EVAPORATIVE COOLING OF APPLE AND PEAR ORCHARDS.

BY

KARI VAN DEN DOOL

Thesis presented in partial fulfilment of the requirements for the degree Master of Science in Agriculture in the Department of Horticultural Science, University of Stellenbosch, Stellenbosch, South Africa.

Supervisor: Dr. S.J.E. Midgley
Co-Supervisor: Dr. W.J. Steyn

December 2006

DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously, in its entirety or in part, been submitted at any university for a degree.
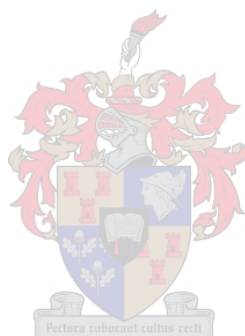

……………………   ……………………

Signature   Date

The most exciting phrase to hear in science, the one that heralds new discoveries, is not "Eureka!" (I found it!) but "That's funny ..."

- Isaac Asimov

SUMMARY

A growing number of fruit producers in warm areas are adopting the use of overtree evaporative cooling (EC) as a technique to reduce sunburn and enhance colour development of red or blushed fruit. Because fruit do not have efficient mechanisms of utilising and/or dissipating solar radiation, fruit surface temperature may rise $10 - 15^{\circ}$C higher than the ambient air temperature, making them very susceptible to sunburn. Sunburn negatively affects the appearance of the fruit, and they cannot be sold for fresh market consumption, which receives the highest prices. Evaporative cooling uses a sprinkler system to cool the trees from above. Energy needed to evaporate the water is extracted from the fruit skin, cooling the fruit down. The air around the trees is cooled, and a more favorable microclimate is created in the orchard. Producers have also found that the use of EC just prior to sundown and sometimes around sunrise has improved colour development on red apples (especially early varieties) before harvest.

In this study, two apple ('Cripps' Pink' and 'Royal Gala') and two pear ('Rosemarie' and 'Forelle') cultivars under EC were compared with control fruit in terms of maturity, colour, sunburn and concentrations of polyphenolics in the skin. Two EC treatments were given; early application starting from the second week in December, and late application starting two to four weeks before harvest. Photosynthetic responses were measured, as well as fruit and leaf temperatures. Underlying physiological responses of trees and fruit to EC were investigated, particularly the phenomenon of acclimation and the potential for colour development and heat stress. Fruit surface temperature of fruit under EC was found to be significantly lower than control fruit. In both apple cultivars a significant increase in fruit skin anthocyanin concentration and a decrease in phenolic content was found as the season progressed. In both pear cultivars there was a significant decrease in both anthocyanin and phenolic. No significant differences were found in anthocyanin content between treatments in either the apple or pear cultivars. In both apple cultivars a higher phenolic content was found in the peel of the EC treatments. A decrease of up to four percent in leaf and fruit surface temperature was found under EC. No significant difference in trunk circumference was found in any of the cultivars. The late EC treatment in 'Cripps' Pink' had a significantly faster rate of budbreak than the control and early EC treatments. Significantly higher transpiration was observed in leaves under EC. 'Royal Gala'

fruit under EC had less sunburn than control fruit. Unfortunately the system broke down on a hot day, causing more sunburn on 'Cripps' Pink' fruit under EC.

Heat tolerance of apple fruit grown under EC was evaluated in 'Cripps' Pink' and 'Royal Gala' by determining the maximum quantum yield of chlorophyll fluorescence (Fv/Fm). Measurements were also made 12 hours after the heat treatments to determine recovery. 'Cripps' Pink' fruit from both EC treatments, but particularly the early EC treatment, were less resistant to heat stress than control (non-EC) fruit at the "threshold" air temperature of 45°C. Apples were able to recover from heat treatments in the range of 32-38°C fruit surface temperature, and generally also recovered fully after 43-45°C fruit surface temperature when exposure did not exceed four hours. This knowledge could be helpful in the management of sunburn, for example when determining the threshold temperature for the activation of evaporative cooling treatments.

Knowledge about the various effects evaporative cooling and the subsequent lowering of ambient temperatures has on fruit trees and fruit could contribute greatly to producers' ability to grow high quality fruit. EC can be used successfully for controlling sunburn and increasing fruit colour, but the system needs to be controlled very carefully and care should be taken that it does not fail on a hot day, as it did during this study.
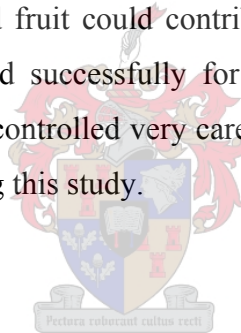
# Verdampingsverkoeling van appel en peer boorde.

OPSOMMING

Verdampingsverkoeling word deesdae deur al hoe meer vrugteprodusente in warm streke gebruik as 'n tegniek om sonbrand te verminder en vrugkleur van rooi vrugte of vrugte met 'n blos te verbeter. Omdat vrugte nie goeie meganismes het om bestraling te gebruik of af te weer nie, kan vrugskiltemperature drasties hoër styg as die omgewingstemperatuur, wat hulle baie dan baie vatbaar maak vir sonbrand. Omdat slegs die hoogste kwaliteit vrugte die mark haal, is dit binne die belang van die produsent om alles binne hulle vermoë te doen om dit te voorkom. Verdampingsverkoeling gebruik 'n sisteem van sproeiers wat bo die bome gemonteer word om die boord van bo af te verkoel. Energie wat nodig is vir die verdamping van die water word uit die vrugskil onttrek en verkoel so die vrug. Die lug rondom die bome word ook afgekoel, en so word 'n gunstige mikroklimaat in die boord geskep. Produsente het ook gevind dat die gebruik van verdampingsverkoeling net voor sonsonder en soms met sonsopkoms kleurontwikkeling by rooi vrugte (spesifiek vroeë kultivars) verbeter.

In hierdie studie is twee appelkultivars en twee peerkultivars onder verdampingsverkoeling vergelyk met kontrolevrugte in terme van rypwording, kleur, sonbrand en die konsentrasie polifenole in die vrugskil. Twee behandelings is gegee; een beginnende vanaf die tweede week in Desember, en 'n laat behandeling twee tot vier weke voor oes. Die effek van die behandelings op fotosintese is gemeet, sowel as op vrug en blaartemperature. Onderliggende fisiologiese reaksie van vrugte en bome op die behandelings is ondersoek, spesifiek die verskynsel van akklimatisasie en die potensiaal vir die ontwikkeling van kleur en hittestres. Vrugskiltemperature van vrugte onder die verkoelingsbehandelings was beduidend laer as die van kontrolevrugte. 'Royal Gala' appels onder die behandelings het ook 'n afname in sonbrand getoon.

Die hittetoleransie van appels onder verdampingsverkoeling is ge-evalueer in 'Cripps' Pink' en 'Royal Gala' deur die bepaling van die maksimum kwantum opbrengs van chlorofil fluorisensie (Fv/Fm). Metings is ook 12 ure na die hittebehandelings gemaak om die herstel van die vrugte te bepaal. 'Cripps' Pink' vrugte van beide verdampingsverkoeling-behandelings, maar in besonder die vroeë behandeling, was minder bestand teen hittestres as

die kontrolevrugte by die "drempel" lugtemperatuur van 45ºC. Vrugte kon herstel van hittebehandelings wat vrugskiltemperature 32-38ºC tot gevolg gehad het, en kon gewoonlik ook herstel van hittebehandelings wat vrugskiltemperature 43-45ºC tot gevolg gehad het, mits die blootstelling nie langer as vier ure geduur het nie. Hierdie kennis kan gebruik word wanneer die drempeltemperature vir die aktivering van die verdampingsverkoeling-behandelings bepaal word.
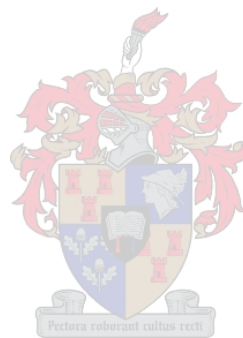
Dedicated to my father Gerrit and my mother Marti, without whose continued support and encouragement none of my studies would have been possible.

ACKNOWLEDGEMENTS

CONTENTS

PAPER 1:

PAPER 2:

General Conclusion

GENERAL INTRODUCTION

High temperatures and light intensities in warm production areas such as the Western Cape cause a high percentage of sunburn damage and poor red colour development in apples and pears produced in these regions (Kotzé *et al.*, 1988; Evans, 1993a; Schrader *et al.*, 2001; Wand *et al.*, 2002). Sunburn negatively affects the appearance and storability of fruit, and they cannot be sold for fresh market consumption, for which the highest prices are attained. Sunburn on apples in South African production areas can amount to 20 – 50% fruit cull in the orchard and up to 10 % rejection of packed cartons thereafter (Bergh *et al.*, 1980). Skin colour of apples is an important factor in consumer acceptance. This is particularly so in red and blushed cultivars, as in most markets red-skinned apples are preferred to others. Skin colour is also an important factor in establishing government grades and standards, because a particular grade must have a certain proportion of the apple skin coloured. Downgrading due to insufficient red colour has limited the profitability of blushed pear cultivars in the Western Cape region of South Africa (Huysamer, 1998).

This study focussed on evaporative cooling provided by an overhead micro jet system as a method to reduce sunburn damage and enhance colour development in two apple (Cripps' Pink and Royal Gala) and two pear (Rosemarie and Forelle) cultivars. It forms part of a bigger study in which other students studied different methods such as reflective kaolin film technology or shadenet covers to attain the aforementioned. In the first part of this study, the effect of evaporative cooling applied early as well as later in the season on the fruit quality of various apple and pear cultivars at harvest under Western Cape growing conditions was evaluated during 2003/2004. Underlying physiological responses of trees and fruit of all four cultivars to EC were investigated, particularly the phenomenon of acclimation and the potential for colour development and heat stress. The effects of early EC (started earlier in the season with the main aim of reducing sunburn) were compared to those of late EC (started two to four weeks before harvest, with the main aim of colour improvement).

In the second part of the study, heat tolerance of apple fruit grown under EC was evaluated in 'Cripps' Pink' and 'Royal Gala' by determining the maximum quantum yield of chlorophyll fluorescence (F_v/F_m) after exposure of fruit to high temperatures (35 to 55 ºC) for up to ten

hours. Measurements were also made 12 hours after the heat treatments to determine permanent damage to thylakoid membranes. Due to time limitation, we focused on the apple cultivars, and these experiments were not done on the two pear cultivars. The knowledge gained from this part of the study could be helpful in the management of sunburn, for example when determining the threshold temperature for the activation of evaporative cooling treatments.

References

Bergh, O., Franken, J., Van Zyl, E. J., Kloppers, F. and Dempers, A. 1980. Sunburn on apples: preliminary results of an investigation conducted during the 1978/79 season. Deciduous Fruit Grower 30: 8-22.

Evans, R. G. 1993a. Designing and operating overtree evaporative cooling systems for apples. Part 1. Good Fruit Grower, June, 23 – 27.

Huysamer, M. 1998. Report of the blushed pear workgroup: Perceptions, facts and questions. Proc. Cape Pomological Association Tech. Symp., Cape Town, South Africa, 2-3 June 1998, 187-192.

Kotzé, W. A. G., Carreira, J. A., Beukes, O. and Redelinghuys, A. U. 1988. Effect of evaporative cooling on the growth, yield and fruit quality of apples. Deciduous Fruit Grower 38: 20 – 24.

Schrader, L. E., Zhang, J. and Duplaga, W.K. 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Online. Plant Health Progress. http://www.planthealthprogress.org/Current/research/sunburn/article.htm

Wand, S. J. E., Steyn, W. J., Mdluli, M. J., Marais, S. J. S. and Jacobs, G. 2002. Overtree evaporative cooling for fruit quality enhancement. SA Fruit Journal, Aug/Sept., 18 – 21.

Literature Review

1. Sunburn and heat stress

In many regions of the world sunburn causes serious economic losses in apples and other fruit (Kotzé *et al.*, 1988; Evans, 1993a; Schrader *et al.*, 2001; Wand *et al.*, 2002). Fruit are more susceptible to sunburn compared to leaves mainly because they do not have efficient mechanisms of utilising and/or dissipating solar radiation (Jones, 1981). As a result, fruit surface temperature may rise $10 - 15^{o}C$ higher than the ambient air temperature (Parchomchuk and Meheriuk, 1996). Sunburn of apples was observed by Bergh *et al.* (1980) when the skin temperature exceeded $50^{o}C$. This happened when air temperature exceeded $36^{o}C$. Sunburn negatively affects the appearance of the fruit, and they cannot be sold for fresh market consumption, which receives the highest prices. Sunburn on apples in South African production areas can amount to $20 - 50\%$ fruit cull in the orchard and up to 10 % rejection of packed cartons thereafter (Berg *et al.*, 1980). According to Van den Ende (1999) almost all apples can burn, regardless of colour, although 'Granny Smith' and other light-skinned apples are amongst the most sensitive to sunburn. Some red varieties may colour over the burnt areas so the damage may not be visually evident, but these apples often have storage problems due to the internal damage (Evans, 1993a).

It is not known whether tissue sensitivity to damage alters with fruit development, or even why some cultivars show less sunburn damage than others (e.g. 'Royal Gala' is less sensitive than 'Braeburn' or 'Granny Smith') (Palmer *et al.*, 2003).

1.1   What is sunburn?

According to the American Phytopathological Society's "Compendium of Apple and Pear diseases" (Jones and Aldwinckle, 1990) sunburn is the term used to describe fruit damaged by solar radiation, whereas sunscald is injury to bark and underlying tissues caused by a combination of high light and freezing. In South Africa the term "superficial scald" is used and refers to damage which only becomes visible during storage, as on 'Granny Smith' in particular.

Two types of sunburn have been identified, 1) sunburn necrosis, and 2) sunburn browning (Schrader *et al*., 2001). Sunburn necrosis is caused by thermal death of epidermal and sub epidermal cells (peel), and causes a necrotic spot on the side of the fruit that was exposed to the sun. This can also happen in the absence of light, when it is caused by heat. Thermal death occurs at surface temperature $52 \pm 1^{o}C$. Electrolyte leakage increases significantly with necrosis, indicating that membrane integrity is lost during thermal death. Sunburn browning is sub-lethal and results in a yellow, bronze, or brown spot on the sun-exposed side of the fruit, and it occurs only in the presence of light. Sunburn browning occurs at a fruit surface temperature of $46-49^{o}C$ and has little effect on membrane integrity. Tests by Schrader *et al*. (2001) indicated that fruit skin temperature is critical to the development of both types of sunburn.

Rabinowitch *et al*. (1986) suggested that sunburn occurs when photosynthesis is disturbed by excessive heat, so that light energy is redirected into damaging photodynamic processes. Sunburn is, therefore, caused by a combination of high temperatures and high light intensities (Glenn *et al.*, 2002; Schrader *et al.*, 2001). The relative contribution of each of these two stresses, however, has not yet been clearly established.

1.2 Effects of high radiation on plant tissue

Irradiance is an important ecological factor on which all photoautotrophic plants depend (Lambers *et al*., 1998). Only the photosynthetically active part of the electromagnetic spectrum (PAR; 400-700 nm) directly drives photosynthesis (Palmer *et al*., 1989; Lakso, 1994). A steady-state response of photosynthesis to irradiance is achieved after exposure of a leaf to constant irradiance for some time until a constant response is reached. Net CO_2 assimilation rate (A) increases asymptotically with increasing irradiance. Below the light-compensation point (A=0) there is insufficient light to compensate for the respiratory release of CO_2 in photorespiration and dark-respiration (Fig 1).
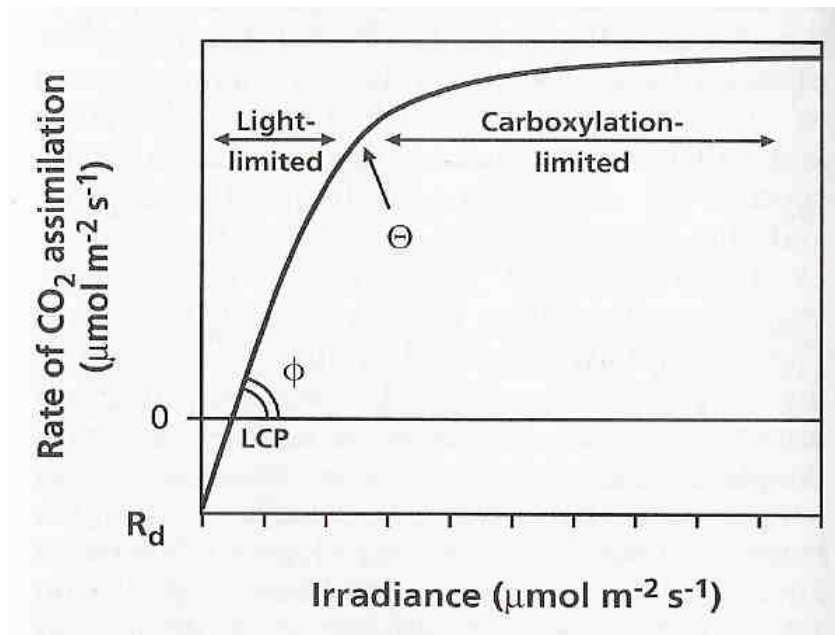
Figure 1. Typical response of photosynthesis to irradiance. The intercept with the x-axis is the light-compensation point (LCP), the initial slope of the line gives the quantum yield (Φ) and the intercept with the y-axis is the rate of dark respiration (R_d). The curvature of the line is described by Θ. At low irradiance, the rate of CO_2 assimilation is light-limited; at higher irradiance A is carboxylation limited. A_{max} is the light-saturated rate of CO_2 assimilation at ambient CO_2 concentration. (Adapted from Lambers et al., 1998)

Thereafter, at low irradiance, A increases linearly with increasing irradiance, with the light-driven electron transport limiting photosynthesis (Lambers et al., 1998). At irradiance levels beyond the linear, light-limited region of the light-response curve, some of the photons absorbed by chlorophyll cannot be used in photochemistry. Plants have mechanisms to dispose of this excess excitation energy safely (Lambers et al., 1998). With these mechanisms at work, the quantum yield of photosynthesis is temporarily reduced. This often occurs at high irradiance in many plants. If the dissipation mechanisms are inadequate, however, the excess excitation energy may cause damage to the photosynthetic membranes (Merzlyak and Solovchenko, 2002). This leads to longer lasting (days) photoinhibition. In extreme cases, this high-light stress may lead to bleaching of fruit or leaves due to a breakdown of chlorophyll (Lambers et al., 1998).

In the absence of dissipation mechanisms, excess energy would be passed on to oxygen via chlorophyll. This causes the formation of toxic oxygen free radicals in the thylakoid membrane. These molecules are either singlet oxygen or superoxide and hydrogen peroxide, and can damage the thylakoid membranes (photooxidative damage). Some plants have a higher photosynthetic capacity than others, and can use more of the available light for photosynthesis, causing them to have lower excess light energy under the same conditions as plants with a lower photosynthetic capacity.

"A life with oxygen, while highly efficient, carries with it potential danger" (Salin, 1988). This chemical paradox arises from the nature of the oxygen molecule, which has a tendency to react with unpaired electrons, giving rise to free radical species (Salin, 1988). These products of the univalent reduction of oxygen are highly reactive and can react with proteins and nucleic acids, potentially causing denaturation or mutagenesis (Alscher, 1989). Toxic oxyradicals are removed through the mobilization of antioxidant reserves. They react both enzymatically and chemically with the toxic molecular species and their products (Alscher, 1989). Chemical constituents have been identified that scavenge free radicals and thus protect photosynthetically active plant cells against oxygen toxicity.

1.3 Ultraviolet radiation

Ultraviolet radiation (especially UV-B; 280-320 nm) can be harmful to plant tissues. It is known to induce oxidative stress in plants and can result in poor fruit quality and crop losses (Forschler *et al.*, 2003). UV radiation intensity is highest in tropical regions and increases with increasing height above sea level. UV radiation is also increasing slowly in several places in the world due to holes in the ozone layer (Kerr and McElroy, 1993). Some researchers suggest that the cause of sunburn is primarily excessive heat damage (Drake *et al.*, 1991; Parchomchuk and Meheriuk, 1996). Others, however, suggest that the high-energy UV-B radiation itself contributes to the incidence of sunburn (Lipton, 1977; Renquist *et al.*, 1989). The greater part of UV radiation penetrating cells is absorbed and causes acute injuries on account of the high quantum energy. In addition to its photooxidative action, UV-B also causes photolesions, particularly in biomembranes. UV-damage to protoplasm consists of the breaking down of the disulfide bridges in protein molecules, and the dimerizing of thymine groups of DNA, resulting in defective transcription. In addition, UV inhibits violaxanthin de-epoxidase, so that the xanthophyll cycle cannot adequately fulfill its role if the light is very

strong. Plants that are sensitive to UV radiation normally have a lower photosynthetic capacity and slower leaf development. UV radiation also results in a decrease in apical dominance, pollen viability, flower development, and acceleration in leaf aging. UV damage to nucleic acids can be reversed, and plants under adequate light conditions usually have the capacity to restore themselves without suffering long-term consequences. Plants can protect themselves against the absorption of UV light by escape movements such as orientating their leaves away from the light, rolling up the shoots (e.g. mosses and pteridophytes), and by chloroplast movements in assimilatory tissue (Lambers *et al.*, 1998). Dense trichome coverings on leaf surfaces or thickened walls in the epidermis and hypodermal tissue (e.g. conifer needles and cacti) act as diffusive filters and lessen the effect of strong radiation. Shiny leaf surfaces can reflect more light. Phenolic compounds, especially flavonoids, effectively absorb UV light in the epidermis before it enters the mesophyll. Plants acclimate by increasing the concentration of absorbing phenolic compounds when they are exposed to increasing intensities of UV-B light (Searles *et al.*., 2001). Anthocyanins in unfolding leaves can also act as darkening filters, shielding the mesophyll.

1.4 Protective mechanisms of plants against high radiation

Plants respond to strong light in remarkable ways, which include changes in pigment content and composition. Several protective mechanisms are employed to avoid light stress caused by high solar radiation: 1) excess energy is dissipated through the xanthophyll cycle (Demmig-Adams et al., 1995; Gilmore, 1997, Müller *et al.*, 2001), 2) induction of antioxidants (e.g., phenols, flavonols and the ascorbate-glutathione cycle) to minimise oxidative damage (Mackerness and Thomas, 1999; Merzlyak and Solovchenko, 2002; Solovchenko and Schmitz – Eiberger, 2003), 3) attenuation by pigments that absorb or reflect light (Mackerness and Thomas, 1999; Merzlyak and Solovchenko, 2002; Steyn, 2003).

1.4.1 Xanthophyll cycle

The xanthophyll cycle is an important mechanism which plants possess to get rid of excess electrons that can cause damage. The xanthophyll cycle in higher plants and green algae consists of several forms of carotenoid pigments: violaxanthin (di-epoxide), antheroxanthin (mono-epoxide) and zeaxanthin (epoxide-free). The three forms are reversibly interconvertible (violaxanthin <==> antheroxanthin <==> zeaxanthin), by the addition or subtraction of an epoxide group (Long and Humphries, 1994). When plants are subjected to

strong light, violaxanthin shows a light-dependant conversion to zeaxanthin via antheroxanthin; this process is then reversed in the dark (Bratt et al., 1995) and both processes are carried out by epoxidase and de-epoxidase enzymes (Gilmore, 1997). It is the de-epoxidized form zeaxanthin that is involved in the protection of the photosynthetic apparatus against damage by excess light (Demmig-Adams and Adams, 1992). It functions similarly to a lightning conduit and by accepting excess electrons from the activated chlorophyll and setting the energy free as heat. Sun leaves have been found to have larger xanthophyll cycle pools than shade leaves and also demonstrate a greater increase in energy dissipation activity within the antenna (Demmig-Adams and Adams, 1992). The percentage of xanthophylls converted to zeaxanthin is greater under light stress conditions. Most excess light energy is worked away by the xanthophyll cycle.

1.4.2 Antioxidant systems

Plants also use the antioxidant ascorbic acid (vitamin C) which they produce themselves, to reduce oxidative cell damage. It is one of the most important nutritional quality factors in many horticultural crops. Various factors such as genotypic differences, preharvest climatic conditions and cultural practices, maturity and harvesting methods can influence the content of vitamin C in fruits and vegetables. Vitamin C content in plant tissues increases with increasing light intensity during the growing season (Lee and Kader, 2000). Plants use vitamin C to defend against ozone, which damages more plants than all other air pollutants combined. Stratospheric, or upper-level ozone protects the earth from damaging UV radiation, but tropospheric, or ground-level ozone is a pollutant. Tropospheric ozone enters plants through their leaves and decomposes into unstable reactive oxygen radicals which must be neutralised by antioxidants. Plants also utilise phenols, e.g. tocopherols, and polyphenols such as quercetin and rutin, as antioxidants. Vinson *et al.* (2001) found them to be stronger antioxidants than the vitamin antioxidants. Polyphenols, particularly the flavonoids, are among the most potent plant antioxidants. Polyphenols can form complexes with reactive metals such as iron, zinc and copper – reducing their absorption. In addition to their chelating effect on metal cations, polyphenols also function as potent free radical scavengers, neutralising free radicals before they can cause cellular damage. Flavanoids occur widely in the plant kingdom, and are especially common in leaves, flowering tissues, and pollen. Glutathione, a tripeptide, is widely distributed in plant cells (Rennenberg, 1982). It appears to be synthesized in both the chloroplast and the cytosol and to occur in both of these subcellular

compartments at relatively high levels (Alscher, 1989). It plays an important role as an antioxidant, and together with ascorbate protects macromolecules against attacks by free radicals and hydrogen peroxide (Alscher, 1989).

1.4.3 Pigments

The pigments in fruit fulfill several important roles. In apples, peel chlorophyll and carotenoids are competent in photosynthesis, and almost as efficient as in leaves (Blanke and Lenz, 1989). Carotenoids participate in light harvesting and are recognized as powerful antioxidants, and excited states and singlet oxygen quenchers are involved in photoprotection (Palett and Young, 1993). The antioxidant actions of carotenoids are based on their singlet oxygen quenching properties and their ability to trap peroxyl radicals. The best documented antioxidant action of carotenoids is their ability to quench singlet oxygen. This results in an excited carotenoid, which has the ability to dissipate newly acquired energy through a series of rotational and vibrational interactions with the solvent, thus regenerating the original unexcited carotenoid, which can be reused for further cycles of singlet oxygen quenching (Sies and Stahl, 1997).

Some lines of evidence suggest that anthocyanins are also involved in the protection of fruit against harmful UV and excessive sun irradiation (Merzlyak and Chivkunova, 2000).
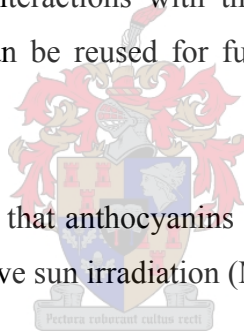
1.5 Effects of high temperatures on plant tissue

High temperatures impair the thermal stability of membranes and proteins. Thylakoid membranes are especially sensitive to heat (Larcher, 1975). Membrane lipids become more fluid and this is correlated with loss of physiological function. The strength of the hydrogen bonds and electrostatic interactions between polar groups of proteins within the aqueous phase of the membrane decreases (Ritenour *et al.*, 2001). High temperatures modify membrane composition and structure and can cause leakage of ions. Membrane disruption causes inhibition of processes such as photosynthesis and respiration. Apparently, the photosystem II complexes located on the thylakoid membranes are the most heat-sensitive part of the photosynthetic mechanism (Santarius and Weiss, 1988; Weiss and Berry, 1988).

