

Sunburn control in apple fruit

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DECLARATION

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

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SUMMARY

Sunburn on apple and pear fruit results in large losses of export volumes in South African orchards. Producers estimate that losses due to sunburn are often about 10-20%. Knowledge of temperature thresholds for sunburn as well as technologies available to manage sunburn is important to increase producer earnings.

The effect of evaporative cooling on fruit surface temperature and sunburn was evaluated on 'Cripps' Pink', 'Royal Gala' and 'Fuji' apples as well as 'Forelle' pears. Evaporative cooling was effective in reducing fruit surface temperature by 0.6-3.7°C. Apple fruit under evaporative cooling treatments appeared to have acclimatised and had significantly higher surface temperatures when dry (0.9-4.7°C) than non-cooled fruit. This acclimitasing effect could be due to a modification of the cuticular wax layer. Futher trials are needed to investigate the cause of this acclimatising effect. The acclimatising effect was not found on 'Forelle' pears. Sunburn was reduced significantly only on 'Royal Gala' apples.

Spraying 'Braeburn', 'Granny Smith', and 'Fuji' apples with reflective kaolin-based particle film (Surround WP) significantly reduced sunburn on 'Braeburn', 'Granny Smith' and 'Fuji' apple cultivars. Surround WP applications reduced leaf stomatal conductance and photosynthetic rate. Four applications of Surround WP with a commercial blower sprayer increased total soluble solids in 'Braeburn'. Four applications with a blower and five applications with a tower sprayer resulted in increased fruit size, higher flesh firmness, and lower percentage starch breakdown in 'Granny Smith'. Surround WP treatments slightly improved fruit colour in 'Granny Smith' apples. Futher trials are needed to establish whether Surround WP can be reliably and economically removed from apple fruit and to evaluate the storage quality of fruit cleansed in this way.

The effect of fruit surface temperatures (24°C, 30°C, 33-35°C, 40-43°C and 48-49°C) for increasing periods of time (two, four, six or eight hours) on maximum quantum yield of fluorescence (F_v/F_m) was measured on exposed and shaded sides of detached 'Cripps' Pink' and 'Royal Gala' apples. Measurements were taken directly

after removal from the chamber as well as 12 hours later to assess recovery. Permanent damage to Photosystem II (PSII) occurred between 48-49°C irrespective of duration, with permanent damage possible at 42-43°C when exposure exceeded six hours. There were no meaningful differences in Fv/Fm between shaded and exposed sides of apple fruit after exposure to high temperature. Duration of exposure was important in lowering PSII efficiency in 'Cripps' Pink' but less so in 'Royal Gala'. 'Cripps' Pink' apples harvested later in the season appeared to be able to recover from heat stress better than apples harvested earlier in the season. However, we only studied the effects of high temperature on apple tissues. More research is needed to establish the reaction of apple tissues to high temperatures in the presence of sunlight.

OPSOMMING

Sonbrand op appel en peervrugte kom jaarliks voor in Suid-Afrikaanse boorde en lei tot groot afnames in vrugte wat geskik is vir die uitvoermark. Producente beraam dat verliese a.g.v. sonbrand jaarliks 10-20% beloop. Kennis van drempelwaarde temperature vir die ontwikkeling van sonbrand sowel as die tegnologieë wat beskikbaar is om sonbrand te bekamp, is van groot belang om uitpakte te verbeter.

Die effek van oorhoofse besproëing op vrugtemperature en sonbrand van 'Cripps' Pink', 'Royal Gala' en 'Fuji' appels sowel as 'Forelle' pere is ondersoek. Oorhoofse besproëing het vrugtemperature betekenisvol verlaag in al die proewe. Dit wil voorkom of appels onder oorhoofse besproëing geakklimatiseer het en dié appels het hoër skil temperature geopenbaar wanneer hulle droog was as die kontrole vrugte. Moontlik is die effek te wyte aan 'n verandering in die kutikulêre waslaag. Verdere proewe om hierdie verskynsel te probeer verklaar is nodig. Die effek is nie waargeneem op die 'Forelle' pere nie. Sonbrand is betekenisvol verminder op 'Royal Gala' appels.

