially shaded 'Red Bartlett' pears towards harvest and enhanced red colour. In addition, even under non-light limiting conditions, shade nets generally promote vegetative growth (Shahak et al., 2004). Therefore if there is need for vegetative manipulation such as summer pruning under nets, it should be synchronised with shoot growth rate so as to achieve a good balance of filtered light in the canopy during the hottest part of the season. It therefore might be possible to delay summer pruning under shade nets.

Plant water status and sunburn

Orchard light manipulation should be augmented with production practices that promote optimal plant performance to minimize susceptibility to sunburn and heat damage. In general, fruit on underperforming trees with limited photosynthetic capacity have reduced tolerance to sunburn (Schrader et al., 2003). Tree water management is one of the most important orchard practices to ensure optimal plant performance. In addition to reducing photosynthetic capacity, water stress can indirectly increase fruit surface temperature, with subsequent increases in sunburn incidence (Makeredza, 2013).

We confirmed that the plant's capacity to utilise light in carbon assimilation is reduced under low plant water potential. There was a general decrease in gas exchange with a decrease in irrigation in our study although a few inconsistences between sites and seasons were observed. This observation was more prominent late in the season compared to the early season. The reduction in photosynthetic light use causes the prevalence of excess light in the system, resulting in photoinhibition (Long and Humphries, 1994; Demmig-Adams et al., 1995). The magnitude of

photoinhibition can be a function of the plant's genetic photosynthetic capacity and the prevailing irradiance (Pastenes et al., 2005). During photoinhibition, the energy that is not used in carbon assimilation can accumulate and overwhelm the photosynthetic system, causing oxidative damage (Demmig-Adams et al., 1995).

We did not obtain any evidence that seemed to suggest there was damage of the photosynthetic system at both the leaf and fruit level according to our measurement of maximum photosynthetic efficiency of PSII (F_v/F_m). The observed F_v/F_m values were within 0.7 and 0.8, considered a range for healthy photosynthesising plant parts (Ritchie, 2006). When photosynthetic damage occurs, the values would drop below 0.6. We, however, observed indications for reduced leaf maximum quantum efficiency of PSII after 14 days of withholding irrigation. For fruit peel, the normal irrigation control consistently resulted in a higher maximum quantum efficiency of PSII than the no irrigation treatment but there were no significant differences. Although withholding irrigation for 14 days in plums reduced carbon assimilation and maximum quantum efficiency of PSII, it did not cause chronic photoinhibition. Chronic photoinhibition may only occur under more prolonged moisture stress.

However, we were certain that reducing irrigation, particularly late in the season towards harvest, played a role in influencing FST and subsequently sunburn. Although it was not very apparent in the first season of withholding irrigation, well-watered trees had lower canopy temperatures. The differences in canopy temperatures were a result of differences in transpiration rates. Expectedly, we observed that conditions of low water potential triggered stomatal closure which in turn decreased transpiration. This can result in an increase in canopy temperature (Colaizzi et al., 2012). FST can exceed ambient temperature by 10-15 °C due to a substantially lower surface to volume ratio compared to leaves (Smart and Sinclair, 1976). If canopy temperature increases, there is less potential for radiative heat loss to the environment, further increasing the fruit surface temperature (Colaizzi et al., 2012). Micro-climatic conditions affecting heat transfer can therefore have a significant role in FST determination.

Most plum growers in the Western Cape Province believe pulse irrigation can help attain cooler micro climatic conditions in the orchard and thereby lower the FST and control sunburn during heat waves (Steyn, personal communication) This involves applying a short pulse of irrigation for a few minutes in cycles ranging from 20-30 minutes to wet the orchard floor when ambient temperatures reach a certain threshold. There is no scientific evidence to support the use of this control measure although it is extensively used in the province. Mupambi (2017) reported a reduction in sunburn

with pulsing irrigation in an under-irrigated orchard of 'Golden Delicious' and 'Granny Smith' apples, with no verification under optimal irrigation conditions. The Western Cape Province is constantly experiencing drought and serious water scarcity, therefore pulsing under optimal irrigation condition to control sunburn should be scientifically justified.