1.6 Protection mechanisms of plants against high temperatures

Plants have several adaptations to protect their leaves against excessive heating. Plants that

are hardy to high temperatures have high protoplasmic viscosity (membranes change from a fluid to a gel), and they are able to synthesise at high rates when temperatures become elevated, allowing synthetic rates to equal breakdown rates and thereby avoid ammonia poisoning (Salisbury and Ross, 1992). Some plants achieve prevention of dangerous overheating of the leaves through the evasion of strong sunlight (Larcher, 1975). Plants orientate their leaves away from the sun, or leaves roll up when the temperature becomes too high.

One of the protective mechanisms against high temperature stress in plant tissues are the so-called heat-shock proteins. Organisms ranging from bacteria to humans respond to high temperatures by synthesising a new set of proteins, the heat-shock proteins (HSPs). When seedlings are suddenly shifted from 25 to 40°C, synthesis of most of the normal mRNAs and proteins is suppressed, while transcription and translation of a set of 30 to 50 other proteins (HSPs) are enhanced (Ritenour et al, 2001). These HSPs appear rapidly, and may become a substantial portion of the total proteins within 30 minutes after heat shock. During the past two decades there has been a considerable interest in the heat-shock response (Key et al., 1985; Kimple and Key, 1985). It is becoming apparent that the HSPs play a vital role in heat tolerance, perhaps by protecting essential enzymes and nucleic acids from heat denaturation. Cells or plants that have been induced to synthesize HSPs show improved thermal tolerance and can tolerate exposure to temperatures that were previously lethal.

1.7 The effects of high temperature on gas exchange

The temperature responses of leaf photosynthesis and respiration differ remarkably (Palmer et $al.$, 2003). Leaf CO_2 assimilation shows a parabolic response to temperature, with a peak at about 30°C, but with a broad shoulder in the 15-35°C range (Lakso, 1994). It drops off rapidly above 35°C, however. In contrast, leaf dark respiration and photorespiration increase exponentially with an increase in temperature. Over the 10 – 30°C range, for each 10°C rise in temperature, the dark respiration rate increases by a factor of 2.5 (Lakso, 1994). The decline in net photosynthesis at temperatures higher than 35°C may be partly due to temperature-induced increases in vapour pressure deficits (VPD) that can affect stomata, but mostly it is due to the increases in photorespiration (Lakso, 1994).

The respiration rate is determined by demand for energy by two main processes, maintenance and growth respiration (Palmer *et al.,* 2003). Maintenance respiration is associated with the energy required for protein turnover and the maintenance of ion gradients. Growth respiration is the energy required for new tissue synthesis. It is primarily maintenance respiration that is temperature sensitive (Palmer *et al.*, 2003). Lakso (1994) explained the high apple yields obtained in New Zealand partly by the combination of high solar radiation, ensuring high rates of photosynthesis, combined with relatively cool temperatures, ensuring low maintenance respiration.

At high temperatures the oxygenating reaction of Rubisco increases more than the carboxylating reaction so that photorespiration becomes proportionally more important (Palmer *et al.*, 2003). Part of the reason for this is that the solubility of CO_2 declines with increasing temperature more strongly than does that of O_2. The effect of temperature on photosynthesis of C_3 plants is also due to the effects of temperature on the kinetic properties of Rubisco. These combined effects cause a decline in net photosynthesis at high temperatures.

2. Evaporative cooling as a mechanism to control sunburn

Because of the high susceptibility of many fruit types to sunburn and the inadequacy of their resistance mechanisms, external intervention from growers is needed to suppress sunburn in fruit. Orchard factors such as row orientation, canopy management, previous exposure history, and summer pruning play a substantial part in susceptibility of fruit to sunburn (Van den Ende, 1999).

Since the 1920's, fruit growers have been looking for ways to avoid or decrease sunburn (Bergh *et al*., 1980). Evaporative cooling, shade net covers and reflective kaolin particle film (KP) are among the several practices that have been used to reduce sunburn in apple orchards. KP involves the use of kaolin particles that are reflective to radiation, especially UV wavelengths that reach the surface of leaves and fruit (Gindaba and Wand, 2005). This helps to lower the leaf and fruit surface temperatures (Glenn *et al*., 2002). Shade net attenuates solar irradiance by shading, thereby lowering the temperature, reducing the wind and increasing the humidity around the trees (Gindaba and Wand, 2005).

This study centres on evaporative cooling (EC), which involves an overtree irrigation system to cool down fruit when air temperature exceeds a certain threshold.

A growing number of fruit producers all over the world are rapidly adopting the use of overtree evaporative cooling as a feasible, chemical-free technique to reduce sunburn and enhance colour development of red or blushed fruit (Evans, 1993a). Apple production is increasing all over the world, also in areas with unfavourable environmental conditions, and growers are moving to higher density plantings. To continue producing maximum yields of high quality fruit, it is becoming critical for growers to alleviate heat and soil water stresses (Unrath and Sneed, 1974). Avoiding extreme leaf and fruit temperatures during the hottest part of the day can greatly reduce the incidence of sunburn on directly exposed fruit. The ability of fruit to utilize or dissipate excess radiation is not as well developed as in leaves (Jones, 1981; Blanke and Lenz, 1989). Producers have also found that the use of EC just prior to sundown and sometimes around sunrise has improved colour development on red apples (especially early varieties) before harvest (Evans, 1993 a, 1999). In South Africa, the profitability of blushed pears (*Pyrus communis)* in the warm production areas of the Western Cape has been limited by insufficient red colour (Steyn *et al.*, 2004). The locally bred early season cultivar Rosemarie is very susceptible to colour loss just prior to harvest. This is due to the net degradation of anthocyanin in response to high temperatures (Steyn *et al*., 2004). EC trials in Stellenbosch have led to improved red blush colour on 'Rosemarie' in some seasons (Wand *et al.*, 2004).

2.1 What is EC?

An EC system applies water above the crop. An overhead sprinkler system is used to wet fruit and leaf surfaces when the temperature rises above a certain threshold point. As the water evaporates from the fruit surfaces, energy is extracted from the skin. If this energy is greater than the total incoming heat energy, the fruit surface temperature decreases (Unrath, 1972, Parchomchuk and Meheriuk, 1996). The air around the trees is also cooled, and the relative humidity increases, thus reducing water loss through transpiration. Often during a day with low humidity, high temperatures and intense sunlight, water is lost to transpiration faster than the root system can replace it from the soil. During fruit development, these conditions can cause water to be withdrawn from the fruit to supply the plant, resulting in smaller fruit,

delayed maturity and reduced fruit quality (Evans, 1993a). By raising the relative humidity in the orchard, EC reduces overall heat stress of the plants.

EC is one of three methods used to cool crops using water. The other two are:
1) Convective cooling: water is evaporated in the air (undertree or overtree) and the circulation of the cooled air is used to reduce fruit temperatures.
2) Hydro-cooling: water is applied to the leaves and fruit. The "cool" water is used to extract heat from the plant organs and carry it away via liquid "runoff".

Of these three methods, EC is by far the most effective (Evans, 1993a). In both the other methods, excessive amounts of water have to be used, where EC uses relatively low volumes of water. Fruit, unlike leaves which cool via transpiration, don't have effective ways of cooling themselves. On a bright sunny day surface temperature of an apple may exceed 50°C, even though the air is 10 to 15°C cooler (Schrader et $al.$, 2001). On an average warm summer's day, the use of EC reduces fruit surface temperatures by about 2-3°C. This can increase to over 10°C on hot days in fruit directly exposed to sunlight (Wand et $al.$, 2002). Wünche et $al.$ (2001) also found that water application treatments to the whole canopy in mid-season caused a reduction of up to 8°C in mean fruit skin temperature.
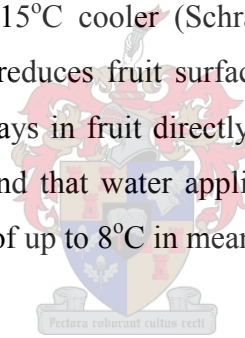
2.2 Mechanics of an EC system

The cost of and difficulty of installing an EC system would, of course, depend on the existing irrigation system and its hydraulic capacity. A properly designed evaporative cooling system is bound to be more expensive than a conventional irrigation system because of increased pipe sizes, pressure control measures, larger pumps, expanded valving needs, control/automation costs, and possible storage dams (Evans, 1993b). It is easier if systems for EC are incorporated into the planning of the orchard, as it can be very expensive and difficult to retrofit existing irrigation systems for EC. System requirements vary from orchard to orchard. A basic system will cost around R6000-R7000/ha, but this can increase to R15000/ha depending on the need for poles, additional pumps, pipes and valves, the type of jets used, equipment for soil water measurements and computers (Wand et $al.$, 2002). According to Evans (1993b), total seasonal water application will be between 25 and 40 percent greater than that used for normal undertree irrigation only.

Effective cooling requires about 2-2.5 mm/hr/ha if it is used on a continuous basis. When pulsing is used, a higher application rate of about 4-5mm/hr/ha is suitable (Wand *et al.*, 2002). This rate ensures large enough droplets to wet all surfaces properly. Pulsed systems at higher flow rates are preferred for their cooling efficiency in reducing sunburn (Evans, 1993b). The system should be designed to cope with the highest required application rate, which is normally for sunburn protection since large amounts of energy must be extracted (Evans, 1993b). Fruit temperatures can be measured by a temperature sensor in the orchard that is connected to a data logger, or by inserting a thermometer into the fruit to measure the fruit temperature. Good control of the system is necessary. Automatic control is usually required to pulse or cycle the water applications based on a time sequence or on fruit temperatures. Evans (1993b) recommends that cycles should be based on fruit core or fruit skin temperature measurements, instead of air temperatures. Research has shown that fruit can warm more quickly and cool off more slowly than the surrounding air temperatures. Basing system controls on ambient air temperatures is therefore a less effective procedure (Evans, 1993b).

According to Wand *et al.* (2002), on- times should be 5 minutes or less, followed by off-times of about 10-15 minutes for best results. Good results can be achieved with longer cycles of 20-30 minutes, as long as the system is activated at least once every hour during the warmest part of the day.

2.3 Effects of evaporative cooling on fruit trees

2.3.1. Colour

Skin colour of apples is an important factor in consumer acceptance. This is particularly so in red and blushed cultivars, as in most markets red-skinned apples are preferred to others. Skin colour is also an important factor in establishing government grades and standards, because a particular grade must have a certain proportion of the apple skin coloured. Downgrading due to insufficient red colour has limited the profitability of blushed pear cultivars in the Western Cape region of South Africa (Huysamer, 1998). Red colour and fruit size are two of the most important parameters for the European Union countries. Even with adequate fruit size, poor fruit colour is an important cause for reduction in grade and is generally associated with poor visual consumer acceptance (Iglesias *et al.*, 2002).

Optimum red colour development in pome fruit depends on both environmental and cultural factors such as adequate sunlight, moderate crop load and moderate vigour. If the orchard is managed well, particularly in regard to fruit thinning, tree training and pruning, irrigation, nitrogen level and weed control, the fruit should colour well. However, the critical environmental influences of light and temperature on fruit colour development usually override cultural practices in warm and hot fruit growing regions (Williams, 1993).

In pome fruit, different shades of red in the peel are thought to be caused through the visual blending of red anthocyanins dissolved in the vacuole in combination with the green to yellow chlorophyll and carotenoids present in the plastids (Lancaster *et al*., 1994). The main anthocyanin in apples and pears is cyanidin-3-galactoside (Iglesias *et al.*, 2002, Dussi *et al.*, 1997). The only secondary pigment in pears is cyanidin-3-arabinoside. Apples also contain cyanidin-3-glucoside and trace amounts of acylated and other cyanidin pigments (Steyn, 2003). Anthocyanins in apples are synthesised in the epidermal and adjacent hypodermal cells of apples, but only in the hypodermal cells of pears (Lancaster *et al*, 1994). Two steps are required for anthocyanin biosynthesis, induction and synthesis. The induction phase is triggered mainly by low temperatures (Christie *et al*., 1994; Curry, 1997). Synthesis depends on the carbon products, mainly carbohydrates, formed during photosynthesis and glucose metabolism. The carbohydrates are formed in the leaves and transported to the fruit. Some of these are eventually transformed into pigments via complex biochemical reactions (Williams, 1993). Since the 1920s, it has been well documented that temperature plays a role in the rate of pigment biosynthesis. Mild days ($20^{o}C$-$25^{o}C$) and cool nights ($<15^{o}C$) are the most conducive for red pigment formation (Curry, 1997). Synthesis of anthocyanin has been associated with an increase in the activity of L-phenylalanine ammonia-lyase (PAL), an enzyme which is critical in the regulation of flavonoid and anthocyanin biosynthesis (Farager, 1983). Farager showed that low temperatures reduce the level of a PAL-inactivating system (PAL-IS). PAL activity and anthocyanin levels were, therefore, higher at lower temperatures. Higher temperatures result in the accumulation of PAL-IS, and thus in a reduction of PAL activity and subsequent anthocyanin accumulation (Farager, 1983).

In pears, red colour peaks early during fruit development and thereafter gradually declines (Dussi et al, 1997). Although good exposure to light is a requirement for all cultivars, the

degree of synthesis and breakdown of the pigment is tightly linked to climatic conditions in some cultivars, notably 'Rosemarie' (Steyn, 2003).

The use of water to cool apple fruit to enhance colour development was first reported in the early 1970's. Unrath and Sneed (1974) in North Carolina tested overtree sprinkler irrigation cycles to promote red colour development in 'Delicious'. Pioneering research in the area of evaporative cooling lay dormant because of the introduction of daminozide (Alar) which delays fruit maturity and reduces shading due to vigour control. However, when Alar was lost from the market in 1989, a research and extension program on the use of EC to promote red colour and reduce sunburn was initiated in the USA (Williams, 1993). Since then, much research done on the use of evaporative cooling to improve colour has had positive results, with colour improvement in both apples and pears. Dussi *et al.* (1997) reported that evaporative cooling increased hue and lightness and colour differences between exposed and shaded fruit surfaces increased with maturity. Fruits from cooled trees matured earlier. At Welgevallen Experimental Farm (Stellenbosch, South Africa), good results for blush improvement of 'Rosemarie' pears were found when the EC system was activated from mid- to late-December (Wand *et al.*, 2004). Activating the system earlier, toward the end of November, did not improve blush at harvest. Colour loss occurred just before harvest. This is thought to be due to earlier EC application leading to the acclimation of the fruit to the lower temperatures. Thus, for blush development, the best results are achieved by using EC for the last 3-4 weeks before harvest. Some growers have reported that application at dusk was most effective for red colour stimulation in 'Cripps' Pink' apples (Iglesias *et al.*, 2000). Much of the latent heat from the day is drawn out of the fruit quickly, and cooler night temperatures prevent them from heating up again. This application has minimal water requirements for potentially large benefits (Evans, 1993a).

2.3.2 Sunburn

Trials by Parchomchuk and Meheriuk (1996) showed that EC can be used successfully to reduce sunburn on apples. Kotzé *et al.* (1988) found a reduction in sunburn of approximately 50% on 'Granny Smith' and 'Golden Delicious' apples under EC in South Africa. The degree of sunburn is reduced allowing for a higher percentage packout, but complete control is usually not possible. As mentioned above, fruit skin is damaged at temperatures above 45°C, and this is easily reached when air temperature is above 35°C. According to Van den Ende

(1999), a critical air temperature threshold is 30 to 32°C. Burning can occur at even more moderate temperatures when there is a lack of air movement. Since more mature fruit are more susceptible to sunburn, this threshold probably decreases with advancing season (Wand *et al.*, 2002). For effective sunburn control, it is advisable to activate the EC system at air temperatures of about 30-32°C earlier in the season and reduce it to 28°C for the last few weeks before harvest (Wand *et al.*, 2002). At Welgevallen Experimental Farm, reductions in sunburn were achieved during the warm 2000/2001 season in both 'Rosemarie' (reduced from 27% to 15%) and 'Cripps' Pink' (reduced from 17% to 6%). Additional measurements on commercial farms during 2001/2002 showed reductions in sunburn on 'Royal Gala' (Nooitgedacht, Ceres) and 'Cripps' Pink' (Vredelust, Villiersdorp) (Wand *et al.*, 2002).

2.3.3 Maturity

The effects of evaporative cooling on maturity are highly variable, and also cultivar dependent. Unrath (1972) found that fruit firmness was increased by cooling irrigation, but only in warm seasons; total soluble solids (TSS) were always higher than in uncooled fruits, and maturity was not delayed. Iglesias *et al.* (2002) reported higher TSS, titratable acidity and fruit firmness under EC treatments. Williams (1993) found that fruit maturity was consistently delayed by 7 to 10 days as indicated by total soluble solids, titratable acidity and starch; furthermore, fruit firmness was slightly higher in cooled fruit. This may be a benefit for controlled atmosphere storage. It may also be used to lengthen harvest intervals by manipulating fruit maturity (Evans, 1993a). In some cases where pear fruit size was increased, firmness was lower and harvest dates were brought forward (Wand *et al.*, 2002). During the 2001/2002 season all cultivars under EC at Welgevallen showed earlier maturation when EC was applied from early in the season. This appeared to go hand in hand with increased fruit size. Because EC was applied in addition to undertree irrigation, the soil under the trees was wetter, probably contributing to these responses (Wand *et al.*, 2002).

2.3.4 Fruit size

There have been differing reports from researchers concerning the size of fruit under EC. Kotzé *et al.* (1988) and Parchomchuk and Meheriuk (1996) found no effect on apple fruit size. In contrast, Unrath and Sneed (1974) found a significant increase in fruit size, as did Iglesias *et al.* (2002). In some studies, soil moisture is increased by the additional use of EC,

and fruit size is increased as a result of improved tree water relations (Wand *et al.*, 2002). Trees also respond to the milder atmosphere by opening their stomata. This, together with optimal temperatures, can increase photosynthesis. Respiration takes place at a slower rate, and fewer carbohydrates are lost. This allows more carbohydrates for fruit growth (Wand *et al.*, 2002). Reductions in size can occur when trees experience moisture stress in the absence of additional undertree irrigation (Wand *et al.*, 2002). Wand *et al.* (2002) have found increased fruit size on almost all the cultivars tested at Welgevallen when EC was applied throughout the season, starting end-November to mid-December. 'Rosemarie' pears, 'Larry Ann' plums and 'Royal Gala' apples were on average 1.6-2.6 mm larger and 14-20 g heavier under EC. 'Cripps' Pink' did not respond during the first season, but fruit were on average 4.3 mm larger and 22 g heavier during the second season.

2.3.5 Effects of evaporative cooling on flowering of the following season

Most deciduous fruit crops initiate their flowers near the end of the summer vegetative growth period in response to physiological age (i.e. days from full bloom), sufficient light intensity and quality, adequate maturity, healthy leaf surface, nutrition, pruning, and the like (Westwood, 1978). Fertilizer, rootstocks, and other cultural practices can alter the time and the intensity of floral initiation. Any practice or combination of practices that will produce a favourable carbohydrate:nitrogen ratio is generally beneficial. There is no literature available on the effects of EC on flowering of the next season, but it is possible that the difference in microclimate and organ surface temperature might affect the floral initiation and therefore the following year's bloom. If the water available to the trees under EC is significantly higher than that of the control trees, the trees under EC have conditions that are more favourable. Wand et al. (2002) suggested that trees respond to additional soil moisture and milder atmosphere under EC by keeping their stomata open for maximum photosynthesis. Milder atmosphere also minimizes respiratory losses, resulting in more available carbohydrates (Palmer *et al.*, 2003). Trees may, however, respond by utilising more energy for leaf and shoot growth instead of flower initiation. Controlled-environment studies by Jonkers (1984) and Tromp (1976) showed that high daytime temperatures (25-27°C) reduced flower formation.

2.4 Potential problems of evaporative cooling

2.4.1 Mineral deposits: It is critical that good quality water should be used for EC. Deposits of calcium carbonates, iron chelates, silicates and other salts on fruit and leaf surfaces can cause serious problems if they reach toxic levels and they are difficult and costly to wash off in packhouses (Evans, 1993a). It is advisable that a chemical analysis (pH, electrical conductivity, calcium, sodium iron and others) is made of the water supply before it is used for EC. It is not feasible to use water with electrical conductivity $>2dS/m^2$ (Evans, 1993a). Water containing high concentrations of salts, particularly calcium, iron and sodium, leads to surface mineral deposition on leaves and fruit (Andrews, 1995). This can cause scorching if toxic levels are reached. If there are large amounts of organic material in the water, it can clog the jets.

2.4.2 Under- or over-irrigation: If undertree irrigation is continued as usual while EC is used, it could lead to waterlogging. This leads to poor root growth and function. Ideally, undertree irrigation should be reduced by 20% or more to account for the extra overhead irrigation. Conversely, if the normal irrigation is discontinued and only EC is used, trees could develop drought stress. As mentioned earlier, EC alone is not enough to supply the trees with adequate water. Tree and fruit growth will be affected negatively (Wand *et al.*, 2002). Producers should make sure that enough water is available to keep both EC and irrigation systems running properly until the end of the season. If the same system is to be used for both cooling and irrigation, a smaller pump can be installed for irrigation purposes and the block watered in smaller sets at night (Evans, 1993b). Relying on EC to keep the soil irrigated as well could result in severe drought at a deeper soil depth.

2.4.3 System down-time: Because fruit become acclimated to lower temperatures, even a temporary system breakdown or power failure can have disastrous effects. Fruit are less resistant and burn very quickly when exposed to stressful conditions when the system is down (Evans, 1993a). Pumps and electricity supply should be reliable and be backed up for emergencies. Discontinuing EC before harvest can result in substantial damage to the fruit due to sunburn.

2.4.4 Pests and diseases: Care must be taken not to humidify the orchard too much after sunset, and trees must be allowed to dry off before nightfall. Only isolated cases of pest or

disease outbreak have been reported for EC orchards (Evans, 1993a). Olcott-Reid *et al.* (1981) compared EC, trickle and no irrigation under a reduced pesticide or no pesticide program for effects on pests of 'Delicious' apples. The appearance of foliar scab and fruit scab were slightly higher in EC orchards that received no fungicides, but fungicides applied to one side of the trees overcame this effect. White rot incidence was not affected by EC. Aphid colonies and damage from external fruit feeders and codling moth increased under EC when populations were high enough to detect differences between the treatments, but this happened only during one season and at one location for each pest (Olcott-Reid *et al.*, 1981).

3. Chlorophyll Fluorescence

The harmful effects of high temperatures on higher plants occur primarily in photosynthetic functions and the thylakoid membranes, particularly the PSII complexes located on these membranes. This is apparently the most heat sensitive part of the photosynthetic mechanism (Krause and Santarius, 1975; Weiss and Berry, 1988). Because of this, fluorescence is a handy tool for studying the effects of heat stress on apple surface tissues.

3.1 A brief overview of chlorophyll fluorescence

Light energy absorbed by chlorophyll molecules can undergo one of three fates (Figure 1): it can be used to drive photosynthesis (photochemistry), excess energy can be dissipated as heat, or it can be re-emitted as light, i.e. chlorophyll fluorescence (DeEll and Toivonen, 2003). These three processes occur in competition, which means that any increase in the efficiency of one will result in the decrease in the yield of the other two. Hence by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be gained (Maxwell and Johnson, 2002).
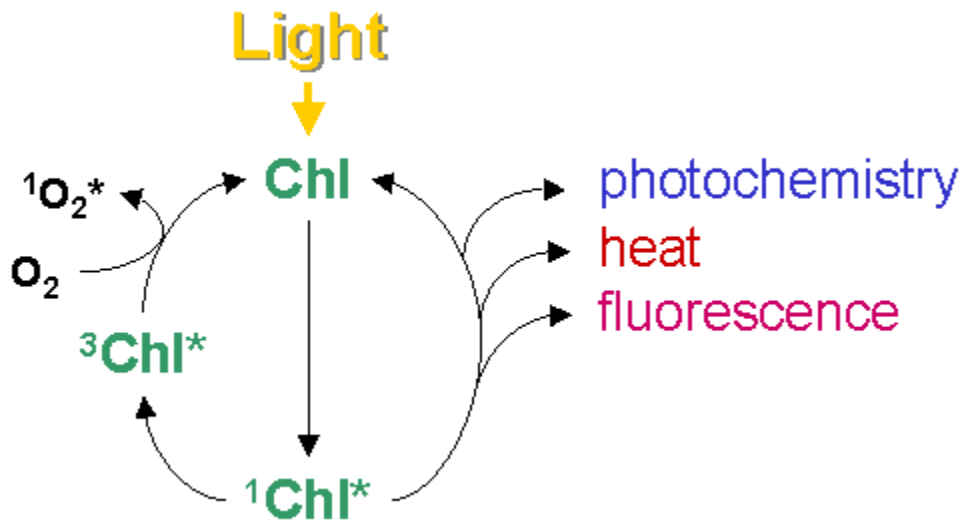
Figure 1: Possible fates of excited chlorophyll. ETH Zürich - Institute of Plant Sciences - Agronomy and Plant Breeding - Dr. J. Leipner. (www.ethz.ch/)

Each quantum of light absorbed by a chlorophyll molecule raises an electron from the ground state to an excited state (Salisbury and Ross, 1992). Upon de-excitation from a chlorophyll a molecule from an excited state to ground state, a small proportion of the excitation energy is dissipated as red fluorescence. Approximately 3-9% of the light energy absorbed by chlorophyll pigments is re-emitted from the first excited state as fluorescence with a peak at 682 nm, and a broad shoulder at about 740 nm (Krause and Weiss, 1984; Salisbury and Ross, 1992; Maxwell and Johnson, 2002) (Figure 2). The emission peak is of a longer wavelength than the excitation energy. This effect was first observed more than 100 years ago by N.J.C. Müller (1874). He noticed that fluorescence changes that occur in green leaves are correlated with photosynthetic assimilation. Lack of appropriate technical equipment, however, prevented a more detailed investigation.
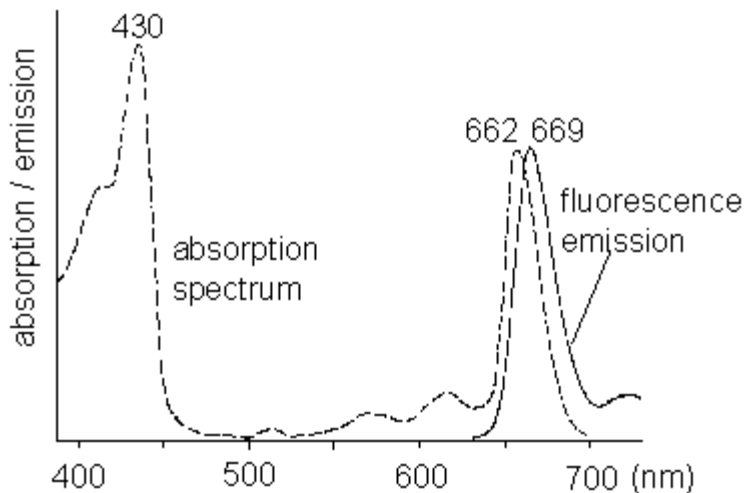
Figure 2: Absorption and emission spectrum of chlorophyll *a*.   ETH Zürich - Institute of Plant Sciences - Agronomy and Plant Breeding - Dr. J. Leipner. (www.ethz.ch/)

Over the last decade, the measurement of chlorophyll fluorescence kinetics has provided considerable information on the organization and function of the photosynthetic apparatus. With the development of instruments that are capable of rapidly resolving the differences in photochemical and non-photochemical quenching, the use of the chlorophyll fluorescence signal as an intrinsic probe of photosynthetic function has become routine in many laboratories. In addition, with the development of smaller electronic components and optical systems, instruments are becoming smaller and more readily usable outside the laboratory, in the greenhouse, and in controlled environment chamber and field situations (Maxwell and Johnson, 2002).