Toediening van weerkaatsende kaolin-gebaseerde partikels (Surround[®] WP) het sonbrand betekenisvol verlaag op 'Braeburn', 'Granny Smith', en 'Fuji' appels. Surround[®] WP toedienings het blaarhuidmondjie-geleiding en die tempo van fotosintese verlaag. Vier toedienings van Surround[®] WP met 'n kommersiële waaierblaser het totale oplosbare vastestowwe (TOVS) beduidend verhoog in 'Braeburn' appels. Vier toedienings met 'n waaierblaser en vyf toedienings met 'n toring spuitkar het gelei tot 'n verhoging in vrugmassa en vrugfermheid, en 'n verlaging in persentasie styselafbraak in 'Granny Smith' appels. Surround[®] WP toedienings het gelei tot effens beter kleur op 'Granny Smith' appels. Verdere proewe is nodig om te bepaal of Surround[®] WP op 'n betroubare en ekonomiese wyse van vrugte verwyder kan word in die pakstoor, en wat die hou-vermoë is van appels wat só behandel is.

Die effek van vrugoppervlaktemperature (24°C, 30°C, 33-35°C, 40-43°C en 48-49°C) vir toenemende tydsdure (twee, vier, ses en agt ure) op maksimum kwantum-opbrengs

van fluoressensie is gemeet op sonkante en skadukante van geplukte 'Cripps' Pink' en 'Royal Gala' appels. Metings is geneem direk na verwydering uit die oonde sowel as 12 ure later om moontlike herstel waar te neem. Permanente skade aan Fotosistiem II (PSII) het plaasgevind tussen 48-49°C ongeag tydsduur, met permanente skade ook moontlik by 42-43°C wanneer blootstelling aan genoemde temperature ses ure oorskry het. Daar was geen betekenisvolle verskille in Fv/Fm tussen skadu- en sonkante van appels na blootstelling aan hoë temperature nie. Die tydsduur van blootstelling was belangrik om PSII effektiwiteit te verlaag in 'Cripps' Pink', maar minder so in 'Royal Gala'. Dit wil voorkom of 'Cripps' Pink' appels later in die seisoen beter daartoe in staat is om te herstel van hitte stres as vroeër in die seisoen. Ons het egter slegs die effek van hoë temperature op appel weefsels ondersoek. Verdere proewe is nodig om die reaksie van appelweefsels op hoë temperature in die teenwoordigheid van sonlig te ondersoek.

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1. LITERATURE REVIEW

1.1 Introduction

What is stress? In 1972 Jacob Levitt proposed a definition of biological stress derived from physical science. Physical stress is any force applied to an object, strain is the change in the object's dimensions caused by the stress (Levitt, 1972). Levitt suggested that biological stress is any change in environmental conditions that might reduce or adversely change a plant's growth or development; biological strain is the reduced or changed plant function (Levitt, 1972). Furthermore, Levitt (1980) distinguished between avoidance and tolerance (hardiness) to any given stress factor. When using avoidance strategies, the organism responds by somehow reducing the impact of the stress factor. If the plant develops tolerance, on the other hand, it simply tolerates or endures the adverse environment.

Larcher pointed out that Levitt's concept works best when we are dealing with individual stress factors, although stress responses are typically caused by more than one stress factor (Larcher *et al.*, 1990). Hot summer weather, for example, may produce stress factors of high light levels (photodestruction of chlorophyll), low humidity, dry soil and high temperatures. Furthermore, stress responses are typically complex, are exhibited by various parts of the plant, and may involve such stress hormones as abscisic acid (ABA) and ethylene, which are distributed throughout the plant (Salisbury & Ross, 1992).

Apple and pear trees grown in South Africa are often exposed to heat stress due to high summer temperature, and light stress due to high light intensity which is a result of the latitude of the growing areas. The aims of this thesis are to study responses to high temperature and intense light on apple and pears, and to evaluate methods of reducing the effects of these climatic conditions on pome fruit. The literature study first discusses heat and light stress, and then reviews sunburn, which is caused by high temperature in the presence of light. The physiology, adaptations to, and the environmental and cultural factors that play a role in the development of sunburn are discussed, after which follows a review on the management options available to minimise sunburn.

1.2 Heat stress

Mention of high temperature stress usually brings to mind studies on thermophilic organisms that inhabit hot springs and seed plants occurring in hot deserts (Turner & Kramer, 1980). These plants can survive temperatures of 50°C or higher (Turner & Kramer, 1980). However, we tend to ignore the plants that suffer high temperature stress at temperatures often encountered during the growing season in temperate zones (Turner & Kramer, 1980). For example, many plants from alpine habitats are quite unable to survive hot summer weather at low altitudes, and cool season crops such as broccoli, lettuce, and English peas do poorly at midsummer (Turner & Kramer, 1980). This is partly because such basic physiological processes as dark respiration and photosynthesis are affected directly by temperature (Turner & Kramer, 1980). Larcher (1995) distinguishes between two types of heat resistance in eukaryotes; heat sensitive species and relatively heat resistant species. Heat sensitive species are injured at 30-40°C, or at the most 45°. Relatively heat resistant plants are from sunny and dry localities and are as a rule able to acquire hardiness to heat and survive heating of 50-60°C for half an hour.