In our study we confirmed that increasing irrigation beyond the general normal agronomic practice did not further reduce sunburn. This also seems to indicate that under optimal irrigation levels, pulsing might not contribute much in terms of reducing canopy temperature. It is important to note that fruit on trees with low water potential have increased sunburn risk and farmers should strive to attain optimal water level to avoid predisposing fruit to sunburn. Proper irrigation practice should therefore not be regarded as a direct sunburn control measure. Orchard practices such as timing of summer pruning and installation of shade nets are more direct control practices. However, when these control measures are undertaken, it is important to ensure trees are maintained at optimal water levels. We observed that shade netting cooled the canopy and lowered the FST by 1-2°C. Therefore, shade nets can potentially save on irrigation due to reduced evapotranspirational water loss (McCaskill et al., 2016).

We observed that maintaining low irrigation levels throughout the season predisposed fruit to sunburn sooner than trees under optimal or high irrigation regimes. Withholding irrigation during the early season did not result in an increase in FST and sunburn at harvest. Fruit sensitivity to photoinhibition increases during fruit development (Steyn et al., 2009). This seems to suggest there must be a specific threshold period in the season where deficit irrigation can aggravate photoinhibition and predispose fruit to sunburn. This requires further investigation and verification. Reverting to normal moisture regimes soon after moisture stress might still increase the chances of escaping sunburn.

Sunburn and fruit quality

The low sunburn class (less severe sunburn browning) was the most predominant at all sites, followed by the high sunburn class (severe sunburn browning). Unmarketable sunburn (necrosis) was low but in cases where it was prevalent, it was mostly in the low and no irrigation treatments and upper canopy fruit. Where we maintained low irrigation for the whole season, unmarketable sunburn was observed as early as a month and more before harvest. In the control and high irrigation treatments, unmarketable sunburn was still yet to be observed at this point. Therefore, in addition to increasing sunburn incidence, low plant water potential seems to increase sunburn

severity. Sunburn severity also increased with an increase in canopy height and resultant increased sunlight exposure.

Studies in apples revealed that apart from affecting the physical appearance of the fruit, sunburn was associated with changes in other textural and chemical qualities of the fruit (Klein et al., 2001; Schrader et al., 2009; Makeredza, 2013). In plums, sunburn resulted in fruit with high TSS, an observation which was consistent with findings in apples (Schrader et al., 2009; Makeredza, 2013). In our study, sunburnt fruit were more prevalent in the upper and exposed canopy positions and treatments of low irrigation. High light conditions have been reported to advance fruit maturity, hence an increase in TSS in the sun exposed upper canopy positions. Hamadziripi et al. (2014) reported a decreasing TSS gradient from the outer to the inner canopy in apples. Several studies have also revealed how reduced irrigation increases TSS in various fruit. This has been attributed to an increase in carbon compounds within the fruit as result of reduced water movement into the fruit or increased transpirational water loss at high vapour pressure deficit (Léchaudal et al., 2013; Rahmati et al., 2015). Opara et al. (1997) reported a decrease in TSS with an increase of irrigation frequency.

Reports in apples associated sunburn with firmer fruit (Racskó et al., 2005; Schrader et al., 2009; Makeredza, 2013). In our study, this was only observed in treatments of low or no irrigation. Kucukyumuk et al. (2013) reported higher fruit firmness under deficit irrigation. In moisture stress studies, low flesh firmness has always been linked to the subsequent smaller fruit size. However, in situations of regular irrigation, sunburn fruit in upper canopy positions had lower flesh firmness. This confirms the effect of light on advancing fruit maturity in stone fruit as indicated by Murray et al. (2005). Fruit maturity of plums in shaded positions can lag as far behind as 14 days (Manganaris et al., 2008). Selective picking based on fruit colour at harvest is therefore advisable to avoid fruit of variable maturity.