Fluorescence measurements are used for:

- Screening for environmental stress tolerance in plant breeding and production programs.
- Air pollution studies and its effect on photosynthesis (ozone, SO_2, NO_x, etc.).
- Studies of herbicide translocation and mode of action.
- Environmental stress studies such as photoinhibition, chilling, freezing, heat stress, nutrient deficiency, etc.

3.2 Quenching and maximum quantum efficiency of PSII (Fv/Fm)

Changes in the yield of chlorophyll fluorescence were first observed as early as 1960 by Kautsky and co-workers. They found that when photosynthetic material was transferred from the dark into light, the yield of chlorophyll fluorescence increased over a period of time of around one second. (Maxwell and Johnson, 2002). This rise has subsequently been explained as a consequence of reduction of electron acceptors in the photosynthetic pathway downstream of PSII, notably plastoquinone and in particular QA. Once PSII absorbs light and QA has accepted an electron, it is not able to accept another until it has passed the first onto a subsequent electron carrier (QB). During this period, the reaction centre is said to be 'closed'. At any point in time, the presence of a proportion of closed reaction centers leads to an overall reduction in the efficiency of photochemistry and so to a corresponding increase in the yield of fluorescence (DeEll and Toivonen, 2003).

The most useful and widely used chlorophyll fluorescence technique is the so-called quenching analysis of modulated fluorescence by the saturation pulse method (Krause and Weiss, 1991). A typical measurement is shown in Figure 3. The progressive closing of PSII reaction centers when a leaf is transferred from darkness into light, gives rise (during the first second or so of illumination) to an increase in the yield of chlorophyll fluorescence. After this the fluorescence level typically starts to fall again over a time-scale of a few minutes (Maxwell and Johnson, 2002). This is called fluorescence quenching, and is explained in two ways. Firstly, there is an increase in the rate at which electrons are transported away from PSII; this is due mainly to the light-induced activation of enzymes involved in carbon metabolism and the opening of stomata. Such quenching is referred to as 'photochemical quenching'. At the same time, there is an increase in the efficiency with which energy is converted to heat. This process is termed 'non-photochemical quenching' (NPQ). In a typical plant, changes in these two processes will be complete within about 15–20 min and an approximate steady-state is attained, although the time taken to reach this state can vary significantly between plant species, and even between different leaves of a plant (Maxwell and Johnson, 2002).

In order to gain useful information about the photosynthetic performance of a plant from measurements of chlorophyll fluorescence yield, it is necessary to be able to distinguish

between the photochemical and non-photochemical contributions to quenching (Maxwell and Johnson, 2002). The usual approach is to 'switch off' one of the two contributors, specifically photochemistry, so that the fluorescence yield in the presence of the other alone can be estimated. This can be achieved *in vitro* by the addition of chemicals, such as the herbicide Diuron (DCMU), that inhibit PSII and thereby reduces photochemistry to zero. This method is, however, both impractical and undesirable in a more physiological context (Maxwell and Johnson, 2002). Instead, a method has been developed that allows the contribution of photochemical quenching to be transiently reduced to zero (Quick and Horton, 1984). In this approach, a high intensity, short duration flash of light is given. This closes all PSII reaction centers. Provided the flash is short enough, no (or a negligible) increase in non-photochemical quenching occurs and no long-term change in the efficiency of photosynthesis is induced (Maxwell and Johnson, 2002). During the flash, the fluorescence yield reaches a value equivalent to that which would be attained in the absence of any photochemical quenching, the maximum fluorescence, F_m. When this value is compared with the steady-state yield of fluorescence in the light (F_t) and the yield of fluorescence in the absence of an actinic (photosynthetic) light (F_0), it gives information about the efficiency of photochemical quenching and by extension, the performance of PSII. The difference between F_m and F_0 is called the variable fluorescence (F_v). The maximum quantum efficiency of photosystem II (PSII) primary photochemistry can then be given as F_v/F_m (DeEll and Toivonen, 2003). F_v/F_m is calculated as $(F_m-F_0)/F_m$. A decrease in F_v/F_m usually is originated by a decrease in F_m in combination with an increase in F_0. The latter is provoked by dissociation of light harvesting pigment system of PSII from the PSII core. It is thought that a decrease in F_v/F_m might be due to both photoprotection and photodamage (Krause and Weiss, 1991; DeEll and Toivonen, 2003).
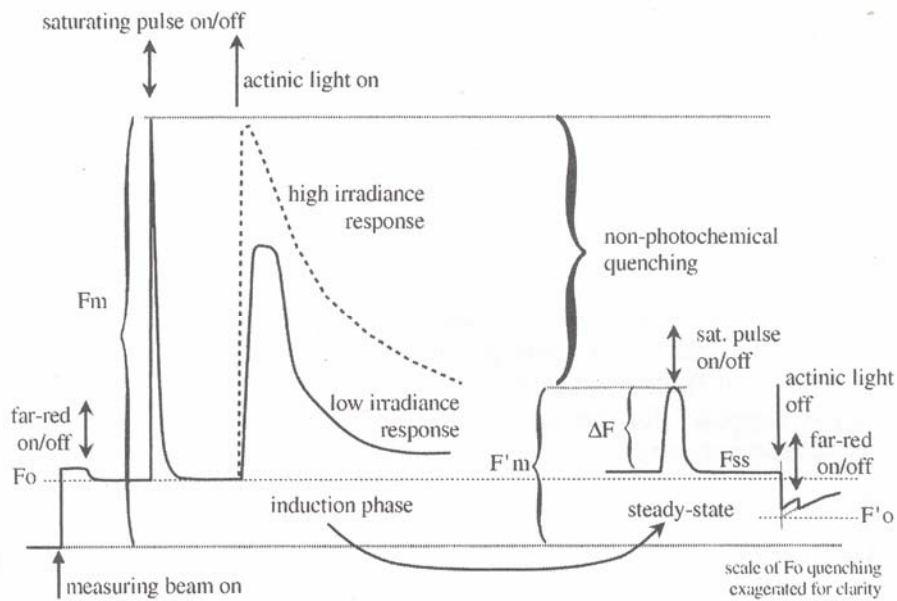
Figure 3: Measurement of chlorophyll fluorescence by the saturation pulse method (adapted from Van Kooten and Snell, 1990).

4. Conclusion

Sunburn causes serious economic losses in warm fruit producing areas all over the world. In South Africa, producers in the Western Cape also lose a substantial percentage of fruit every year due to sunburn and related disorders, including insufficient colouring of blushed cultivars. In order to produce superior quality fruit, the producer must have a basic knowledge of the biology of and the factors affecting sunburn, as well as the different management practices influencing fruit colour. If used correctly, evaporative cooling could lower the incidence of sunburn on fruit in warm production areas, as well as improving colour of blushed cultivars. The system will, however, necessitate good control. A good backup system should be in place in case of power failures or breakdown. Care should be taken to adapt irrigation scheduling to prevent under- or over-irrigation. The use of water for cooling is a luxury consumption of water. Because of frequent water shortages in the Western Cape, producers should be aware of pressure on the industry to conserve water.

5. References

Alscher, R. G. 1989. Biosynthesis and antioxidant function of glutathione in plants. Physiol. Plant. 77: 457-464.

Andrews, P. K. 1995. Evaporative cooling of Fuji apples. Good Fruit Grower. July, 32 – 34.

Bergh, O., Franken, J., Van Zyl, E. J., Kloppers, F., and Dempers, A. 1980. Sunburn on apples: preliminary results of an investigation conducted during the 1978/79 season. Deciduous Fruit Grower 30: 8-22.

Blanke, M. M. and Lenz, D. H. 1989. Fruit photosynthesis. Plant Cell Environ. 12: 31 – 46.

Bratt, C. E., Arvidsson, P. O., Carlsson, M. and Akerlund, H-E. 1995. Regulation of violaxanthin de-epoxidase activity by pH and ascorbate concentration. Phot. Res. 45: 169-175.

Christie, P. J., Alfenito, M. R. and Walbot, V. 1994. Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: Enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. Planta 194: 541 – 549.

Curry, E. A. 1997. Temperatures for optimal anthocyanin accumulation in apple tissue. J. Hort. Sci. 72: 723 – 729.

DeEll, J. R., and Toivonen, P. M. A. (eds) Practical Applications of Chlorophyll Fluorescence in Plant Biology. 2003. Kluver Academic Publishers, Boston, USA.

Demmig-Adams, B. and Adams III, W.W. 1992. Photoprotection and other responses of plants to high light stress. Ann. Rev. Plant Physiol. Plant Mol. Biol. 43: 599-626.

Demmig-Adams, B., Adams III, W. W., Logan, B. A. and Verhoeven, A. S. 1995. Xanthophyll cycle-dependent energy dissipation and flexible photosystem II efficiency in plants acclimated to light stress. Aust. J. Plant Physiol. 22:249-260.

Drake, S. R., Larson, F. E. and Higgins, S. S. 1991. Quality and storage of 'Granny Smith' and 'Greenspur' apples on seedling, M26, and MM 111 rootstocks. J. Amer. Soc. Hort. Sci. 116: 261-264.

Dussi, M. C., Sugar, D., Azarenko, A. N. and Righetti, T.L. 1997. Effects of cooling by over-tree sprinkler irrigation on fruit colour and firmness in 'Sensation Red Bartlett' pear. HortTechnology 7: 55 – 57.

Evans, R. G. 1993a. Designing and operating overtree evaporative cooling systems for apples. Part 1. Good Fruit Grower, June, 23 – 27.

Evans, R. G. 1993b. Assessing the mechanics of evaporative orchard cooling. Part 2. Good Fruit Grower, June, 29 – 32.

Farager, J. D. 1983. Temperature regulation of anthocyanin accumulation in apple skin. J. Exp. Bot. 34: 1291 – 1298.

Forschler, A. F., Schmitz-Eiberger, M. A. and Noga, G. J. 2003. Reduction of UV-B injury on Phaseolus vulgaris leaves and Malus domestica fruits by application of protecting agents. J. App. Bot. 77: 75-81.

Gilmore, A. M. 1997. Mechanistic aspects of xanthophyll cycle-dependant photoprotection in higher plant chloroplasts and leaves. Physiol. Plant. 99: 197-209.

Gindaba, J., and Wand, S. J. E. 2005. Comparative effects of evaporative cooling, kaolin particle film and shade net on sunburn and fruit quality in apples. HortScience 40 (3): 592-596.

Glenn, D. M., Prado, E., Erez, A., McFerson, J. and Puterka, G. J. 2002. A reflective processed-kaolin particle film affects fruit temperature, radiation reflection and solar injury in apple. J. Amer. Soc. Hort. Sci. 127: 188-193.

Huysamer, M. 1998. Report of the blushed pear workgroup: Perceptions, facts and questions. Proc. Cape Pomological Association Tech. Symp., Cape Town, South Africa, 2-3 June 1998, 187-192.

Iglesias, I., Graell, J., Echeverria, G. and Vendrell, M. 2000. Orchard cooling with overtree sprinkler irrigation to improve fruit colour of 'Delicious' apples. HortScience 35: 1207 – 1208.

Iglesias, I., Salvia, J., Torguet, L. and Cabus, C. 2002. Orchard cooling with overtree microsprinkler irrigation to improve fruit colour and quality of 'Topred Delicious' apples. Scientia Hort. 93: 39 – 51.

Jones, A. L., and Aldwinckle, H. S., eds. 1990. Compendium of Apple and Pear Diseases. American Phytopathological Society, St. Paul.

Jones, H. G. 1981. Carbon dioxide exchange of developing apple (Malus domestica Mill.) fruits. J. Exp. Bot. 32: 1203-1210.

Jonkers, H. 1984. Effect of temperature on formation of flower buds in two apple cultivars. Acta Hort. 149: 49 – 51.

Kerr, J. B. and McElroy, C. T. 1993. Evidence for large upward trends of UV-B radiation linked to ozone depletion. Science 262: 1032 – 1034.

Key, J. L., Kimpel, J., Vierling, E., Lin, C., Nagao, R. T., Czarnecka, E. and Schoffl, F. 1985. Physiological and molecular analysis of the heat shock response in plants. pp. 237-348 in B. G. Atkinson and D. B. Walden (eds.), Changes in eucariotic gene expression in response to environmental stress. Academic press, New York.

Kimple, J. A. and Key, J. L. 1985. Heat shock in plants. Trends in Biochem. Sci. 117: 353-357.

Kotzé, W. A. G., Carreira, J. A., Beuker, O. and Redelinghuys, A. U. 1988. Effect of evaporative cooling on the growth, yield and fruit quality of apples. Deciduous Fruit Grower 38: 20 – 24.

Krause, G. H. and Santarius, K. A. 1975. Relative thermostability of the chloroplast envelope. Planta (Berl) 127: 285-299.

Krause, G. H. and Weiss, E. 1991. Chlorophyll fluorescence and photosynthesis: the basics. Ann. Rev. of Plant Mol. Biol.: 42: 313 – 249.

Lambers, H., Chapin III, F. S. and Pons, T. L. 1998. Plant physiological ecology. Springer-Verlag, New York.

Lakso, A. N. 1994. Apple. In: Scaffer, B. S. and Andersen, P. C. **(eds.)** Handbook of Environmental Physiology of Fruit Crops, Vol. 1. CRC Press, Boca Raton, pp. 3 – 42.

Lancaster, J. E., Grant, J. E. Lister, C. E. and Taylor, M. C. 1994. Skin colour in apples – influence of copigmentation and plastid pigments on shade and darkness of red colour in five genotypes. J. Amer. Soc. Hort. Sci. 119:63-69.

Larcher, W. 1975. Physiological Plant Ecology. Third edition. Springer-Verlag, Berlin Heidelberg.

Lee, S. K., and Kader, A. A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. 2000. Posth. Biol. and Techn. 20: 207 – 220.

Lipton, W. J. 1977. Ultraviolet radiation as a factor in sunburn and vein tract browning of cantaloupes. J. Amer. Soc. Hort. Sci. 102: 32-36.

Long, S. P. and Humphries, S. 1994. Photoinhibition of photosynthesis in nature. Ann. Rev. Plant Physiol. Plant Mol. Biol. 45: 633-662.

Mackerness, S. A. H. and Thomas, B. 1999. Effects of UV-B radiation on plants: gene expression and signal transduction pathways, p.17-24. In: Smallwood, M. F., Calvert, C. M., and Bowles, D. J. (eds.) Plant responses to environmental stresses. Bios Scientific Publishing, Oxford.

Maxwell, K. and Johnson, G. 2002. Chlorophyll fluorescence – a practical guide. J. Exp. Bot. 51: 659 – 668.

Merzlyak, M. N. and Chivkunova, O. B. 2000. Light-induced pigment changes and evidence for anthocyanin photoprotection in apples. J. Photochem. Photobiol. (B) 55: p.155 - 163.

Merzlyak, M. N. and Solovschenko, A. E. 2002. Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. Plant Biochem. and Physiol. 40: 679 – 684.

Müller, P., Li, X. P. and Niyogi, K. K. 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125: 1558 – 1566.

Olcott-Reid, B., Sutton, T. B. and Unrath, C. R. 1981. Evaporative cooling irrigation influences disease, insect, and mite pests of 'Delicious' apples. Amer. Soc. Hort. Sci. 106: 469 - 474.

Palett, K.E. and Young, A.J., 1993. Carotenoids. In: Alscher, R.G. and Hess, J.L. (Eds.), Antioxidants in Higher Plants. CRC Press, Boca Raton, pp. 60 - 89.

Palmer, J. W., Bunemann, G., Sansavini, S., Wagenmakers, P. S. and Winter, F. 1989. The international planting systems trial. Acta Hort. 243: 231 – 241.

Palmer, J. W., Privé, J. P. and Tustin, D. S. 2003. Temperature p217 – 236 in: Apples, botany, production and uses. Ferree D. C. and Warrington, I. J. (eds.) CABI publishing, Wallingford, UK.

Parchomchuk, P. and Meheriuk, M. 1996. Orchard cooling with pulsed overtree irrigation to prevent solar injury and improve fruit quality of 'Jonagold' apples. HortScience 31: 802 – 804.

Quick, W. P. and Horton, P. 1984. Studies on the induction of chlorophyll fluorescence in barley protoplasts. I. Factors affecting the observation of oscillations in the yield of chlorophyll fluorescence and the rate of oxygen evolution. Proc. of the Royal Soc. of London. B 220: 361 – 370.

Rabinowitch, H. D., Ben-David, B. and Friedmann, M. 1986. Light is essential for sunscald induction in cucumber and pepper fruits, whereas heat conditioning provides protection. Scientia Hort. 29:21 – 29.

Rennenberg, H. 1982. Glutathione metabolism and possible biological roles in the higher plant. Phytochemistry 21: 2771 – 2781.

Renquist, A. R., Hughes, H. G. and Rogoyski, M. K. 1989. Combined high temperature and ultraviolet radiation injury of red raspberry fruit. HortScience 24: 597 – 599.

Ritenour, M. A., Kochhar, S., Schrader, L. E., Hsu, T. and Ku, M. 2001. Characterization of heat shock protein expression in apple fruit peel under field and laboratory conditions. Jnl. Amer. Soc. Hort. Sci. 126(5):564-570.

Salin, M. L. 1988. Toxic oxygen species and protective systems of the chloroplast. Physiol. Plant. 72: 681 – 689.

Salisbury, F. B. and Ross, C. W. 1992. Stress physiology, p575 – 600 in Plant Physiology. Wadsworth Publishing Company, Belmont, California.

Santarius, K. A. and Weiss, E. 1988. Heat stress and membranes, p 707 – 706 in J. L. Harwood and T. J. Walton (eds.), Plant Membranes – structure, assembly and function. The Biochemical Society, London.

Schrader, L. E., Zhang, J. and Duplaga, W.K. 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Online. Plant Health Progress. http://www.planthealthprogress.org/Current/research/sunburn/article.htm

Searles, P. S., Kropp, B. R., Flint, S. D., and Caldwell, M. M. 2001. The influence of solar ultraviolet-B on some microbial communities of Tierra del Fuego, Arg. New Phytol. 152: 213-221.
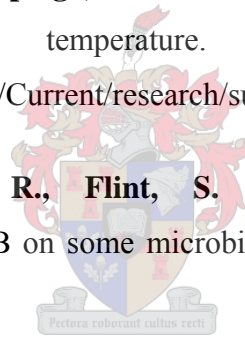
Sies, H. and Stahl, W. 1997. Carotenoids and intercellular communication via gap junctions. Int. J. Vitam. Nutr. Res 67: 364–367.

Solovchenko, A. E. and Schmitz – Eiberger, M. 2003. Significance of skin flavonoids for UV-B protection in apple fruits. J. Exp. Bot. 54: 1977 – 1984.

Steyn, W. J. 2003. Red colour development and loss in pear fruit. PhD Thesis. University of Stellenbosch, South Africa.

Steyn, W. J., Wand, S. J. E., Holcroft, D. M. and Jacobs, G. 2001a. Climate and red colour development in blushed apples & pears. Part 1: Apples. Deciduous Fruit Grower, September, 43 – 45.

Steyn, W. J., Wand, S. J. E., Holcroft, D.M. and Jacobs, G. 2001b. Climate and red colour development in blushed apples & pears. Part 2: Pears. Deciduous Fruit Grower, October, 20 – 21.

Tromp, J. 1976. Flower-bud formation and shoot growth in apple as affected by temperature. Sci. Hort. 5: 331-338.

Unrath, C. R. 1972. The quality of 'Red Delicious' apples as affected by overtree sprinkler irrigation. J. Amer. Soc. Hort. Sci. 97: 58 – 61.

Unrath, C. R. and Sneed, R. E. 1974. Evaporative cooling of 'Delicious' apples – the economic feasibility of reducing environmental heat stress. J. Amer. Soc. Hort. Sci. 99: 372 – 375.

Van den Ende, B. 1999. Sunburn management. The Compact Fruit Tree 32 (1), 13 – 14.

Van Kooten, 0. and Snel, J. F. H. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. Phot. Res. 25: 147-150

Vinson, J. A., Hao, Y. and Zubic, S. K. 2001. Food antioxidant quantity and quality in foods: vegetables. Jnl. of Agric. Food Chemistry 46: 3630–3634.

Wand, S. J. E., Steyn, W. J., Holcroft, D. M., Mdluli, M. J., van den Dool, K., Jacobs, G. 2005. Use of evaporative cooling to improve 'Rosemarie' and 'Forelle' pear fruit blush colour and quality. Acta Hort. 671: 103-111.

Wand, S. J. E., Steyn, W. J., Mdluli, M. J., Marais, S. J. S. and Jacobs, G. 2002. Overtree evaporative cooling for fruit quality enhancement. SA Fruit Journal, Aug/Sept., 18 – 21.

Weiss, E. and Berry, J. A. 1988. Plants and high temperature stress, p 329 – 346 in S. P. Long and F. I. Woodward (eds.), Plants and Temperature. Symposia of the Society for Experimental Biology, No. 42. The Company of Biologists Limited, Cambridge.

Westwood, M. N. 1978. Fruit and nut species. In: Temperate-Zone Pomology, M.N. Westwood (ed) Chapt. 3, W.H. Freeman and Co., San Francisco CA, pp. 41-76.

Williams, K. M. 1993. Use of evaporative cooling for enhancing apple fruit quality. Part three. Good Fruit Grower, August, 23 – 27.

Wünsche, J. N., Greer, D. H., Palmer, J. W., Lang, A. and McGhie, T. 2001. Sunburn: the cost of high light environment. Acta Hort. 557:349-356.
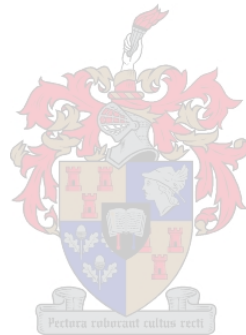
Paper 1

Effect of evaporative cooling on microclimate, tree growth and fruit quality of apples and pears.

Abstract

Evaporative cooling (EC) is an orchard practice used to decrease fruit temperature during the hottest part of the day. Fruit sunburn occurs easily when temperatures and light intensity are high, and this decreases packouts. EC has also been used successfully to improve colour of blushed apple and pear cultivars. The response of different apple and pear cultivars to EC was studied during 2003/2004 at Welgevallen Experimental Farm, Stellenbosch. EC was activated during the day at temperatures above $30^{o}C$ to reduce sunburn and between 18h00 and 21h00 at temperatures above $22^{o}C$ to improve colour development. Three treatments were evaluated: Control without EC, early application starting from the second week in December 2003, and late application starting 2 - 4 weeks before harvest, 2004. Experiments were done on two apple cultivars ('Royal Gala' and 'Cripps' Pink') and two pear cultivars ('Forelle' and 'Rosemarie'). Generally, fruit under EC had more sunburn than control fruit, due to failure of the system on a hot day. Underlying physiological responses of trees and fruit to EC were also investigated, particularly the phenomenon of acclimation and the potential for colour development and heat stress. In both apple cultivars a significant increase in fruit skin anthocyanin concentration and a decrease in phenolic content was found as the season progressed. In both pear cultivars there was a significant decrease in both anthocyanin and phenolic content. No significant differences were found in anthocyanin content between treatments in either the apple or pear cultivars. In both apple cultivars a higher phenolic content was found in the peel of the EC treatments. A decrease of up to four percent in leaf and fruit surface temperature was found under EC. No significant difference in trunk circumference was found in any of the cultivars. The late EC treatment in 'Cripps' Pink' had a significantly faster rate of budbreak than the control and early EC treatments. Significantly higher stomatal conductance and transpiration was observed in leaves under EC. Knowledge about the various effects evaporative cooling and the subsequent lowering of ambient temperatures has on fruit trees and fruit could contribute greatly to producers' ability to grow high quality fruit.

Introduction

The use of evaporative cooling (EC) to reduce sunburn and enhance colour development of red or blushed fruit is becoming more popular among growers in arid regions all over the world. As apple production increases in areas where unfavourable environmental conditions are common and growers move to higher density plantings, the need to alleviate heat and soil moisture stress becomes more critical for commercial production of maximum yields of high quality fruit (Unrath and Sneed, 1974). The emergence of new varieties and new training systems that allow more light into the trees also play a role. Factors such as cultivar, climate fluctuations and orchard management practices also have an influence on the damage.

Sunburn on apples in South African production areas can amount to 20 – 50% fruit cull in the orchard and up to 10 % rejection of packed cartons thereafter (Bergh *et al.*, 1980). Gindaba and Wand (2005) estimated the incidence of sunburn in 'Cripps' Pink' and 'Royal Gala' apples to 15-20% during the 2003/2004 summer.

Two types of sunburn have been identified, 1) sunburn necrosis, and 2) sunburn browning (Schrader *et al.*, 2001). Sunburn browning occurs at a fruit surface temperature of 46-49°C, is sub-lethal and results in a yellow, bronze, or brown spot on the sun-exposed side of the fruit. It occurs only in the presence of light, and has little effect on membrane integrity. Sunburn necrosis is caused by thermal death of epidermal and sub epidermal cells (peel), and causes a necrotic spot on the side of the fruit that was exposed to the sun. This can also happen in the absence of direct light, when it is caused by heat. Thermal death occurs at surface temperature 52 ± 1°C. Tests by Schrader *et al*. (2001) indicated that fruit skin temperature is critical to the development of both types of sunburn.

The incidence of sunburn on exposed fruit can be greatly reduced by avoiding excessive fruit temperatures during the hottest part of the day (Savage *et al*., 1997). On a hot day, surface temperature of an apple may exceed 50°C, even though the air is 10 to 15°C cooler (Schrader *et al.*, 2001).

An evaporative cooling system applies water above the crop. An overhead sprinkler system is used to wet fruit and leaf surfaces when the temperature rises above a certain threshold point. Water is pulsed on and off so that free water is continually evaporating. As the water

evaporates from the fruit surfaces, energy is extracted from the skin. If this energy is greater than the total incoming heat energy, the fruit surface temperature decreases (Unrath, 1972; Parchomchuk and Meheriuk, 1996). The air around the trees is also cooled, and the relative humidity increases, thus reducing water loss through transpiration. Often during a day with low humidity, high temperatures and intense sunlight, water is lost to transpiration faster than the root system can replace it from the soil. During fruit development, these conditions can cause water to be withdrawn from the fruit to supply the plant, resulting in smaller fruit, delayed maturity and reduced fruit quality (Evans, 1993). By raising the relative humidity in the orchard, EC reduces overall heat stress of the plants (Evans, 1999). On an average warm summer's day, the use of EC reduces fruit surface temperatures by about 2 to 3°C. This can increase to over 10°C on hot days in fruit directly exposed to sunlight (Wand *et al.*, 2002). For effective sunburn control, it is advisable to activate the EC system at air temperatures of about $30\text{-}32^{\circ}$C earlier in the season and reduce it to 28°C for the last few weeks before harvest (Wand *et al.*, 2000).