1.2.1 Physiological effects of heat stress on plants

The effect of temperature on photosynthesis depends on the species, the environmental conditions under which the plant was grown, and the environmental conditions during measurement (Turner & Kramer, 1980). The maximum rate of photosynthesis occurs at 20 to 30°C in most temperate zone crop plants and as high as 35°C in some tropical plants (Turner & Kramer, 1980). However, the rate of dark respiration tends to increase with rising temperature up to a point at which injury to the protoplasm begins to occur (Turner & Kramer, 1980). Desert species have higher temperature optima than do arctic or alpine species, and desert annuals that grow during the hot summer months (mostly C₄ species) have higher optima than those that grow during the winter months (mostly C₃ species) (Turner & Kramer, 1980). On apple trees, temperatures of around 35°C in early spring may be stressful for apple trees with tender foliage while these effects could be minimal in mid-summer (Lakso, 1994). In general, optimum temperatures for photosynthesis are similar to the daytime temperatures at which the plants normally grow, except that in cold environments the optima are usually higher than air temperatures (Salisbury & Ross, 1992). When temperatures are above the optimum for photosynthesis, there is a strong decline in net gas exchange for the whole tree due to the

simultaneous decline in carbon fixation and increase in respiration. The response of the tree to such a potential limitation is dependant on the state of the carbon balance at the time of the heat stress (Lakso, 1994). If the trees are exposed to heat stress up to twenty days after full-bloom, fruit size may be reduced (Bergh, 1990).

Normal temperature increases have little influence on the light driven splitting of H₂O or on diffusion of CO₂ into the leaf, but they more markedly influence biochemical reactions of CO₂ fixation and reduction (Hall & Keys, 1983). Thus increases in temperature usually increase photosynthetic rates until enzyme denaturation and photosystem destruction begin (Hall & Keys, 1983). However, respiratory CO₂ loss also increases with temperature, and this is especially pronounced for photorespiration, largely because a temperature rise increases the ratio of dissolved O₂ to CO₂ (Hall & Keys, 1983).

Plants typically die when exposed to temperatures of 44 to 50°C, but some can tolerate higher temperatures (Salisbury & Ross, 1992). Eukaryotic organisms have not been found growing at temperatures above 56 to 60°C (temperatures of hot springs that support green algae) (Salisbury & Ross, 1992). Several dry spores and seeds of higher plants will survive temperatures well above 100°C, but these do not actively grow at such temperatures. In general, dry and dormant structures withstand various stresses well (Salisbury & Ross, 1992).

The harmful effects of high temperatures on higher plants occur primarily in photosynthetic functions, and the thylakoid membranes, particularly the photosystem II complexes located on these membranes, are apparently the most heat sensitive part of the photosynthetic mechanism (Weiss & Berry, 1988). Long-term acclimations can be superimposed on a more adaptive adjustment to high temperatures that occur in a few hours (Salisbury & Ross, 1992). In addition to the heat effects on primary photochemical reactions, there is evidence that Rubisco and other enzymes of carbon metabolism are adversely affected (Salisbury & Ross, 1992). Energy consumed by photorespiration can exceed that accumulated by CO₂ assimilation (Salisbury & Ross, 1992). Light and other factors can cause an increase in tolerance to heat, but plants' acclimation to high temperatures is minimal (a few degrees), compared to acclimation to drought or to freezing temperatures (Salisbury & Ross, 1992). For example, soybean seedlings exposed

for 2 h to 40°C are subsequently able to survive an otherwise lethal 2 h exposure to 45°C (Salisbury & Ross, 1992). This small acclimation could nevertheless be significant because high temperature extremes may exceed normal high temperatures by only a few degrees (Salisbury & Ross, 1992).

Plants that are hardy to high temperatures exhibit high levels of water hydration and high protoplasmic viscosity, characteristics that are also exhibited by xerophytes (plants that have physical adaptations to low moisture environments and are adapted to several dry spells per year) (Salisbury & Ross, 1992). High temperature adapted plants also are able to synthesise heat shock proteins at high rates when temperatures become elevated, allowing synthetic rates to equal breakdown rates and thereby avoiding ammonia poisoning (Salisbury & Ross, 1992). These features were observed long ago, and some of them may be functions of heat shock proteins (Salisbury & Ross, 1992).