We expected sunburn to be associated with low TA as reported by Schrader et al. (2009). Our observations were however inconsistent. Under regular irrigation, there was no significant difference in TA between sunburnt upper canopy fruit and shaded non-burnt inner canopy fruit. No significant differences in TA were also observed when irrigation was manipulated to give low, high and normal deliveries. However when irrigation was withheld for 14 days towards harvest, there was a significant decrease in TA. Early season withholding of irrigation did not result in a decrease in TA. It therefore appears that TA levels did not seem to be associated with sunburn. Although it seemed to respond to irrigation levels, the responses were not highly sensitive.

Internal heat damage

Although there were variations between cultivars and harvest maturities, we generally observed that an increase in temperature and duration of exposure to high temperature increased metabolic processes such as respiration and ethanol evolution. Further increases were, however, inhibitory to varying extents among the cultivars. In all cultivars we observed no significant differences in respiration after cold storage. Klein and Lurie (1990) explained that high respiration rates revert to normal respiration when fruit are moved from a high temperature environment to ambient or lower temperature conditions.

Ethanol is a product of anaerobic respiration under conditions of low O_2 concentration. A reduction in internal fruit O_2 to CO_2 ratio results in an increase in fruit ethanol levels (Paul and Pandey, 2014; Kader, 1987). This ratio tends to decrease as fruit advance in maturity (Bufler and Bangerth, 1982; Paul and Pandey, 2014), hence the observed higher ethanol levels in more mature fruit of the sensitive cultivars, Laetitia and Fortune. Increased heat exposure and duration further increased accumulation of ethanol although this tended to decline at high temperature such as 45°C. Mitcham and McDonalds (1993) reported an increase in internal CO_2 and decrease in O_2 concentration with subsequent increase in ethanol concentration in mangoes treated at 46°C or 48°C. In stone fruit, if such heat treatments concurrently occur with oxidation of phenolic compounds, dark discolourations of internal heat damage manifest (Amiot et al., 1997).

In our study, internal heat damage symptoms were prevalent after cold storage although they were generally related to respiration and ethanol evolution at harvest. This is consistent with observations in the Western Cape where the problem of internal heat damage is not immediately apparent in the orchard even if heat waves are experienced. This poses a problem to growers as internal heat damage is an unpredictable disorder that is not consistently observed every season and the exact conditions leading up to its manifestations are not clear. De Kock (2015) suggested ambient temperatures of about 40°C experienced in just a single day or 35°C or above over three-day periods or longer would result in internal heat damage. Our attempts to assess internal heat damage in 2012/13 were unsuccessful as no symptoms developed in the orchard, after cold storage and shelf life.

In the subsequent seasons we set out to test the factors that contribute to pitburn as suggested by De Kock (2015) under simulated field conditions in the laboratory. In addition, we attempted to increase heat load on fruit by applying heat absorbing black stickers on the fruit surface. In all cases

it was not really possible to simulate pitburn in response to field heat in the laboratory as we got clear indications of curing taking place for high temperatures (45°C) in cold storage.

Gel breakdown was the predominant form of internal heat damage manifesting after cold storage and shelf life. Immediately after treatments at harvest, there were no internal defects in 'Laetitia' and a very low incidence of pitburn in 'Fortune'. It is therefore important to note that cold storage conditions play a significant role in the ultimate manifestation of the internal heat damage symptoms.

The most susceptible 'Laetitia' fruit were more mature fruit exposed to temperatures of 30°C and 40°C. Fruit exposed to the highest temperature of 45°C had significantly lower internal defects for both harvest maturities. Similarly for 'Fortune', advance maturity fruit were more susceptible to internal defects but when these fruit were exposed to the high temperature regime of simulated heat wave conditions, there was lower incidence of internal defects after cold storage. As the threshold pre-conditioning temperature appears to be around 45 °C, heat waves that do not exceed this threshold point particularly in the late harvesting window are bound to be more detrimental.