Producers have also found that the use of EC just prior to sunset and sometimes around sunrise has improved colour development on red apples (especially early varieties) before harvest (Evans, 1993). The external appearance is one of the most important factors that affect the value of the fruits. Consumers have developed distinct correlations between colour and the overall quality of the specific product. They are attracted by the colour and its distribution on the surface. As red-skinned apples are preferred in most markets, the skin colour of these and blushed cultivars are especially important. Skin colour is also an important factor in establishing government grades and standards, because a particular grade must have a certain proportion of the apple skin coloured. Downgrading due to insufficient red colour has also limited the profitability of blushed pear cultivars in the Western Cape region of South Africa (Huysamer, 1998).

Research has also shown EC to have a marked effect on fruit colour (Unrath and Sneed, 1974; Dussi *et al.*, 1997; Coetsee, 2000; Iglesias *et al.*; 2002, Wand *et al.*, 2004). The main anthocyanin pigment responsible for red colour in apples is cyanidin-3-galactoside (idaein). Development of red colour is regulated directly by light (Arakawa, 1988; Saure, 1990; Lancaster, 1992) and temperature. Farager (1983), Arakawa (1991), and Iglesias *et al.* (2002) reported that anthocyanin levels in apple skin were inversely related to field temperature. Development of idaein generally occurs in the temperature range from 5ºC to about 30ºC, with an optimum at about 21ºC (Evans, 1999; Steyn, 2003). The amount of coloring will be in

direct proportion to the amount of time that fruit is in this range (Evans, 1999). The activity of the enzyme phenylalanine ammonia-lyase (PAL), which catalyses the reactions forming anthocyanins, is increasingly inhibited by high temperatures (Tan, 1980; Faragher, 1983). Thus apples have better colour when the days are clear, bright and moderate and the nights are cool. Night temperatures below 18°C and moderate day temperature (20-25°C) generally enhance red colour formation, but excessive day temperatures can negate the positive effect of cold night temperatures (Tan, 1979; Williams, 1993). It is generally accepted that water should be applied in the evening for better colour. Growers are applying water over the fruit and canopy starting 4 to 6 weeks before harvest (Evans, 1999). It is believed that, depending on the rate of application and uniformity, optimum benefits will occur by starting EC about 30 minutes before and continuing about 20 to 40 minutes after sundown. Some growers also apply water again at sunrise for about an hour to extend the lower fruit temperature periods (Evans, 1999).

EC may also increase harvested fruit size due to reduced water stress levels and improved management of soil water status throughout the season (Evans, 1999). Unrath and Sneed (1974) found that fruit temperature reduction improved fruit quality and increased fruit size and soluble solids. Most increases in fruit size will be primarily due to improved water management. Many effects of soil type, depth, and nutrient status variability on sizing may be reduced by improved water management under high frequency water applications (Evans, 1999). Photosynthesis of plant organs has an optimum range (16°C – 27°C), and will begin to decrease above and below this range. If EC can be utilized to reduce plant water stress due to high temperatures, and maintain plant organs closer to their optimum photosynthesis range, theoretically fruit size should be increased. On the other hand fruit sizes may be reduced if growers do not actively and adequately manage soil water status. Crop irrigation requirements cannot be met by EC, and soils may become either too dry if normal irrigation is ceased, or waterlogged (Evans, 1999). Special care should be taken that the system does not break down and that pumps and electricity supply are backed up in case this happens. Fruit acclimatize to lower temperatures, and extensive sunburn damage can occur because of a system breakdown on a hot day.

The purpose of this study was to evaluate the effect of evaporative cooling applied early as well as later in the season on the fruit quality of various apple and pear cultivars at harvest under Western Cape growing conditions during 2003/2004, as well as to investigate some underlying physiological responses in relation to reduced heat stress. The effects of early EC

(started earlier in the season with the main aim of reducing sunburn) were compared to those of late EC (started two to four weeks before harvest, with the main aim of colour improvement).

Materials and Methods

Plant material and EC system

The experiments took place at Welgevallen Experimental Farm, Stellenbosch, South Africa (33° 55' S, 18° 53'E). Experiments were conducted on four different cultivars: 'Cripps' Pink' apples (on M793 rootstock, 4 m x 1.5 m spacing, planted 1998), 'Royal Gala' apples (M793 rootstock, 4 m x 1.5 m spacing, planted 1998), 'Rosemarie' pears (on BP1 rootstock, 4.5 m x 2 m spacing, planted 1991) and 'Forelle' pears (on Quince A rootstock, 4 m x 1.25 m spacing, planted 1998). Row orientation for all the orchards was north-east by south-west.

The EC system was installed in 2001/2002. Micro sprinklers (DAN 2001 jets) with a 28 L.h^{-1} discharge were installed on 4.5 m poles. The poles were spaced 8 m apart with jets every 2.5 m along a suspended pipe to discharge water over the trees at a height of 3.5 m. The radius of each jet was 1.5 m, and they gave a precipitation rate of 4.5 mm.h^{-1}. Pulsing cycles of 5 minutes on, 15 minutes off were used, and the system was activated at air temperatures of 30ºC and above during the day (between 06h00 and 18h00), and 22°C during the early evening (between 18h00 and 21h00). Air temperature was measured by means of a shielded temperature sensor connected to the irrigation computer. Normal undertree irrigation was given to all the orchards. The trees were irrigated with micro-jet sprinklers scheduled using neutron moisture probe measurements in non-EC parts of the orchards.

Treatments and experimental design

Treatments evaluated were (Table 1): 1) control without EC, 2) early EC application starting on 8 December 2003, and 3) late EC application starting about four weeks before harvest, except 'Forelle' (two weeks before harvest). A complete randomised block design with six to ten replicates was used. Nine blocks were used for 'Cripps' Pink', six for 'Royal Gala', eight for 'Rosemarie', and ten for 'Forelle'. For the pears, a block consisted of three rows of which only the middle row was used. For the apples, a block consisted of two rows of which only the north-western row was used, due to the prevailing south-easterly wind. Three trees were used per replicate, except for 'Rosemarie' where 5 trees were used. Treated trees within rows

were separated by at least two trees that acted as buffers to avoid water from the overhead sprinklers reaching adjoining treatments.

Fruit surface temperature

Fruit surface temperatures of 'Cripps' Pink' and 'Royal Gala' apples (6 apples per treatment per block) were measured on 25 January 2004 with an infra-red thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, California) to determine the difference in fruit temperature between treatments and the control. Only sun-exposed apples were used, and measurements were taken during the hottest part of the day, between 13:30 and 14:00. Measurements were taken block by block to eliminate time effects. Fruit surface temperature of 3 individual 'Cripps' Pink' apples was also measured on 26 January 2004 at one minute intervals over a 40 minute period to determine the rise and fall of the temperature when the sprinklers were on or off. Measurements were done from 13:00 to 13:40. This experiment was only done on 'Cripps' Pink', because similar results were expected in the other cultivars.

Ambient air temperature and relative humidity were measured under EC and under control blocks using a data logger (CR10X, Campbell Scientific, Logan, UT, USA). The hue of 9 fruit per block was measured with a chromameter (Nippon Denshoku, Tokyo, Japan, model: NR 3000) three times a week for each cultivar to determine the seasonal changes in hue of the fruit. Measurements started on 1 December and continued until harvest. There were two cold fronts and two heat waves during the time that measurements were taken. Hue was plotted against maximum and minimum temperatures.

Gas exchange

Light-saturated net CO_2 assimilation rate (A_{max}), stomatal conductance (g_s) and transpiration rate (E) of 'Cripps' Pink' apple leaves were measured under ambient temperature conditions using a LI-6400 infrared gas analyser, (Li-Cor, Lincoln, Nebraska, USA). Cuvette CO_2 concentration was controlled using the LI-6400 CO_2 injection system and compressed CO_2-cylinders (at 380 μmol mol^{-1}). Photosynthetic photon flux density (PPFD) was set at 1500 μmol m^{-2}s^{-1}. After measurement of A_{max}, the cuvette CO_2 concentration was increased to 1000 μmol mol^{-1} for the measurement of light- and CO_2- saturated rate of net CO_2 assimilation (A_{sat}). For the measaurement of R_D (dark respiration rate), CO_2 concentration was brought back down to 380 μmol mol^{-1} and the lamp was turned off. The measurements were made between 10:00 and 16:00 on 17 March and 20 April on two sun-exposed leaves per tree per block. Ambient leaf temperature was 26-33ºC on both days. Measurements were done on

control and early EC treatments. There were no significant differences in all parameters between the two dates, so the data was pooled and analysed as one data set.

Trunk growth

Trunk circumference of all cultivars was measured twice, once at the beginning of the season (24 November 2003), and again at the end (18 September 2004). Trunk growth was calculated as % growth.

Fruit quality at harvest

Thirty six fruit were harvested per replication, except for 'Forelle', where 60 fruit were harvested per replication. All fruit were harvested on the north-western side of the trees. Fruit mass was recorded, and fruit diameter was measured with a digital calliper (CD-6"c, Mitutayo, Absolute digimatic). Ground colour of all cultivars was measured by means of the colour chart for apples and pears, Unifruco Research Services Ltd., where 0 is green and 5 is yellow. Blush colour of 'Rosemarie' was assessed with the P.23 colour chart from Unifruco, where 1 has the biggest percentage blush and 12 the least. Blush colour of 'Forelle' was measured with the P.25 chart from Unifruco where A1 has the biggest percentage blush and B6 the least; of 'Royal Gala' with the A.42 chart of the DFB where 1 is red and 12 is yellow, and of 'Cripps' Pink' with the 'Pink Lady' chart from Topfruit Ltd. where 1 is green and 12 is red. The colour chart rates fruit from 1 – 12 with one the best and 12 the worst coloured fruit. The hue of the blushed side of the fruit was measured with a chromameter (Nippon Denshoku, Tokyo, Japan, model: NR 3000) at the reddest position on the fruit. Flesh firmness was recorded with a penetrometer (Southtrack fruit pressure tester, model FT 327, Alphonsine, Italy). An 11 mm tip was used for apples and an 8 mm tip for pears. Readings were taken on opposite sides of the fruit from which the skin had been pared. Total soluble solids (TSS) in a composite juice sample was measured by cutting a slice out of both sides of each fruit, blending the pieces in a liquidiser and measuring TSS of the juice with a refractometer (TSS 0-32%, Model N1, Atago, Tokyo, Japan). Total titratable acidity (TA) of the apples was measured on the same juiced sample using a Metrohm Titrino automatic titrator (model: 719 S, Metrohm AG, Hersiau, Switzerland). Starch conversion of apples was assessed using the Unifruco Research Services starch conversion chart for circular type pome fuit. Sunburn was assessed visually according to the absence or presence of sunburn where sunburn includes sunburn browning and sunburn necrosis. Sunburn was expressed as a percentage of all the fruit in a replication. Insect damage, decay and occurrence of *Fusarium*

verticillioides damage were recorded visually as present or absent, and expressed as a percentage.

Fruit skin anthocyanin and phenolic concentration

Fruit skin was analysed for anthocyanin and total phenolic concentrations. 90 Fruit (30 per treatment) from each cultivar were picked on different dates: 'Rosemarie' on 9 December 2003 and 22 January 2004, 'Forelle' on 9 December 2003 and 10 March 2004, 'Royal Gala' on 9 December 2003, 28 January and 2 March 2004, and 'Cripps' Pink' on 9 December 2003, 3 February and 12 April 2004. Fruit were peeled with a potato peeler, and the skin was placed first in liquid nitrogen and then in a -80ºC freezer where it was kept until pigment analysis. The frozen peel was crushed with a mortar and pestle, and 1.5 g fruit peel was mixed with 10 ml 99:1 (v/v) MeOH/HCl. The samples were placed on a mixer inside a refrigerator at 4ºC in the dark for 12 hours. The solution was then centrifuged for 10 minutes at 10000 x g, decanted, and 5 ml MeOH/HCl was added and mixed. This was centrifuged again for 10 minutes, decanted, filtered through 0.45 μm filters (Millex-HV; Millipore Corporation, Milford, MA). Absorbance of the supernatant was measured at 530 nm on a 'Beckman DU – 64' spectrophotometer for anthocyanin concentration, and at 280 nm for total phenolic concentration. The phenolic solutions were diluted 20 times before readings were taken. For anthocyanin, the readings were multiplied by 0.25 according to the method of Mancinelli (Mancinelli *et al*., 1975). A standard curve was obtained with the anthocyanin idaein chloride (cy 3-gal) (Carl Roth GmbH and Company, Germany).

Spring Budbreak

Budbreak of all cultivars was measured during early spring 2004, by counting the number of buds at greentip stage (first visible signs of green expanding leaves) three times a week. Three trees per block and three branches per tree were tagged. Counting for 'Forelle' started on 13 September and ended on 4 October 2004, for 'Cripps' Pink' counting was done between 1 October and 22 October, for 'Rosemarie' between 20 September and 11 October, and for 'Royal Gala' between 27 September and 18 October. The rate of budbreak was determined from the inverse of the time for 50 percent budburst to occur.

Statistical Analysis.

Analysis of variance (ANOVA) with blocks was performed using the General Linear Model procedure of SAS (Enterprise Guide version 1.3, Statistical Analysis Systems Institute, 1996,

Cary, NC). Means were separated using the LSD multiple comparison test, with significance set at $P \leq 0.05$. For the pigment analysis, a two-way ANOVA was performed, with treatment and date as factors.

Results

Fruit surface temperature

In both 'Cripps' Pink' and 'Royal Gala' apples, fruit skin temperature of control fruit on 25 January 2004 was higher than that of fruit under EC although not statistically significant in 'Royal Gala' (Table 2). Fruit skin temperature of 'Cripps' Pink' apples under EC was reduced on average by $4.7^{\circ}C$ and of 'Royal Gala' by $2^{\circ}C$. Fruit skin temperature of 'Cripps' Pink' varied by $6 - 6.5^{\circ}C$ between EC cycles (Fig.1). Seasonal variations in hue (Fig.2, 3) is not clear. Colour variations follow the normal pattern of colour formation for apples. Two weeks before harvest the signal becomes genetic and the temperature at which anthocyanin accumulates increases.

Gas exchange

We measured gas exchange two weeks before harvest and two weeks after harvest in 'Cripps' Pink'. Several studies have shown that net photosynthesis is downregulated after harvest mainly due to reduced sink strength (Pretorius, 2006; Fujii and Kennedy, 1985). However, since there were no significant differences in gas exchange parameters between the two dates in 'Cripps' Pink', the data was pooled (Table 3). There were no significant differences in light- saturated rate of net CO_2 assimilation (A_{max}), dark respiration rate (R_D) or light and CO_2-saturated rate of net CO_2 assimilation (A_{sat}) between the control and EC treatments. Stomatal conductance (g_s) was almost significant ($P=0.0517$), with the early EC treatment showing higher g_s than the control. Transpiration rate (E) was significantly higher under the early EC treatment than it was for the control.

Trunk growth

No significant differences were found in percentage growth of trunk circumference over one season between control and EC trees in any cultivar (Table 4).

Fruit quality at harvest

In 'Royal Gala' (Table 5), early EC had significantly lower percentage sunburn than control or late EC fruit. The hue angle of control fruit was lower (but not significantly at $P = 0.0750$)

than that of late EC fruit. Early EC reduced fruit firmness compared to control and late EC treatments. Decay was more prevalent on early EC fruit compared to control and late EC. In 'Cripps' Pink' (table 6), control fruit had a significantly lower percentage sunburn than early and late EC.

In 'Rosemarie' (table 7), sunburn was reduced from 63.9% on late EC fruit and 51.2 % on early EC fruit to 31.4% on control fruit. A significantly higher percentage of *Fusarium verticillioides* damage was found on late EC fruit than on control fruit. In 'Forelle' (Table 8), sunburn on control fruit was higher (but not significantly at $P = 0.2941$) than that of the EC treatments. The hue, as well as the ground colour, of early EC fruit was significantly lower than that of control or late EC fruit. Control fruit had a significantly lower percentage of insect damage than early EC fruit.

Fruit skin anthocyanin and phenolic concentration

Anthocyanin concentration of fruit skin increased significantly in 'Royal Gala' and 'Cripps' Pink' as the season progressed (Table 9), while phenolic content showed a significant decrease. In both cultivars there was a significant difference in phenolic concentration between treatments. In 'Cripps' Pink', the early EC treatment had a higher phenolic content than control and late EC treatments. In 'Royal Gala', both EC treatments had a higher phenolic concentration than the control treatment. In 'Cripps' Pink' there was also a significant difference in the two-way interaction between treatment and date.

In 'Rosemarie' and 'Forelle', there was a significant decrease in anthocyanin concentration between the start and the end of the growing season (Table 10), as well as a decrease in phenolic concentration. There were no significant differences in anthocyanin and phenolic concentrations between treatments.

Spring budbreak

Only 'Cripps' Pink' showed significant difference in rate of budbreak between treatments (Table 11). The late EC treatment had a significantly faster rate of budbreak than the control and early EC treatments.

Discussion

As suspected, temperatures of fruit under EC treatments were considerably lower than control fruit. The reductions in fruit skin temperatures under EC correlates with the findings of Evans (1999) and Wand *et al.* (2002).

The increased g_s observed in EC leaves correlates with the findings of Gindaba and Wand (2005). They observed a significant increase in g_s of EC leaves in two of five measuring dates. We observed no significant difference in net CO_2 assimilation, however, which differs from the findings of Gindaba and Wand (2005). They found a significant increase in net CO_2 assimilation on three of five measurement dates. The increase in A and g_s could be due to reduced leaf temperature and improved plant water relations (Gindaba and Wand, 2005). Wand *et al*. (2002) suggested that in response to additional soil moisture and milder atmosphere (lower temperature and higher humidity) under EC, leaf stomata is kept open longer for optimum photosynthesis. This could also explain our results of higher E under EC.

Heat stress reduction and improved tree water relations can stimulate shoot and trunk growth (Kotzé *et al.,* 1988). This can be seen as a positive effect where trees are growing sub-optimally. We had no significant results as far as tree growth was concerned. This is probably a direct result of there being no significant differences in photosynthesis, so no extra carbohydrates were formed that could be allocated to growth.

As expected, the percentage of sunburnt fruit in 'Royal Gala' and 'Forelle' was higher on control fruit than on EC fruit. The fruit surface temperature of EC fruit was lower than that of the control fruit, resulting in a lower percentage of sunburn. These results differ from those of the 2002/2003 season, when no reduction in sunburn was found on 'Royal Gala', and higher percentage sunburn was found on EC 'Forelle'. The lower firmness of 'Royal Gala' fruit under early EC supports the results of the 2001/2002 and 2002/2003 seasons, when all cultivars under early EC showed earlier maturation (Wand *et al*. (2002). Because EC in our experiments was applied in addition to normal undertree irrigation, the soil under the trees was wetter and more water was taken up by the fruit. EC reduces the actual water use of the tree on the order of 15%-20% depending on climatic conditions (Evans, 1999). Unless earlier harvest is desirable, undertree irrigation should ideally be reduced where EC is applied, in line with total water requirements. This should alleviate the effects on fruit maturity (firmness and TSS). Our results differ from those of Iglesias *et al.* (2002). They found that fruit firmness, fruit size and soluble solids concentration were significantly higher in cooled fruit than

control fruit. It might be that they reduced undertree irrigation, or that rainfall in Stellenbosch was higher, resulting in more water in the orchard. Unrath (1972) also found lower fruit firmness in apples under EC. He attributed it to their greater size. Parchomchuk and Meheriuk (1996) found no differences in fruit size, firmness or colour between cooled and control fruit of 'Jonagold' apples. They did, however, find reduced soluble solids in fruit under EC.

The reason for the substantially higher percentage sunburn on early and late EC 'Cripps' Pink' and 'Rosemarie' fruit is not clear, but it might be because of the substantial nature of the sampling. Late EC fruit might also have already been damaged before the system came on four weeks before harvest.

Evans (1999) recommends that EC systems should be cycled based on fruit core or fruit skin temperatures, instead of on air temperatures. Available information shows that starting EC based on air temperatures is a very poor procedure (Evans, 1999). Research has shown that fruit can warm much more quickly (eg. 10°C to 15°C warmer) and cool off more slowly than ambient air temperatures. In our trails EC cycles were based on air temperatures, which might also explain why the results were poorer than expected.

The hue of 'Forelle' under early EC was lower, indicating that fruit were redder and less green. Similar results were obtained in 'Sensation Red Bartlett' pears under EC (Dussi *et al*, 1997). Iglesias *et al.* (2000 and 2002), found that increased red colour and higher anthocyanin content resulted from sprinkler irrigation, especially when applied at sunset. These results show the possibility that EC could enhance the red blush on blushed cultivars, which would make them more acceptable for the consumer market. Unfortunately we obtained this desired result only in 'Forelle'. It might be that low temperatures prior to harvest masked the effects of EC on fruit colour in our experiments. EC has a better effect on fruit colour in warm regions. It might be that average temperatures in Spain were higher during the experiments than average temperatures of Stellenbosch.

In pears, red colour (anthocyanin pigments) peaks early during fruit development and thereafter gradually declines (Steyn *et al.* 2004). This is reflected in our results for fruit skin anthocyanin content of 'Rosemarie' and 'Forelle'. Although good exposure to light is a requirement for all cultivars, the degree of synthesis and breakdown of the pigment is tightly linked to climatic conditions in some cultivars, notably 'Rosemarie'. Cool nights (<15°C) and mild days (20-25°C) are ideal for good colour development, but temperatures above 30°C are

detrimental. This could help to explain the poor colour of 'Rosemarie' in the Western Cape, as temperatures in this region is often above this threshold.

The increase that we found in fruit skin anthocyanin content of 'Royal Gala' and 'Cripps' Pink' over the season is consistent with literature, since apples exhibit a pre-harvest burst of anthocyanin synthesis (Solovchenko and Schmitz-Eiberger, 2003). Due to this fact, apples may be more responsive to a late application of EC. We did not find this to be the case, however. The decrease in phenolic content over the season is also consistent with literature (Solovchenco and Schmitz-Eiberger, 2003; Merzlyak and Solovchenko, 2002).

Only isolated incidences of pest or disease outbreaks have been reported for EC orchards (Evans 1993). Coetsee (2000) reported no increases in diseases. Care must be taken not to humidify orchards too much. The higher incidence of insect damage, primarily codling moth, on early EC 'Forelle' fruit could be because the EC treatment interfered with the spray programme and/ or washed off the pesticides.

The faster rate of budbreak that was found in the late EC treatment could be caused by a higher amount of chilling units that was accumulated because of the lower temperatures under EC. Chilling units are usually accumulated in April, and as the late EC was started in March, it could have resulted in the accumulation of more chilling units for EC treatments. We only found a significantly higher budbreak in the late EC treatment of 'Cripps' Pink', however. One would expect the early EC treatment to show the same trend. More research is needed to explain these results.

References

Arakawa, O. 1988. Photoregulation of anthocyanin synthesis in apple fruit under UV-B and red light. Plant Cell Physiol. 29(8):1385-1389.

Arakawa, O. 1991. Effect of temperature on anthocyanin accumulation in apple fruit as affected by cultivar, stage of fruit ripening and bagging. J. Hort. Sci. 56: 763-768.

Bergh, O., Franken J., Van Zyl, E. J., Kloopers F., and Dempers A. 1980. Sunburn on apples: preliminary results of an investigation conducted during the 1978/79 season. Deciduous Fruit Grower 30: 8-22.

Coetsee, J. 2000. Kunsmatige koue verbeter appeloes dramaties. Landbouweekblad, 9 Junie, 28 – 29.

Dussi, M. C., Sugar, D., Azarenko, A. N. and Righetti, T. L. 1997. Effects of cooling by over-tree sprinkler irrigation on fruit colour and firmness in 'Sensation Red Bartlett' Pear. HortTechnology 7: 55 – 57.

Evans, R. G. 1993. Assessing the mechanics of evaporative orchard cooling. Part Two. Good Fruit Grower, June, 29 – 32.

Evans, R.G. 1999. Overtree evaporative cooling system design and operation for apples in the PNW. Online. http://www.sidney.ars.usda.gov/Site_Publisher_Site/pdfs/personnel/Overtree%20Evaporative %20Cooling%20System%20Design.pdf.

Farager, J. D. 1983. Temperature regulation of anthocyanin accumulation in apple skin. J. Exp. Bot. 34: 1291 – 1298.

Fujii, J. A., and Kennedy, R.A. 1985. Seasonal changes in the photosynthetic rate in apple trees. A comparison between fruiting and non-fruiting trees. Plant Physiol. 78: 519-524.

Gindaba, J., and Wand, S. J. E. 2005. Comparative effects of evaporative cooling, kaolin particle film and shade net on sunburn and fruit quality in apples. HortScience 40 (3): 592-596.

Huysamer, M. 1998. Report of the blushed pear workgroup: Perceptions, facts and questions. Proc. Cape Pomological Association Tech. Symp., Cape Town, South Africa, 2-3 June 1998, 187-192.

Iglesias, I., Graell, J., Echeverria, G. and Vendrell, M. 2000. Orchard cooling with overtree sprinkler irrigation to improve fruit colour of 'Delicious' apples. HortScience 35: 1207 – 1208.

Iglesias, I., Salvia, J., Torguet, L. and Cabus, C. 2002. Orchard cooling with overtree microsprinkler irrigation to improve fruit colour and quality of 'Topred Delicious' apples. Scientia Hort. 93: 39 – 51.

Krauss, P., Markstädter, C. and Riederer, M. 1997. Attenuation of UV radiation by plant cuticles from wooden species. Plant Cell Environ. 20: 1079-1085.

Lancaster, J. E. 1992. Regulation of skin colour in apples. Crit. Rev. Plant Sci. 10: 487 – 502.

Mancinelli, A. L., Chia-Ping H. Y., Lindquist, P., Anderson, O. R. and Rabino, I. 1975. Photocontrol of anthocyanin synthesis. Plant Physiol. 55: 251 - 257.

Mazza, C. A., Boccalandro, H. E., Giordano, C. V., Battista, D., Scopel, A. L. and Ballaré, C. L., 2000. Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown soybean crops. Plant Physiol. 122: 33-43.

Merzlyak, M. N. and Solovschenko, A. E. 2002. Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. Plant Biochem. and Physiol. 40: 679 – 684.

Parchomchuk, P. and Meheriuk, M. 1996. Orchard cooling with pulsed overtree irrigation to prevent solar injury and improve fruit quality of 'Jonagold' apples. HortScience 31: 802 – 804.

Pretorius, J. J. B. 2006. Environmental and endogenous influences on carbohydrate assimilation and allocation of apple trees (*Malus domestica* Borkh.). PhD Thesis. University of Stellenbosch, South Africa.

Saure, M. C. 1990. External control of anthocyanin formation in apple. Scientia Hort. 42: 181 – 218.

Savage, M. J., Allan, P. and Lightbody, K. E. 1997. The temperature response of mature macadamia canopies to intermittent overhead sprinkling. J. S. Afr. Soc. Hort. Sci. 7 (2): 74 – 78.