1.2.2 Heat shock proteins

Organisms ranging from bacteria to humans respond to high temperatures by synthesising a new set of proteins, the heat shock proteins (HSP's) (Salisbury & Ross, 1992). Some HSP's have relatively high molecular mass, as much as 70 kilodaltons, but are usually in the region of 15-27 kDa (Salisbury & Ross, 1992). Proteins with a molecular mass of 15-42 kDa are designated small heat shock proteins (sHSP's) and due to the rapidity with which they can be expressed they seem to be important in helping plants adapt quickly to changing conditions (De Rocher *et al.*, 1991; Waters *et al.*, 1996). The HSP's appear rapidly, often becoming a substantial portion of the total proteins within 30 minutes after an abrupt shift from, for example, 28°C to 41°C. Their synthesis continues during the next three to four hours, but after eight hours the pattern of synthesis is essentially the same as it was at the initial low temperature. The HSP's also appear when the increase in temperature is more gradual, as might occur under natural conditions. Three or four hours after return to the normal temperature, HSP's are no longer produced, but many of the HSP's are still present, indicating that they are quite stable. Heat shock mRNA's have also been studied. The kinetics of their appearance and disappearance matches that of the HSP's in the expected way (Salisbury & Ross, 1992).

It is becoming apparent that the HSP's play a role in heat tolerance, perhaps by protecting essential enzymes and nucleic acids from heat denaturation (Burke & Orzech, 1988). The role played by HSP's in heat tolerance is apparently that of stabilising chromatin structures and membranes and promoting repair mechanisms (Larcher, 1995). Without such protection, nucleic acids might be cleaved by specific metal ions that leak into the cytoplasm from outside (or from the vacuole) as membranes become more permeable at higher temperatures (Burke & Orzech, 1988).

In a study on *Prosopis chilensis* and *Glycine max* 'McCall', Ortiz & Cardemil (2001) found that both species had a lethal temperature of 50°C when germinated at 25°C, but both species were able to grow at 50°C after germination at 35°C. Membrane damage determinations in leaves showed that *P. chilensis* has an LT50 (temperature at which fifty percent of a population would die) 6°C higher than that of soybean. There were no differences in the quantum yield of fluorescence (Fv/Fm) between both plants when the temperatures were raised. *P. chilensis* showed higher relative levels of free ubiquitin and HSP70 than soybean seedlings when the temperatures were raised. Time course studies of accumulation rates of ubiquitin, conjugated ubiquitin and HSP70 were higher in *P. chilensis* than in soybean. In both plants, free ubiquitin decreased during the first 5 min and increased after 30 min of heat shock, conjugated ubiquitin increased after 30 min and HSP70 began to increase dramatically after 20 min of heat shock. The authors concluded from these findings that *P. chilensis* is more resistant to heat stress than soybean. Al-Niemi & Stout (2002) found that sHSPs as well as HSP's were expressed after exposure to 40°C for two hours in the perennial grass *Dichanthelium lanuginosum*, and that these elevated levels of sHSP's and HSP's persisted for 5-7 days after the heat treatment.

In a study on papayas, Paull & Jung Chen (1990) found that exposing fruit to 42°C for 4 hours or 38-42°C for one hour followed by three hours at 22°C resulted in the development of thermotolerance to the otherwise injurious heat treatment of 49°C for 70 minutes. Paull & Jung Chen (1990) found that polypeptides with molecular weights of 17, 18, and 70 kDa showed marked increases after two hours at 38°C. Takeda *et al.* (2003) found that HSP's were expressed at 45°C in strawberries. In a study on 'Fuji', 'Jonagold' and 'Gala' apples, Ritenour *et al.* (2001) found that apples exposed to direct sunlight accumulated higher levels of sHSP's than apples

grown in shaded parts of the canopy. Ritenour *et al.* (2001) also found that sHSP's started to disappear first in 'Gala', and last in 'Fuji', suggesting that maturity could play a role in regulating sHSP accumulation.