We deduced that pre-storage heat exposure at the threshold temperature initiated tolerance to cold storage-induced internal heat damage in 'Laetitia' and 'Fortune' plums. This phenomenon has been reported in several fruit (Ferguson et al., 1999). In avocado, exposing the fruit to temperatures of up to 50°C resulted in a reduction in cold storage disorders (Woolf et al., 1995). Cao et al. (2010) reported tolerance to cold storage disorders in different peach cultivars that had been treated to high temperatures prior to cold storage. This could be a result of the accumulation of heat shock proteins (HSP) (Ferguson et al., 1994). Although the HSP precondition the fruit for protection against recurring heat damage, they also have a protective role against low temperature disorders (Woolf et al., 1995; Lurie, 1998).

'African Delight' did not show any symptoms of internal heat damage. This could be linked to fruit peel gas permeability of this cultivar. African Delight is 20% more permeable to gas compared to the susceptible cultivar Laetitia (Theron, 2015). With high gas permeability, it is therefore possible that high respiration rate at high temperatures might not deplete O_2 to levels low enough to initiate hazardous accumulation of ethanol and manifestation of internal damage symptoms. The fruit peel has numerous open hairline concentric cracks which are prominent on the stem end of the fruit (Theron, 2015). These cracks enhance gaseous exchange between the internal fruit environment and the external atmosphere and explain why 'African Delight' is very susceptible to moisture loss and

shriveling during storage (Theron, 2015). Therefore, at high temperatures when respiration rate increases, internal O_2 may not become depleted.

Although it was tolerant to internal damage, 'African Delight' was more susceptible to peel damage than 'Laetitia' and 'Fortune'. The incidence of peel damage was also higher in fruit that we observed to have been heat cured for internal damage in 'Laetitia' and 'Fortune'. This detracts from the heat curing potential in cold storage. It is important to note that external fruit appearance considerably influences consumer appeal. Therefore, there is need to minimise external fruit damage, if high temperature treatments are to be used in pre-conditioning fruit against internal heat damage.

Fruit exposed to high temperature treatments and longer heat exposure durations before cold storage can potentially store longer. This can be a result of reduced loss of firmness, one of the ripening processes that we observed to be interrupted by ethylene synthesis inhibition due to high temperatures. 'Fortune' exposed to the heat wave temperature regime for 3 days were firmer after cold storage. Similar heat treatment observations were reported in stone fruit (Malakou and Nanos 2005) and avocados (Woolf and Ferguson, 2000). Further research in this area could contribute immensely towards improved fruit storability. In addition, a clear understanding of the behaviour of other fruit quality attributes should be concurrently monitored. We observed a general increase in TSS with heat treatments. High TSS render good eating attributes and often correlate positively with increased consumer perception of fruit.

Conclusion

We conclude from this study that fruit, which on average receive more than 50% of the full sun, and attain an average temperature of 35°C or higher during the day, will develop sunburn. Fruit that are in the upper and exposed canopy positions are more susceptible although they are more likely to be bigger, redder and have higher TSS. Early summer pruning is an effective cultural practice to control sunburn as it enables vegetative regrowth before the hottest part of the season. Refraining from summer pruning results in excessive growth of water shoots that shade the fruit and delay fruit maturity and is therefore not a viable option. A shade net during the hottest part of the season decreases irradiance reaching the fruit, lowering its FST by up to 2°C and thereby reducing sunburn. We ascertained that low plant water potential increases FST and sunburn possibly due to canopy heating and loss of convectional cooling and concluded that excessive irrigation does not reduce sunburn. We confirmed tolerance of 'African Delight' to internal heat damage. In the susceptible cultivars, Laetitia and Fortune, advanced maturity seemed to render fruit more susceptible to heat

damage but at high temperatures of up to 45°C, curing occurred in cold storage. However, such high temperatures increase the prevalence of peel damage. ‘African Delight’ was more susceptible to peel damage compared to ‘Laetitia’ and ‘Fortune’.

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