Schrader, L. E., Zhang, J. and Duplaga, W.K. 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Online. Plant Health Progress. http://www.planthealthprogress.org/Current/research/sunburn/article.htm

Solovchenko, A. E. and Schmitz – Eiberger, M. 2003. Significance of skin flavonoids for UV-B protection in apple fruits. J. Exp. Bot. 54: 1977 – 1984.

Steyn, W. J. 2003. Red colour development and loss in pear fruit. PhD Thesis. University of Stellenbosch, South Africa.

Tan, S. C. 1979. Relationships and interactions between phenylalanine ammonia-lyase, phenylalanine ammonia-lyase inactivating system, and anthocyanin in apples. J. Amer. Soc. Hort. Sci. 104: 581 – 586.

Tan, S. T. 1980. Phenylalanine ammonia-lyase and the phynylalaninie ammonia-lyase inactivating system: Effects of light, temperature and mineral deficiencies. Aust. J. Plant Physiol. 7:159-167.

Unrath, C. R. 1972. The quality of 'Red Delicious' apples as affected by overtree sprinkler irrigation. J. Amer. Soc. Hort. Sci. 97: 58 – 61.

Unrath, C. R. and Sneed, R.E. 1974. Evaporative cooling of 'Delicious' apples – the economic feasibility of reducing environmental heat stress. J. Amer. Soc. Hort. Sci. 99: 372 – 375.

Wand, S. J. E., Steyn, W. J., Mdluli, M. J., Marais, S. J. S. and Jacobs, G. 2002. Overtree evaporative cooling for fruit quality enhancement. SA Fruit Journal, Aug/Sept., 18 – 21.

Wand, S. J. E., Steyn, W. J., Holcroft, D. M., Mdluli, M. J., van den Dool, K., Jacobs, G. 2005. Use of evaporative cooling to improve 'Rosemarie' and 'Forelle' pear fruit blush colour and quality. Acta Hort. 671: 103-111.

Williams, K.M. 1993. Use of evaporative cooling for enhancing apple fruit quality. Part three. Good Fruit Grower, August, 23 – 27.

Table 1: Cultivars and dates of full bloom, start of evaporative cooling (EC) treatments and harvest.

Cultivar	Full bloom	Early EC	Late EC	Harvest date
Rosemarie	20/09/2003	08/12/2003	21/12/2003	20/ 01/2004
Royal Gala	20/09/2003	08/12/2003	08/01/2004	05/02/2004
Forelle	16/09/2003	08/12/2003	28/01/2004	09/02/2004
Cripps' Pink	20/09/2003	08/12/2003	08/03/2004	02/04/2004
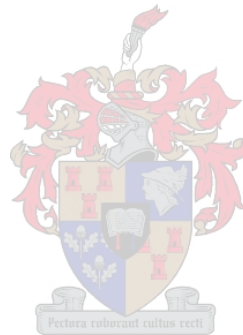
Table 2: Effects of evaporative cooling (EC) on fruit surface temperature of 'Cripps' Pink' and 'Royal Gala' apples on 25 January 2004. Values are means with standard errors. Means were separated by LSD (5%) where the F-value was significant.

Cultivar	Control	EC	P value
Cripps' Pink	41.9 ± 0.54 [Z]	37.2 ± 0.78	0.0018
Royal Gala	42.2 ± 0.82 [NS]	40.2 ± 1.11	0.1132

[NS] Not significant
[Z] Means with different letters differ significantly at $P \leq 0.05$.
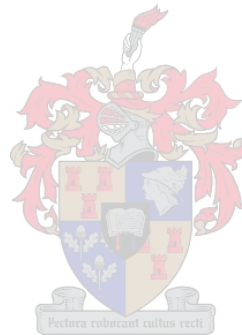
Table 3: Effects of early evaporative cooling (early EC) on gas exchange of 'Cripps' Pink' apple leaves by use of an infra-red gas analysis (IRGA) system. A_{max} = light- saturated rate of net CO_2 assimilation, g_s = stomatal conductance, E = transpiration rate, R_D = dark respiration rate, A_{sat} = light-and CO_2-saturated rate of net CO_2 assimilation. Means were separated by LSD (5%) where the F-value was significant.

	Control	Early EC	P value
A_{max} (μmol m^{-2} s^{-1})	11.1 [NS]	11.5	0.5098
gs (mol m^{-2} s^{-1})	0.204 [NS]	0.260	0.0517
E (mol m^{-2} s^{-1})	4.26 [Z]	5.29	0.0184
R_D (μmol m^{-2} s^{-1})	3.62 [NS]	3.78	0.7384
A_{sat} (μmol m^{-2} s^{-1})	22.7 [NS]	24.4	0.1411

[NS] Not significant
[Z] Means with different letters differ significantly at P≤0.05.

Table 4: Effects of evaporative cooling (EC) on percentage growth in trunk circumference of 'Cripps' Pink' and 'Royal Gala' apples and 'Forelle' and 'Rosemarie' pears over one season. Values are means with standard errors. Means were separated by LSD (5%) where the F-value was significant.

Cultivar	Control	Early EC	Late EC	P value
Cripps' Pink	6.5 ± 0.95 [NS]	7.2 ± 1.32	7.2 ± 0.76	0.8569
Royal Gala	5.2 ± 0.51 [NS]	5.6 ± 0.59	5.1 ± 0.53	0.7134
Forelle	2.5 ± 0.63 [NS]	2.1 ± 0.29	2.4 ± 0.33	0.6598
Rosemarie	1.1 ± 0.15 [NS]	2.2 ± 0.46	2.0 ± 0.44	0.1036

[NS] Not significant
[Z] Means with different letters differ significantly at P≤0.05.
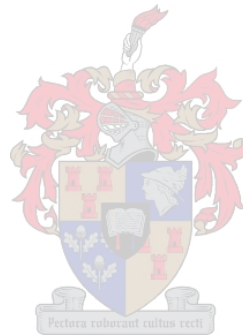
Table 5: Effects of evaporative cooling (EC) on fruit quality parameters of 'Royal Gala' apples. Means were separated by LSD (5%) where the F-value was significant.

Parameter	Control	EC early	EC late	P value
Mass (g)	99.4 ± 3.35 [NS]	106.8 ± 3.10	104.7 ± 2.46	0.2988
Fruit diameter (mm)	60.6 ± 0.92 [NS]	61.7 ± 0.52	61.6 ± 0.37	0.4537
Blush colour (chart value)	5.2 ± 0.45 [NS]	6.0 ± 0.30	6.4 ± 0.33	0.1022
Ground colour (chart value)	3.7 ± 0.02 [NS]	3.6 ± 0.03	3.6 ± 0.05	0.1053
Hue (°)	37.7 ± 2.0 [NS]	42.5 ± 2.1	44.0 ± 2.0	0.0750
Firmness (kg)	9.9 ± 0.2 [Z]	8.7 ± 0.1	9.6 ± 0.12	0.0003
TSS (%)	13.1 ± 0.34 [NS]	13.7 ± 1.62	12.5 ± 0.27	0.6633
TA (%)	0.52 ± 0.02 [NS]	0.5 ± 0.00	0.5 ± 0.00	0.2278
Starch conversion (%)	26.5 ± 3.7 [NS]	21.0 ± 3.0	19.1 ± 3.8	0.3220
Sunburn (%)	72.7 ± 11.8 [Z]	40.4 ± 4.0	68.5 ± 4.0	0.0416
Decay (%)	0.4 ± 0.4 [Z]	4.7 ± 1.8	0.4 ± 0.4	0.0341
Insect damage (%)	0.4 a ± 0.4 [NS]	1.7 ± 0.8	1.3 ± 0.9	0.5365

[NS] Not significant
[Z] Means with different letters differ significantly at P≤0.05.

Table 6: Effects of evaporative cooling (EC) on fruit quality parameters of 'Cripps' Pink' apples. Means were separated by LSD (5%) where the F-value was significant.

Parameter	Control	EC early	EC late	P value
Mass (g)	110.8 ± 3.49 ^{NS}	109.1 ± 2.90	107.2 ± 2.94	0.7041
Fruit diameter (mm)	63.2 ± 0.60 ^{NS}	62.9 ± 0.59	62.2 ± 0.60	0.4782
Blush colour (chart value)	10.0 ± 0.29 ^{NS}	9.2 ± 0.43	8.8 ± 0.42	0.0980
Ground colour (chart value)	3.9 ± 0.09 ^{NS}	3.8 ± 0.04	3.9 ± 0.06	0.6619
Hue (^{o})	26.1 ± 0.8 ^{NS}	27.0 ± 0.7	27.3 ± 0.63	0.3970
Firmness (kg)	8.5 ± 0.2 ^{NS}	8.5 ± 0.1	8.4 ± 0.1	0.9127
TSS (%)	15.3 ± 0.18 ^{NS}	15.3 ± 0.19	15.6 ± 0.18	0.3443
TA (%)	0.80 ± 0.03 ^{NS}	0.90 ± 0.02	0.8 ± 0.02	0.4181
Starch conversion (%)	43.2 ± 4.4 ^{NS}	43.2 ± 2.3	39.6 ± 3.9	0.9043
All sunburn (%)	18.1 ± 4.4 ^{Z}	26.3 ± 2.2	33.4 ± 3.9	0.0257
Insect damage (%)	0.0 ^{Z}	1.0 ± 0.5	0.0	0.0601

^{NS} Not significant
^{Z} Means with different letters differ significantly at P≤0.05.

Table 7: Effects of evaporative cooling (EC) on fruit quality parameters of 'Rosemarie' pears. Means were separated by LSD (5%) where the F-value was significant. Means with the same letter are not significantly different (LSD-test, P ≤ 0.05).

Parameter	Control	EC early	EC late	P value
Mass (g)	115.5 ± 3.76 ^{NS}	121.3 ± 7.33	117.6 ± 3.16	0.7057
Fruit diameter (mm)	58.2 ± 0.53 ^{NS}	57.6 ± 0.96	58.3 ± 0.75	0.8140
Blush colour (chart value)	9.6 ± 0.32 ^{NS}	9.5 ± 0.46	9.4 ± 0.32	0.9405
Ground colour (chart value)	3.4 ± 0.02 ^{NS}	3.5 ± 0.03	3.4 ± 0.02	0.4337
Hue (°)	83.6 ± 2.4 ^{NS}	82.0 ± 3.2	79.0 ± 1.8	0.4655
Firmness (kg)	6.3 ± 0.1 ^{NS}	6.2 ± 0.1	6.35 ± 0.1	0.2216
TSS (%)	13.9 ± 0.23 ^{NS}	13.4 ± 0.22	13.9 ± 0.28	0.1845
Decay (%)	0.3 ± 0.32 ^{NS}	1.9 ± 0.80	1.3 ± 0.97	0.3925
Sunburn (%)	31.4 ± 5.4 ^{Z}	51.2 ± 8.9	63.9 ± 6.5	0.0005
Insect damage (%)	0.6 ± 0.4 ^{NS}	1.6 ± 0.5	0.0	0.1820

^{NS} Not significant
^{Z} Means with different letters differ significantly at P≤0.05.

Table 8: Effects of evaporative cooling (EC) on fruit quality parameters of 'Forelle' pears.
Means were separated by LSD (5%) where the F-value was significant.

Parameter	Control	EC early	EC late	P value
Mass (g)	100.2 ± 2.40 [NS]	98.6 ± 2.34	100.1 ± 1.46	0.7466
Fruit diameter (mm)	54.2 ± 0.48 [NS]	54.2 ± 0.57	54.7 ± 0.31	0.6023
Blush colour (chart value)	10.0 ± 0.29 [NS]	9.2 ± 0.43	8.8 ± 0.42	0.0980
Ground colour (chart value)	2.9 ± 0.03 [Z]	2.2 ± 0.07	2.9 ± 0.04	< 0.0001
Hue (^{o})	59.7 ± 2.1 [Z]	54.1 ± 2.3	60.1± 1.8	0.0032
Firmness (kg)	5.9 ± 0.1 [NS]	5.8 ± 0.1	5.8 ± 0.1	0.2858
TSS (%)	15.5 ± 0.32 [NS]	14.7 ± 0.27	15.3 ± 0.15	0.1031
All sunburn (%)	1.0 ± 0.4 [NS]	0.2 ± 0.2	0.5 ± 0.5	0.2941
Insect damage (%)	16.4 ± 3.0 [Z]	28.4 ± 3.6	23.3 ± 4.2	0.0380

[NS] Not significant
[Z]   Means with different letters differ significantly at P≤0.05.
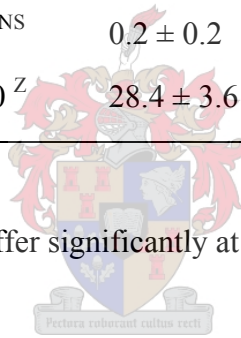
Table 9: Effects of evaporative cooling (EC) on fruit skin concentrations of total anthocyanin (measured against a standard curve) and total phenolics (absorbances at 280 nm) in 'Cripps' Pink' and 'Royal Gala' apple. Means were separated by LSD (5%) where the F-value was significant.

Cultivar	Variable	Anthocyanin (μg/g)	Phenolics 280 nm (absorbance)
Cripps' Pink	Date		
	09/12/2003	6.1 [Z]	1.055
	10/03/2004	17.2 [Z]	0.341
	20/04/2004	222.6 [Z]	0.536
	Treatment		
	Control	74.3 [NS]	0.619
	Early EC	90.2 [NS]	0.684
	Late EC	81.4 [NS]	0.630
	Pr > F		
	Date	< 0.0001	< 0.0001
	Trt	0.1659	0.2527
	Date*Trt	0.1423	0.3907
Royal Gala	Date		
	09/12/2003	19.9 [Z]	1.095
	28/01/2004	54.7 [Z]	0.519
	06/02/2004	87.6 [Z]	0.595
	Treatment		
	Control	51.6 [NS]	0.688
	Early EC	48.6 [NS]	0.685
	Late EC	62.0 [NS]	0.836
	Pr > F		
	Date	< 0.0001	< 0.0001
	Trt	0.0973	0.1696
	Date*Trt	0.0390	0.3743

[NS] Not significant
[Z] Means with different letters differ significantly at P≤0.05.

Table 10: Effects of evaporative cooling (EC) on fruit skin concentrations of total anthocyanin (measured against a standard curve) and total phenolics (absorbances at 280 nm) in 'Rosemarie' and 'Forelle' pear skin. Means were separated by LSD (5%) where the F-value was significant.

Cultivar	Variable	Anthocyanin (µg/g)	Phenolics 280 nm (absorbance)
Rosemarie	Date		
	09/12/2003	45.5 [Z]	0.946
	22/01/2004	27.3 [Z]	0.538
	Treatment		
	Control	34.9 [NS]	0.738
	Early EC	42.6 [NS]	0.787
	Late EC	31.9 [NS]	0.701
	Pr > F		
	Date	0.0239	< 0.0001
	Trt	0.4908	0.6985
	Date*Trt	0.5034	0.2524
Forelle	Date		
	09/12/2003	147.9 [Z]	1.422
	10/03/2004	30.7 [Z]	0.784
	Treatment		
	Control	87.4 [NS]	0.090
	Early EC	92.7 [NS]	0.083
	Late EC	89.7 [NS]	0.083
	Pr > F		
	Date	< 0.0001	< 0.0001
	Trt	0.9097	0.7359
	Date*Trt	0.6918	0.3245

[NS] Not significant
[Z] Means with different letters differ significantly at P≤0.05.

Table 11: Growth potential of 'Cripps' Pink' and 'Royal Gala' apples, 'Rosemarie' and 'Forelle' pears under three treatments: control, early evaporative cooling (EC) and late evaporative cooling. Growth potential is determined as the reciprocal of the number of days from when the first bud on marked shoots sprouted until 50% of buds on the shoot have sprouted. Means were separated by LSD (5%) where the F-value was significant.

Cultivar	Control	Early EC	Late EC	P value
Rosemarie	0.109 ^{NS}	0.123	0.113	0.5877
Royal Gala	0.061 ^{NS}	0.144	0.143	0.1524
Forelle	0.103 ^{NS}	0.126	0.107	0.2622
Cripps' Pink	0.115 ^{Z}	0.093	0.223	0.0099

^{NS} Not significant

^{Z} Means with different letters differ significantly at P≤0.05.
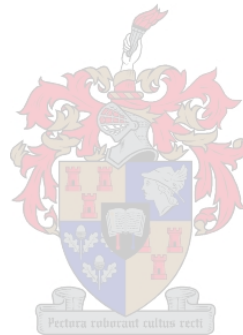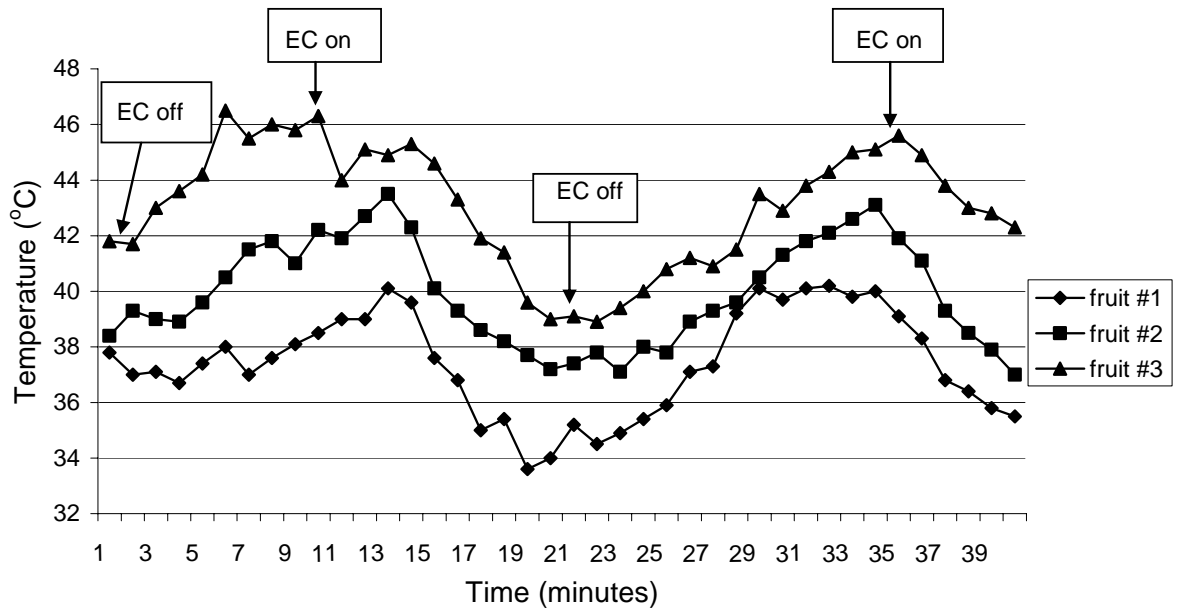
Fig 1: Fruit skin temperature of three 'Cripps' Pink' apples under EC and exposed to full sunlight measured in response to system 'on' and 'off' times over a period of 40 minutes at one minute intervals. Measurement commenced at 13:00 on 27 January 2004.
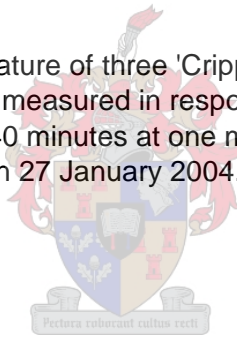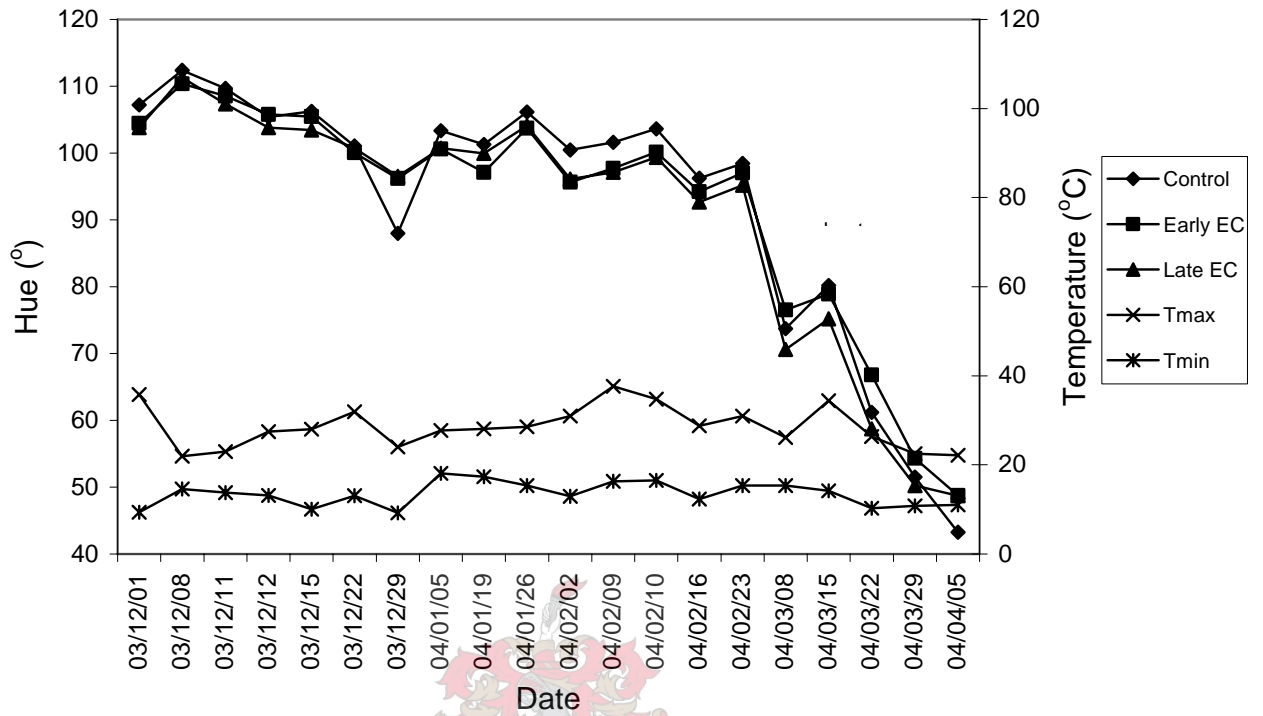
Fig 2: Seasonal changes in hue angles of 'Cripps' Pink' apples during the 2003/2004 season where 0° is red-purple and 90° is yellow. Tmax = daily maximum air temperature and Tmin = daily minimum air temperature.
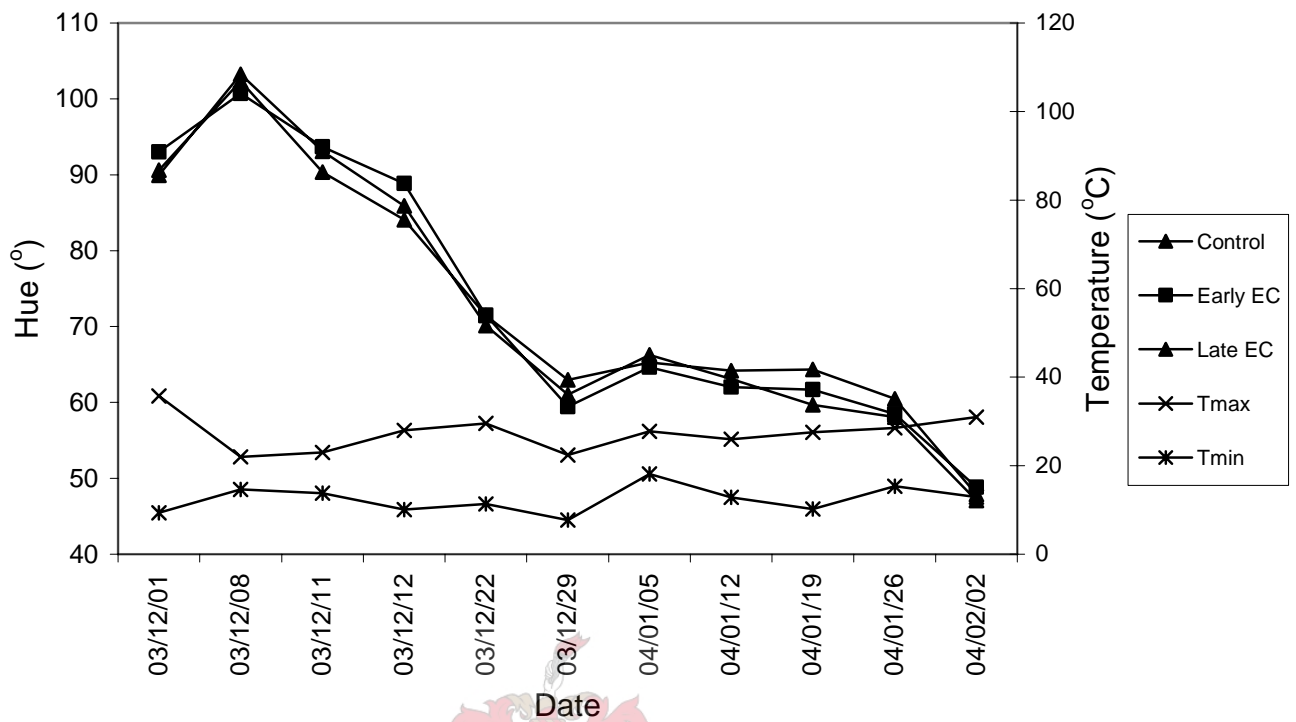
Fig 3: Seasonal changes in hue of 'Royal Gala' apples during the 2003/2004 season where 0° is red-purple and 90° is yellow. Tmax = daily maximum air temperature and Tmin = daily minimum air temperature.

Paper 2

Heat stress resistance of 'Cripps' Pink' and 'Royal Gala' apple fruit grown under evaporative cooling as measured using chlorophyll fluorescence (Fv/Fm)

Abstract

Heat tolerance of apple fruit grown under an evaporative cooling (EC) system was evaluated in 'Cripps' Pink' (three times) and 'Royal Gala' (twice) by determining the maximum quantum yield of chlorophyll fluorescence (Fv/Fm). A control and two evaporative cooling (EC) treatments, one that started earlier in the season (early EC) and one that started later in the season (late EC) were used. Apples picked at harvest or up to ten days earlier were exposed to different air temperatures ($35^{o}C$, $40^{o}C$, $45^{o}C$, $50^{o}C$ or $55^{o}C$) for increasing periods of time. Measurements of fruit surface temperature (FST) and Fv/Fm were taken directly after the treatments, as well as 12 hours later to determine their recovery. The heat treatments resulted in mean FST of about 33°C, 37°C, 43°C, 48°C and 51°C, respectively. Fruit from both cultivars exposed to 35 and $40^{o}C$ recovered fully for all time periods of exposure. 'Cripps' Pink' fruit exposed to $45^{o}C$ recovered well after shorter periods of exposure, but after four to six hours of exposure and thereafter the recovery steadily declined, especially during the first experiment. 'Royal Gala' apples exposed to 45°C recovered almost fully for eight hours of treatment. Partial recovery of 'Cripps' Pink' fruit occurred only after two hours of exposure at 50 and $55^{o}C$, with very little or no recovery (permanent damage) after four hours of exposure. 'Royal Gala' fruit exposed to 50 and 55°C recovered better than 'Cripps' Pink' fruit. 'Cripps' Pink' fruit from both EC treatments, but particularly the early EC treatment, were less resistant to heat stress than control (non-EC) fruit at the "threshold" air temperature of 45°C. In 'Royal Gala', early EC fruit were also less resistant to heat stress than late EC fruit during the first experiment, but not the second experiment at harvest. 'Cripps' Pink' apples picked on 8 April following a very hot day (35°C maximum air temperature) appeared to be able to recover better from heat stress than apples harvested five days earlier.

Introduction

Chlorophyll fluorescence is a very useful tool to study the effects of environmental stress on plants, since photosynthesis is often reduced in plants experiencing adverse conditions (Fracheboud *et al.*, 2004). Heat stress influences the structure and composition of the thylakoid membrane. Chlorophyll fluorescence can be used as a non-destructive tool to monitor the functioning of the light-capturing reactions within the thylakoid membranes (Schreiber and Bilger, 1993).

When the protective mechanisms that exist in the fruit skin are overcome by excess ultraviolet or visible and/or thermal radiation, fruit will experience injury (Andrews and Johnson, 1996). They postulated that solar injury (sunburn) is caused directly by photo-oxidative damage from high energy visible and ultraviolet (UV) light and that fruit are "preconditioned" for this damage by high temperatures. Heat effects on the light-dependant reactions of photosynthesis are primarily responsible for irreversible damage due to heat exceeding a critical temperature (Weiss and Berry, 1988). As each quantum of light is absorbed by a chlorophyll molecule, it raises an electron from the ground state to an excited state (Salisbury and Ross, 1992). A plant only has a certain capacity for photochemistry. This capacity depends upon a range of factors, including environmental stresses. Excess energy exceeding the capacity for photochemistry must be efficiently dissipated by non-photochemical processes. Such processes include the emission of heat and the re-emission of small but diagnostically significant amounts of the absorbed radiation as longer wavelength red/far red energy (Salisbury and Ross, 1992; Fracheboud *et al.*, 2004). The maximum quantum efficiency of Photosystem II (PSII) is given by the measurement Fv/Fm, which in healthy leaves and other chlorophyllous tissues is close to 0.75-0.80, irrespective of the plant species studied (Fracheboud *et al.*, 2004). According to Van Kooten and Snel (2002), Fv/Fm is one of the fastest and most powerful measurements to monitor physiological injury.

The harmful effects of high temperature on higher plants occur primarily in photosynthetic functions and the thylakoid membranes, particularly in the PSII complexes located on these membranes (Krause and Santarius, 1975; Weiss and Berry, 1988). This makes the measurement of photosynthesis an efficient means of measuring the effects of heat stress on apple surface tissues. As there is such a high incidence of sunburn on apples in warm regions all across the

world, there is a great need for effective strategies to combat damage on apples (and other fruit). Understanding more about how apples react to high temperature and high levels of irradiance will help to formulate such strategies.

Evaporative cooling (EC) is used commercially to alleviate heat stress and lessen the effect of high temperature on fruit quality in warm production regions (Wand *et al.*, 2005). EC can reduce peel temperature by up to 8ºC in apple (Unrath, 1972; Parchomchuk and Meheriuk, 1996; Wünsche *et al.*, 2001). An overtree EC system applies water above the crop. An overhead sprinkler system is used to wet fruit and leaf surfaces when the temperature rises above a certain threshold point. As the water evaporates from the fruit surfaces, energy is extracted from the skin. If this energy is greater than the total incoming heat energy, the fruit surface temperature (FST) decreases (Unrath, 1972; Parchomchuk and Meheriuk, 1996). The air around the trees is also cooled, and the relative humidity increases, thus reducing water loss through transpiration. Often during a day with low humidity, high temperatures and intense sunlight, water is lost to transpiration faster than the root system can replace it from the soil. During fruit development, these conditions can cause water to be withdrawn from the fruit to supply the plant, resulting in smaller fruit, delayed maturity and reduced fruit quality (Evans, 1993). By raising the relative humidity in the orchard, EC reduces overall heat stress of the plants.

Fruit burn easily when they are suddenly exposed to high temperatures and intense sunlight after developing under more protected conditions, for example after summer pruning or selective picking (Wünche *et al.*, 2001). When there is a gradual increase in temperature and solar radiation, fruit can acclimatise, and damage is less likely to occur or reduced. Fruit have mechanisms that protect them against high temperatures and irradiances. One of these mechanisms that could explain the acclimation effect is the development of heat shock proteins (HSP's), a group of proteins that are expressed rapidly when cells undergo heat stress situations. Several researchers found that, by exposing fruit or perennial grass to a series of higher temperatures over a certain period of time before introducing a heat shock treatment, the fruit/grass suffered less damage (Paull, 1990; Woolf and Laing, 1996; Al-Niemi and Stout, 2002; Gulen and Eris, 2003). It is postulated that the increase of HSP's help to protect the tissue from damage during the heat shock treatment. Another protection mechanism against high radiation levels is excess energy dissipation through the xanthophyll cycle (Demmig-Adams *et al.,* 1995; Gilmore, 1997; Müller *et al.*, 2001). Demmig-Adams *et al.* (1995) found that sun leaves have

larger xanthophyll cycle pools than shade leaves, and also demonstrate a greater increase in energy dissipation activity. It seems that this is another way for plant tissues to acclimatize to higher temperatures and radiation levels. Plants also use the antioxidant ascorbic acid (vitamin C) which they produce themselves, to reduce oxidative cell damage. Vitamin C content in plant tissues increases with increasing light intensity during the growing season (Lee and Kader, 2000).

The purpose of this study was to investigate the effects of high temperature on maximum quantum yield of fluorescence (Fv/Fm) on 'Cripps' Pink' and 'Royal Gala' apples in order to establish temperature thresholds for heat stress injury, and to study the possible acclimation of fruit grown under EC to lower temperatures.

Materials and Methods

Experiments were conducted on two apple (*Malus domestica* Borkh.) cultivars, Cripps' Pink and Royal Gala, in a mixed orchard established on Welgevallen Experimental Farm in Stellenbosch (33^o56'S, 18^o51'E) in 1998. Both cultivars were grafted on M793 rootstock, and planted at 4 m x 1.5 m spacing. Row orientation was approximately north-east by south-west.

For the evaporative cooling system, micro sprinklers (DAN 2001 jets) with a 28 L.h^{-1} discharge were installed on 4.5 m poles. The poles were spaced 8 m apart with jets every 2.5 m along a suspended pipe. The radius of each jet was 1.5 m, and they gave a precipitation rate of 4 mm.h^{-1}. Pulsing cycles of 5 minutes on, 15 minutes off were used. Between 08h00 and 18h00, the system was activated at air temperatures of 30°C and higher, and between 18h00 and 21h00, the system was activated at temperatures above 22°C. Temperature was measured by means of a temperature sensor positioned in the orchard and connected to the irrigation computer. Normal undertree irrigation was given to all the orchards. Treatments evaluated were: 1) control without EC, 2) early EC application from the first week in December 2003, and 3) late EC application starting two to four weeks before harvest. A complete randomized block design was used, with nine blocks for 'Cripps' Pink' and six for 'Royal Gala'. A block consisted of two rows of which only the north-western row was used, due to the prevailing south-easterly wind. Three trees were used per replicate. Treated trees within rows were separated by at least two trees not used.

One hundred and eighty 'Cripps' Pink' apples (60 per field treatment) were harvested on three different dates, 3 April 2004, 8 April 2004 and 13 April 2004 (commercial harvest date). 180 'Royal Gala' apples were harvested on two different dates, 28 January 2004 and 3 February 2004 (commercial harvest). Sun-exposed apples were harvested on the south-eastern side of the trees.

Thirty six fruit (12 for each field treatment) were placed in five different ovens in the dark at air temperatures of $35^{\circ}C$, $40^{\circ}C$, $45^{\circ}C$, $50^{\circ}C$ or $55^{\circ}C$. Maximum quantum yield of fluorescence (Fv/Fm) as well as FST of all fruit was measured at room temperature under weak light before being placed in the ovens. Fv/Fm was measured using a pulse modulated fluorometer (FMS2, Hansatech Instruments Ltd., King's Lynn, Norfolk, England), and FST was measured using a hand held infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, CA).

On all the above dates except 28 January and 3 April, three apples were taken out of each oven every two, four, six or eight hours, and FST and Fv/Fm were measured immediately after removal. Fv/Fm was again measured after a recovery period of 12 hours at room temperature under weak light. On 28 January and 3 April two apples were taken out of each oven every two hours for up to twelve hours.

Data was analysed using a three-way ANOVA ($Pr \leq 0.05$) with field treatment (trt), temperature of heat treatment (temp) and duration of heat treatment (time) as factors (Enterprise Guide VI, release 1.3 SAS Institute, Cary, NC, USA, 2001).

Results

Fruit surface temperature (FST)
FST was measured as soon as fruit were removed from the ovens. Mean FST's were 32-34°C, 36-38°C, 43-45°C, 46-49°C and 49-53°C for oven air temperatures of 35, 40, 45, 50 and 55°C, respectively (Table 1).

'Cripps' Pink'
3 April 2004: There was significant 3-way interaction between the factors. However, this was the only case of a 3-way interaction in all the experiments, and to avoid confusion only 2-way

interactions and main effects will be discussed hereafter. There was significant interaction (Pr<0.0001) between duration of heat treatment and temperature (Table 2a, Fig. 1a). At 35°C, Fv/Fm remained relatively constant at >0.7 for the full duration of the experiment, whereas Fv/Fm decreased from initial values of >0.75 after 10 hours (40°C) or after 2 hours (45, 50 and 55°C). Very low Fv/Fm (<0.1) was reached after 6 hours (45°C) or 2 hours (50 and 55°C). There was also significant interaction (P=0.0049) between field treatments and temperature (Table 2a, Fig. 1b). Fv/Fm of the control (non-EC) treatment was higher at 35-45°C than the two EC treatments. At 50 and 55°C there were no differences between the three field treatments. Significant interaction (P<0.0001) between field treatments and duration of exposure (Table 2a, Fig. 1c) was due to the maintenance of higher Fv/Fm values in the control (non-EC) compared to EC treatments after lengthy exposure times (>6 hours).

Following the 12-hour recovery period (Table 2b), fruit subjected to 35 and 40°C showed full recovery, whereas fruit exposed to 45°C recovered almost fully when exposed for up to 4 hours (Fig. 2a). After 6 hours of exposure, fruit recovered partially, but recovery was poor after 8-12 hours of exposure. Fruit exposed to 50 or 55°C did not recover. Control (non-EC) fruit recovered better than EC fruit at 45°C but not at higher temperatures (Table 1b, Fig. 2b). There was no significant interaction between field treatments and duration of exposure (Table 1b, Fig. 2c).

8 April 2004: There was significant interaction (P<0.0001) between duration of exposure and temperature (Table 3a, Fig. 3a). At 35 and 40°C, Fv/Fm remained constant at >0.8 for the full duration of the experiment, whereas at 45°C Fv/Fm slowly started decreasing from initial values after 2 hours, or more rapidly at 50 and 55°C. Minimum Fv/Fm (<0.005) was reached after 8 hours (50 and 55°C). There was also significant interaction (P = 0.0003) between field treatments and temperature (Table 3a, Fig. 3b). At 35 and 40°C there were no differences between the two field treatments. At 45°C, Fv/Fm of the EC treatment was significantly lower at 0.44 than Fv/Fm of the control (non-EC) treatment at 0.56. At 50°C there was again no difference between the two treatments. There was no significant interaction between field treatments and duration of exposure (Table 3a, Fig. 3c).

Following the 12-hour recovery period (Table 3b), fruit subjected to 35 and 40°C showed full recovery, whereas fruit exposed to 45°C for up to 4 hours recovered almost fully (Fig. 4a). Fruit exposed for 6 and 8 hours recovered partially but still relatively well. Fruit subjected to 50°C

recovered fully after 2 hours of exposure, but did not recover after 4 hours of exposure or longer. Fruit subjected to 55°C did not recover. There was again significant interaction (P = 0.0229) between field treatments and temperature treatments (Table 3b, Fig. 4b), with EC fruit exposed to 45°C showing poorer recovery than non-EC fruit. There was no significant interaction between field treatments and duration of exposure (Fig. 4c).

13 April 2004: There was significant interaction (P<0.0001) between duration of exposure and temperature (Table 4a, Fig. 5a). At 35 and 40°C, Fv/Fm stayed more or less constant at >0.65. Fv/Fm of fruit subjected to 45°C decreased from values of 0.58 after two hours to 0.16 after 8 hours. Fv/Fm of fruit subjected to 50 and 55°C declined to values of 0.25 and 0.3, respectively, during the first two hours, and thereafter declined to values of <0.13. There was no significant interaction between field treatments and temperature (Fig. 5b), or between duration of exposure and field treatments (Fig. 5c). However, the main effect of field treatment was significant (Table 4a) with the late EC fruit showing higher heat tolerance than the early EC fruit.

Following the 12-hour recovery period (Table 4b), fruit subjected to 35 and 40°C showed full recovery, whereas fruit exposed to 45°C recovered almost fully when exposed for up to 4 hours (Fig. 6a). After 6 and 8 hours of exposure, fruit exposed to 45°C recovered partially to a maximum Fv/Fm of 0.66. Fruit subjected to 50 and 55°C for two hours recovered well, but after 4 hours of exposure they did not recover (Fig. 6a). There was no significant interaction between field treatments and temperature (Fig. 6b), or between duration of exposure and field treatments (Fig. 6c). However, the main effect of field treatment (Table 4b) once again showed that late EC fruit were better able to recover than the early EC fruit.

'Royal Gala'

28 January 2004: There was significant interaction (Pr=0.0410) between duration of exposure and temperature (Table 5a, Fig. 7a). At 35 and 40°C, Fv/Fm stayed constant at >0.75 for the full duration of the experiment, whereas at 45°C, Fv/Fm decreased gradually from 0.67 after 2 hours of exposure to 0.33 after 12 hours. At 50°C, Fv/Fm varied between 0.06 and 0.38 with no consistent trend with regard to duration of exposure. At 55°C, Fv/Fm increased from 0.05 after 2 hours to a maximum of 0.3 after 6 hours, from where it decreased again to 0.05 after 12 hours of exposure. There was no significant interaction between field treatments and temperature (Fig. 7b), or between duration of exposure and field treatments (Fig. 7c).

Following the 12-hour recovery period (Table 5b), fruit subjected to 35 and 40°C showed full recovery, whereas fruit exposed to 45°C recovered almost fully when exposed for up to 8 hours (Fig. 8a). Fruit exposed for 10 and 12 hours did not recover fully, but still relatively well. Fruit exposed to 50°C recovered to between 0.52 (2-6 hours) and 0.34 (8-12 hours), and fruit exposed to 55°C remained at between 0.25 and 0.06 (all durations). There was no significant interaction between field treatments and temperature (Fig. 8b). Late EC treatment fruit generally recovered better than early EC fruit (Fig. 8c).

4 February 2004:

There was significant interaction ($P<0.001$) between duration of exposure and temperature (Table 6a, Fig. 9a). At 35 and 40°C, Fv/Fm stayed constant at >0.8 for the full duration of the experiment, whereas Fv/Fm of fruit subjected to 45°C decreased from 0.81 after 2 hours to 0.60 after 8 hours. At 50°C, Fv/Fm decreased from values of 0.54 after two hours to 0.2 after 8 hours, and at 55°C the values decreased from 0.78 to 0.14 after 8 hours. There was no significant interaction between field treatments and temperature (Fig. 9b), or between duration of exposure and field treatments (Fig. 9c). There was no main effect of field treatment (Table 6a).

Following the 12-hour recovery period (Table 6b), fruit subjected to 35, 40 and 45°C showed full recovery (Fig. 10a). Fruit subjected to 50°C recovered well after 2 hours of exposure, but recovered only partially to between 0.23 and 0.41 thereafter. Fruit exposed to 55°C recovered well after 4 hours of exposure, but recovery was less after 6 hours and after 8 hours there was no recovery. There was no significant interaction between field treatments and temperature (Fig. 10b), or between duration of exposure and field treatments (Fig. 10c). There was no main effect of field treatment (Table 6b).

Discussion

It was established in these trials that the main cause of irreversible heat damage to apple fruit was exposure to above-threshold temperature, but that longer times of exposure resulted in more serious damage at temperatures just below the lethal threshold. These findings confirm those of Marais (2005). PSII operating efficiency was not affected by heat treatments of 35-40°C (32-

38°C FST) in either 'Royal Gala' or 'Cripps Pink'. When exposed to 45°C (43-45°C FST), Fv/Fm was significantly reduced in both cultivars, but more so after longer treatment periods. Fruit that were exposed for up to four hours generally recovered almost fully. For longer exposure times, recovery was less effective, but minimum recovery was still above 0.45, with the exception of 'Cripps' Pink' picked on 3 April 2004. Exposure to temperatures of 50-55°C (46-53°C FST) strongly reduced Fv/Fm values in both cultivars. In 'Cripps' Pink' these fruit did not recover at all or only weakly, indicating irreparable damage to PSII, especially after more than two hours of heat treatment. Schrader *et al.* (2001) found that apple FST exceeding 46-49°C in the presence of light resulted in sunburn browning. The threshold was cultivar-specific, being lower in 'Fuji' and 'Granny Smith" than in 'Cripps' Pink' and 'Gala' (Schrader *et al.*, unpublished). Sunburn necrosis was reported to occur at FST >52°C in the absence of light, thus a heat damage effect.

In 'Royal Gala', fruit exposed to 50-55°C showed better recovery than in 'Cripps' Pink'. Marais (2005) also found a more significant interaction between duration of exposure and the lowering of PSII efficiency in 'Cripps' Pink' than in 'Royal Gala', and 'Royal Gala' fruit seemed to recover better than 'Cripps' Pink', especially later in the season. The possible reasons for this difference in sensitivity are unclear, but could be cultivar related.

Song *et al*. (2001) found that a sharp decrease in Fv/Fm of 0.3 or more was a good indicator of irreparable damage expressed as flesh browning on apple fruit that was exposed to heat treatments for more than four hours. A reduction in chlorophyll fluorescence has also been found in other crops following heat treatments. In heat-treated broccoli, an immediate decline in Fv/Fm by 0.3 or more was used to discriminate between beneficial or detrimental heat treatments (Tian *et al*., 1996). A sudden drop in Fv/Fm was also used to indicate heat stress in mangos (Joyce and Shorter, 1994).

Heat shock proteins (HSP's) are a group of proteins that are present in all cells in all life forms (Salisbury and Ross, 1992; Larcher, 2003). They appear to be molecular 'chaperones' for protein molecules, and perform functions in various intra-cellular processes, such as helping to stabilize partially unfolded proteins, and so aiding in the transportation of proteins across membranes within the cell (Larcher, 2003). Their expression is increased when a cell undergoes various types of environmental stresses such as heat, cold and oxygen deprivation. According to Ritenour

et al. 2001, HSP's appear rapidly, often becoming a substantial portion of the total proteins within 30 minutes after an abrupt shift from moderate (28°C) to high (41°C) temperature. It has not been discovered exactly how heat-shock (or other environmental stressors) activates the heat-shock factor. However, some studies suggest that an increase in damaged or abnormal proteins brings HSPs into action (Ritenour *et al*, 2001; Key *et al*.).

Al-Niemi and Stout (2002) found in their experiments with the perennial grass *Dichantehelium lanuginosum* that HSP's were expressed after two hours of exposure to 40°C, and that the levels of HSP's remained elevated for 5-7 days after the heat treatment. Gulen and Eris (2003) studied the effects of gradual heat and heat shock on the activity of peroxidase (PRX) isozyme in strawberries. They acclimatised half of the plants by gradually increasing the heat of the chamber, from 25°C to 45°C, and the rest of the plants were brought into the growth chamber from outside at every heat step. They found that acclimatised plants showed significantly higher activities of PRX enzyme in response to high temperature compared to those from the leaves of plants that were brought into the chamber from outside, and therefore exposed to a single heat shock. It could be that PRX is one of the so-called heat shock proteins. From research done by Zhang *et al*., it seems that PRX plays an important role in protection against ionising radiation.

Fruit under EC acclimatise to lower temperatures, and when the temperature rises suddenly they can be more prone to sunburn. The results of this study confirm that this acclimation renders apple fruit grown under EC more susceptible to heat damage at high but not lethal fruit temperatures. The EC system should be monitored very carefully, because if it malfunctions or does not activate on a hot day, many fruit could be lost due to sunburn.

It seems that HSP's are expressed in plants when the temperature exceeds about 40°C (Ritenour *et al.* (2001). This temperature threshold, as well as the known persistence of the HSP's, could explain the results of this study, where FST above 43°C but not 38°C or below, significantly reduced Fv/Fm. Fruit experiencing 43-45°C FST for up to four hours generally recovered almost completely after twelve hours, but fruit experiencing these temperatures for longer than four hours recovered less effectively. It is possible that the expression of HSP's reaches a peak after four hours and that these proteins are then unable to limit further damage. Ritenour *et al.* (2001) found that accumulation of small HSP's was maximal after a four-hour heat treatment in 'Fuji' apples.

Woolf and Laing (1996) found that external browning that resulted from exposure of avocados to 50°C could be significantly reduced by pre-treatment of the avocados with hot water (38°C) for one hour. Chen and Paull (1990) also found development of thermotolerance to otherwise injurious heat treatment in papayas when they were exposed to 42°C for four hours or 38-42°C for one hour followed by three hours at 22°C. This could help explain our findings on 'Cripps' Pink' where fruit harvested on 8 April suffered less heat damage than fruit harvested on 3 April. It seems that the heat event on the previous day (7 April), when maximum air temperature reached 35°C, could have caused the fruit skin to express HSP's that could stabilize the membranes, allowing them to recover. Fruit did not recover from FST higher than 46°C, indicating that these temperatures were too extreme for the HSP's to continue to have a protective function.

'Cripps' Pink' fruit under EC showed more damage than control fruit (3 and 8 April), and recovery of EC fruit after the 45°C heat treatment was poorer than in control fruit (4 and 9 April). We suspect that because the fruit under the EC treatment had acclimatized to the lower temperatures, they were injured more easily under warm conditions. The fact that in 'Cripps' Pink' (13 April), and in 'Royal Gala' (28 January), early EC fruit had more damage than late EC or control fruit supports this suggestion. The early EC fruit had the longest time to acclimatize; therefore they had a higher percentage of heat damage. It could be that control fruit, and to a lesser extent late EC fruit, had acclimatised to higher temperatures by means of expression of HSP's or membrane changes, and were therefore more protected against the higher temperatures later in the season, or heat shock on exceptionally hot days. These findings could mean that, depending on the amount of very hot days or heat waves in a season, EC could be detrimental to an orchard instead of beneficial if the risks of system failure are not properly managed.

Apples were thus able to recover from heat treatments in the range of 32-38°C FST, and generally also recovered fully after 43-45° FST when exposure did not exceed four hours. This knowledge could be helpful in the management of sunburn, for example when determining the threshold temperature for the activation of evaporative cooling treatments (Marais, 2005). It should be noted, however, that in this study heat treatments took place in the absence of light. Due to the fact that light is also a requirement for the development of sunburn browning (Schrader *et al.*, 2001), further experiments would be required to evaluate the effects of high temperatures in

conjunction with various light intensities to understand the roles of these two factors in the development of sunburn and heat stress in apples. Further research should be conducted *in situ* due to the different reactions that could be expected from fruit with an intact xylem water supply compared to picked fruit.

References

Al-Niemi, T. S. and Stout, R. G. 2002. Heat-shock protein expression in a grass commonly associated with active geothermal areas in western North America. Jnl. Thermal Biol. 27(7): 547-553.

Andrews, P. K. and Johnson, J. R. 1996. Physiology of sunburn development in apples. Good Fruit Grower 47(12): 32-36.

Armond, P. A., Schreiber, U. and Björkman, O. 1978. Photosynthetic acclimation to temperature in the desert shrub Larrea divaricata. Plant Physiol. 61:411-415.

Aro, E. M., McCaffery, S. and Anderson, J. M. 1994. Recovery from photoinhibition in peas (Pisum sativum L.) acclimated to varying growth irradiances. Plant Physiol. 104(3):1033-1041.

Aro, E. M., Virgin, I. and Andersson, B. 1993. Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. Biochim. Biophys. Acta 1143(2):113-134.

Berry, J. and Björkman, O. 1980. Photosynthetic response and adaptation to temperature in higher plants. Ann. Rev. Plant Physiol. 31:491-543.

Chen, N. J. and R. E. Paull. 1990. Heat shock response in field grown papaya fruit. J. Amer. Soc. Hort. Sci. 115:623-631.

Demmig-Adams, B., Adams III, W. W., Logan, B. A. and Verhoeven, A. S. 1995. Xanthophyll cycle-dependent energy dissipation and flexible photosystem II efficiency in plants acclimated to light stress. Aust. J. Plant Physiol. 22:249-260.

Evans, R. G. 1993. Designing and operating overtree evaporative cooling systems for apples. Part 1. Good Fruit Grower, 44(11): 23-27.

Falcone, D. L., Ogas, J. P. and Somerville, C. R. 2004. Regulation of membrane fatty acid composition by temperature in mutants of Arabidopsis with alterations in membrane lipid composition. BMC Plant Biol. 4:17.

Fracheboud, Y., Jompuk, C., Ribaut, J. M., Stamp, P. and Leipner, J. 2004. Genetic analysis of cold tolerance of photosynthesis in maize. Plant Mol. Biol. 56: 241-253.

Gilmore, A. M. 1997. Mechanistic aspects of xanthophyll cycle-dependant photoprotection in higher plant chloroplasts and leaves. Physiol. Plant. 99: 197-209.

Gulen, H. and Eris, A. 2003. Some physiological changes in strawberry (Fragaria x ananassa 'Camarosa') plants under heat stress. J. Hort. Sci. Biotech. 78: 894-898.

Harwood, J. L., Jones, A. L., Perry, H. J., Rutter, A. J., Smith, K. M. and Williams, M. 1994. Changes in plant lipids during temperature adaptation. In: Temperature adaptation of biological membranes (Edited by: Cossins, A.R.). London: Portland Press, 107-117.

Joyce, D. C. and Shorter, A. J. 1994. High temperature conditioning reduces hot water treatment injury of 'Kensington Pride' mango fruit. HortScience 29: 1047 – 1051.

Krause, G. H. and Santarius, K. A. 1975. Relative stability of the chloroplast envelope. Planta 127: 285 – 299.

Larcher, W. 2003. Physiological Plant Ecology. Fourth edition. Springer-Verlag, Berlin Heidelberg.

Lee, S. K. and Kader, A. A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Posth. Biol. and Technol. 20: 207 – 220.

Marais, S. J. S. 2005. Sunburn control in apple fruit. MScAgric thesis, University of Stellenbosch, Stellenbosch, South Africa.

Müller, P., Li, X. P. and Niyogi, K. K. 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125: 1558 – 1566.

Paull, R.E. 1990. Postharvest heat treatments and fruit ripening. Postharv. News Info. 1:355-363.

Parchomchuk, P. and Meheriuk, M. 1996. Orchard cooling with pulsed overtree irrigation to prevent solar injury and improve fruit quality of 'Jonagold' apples. HortScience 31: 802 – 804.

Pearcy, R. W. 1978. Effect of growth temperature on the fatty acid composition of the leaf lipids in Atriplex lentiformis (Torr.) Wats. Plant Physiol. 61:484-486.

Ritenour, M. A., Kochhar, S., Schrader, L.E., Hsu, T. and Ku, M. 2001. Characterization of heat shock protein expression in apple fruit peel under field and laboratory conditions. J. Amer. Soc. Hort. Sci. 126(5):564-570.

Salisbury, F. B. and Ross, C. W. 1992. Stress physiology. In: Plant Physiology. Wadsworth Publishing Company, Belmont, California, pp. 575 – 600.

Schrader, L. E., Zhang, J. and Duplaga, W.K., 2001. Two types of sunburn in apple caused by high fruit surface (peel) temperature. Online. Plant Health Progress. http://www.planthealthprogress.org/Current/research/sunburn/article.htm

Schreiber, U., Bilger, W. 1993. Progress in chlorophyll fluorescence research: Major developments during the past years in retrospect. Prog. Bot. 54: 151 – 173.

Song, J., Fan, L., Forney, C. F. and Jordan, M. A. 2001. Using volatile emissions and chlorophyll fluorescence as indicators of heat injury in apples. J. Amer. Soc. Hort. Sci. 126: 771 – 777.

Tian, M. S., Woolf, A. B., Bowen, J. H. and Ferguson, L. E. 1996. Changes in color and chlorophyll fluorescence of broccoli florets following hot water treatment. J. Amer. Soc. Hort. Sci. 21: 310 – 313.

Unrath. C. R. 1972. The quality of 'Red Delicious' apples as affected by overtree sprinkler irrigation. J. Amer. Soc. Hort. Sci. 97: 58 – 61.

Van Kooten, 0. and Snel, J. F. H. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. Phot. Res. 25: 147-150.

Wand, S. J. E., Steyn, W. J., Holcroft, D. M., Mdluli, M. J., van den Dool, K. and Jacobs, G. 2005. Use of evaporative cooling to improve 'Rosemarie' and 'Forelle' pear fruit blush colour and quality. Acta Hort. 671: 103-111.

Weiss, E. and Berry, J. A. 1988. Plants and high temperature stress, pp 329 – 346 In: Long SP, Woodward FI, eds. Plants and temperature. Cambridge: The Company of Biologists Limited. Symposia of the Society for Experimental Biology, No. 42.

Williams, J., Khan, M., Mitchell, K. and Johnson, G. 1988. The effect of temperature on the level and biosynthesis of unsaturated fatty acids in diacylglycerols of Brassica napus leaves. Plant Physiol. 87:904-910.

Woolf, A. B. and Laing, W. A. 1996. Avocado fruit skin fluorescence following hot water treatments and pretreatments. J. Amer. Soc. Hort. Sci. 121: 147 – 151.

Wünsche, J. N., Greer, D. H., Palmer, J. W., Lang, A. and McGhie, T. 2001. Sunburn—The cost of a high light environment. Acta Hort. 557:349-356.
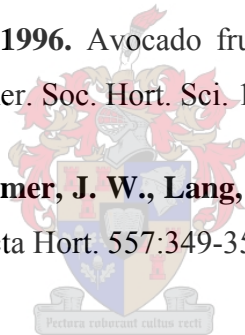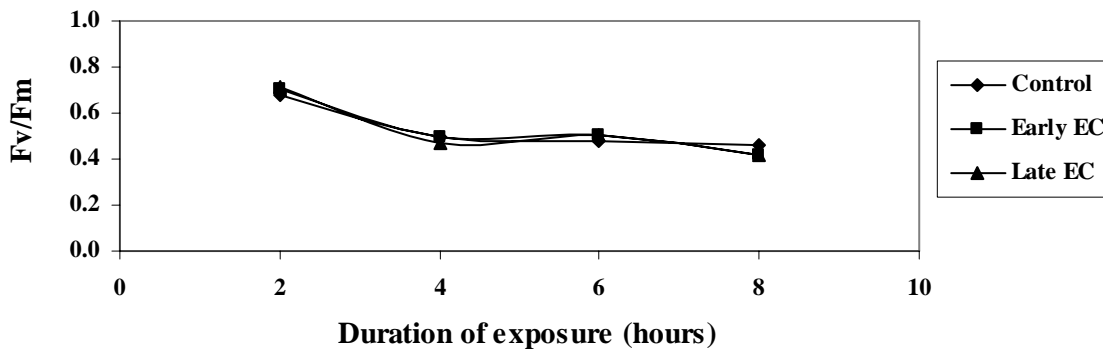
Figure 1a: The effect of duration of exposure to a range of temperatures on Fv/Fm of 'Cripps' Pink' apples on 3 April 2004 (Time*Temp Pr>F <0.0001)
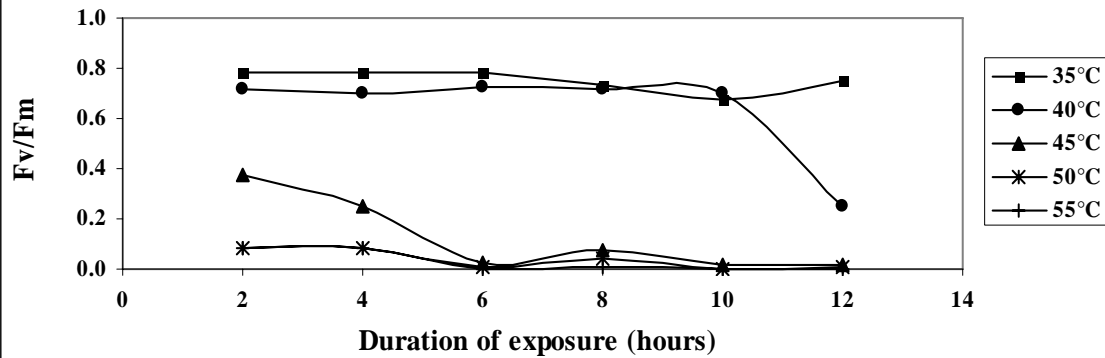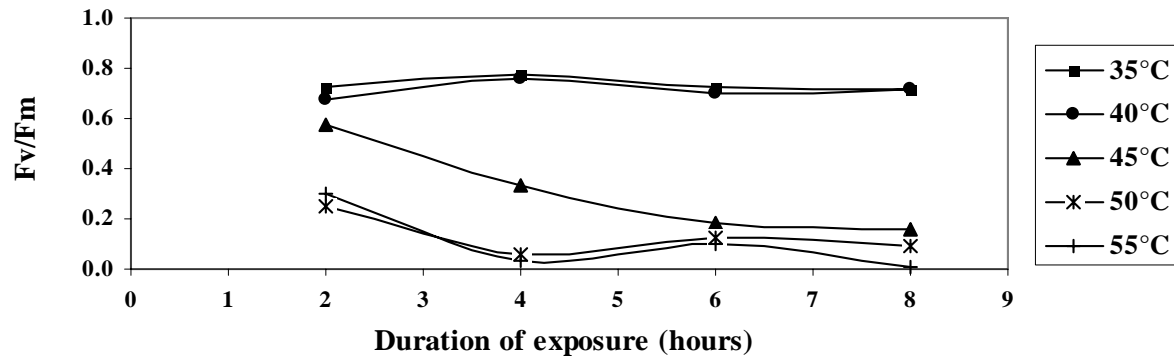
Figure 1b: The effects of temperature on Fv/Fm of 'Cripps' Pink' apples grown under three treatments (Trt*Temp Pr>F 0.0049)
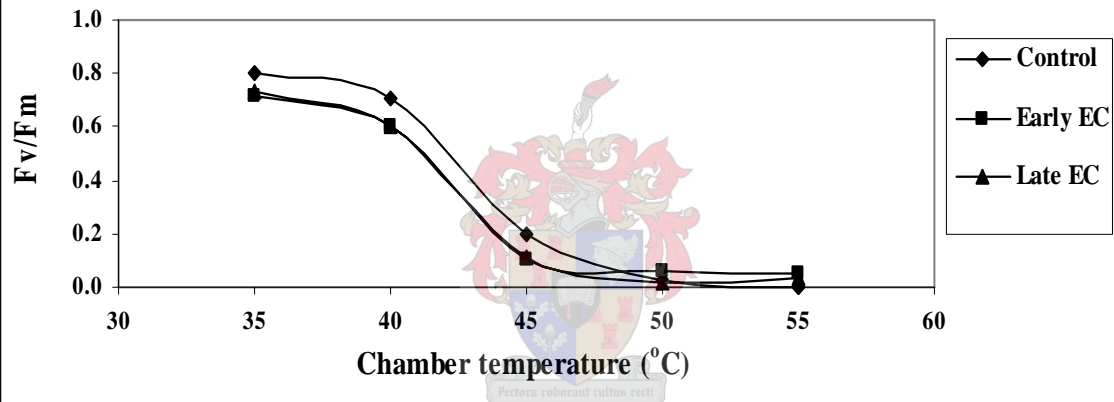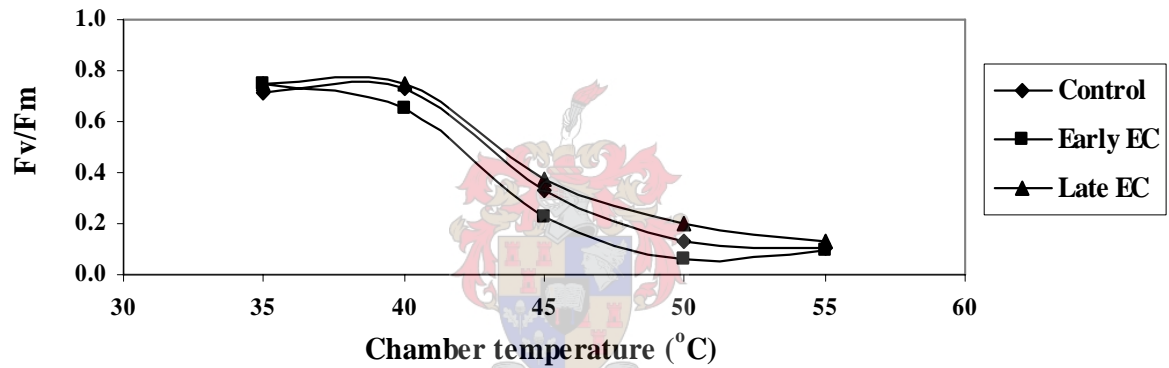
Figure 1c: The effect of duration of exposure on Fv/Fm of 'Cripps' Pink' apples grown under three treatments (Trt*Time Pr>F 0.0205)
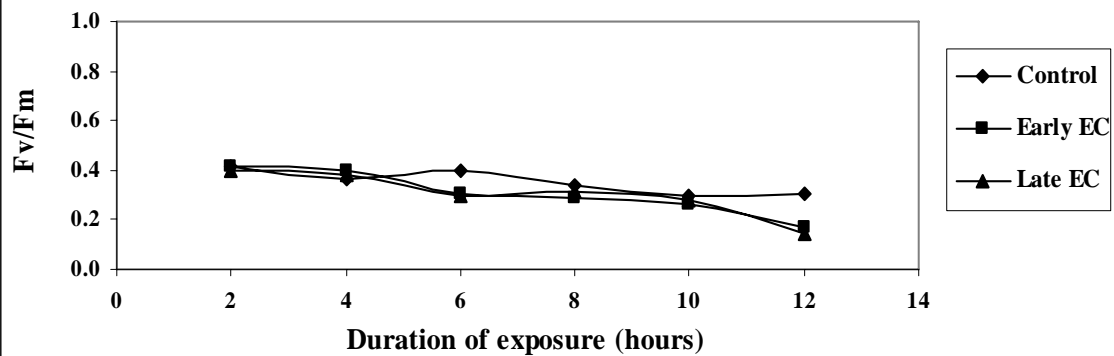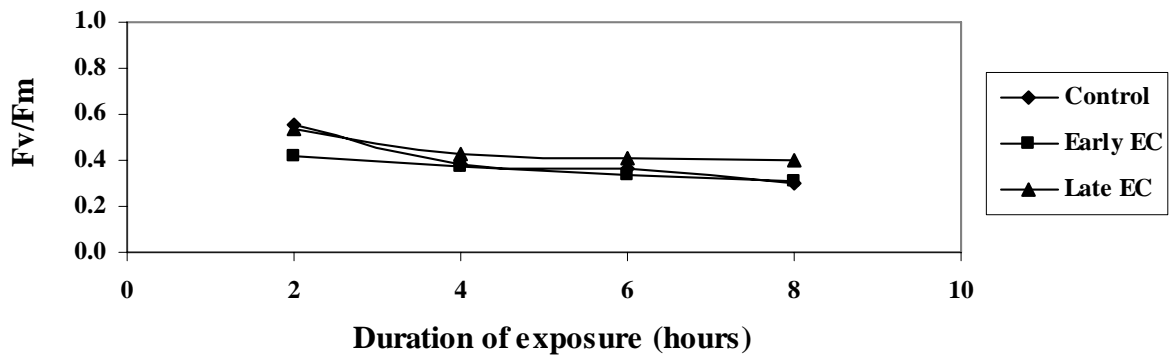
Figure 2a: Effect of exposure to different chamber temperatures on recovery of Fv/Fm on 4 April 2004 of 'Cripps' Pink' apples following treatments on 3 April 2004 (Time*Temp Pr>F <.0001)
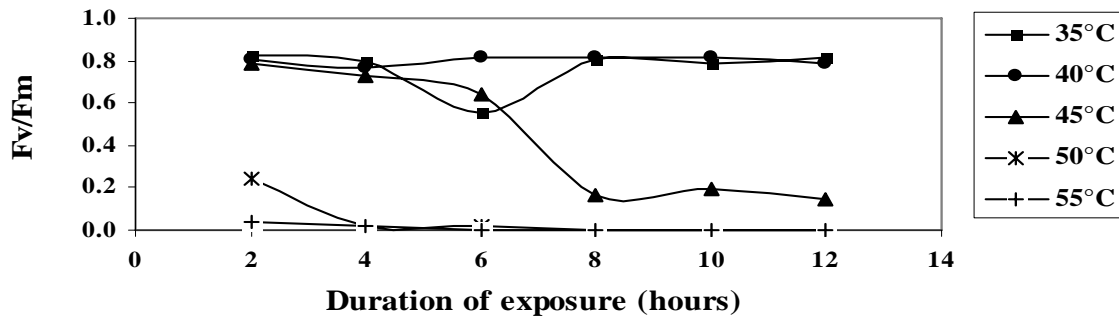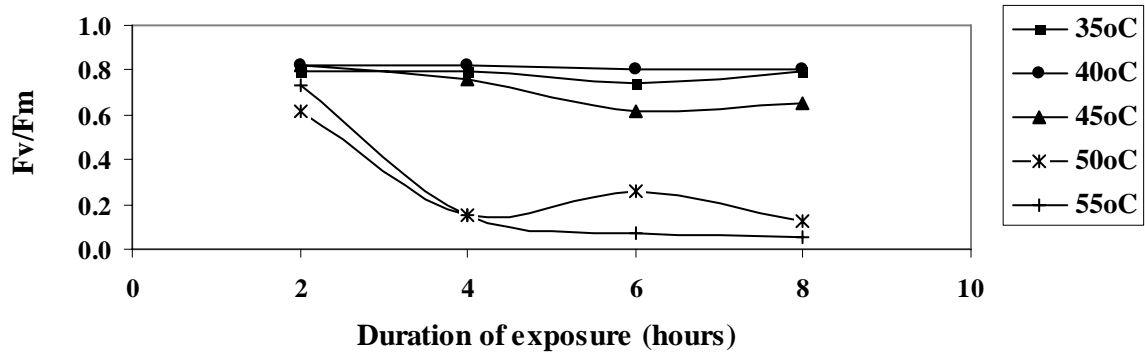

Figure 2b: Effect of different chamber temperatures on recovery of Fv/Fm on 4 April 2004 of 'Cripps' Pink' apples following treatments on 3 April 2004 (Trt*Temp Pr>F 0.0004)
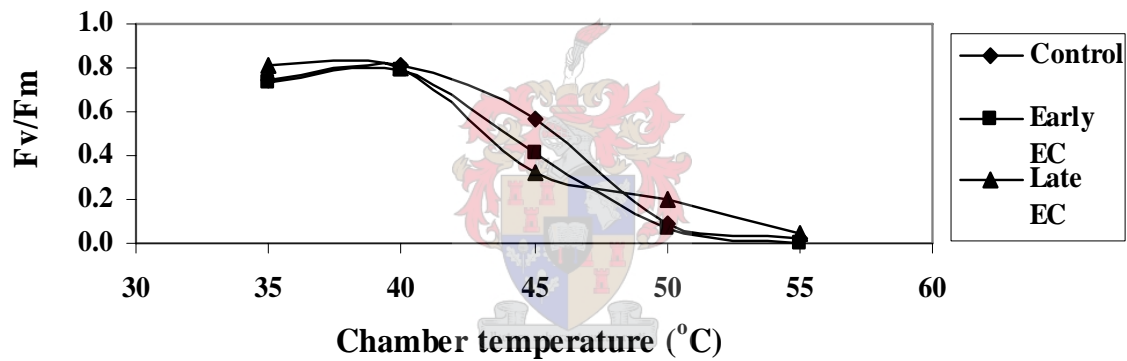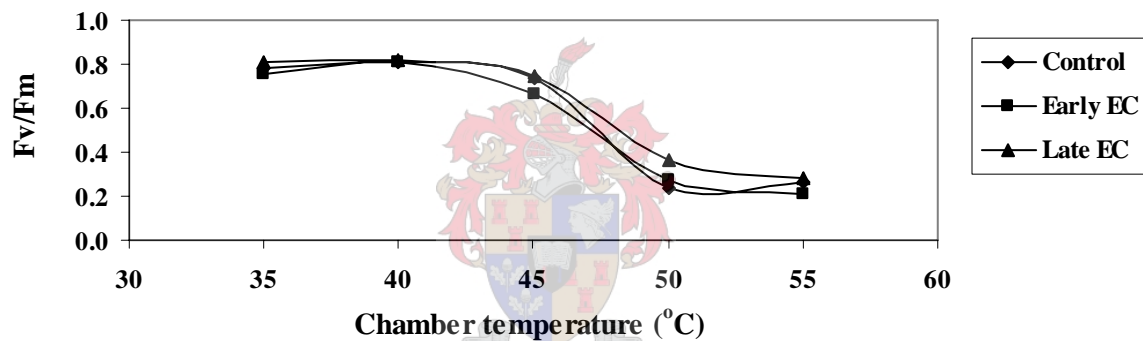

Figure 2c: Effect of duration of exposure to high temperatures on the recovery of Fv/Fm of control, early EC and late EC 'Cripps' Pink' apples on 4 April 2004 following treatments on 3 April 2004 (Time*Trt Pr>F 0.8627)
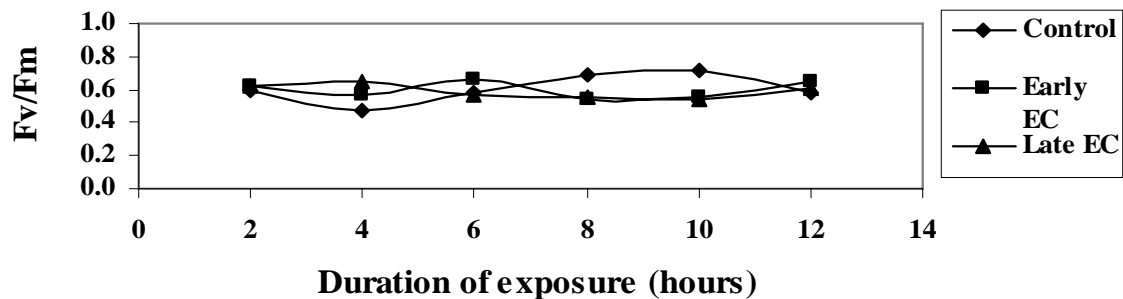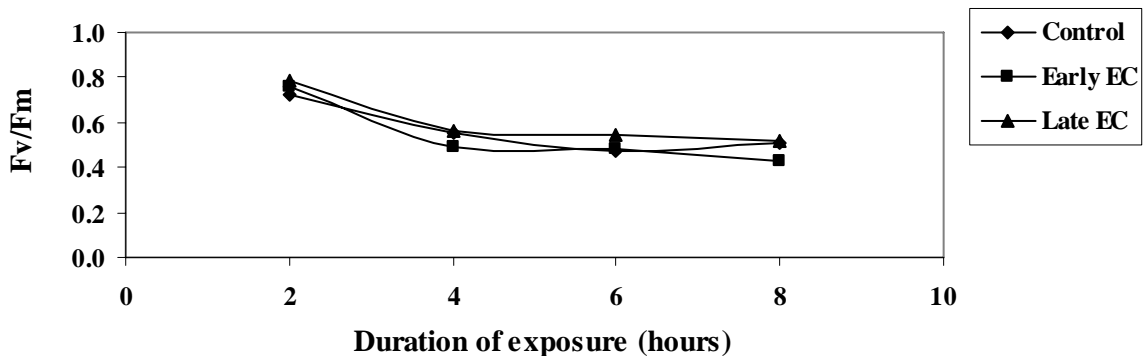
Figure 3a: The effect of duration of exposure to a range of temperatures on Fv/Fm of 'Cripps' Pink' apples on 8 April 2004 (Time*Temp Pr>F <0.0001)
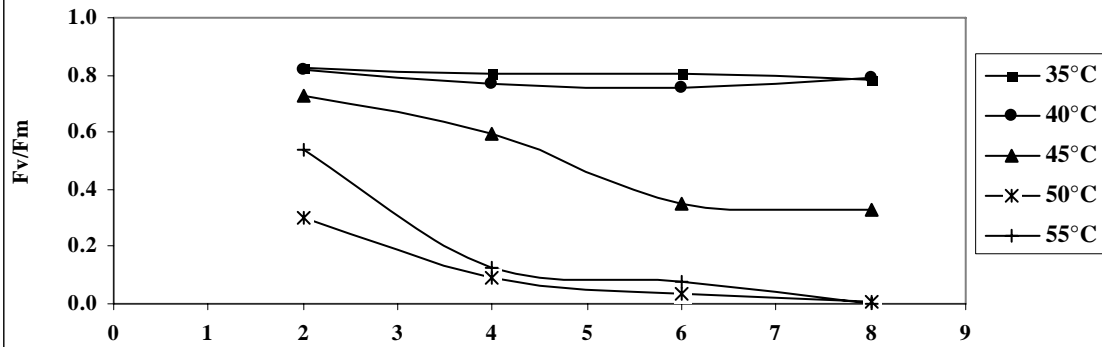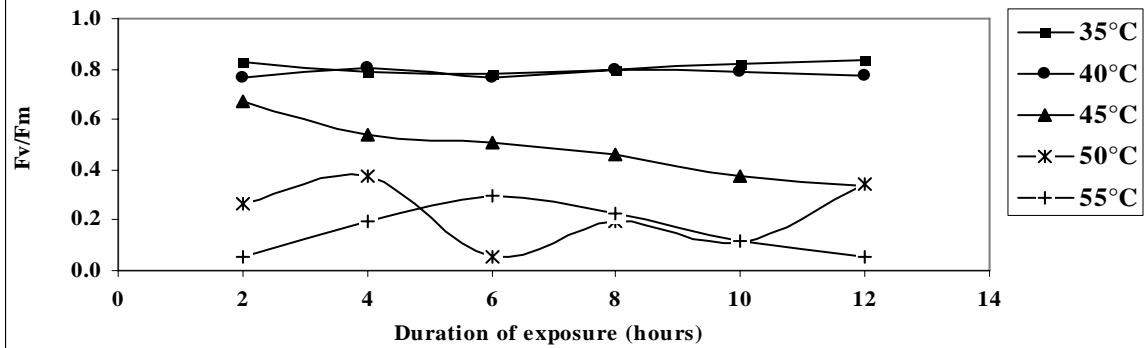
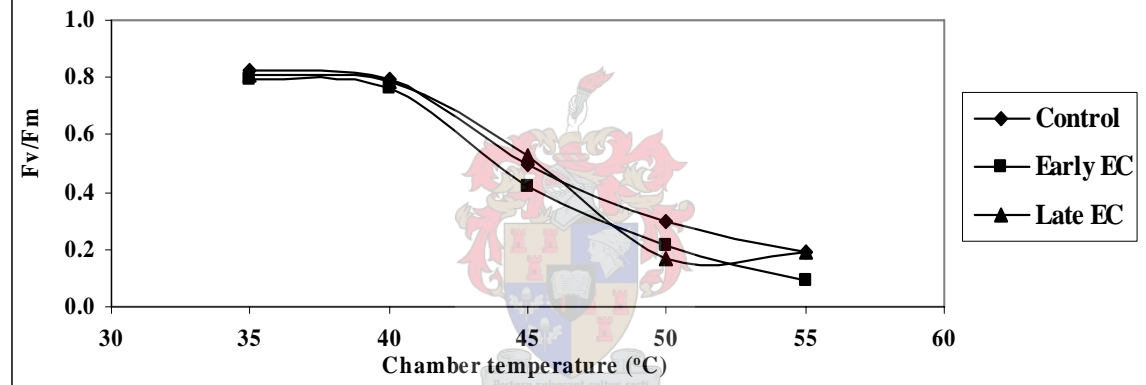Figure 3b: The effects of temperature on Fv/Fm of 'Cripps' Pink' apples grown under three treatments (Trt*Temp Pr>F 0.0003)
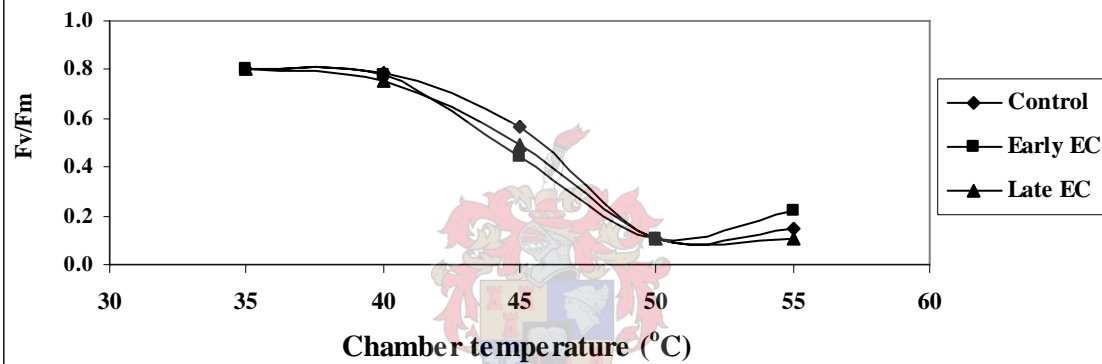
Figure 3c: The effect of duration of exposure on Fv/Fm of 'Cripps' Pink' apples grown under three treatments (Time*Trt Pr>F 0.0954)
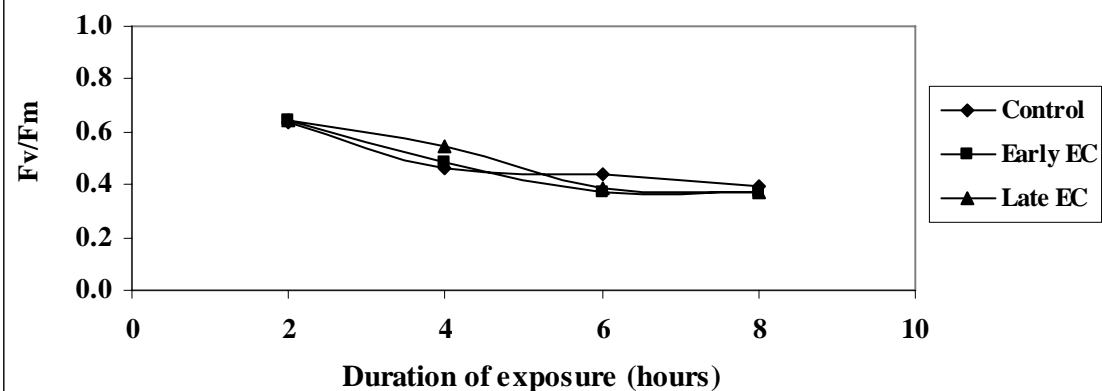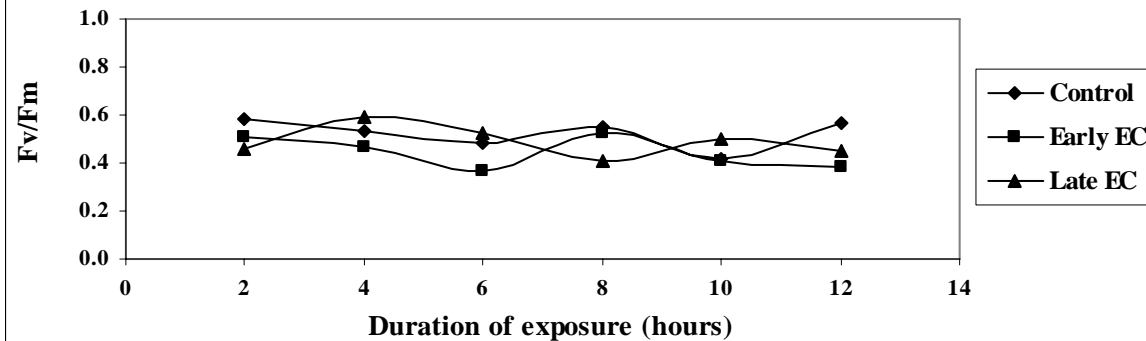
Figure 4a: Effect of duration of exposure to different chamber temperatures on recovery of Fv/Fm on 9 April 2004 of 'Cripps' Pink' apples following treatments on 8 April 2004 (Time*Temp Pr>F <.0001)
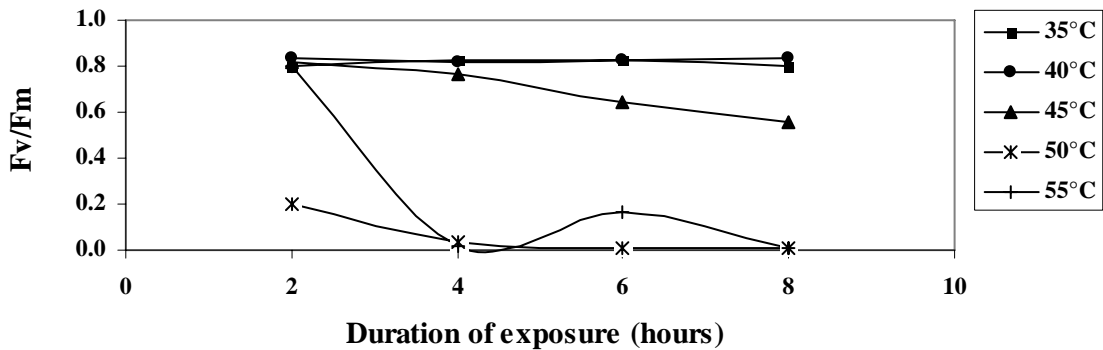
Figure 4b: Effect of different chamber temperatures on recovery of Fv/Fm on 9 April 2004 of 'Cripps' Pink' apples following treatments on 8 April 2004 (Trt*Temp Pr>F 0.0229)
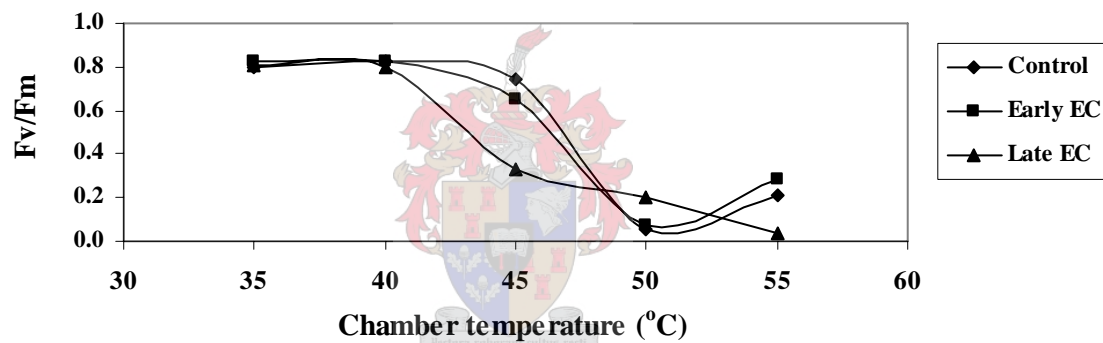
Figure 4c:Effect of duration of exposure to high temperatures on the recovery of Fv/Fm of control, early EC and late EC 'Cripps' Pink' apples on 9April 2004 following treatments on 8 April 2004 (Time*Trt Pr>F 0.3180).

Figure 5a: The effect of duration of exposure to a range of temperatures on
Fv/Fm of 'Cripps' Pink' apples on 13 April 2004 (Time*Temp Pr>F <0.0001)

Figure 5b: The effects of temperature on Fv/Fm of 'Cripps' Pink' apples grown
under three treatments (Trt*Temp Pr>F 0.2352)

Figure 5c: The effect of duration of exposure on Fv/Fm of 'Cripps' Pink'
apples grown under three treatments (Time*Trt Pr>F 0.1496)

Figure 6a: Effect of duration of exposure to different chamber temperatures on recovery of Fv/Fm on 14 April 2004 of 'Cripps' Pink' apples following treatments on 13 April 2004 (Time*Temp Pr>F <.0001)
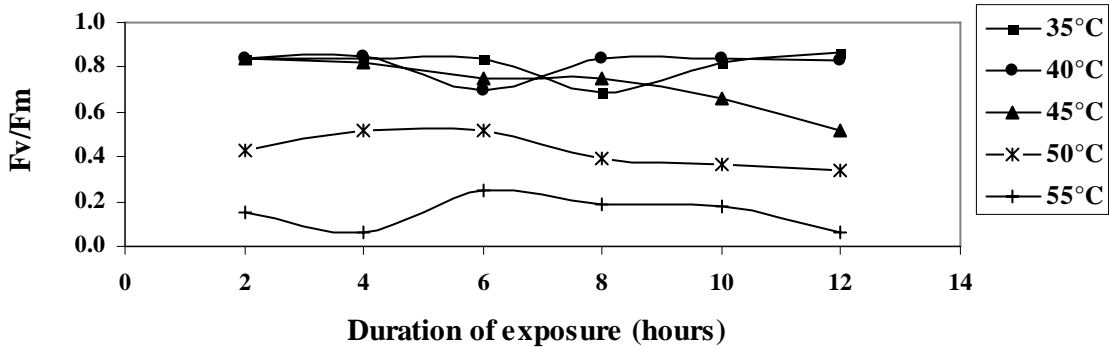
Figure 6b: Effect of different chamber temperatures on recovery of Fv/Fm on 14 April 2004 of 'Cripps' Pink' apples following treatments on 13 April 2004 (Trt*Temp Pr>F 0.6425)
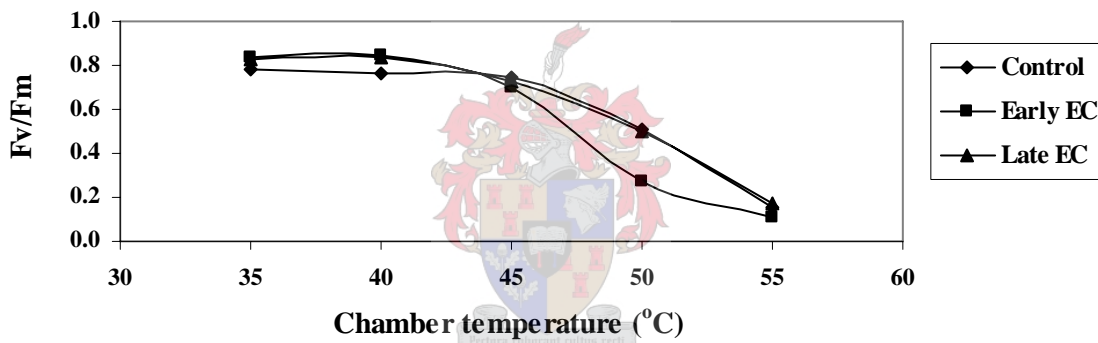
Figure 6c: Effect of duration of exposure to high temperatures on the recovery of Fv/Fm of control, early EC and late EC 'Cripps' Pink' apples on 14 April 2004 following treatments on 13 April 2004 (Time*Trt Pr>F 0.0954)
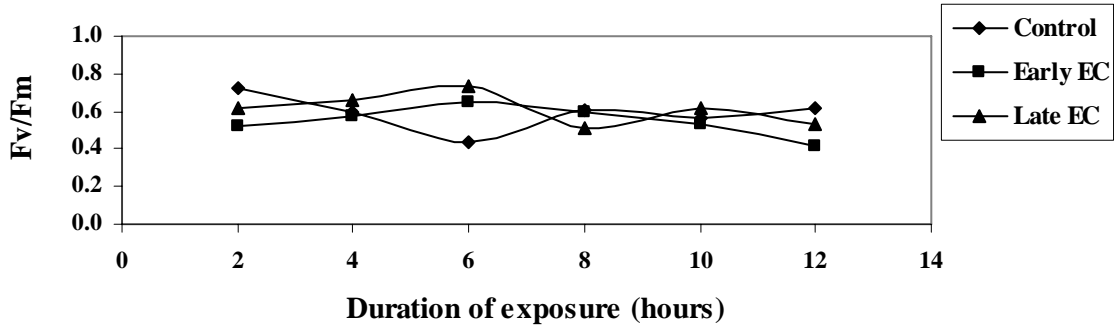
Figure 7a: The effect of duration of exposure to a range of temperatures on Fv/Fm of 'Royal Gala' apples on 28 January 2004 (Time*Temp Pr>F 0.0410)
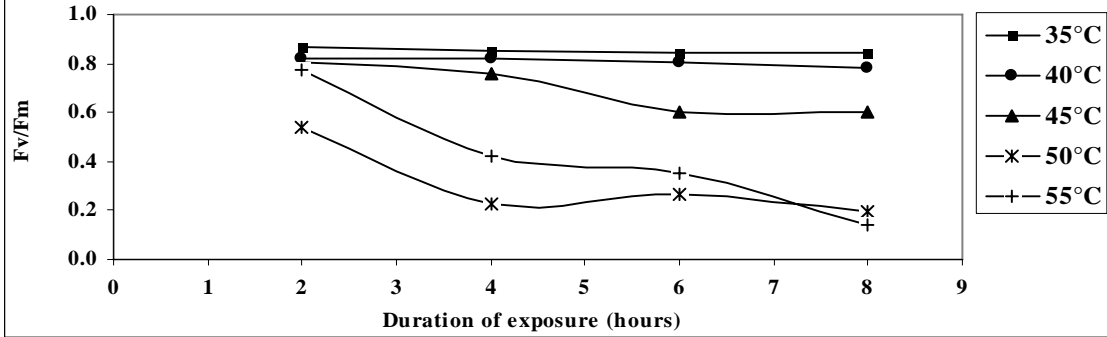
Figure 7b: The effects of temperature on Fv/Fm of 'Royal Gala' apples grown under three treatments (Trt*Temp Pr>F 0.8085)

Figure 7c: The effect of duration of exposure on Fv/Fm of 'Royal Gala' apples grown under three treatments (Time*Trt Pr>F 0.1655)

Figure 8a: Effect of duration of exposure to different chamber temperatures on recovery of Fv/Fm on 29 January 2004 of 'Royal Gala' apples following treatments on 28 January 2004 (Time*Temp Pr>F 0.2483)

Figure 8b: Effect of different chamber temperatures on recovery of Fv/Fm on 29 January 2004 of 'Royal Gala' apples following treatments on 28 January 2004 (Trt*Temp Pr>F 0.1313)

Figure 8c: Effect of duration of exposure to high temperatures on the recovery of Fv/Fm of control, early EC and late EC 'Royal Gala' apples on 29 January 2004 following treatments on 28 January 2004 (Time*Trt Pr>F 0.0029)

Figure 9a: The effect of duration of exposure to a range of temperatures on Fv/Fm of 'Royal Gala' apples on 4 February 2004 (Time*Temp Pr>F <0.0001

Figure 9b: The effects of temperature on Fv/Fm of 'Royal Gala' apples grown under three treatments (Trt*Temp Pr>F 0.7715)
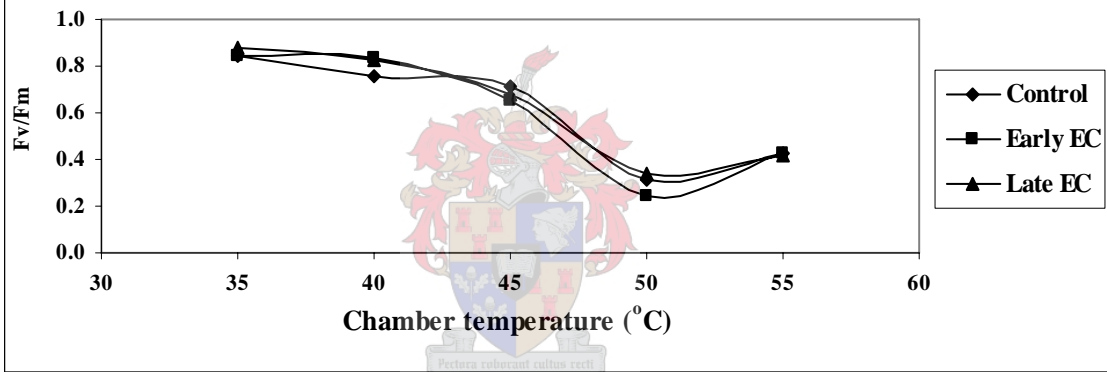
Figure 9c: The effect of duration of exposure on Fv/Fm of 'Royal Gala' apples grown under three treatments (Time*Trt Pr>F 0.9482)
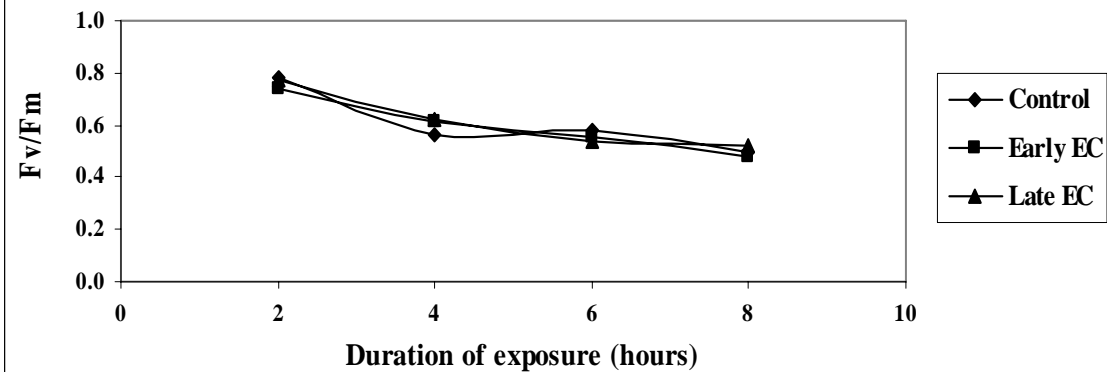
Figure 10a: Effect of duration of exposure to different chamber temperatures on recovery of Fv/Fm on 5 February 2004 of 'Royal Gala' apples following treatments on 4 February 2004 (Time*Temp Pr>F < 0.0001)
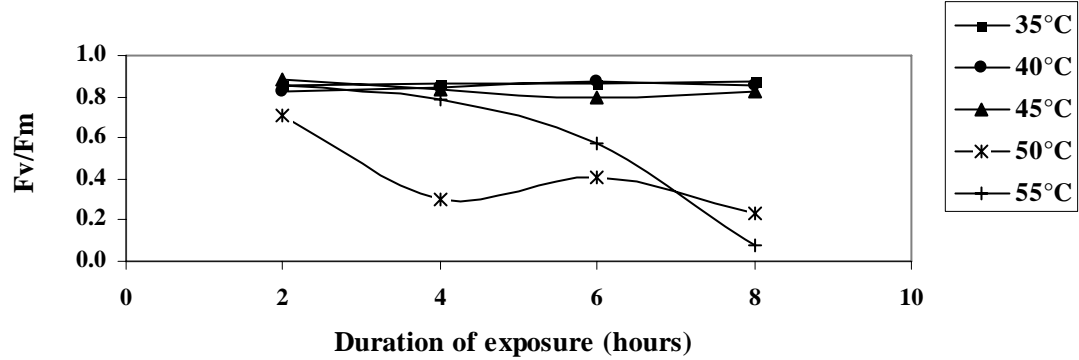
Figure 10b: Effect of different chamber temperatures on recovery of Fv/Fm on 5 February 2004 of 'Royal Gala' apples following treatments on 4 February 2004 (Trt*Temp Pr>F 0.4131)
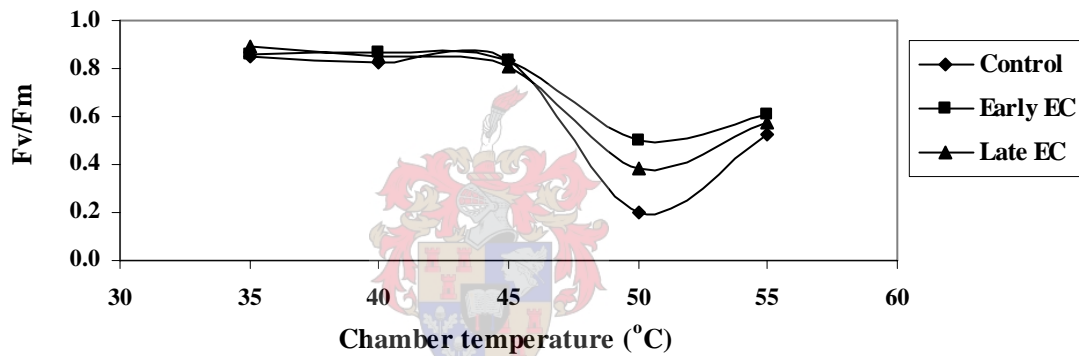
Figure 10c: Effect of different chamber temperatures on recovery of Fv/Fm on 5 February 2004 of 'Royal Gala' apples following treatments on 4 February 2004 (Time*Trt Pr>F 0.8701)
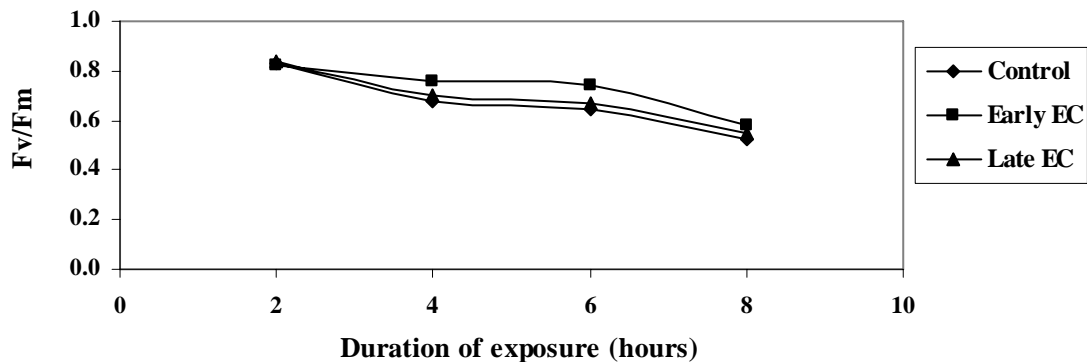
Table 1. Fruit surface temperature (°C) measured immediately after removal from ovens.

| Experiment	Oven set air temperature 35°C	Oven set air temperature 40°C	Oven set air temperature 45°C	Oven set air temperature 50°C	Oven set air temperature 55°C
'Cripps' Pink' 3 April 2004	33°C	38°C	45°C	48°C	52°C
'Cripps' Pink' 8 April 2004	32°C	37°C	43°C	49°C	50°C
'Cripps' Pink' 13 April 2004	34°C	38°C	43°C	48°C	53°C
'Royal Gala' 28 January 2004	32°C	37°C	43°C	46°C	52°C
'Royal Gala' 3 February 2004	32°C	36°C	43°C	47°C	49°C
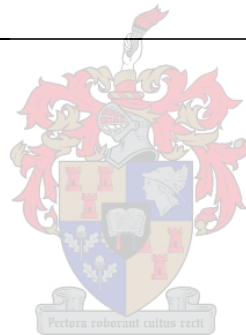
Table 2a. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), early and late evaporative cooling (trt) and chamber temperature (temp) for 'Cripps' Pink' PSII efficiency on 3 April 2004.

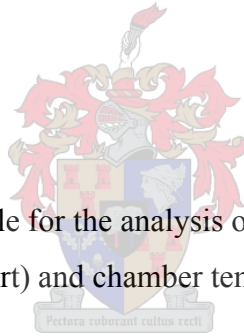|	Degrees of freedom	F-value	Pr>F
Model	89	76.8	<.0001
Time	5	55.6	<.0001
Trt	2	12.5	0.0004
Temp	4	1581.7	<.0001
Time*Trt	10	4.8	0.0205
Time*Temp	20	3.64	<.0001
Trt*Temp	8	1.42	0.0049
Time*Trt*Temp	40	1.9	<.0001

Table 2b. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), early and late evaporative cooling (trt) and chamber temperature (temp) for 'Cripps' Pink' PSII efficiency recovery on 4 April 2004.

	Degrees of freedom	F-value	Pr>F
Model	89	19.8	<.0001
Time	5	11.7	<.0001
Trt	2	3.52	0.0339
Temp	4	362.8	<.0001
Time*Trt	10	0.53	0.8627
Time*Temp	20	7.97	<.0001
Trt*Temp	8	4.03	0.0004
Time*Trt*Temp	40	1.27	0.1777

Table 4a. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), early and late evaporative cooling (trt) and chamber temperature (temp) for 'Cripps' Pink' PSII efficiency on 13 April 2004.

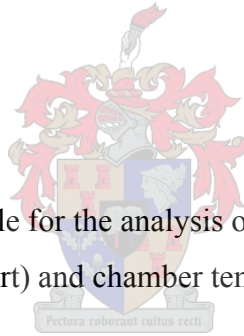|	Degrees of freedom	F-value	Pr>F
Model	59	22.7	<.0001
Time	3	19.9	<.0001
Trt	2	8.85	0.0003
Temp	4	280.8	<.0001
Time*Trt	6	1.61	0.1496
Time*Temp	12	6.92	<.0001
Trt*Temp	8	1.33	0.2352
Time*Trt*Temp	24	1.57	0.0587

Table 4b. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), early and late evaporative cooling (trt) and chamber temperature (temp) for 'Cripps' Pink' PSII efficiency recovery on 14 April 2004.

	Degrees of freedom	F-value	Pr>F
Model	59	17.7	<.0001
Time	3	47.3	<.0001
Trt	2	3.96	0.0215
Temp	4	177.3	<.0001
Time*Trt	6	0.89	0.5016
Time*Temp	12	12.2	<.0001
Trt*Temp	8	0.76	0.6425
Time*Trt*Temp	24	1.24	0.2253

Table 5a. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), early and late evaporative cooling (trt) and chamber temperature (temp) for 'Royal Gala' PSII efficiency on 28 January 2004.

	Degrees of freedom	F-value	Pr>F
Model	89	5.82	<.0001
Time	5	1.25	0.2937
Trt	2	2.69	0.0732
Temp	4	106.42	<.0001
Time*Trt	10	1.47	0.1655
Time*Temp	20	1.74	0.0410
Trt*Temp	8	0.56	0.8085
Time*Trt*Temp	40	0.67	0.9188

Table 5b. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), early and late evaporative cooling (trt) and chamber temperature (temp) for 'Royal Gala' PSII efficiency recovery on 29 January 2004.

	Degrees of freedom	F-value	Pr>F
Model	89	5.78	<.0001
Time	5	1.19	0.3208
Trt	2	1.83	0.1665
Temp	4	96.1	<.0001
Time*Trt	10	2.97	0.0029
Time*Temp	20	1.23	0.2483
Trt*Temp	8	1.62	0.1313
Time*Trt*Temp	40	1.32	0.1420

Table 6a. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), early and late evaporative cooling (trt) and chamber temperature (temp) for 'Royal Gala' PSII efficiency on 4 February 2004.

	Degrees of freedom	F-value	Pr>F
Model	59	7.59	<.0001
Time	3	20.7	<.0001
Trt	2	0.21	0.8086
Temp	4	76.3	<.0001
Time*Trt	6	0.27	0.9482
Time*Temp	12	4.36	<.0001
Trt*Temp	8	0.61	0.7715
Time*Trt*Temp	23	0.88	0.6293

Table 6b. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), early and late evaporative cooling (trt) and chamber temperature (temp) for 'Royal Gala' PSII efficiency recovery on 5 February 2004.

	Degrees of freedom	F-value	Pr>F
Model	58	9.83	<.0001
Time	3	27.8	<.0001
Trt	2	2.62	0.0775
Temp	4	77.7	<.0001
Time*Trt	6	0.41	0.8701
Time*Temp	12	12.1	<.0001
Trt*Temp	8	1.04	0.4131
Time*Trt*Temp	23	0.62	0.9049

GENERAL CONCLUSION

Sunburn causes serious economic losses in warm fruit producing areas all over the world. In South Africa, deciduous fruit producers in the Western Cape also lose a substantial percentage of fruit every year due to sunburn and related disorders, including insufficient colouring of blushed cultivars. In order to produce superior quality fruit, the producer must have a basic knowledge of the biology of and the factors affecting sunburn, as well as the different management practices influencing fruit colour. If used correctly, evaporative cooling could lower the incidence of sunburn on fruit in warm production areas, as well as improving colour of blushed cultivars. For apples, the system could be activated earlier in the season for colour, as anthocyanin synthesis increases with the season. Because pears tend to lose their colour before harvest, it is advisable to have an active system in the month before harvest. The system will, however, necessitate good control. Fruit acclimatise to lower temperatures and burn very easily when the system fails when temperatures are high. A good backup system should be in place in case of power failures or breakdown. Care should be taken to adapt irrigation scheduling to prevent under- or over-irrigation. The use of water for cooling is a luxury consumption of water. Because of frequent water shortages in the Western Cape, producers should be aware of pressure on the industry to conserve water.

There are many areas that can be improved upon in further studies. Colour measurements can be done on apples and pears, instead of just on apples as in this study. It is advisable to measure the colour, as well as ambient air temperatures every day for such measurements, as averages are not very reliable. It is also advisable to use temperature probes inserted into the fruit for measurement of fruit surface temperature. Temperatures of fruit exposed to direct sunlight can be significantly higher than that of the surrounding air, and it is therefore not reliable enough to measure ambient temperatures only. The experiments of paper two to determine temperature thresholds can be done on pears as well in further studies.