1.3 High light stress

Even though light is essential for plants, sunlight also contains harmful short wavelength ultraviolet rays which are potentially dangerous due to their destructive effects on amino acids, nucleic acid bases, or membrane lipids. About 4% of the energy contained in sunlight is present in wavelengths shorter than 400 nm, and are referred to as ultraviolet (UV) rays. Photochemical damage in cells start when a UV absorbing molecule absorbs the electronic energy of a photon. Photons absorbed from UV wavelengths have sufficient energy to break covalent bonds, although it is unusual for their energy to be absorbed that efficiently. Usually the absorbed energy transforms the target molecule to an electronically excited state, often with a different and rather unstable electron configuration. This initial excited state can be deactivated by fluorescence, it may react with neighbouring molecules, or it may be transformed into a longer lived triplet excited stage. This triplet stage has the potential to interact with molecular oxygen and form singlet oxygen, $^1\text{O}_2$, which is harmful to several amino acids, and reacts with polyunsaturated fatty acids to form lipid hydroperoxides. These peroxides are likely to damage cell and organelle membranes (Larson, 1988). Plants have developed defence mechanisms such as antioxidants (flavonoids, β -carotene, vitamins C and E), and enzymes (superoxide dismutase, catalase, peroxidase) which can "scavenge" free radicals (Wünsche *et al.*, 2004). The role of antioxidants in protecting plant tissues is discussed in section 4.2.

1.4 Sunburn

The terms sunburn and sunscald have been used interchangeably in the literature, and this has caused confusion. The American Phytopathological Society defined sunburn as damage to fruit by exposure to solar radiation, and defined sunscald as injury to the bark and underlying tissues caused by freezing (Schrader *et al.*, 2001) A closely related term is superficial scald, a physiological disorder which sometimes occurs in apples after prolonged storage. Schrader *et al.* (2001) describes sunburn as a physiological disorder that causes a necrotic lesion on the fruit and a browning of the flesh underneath. Sunburn symptoms usually appear on the exposed side of the

fruit, are initially white, tan or yellowed patches and severely injured areas can turn dark brown with cortex tissue that become spongy and sunken (Wünsche *et al.*, 2004).

Sunburn on apples occurs annually in our orchards to a larger or lesser degree (Bergh *et al.*, 1980). The most obvious symptom is discolouration of the skin exposed to the intense midday sun (Andrews & Johnson, 1996). Sunburn injury is worst on the apple surface exposed to the direct rays of the sun. In some cultivars, notably 'Fuji', susceptibility to sunburn escalates once the skin colour begins to change from green to red (Wilton, 1999). As a general rule sunburn sensitivity increases as harvest approaches. Early cultivars such as 'Royal Gala' show sunburn symptoms at earlier maturity than later varieties (Wilton, 1999). Severest damage occurs on fruit which becomes suddenly exposed. The fruit is not as well acclimatised to direct sun as that which has developed for the whole growing season in direct sunlight (Wilton, 1999). Branch movement occurs as fruit size up; this alters fruit exposure to light and causes sheltered fruit to be suddenly exposed to direct sunlight (Wilton, 1999). The fruit burns more quickly than fruit which has been well exposed to sun for most of the growing season. Data gathered on fruit surface temperatures show that fruit surfaces exposed to direct sunlight can be as much as 15-20 percent higher than ambient air temperatures (Wilton, 1999).

During the 1970's, losses as a result of sunburn amounted to between 3-10% of the rejections of packed cartons (Bergh *et al.*, 1980). Statistics obtained from the Deciduous Fruit Board for the 1977/78 season showed that rejections for skin discolouration amounted to approximately 12% of total rejections (Bergh *et al.*, 1980). Another drawback of sunburn damage is the change in colour during cold storage from white to pale yellow, particularly in 'Granny Smith' (Bergh *et al.*, 1980). This causes an unattractive appearance and further losses after storage could amount to as much as 3.4% (Bergh *et al.*, 1980). It is estimated that sunburn could cost Washington State producers \$100 million a year (Warner, 1997a). In Australia in the 1990-91 season sunburn accounted for 3.22% of downgrading in Delicious, 6.66% in 'Granny Smith', 3.54% in 'Jonathan' and 5.10% in 'Bonza' (Dodds *et al.*, 1994). In all cases the amount of downgrading due to sunburn was higher than that due to other factors such as codling moth, bitter pit or poor colour. A major problem in assessing sunburn damage is the large number of fruit that is culled in

the orchard, producers estimate that losses due to sunburn may be 10-20%, with another 3-10% being culled in the packhouse.

1.4.1 Physiology of sunburn

Andrews & Johnson (1996) state that sunburn occurs when a series of complementary protective mechanisms existing within the fruit skin are overcome by a combination of excess ultraviolet, visible, and/or thermal solar radiation. As these protective mechanisms are overcome by extremes of light and heat, anatomical and metabolic changes occur within the fruit (Andrews & Johnson, 1996). They postulate that sunburn injury is caused directly by photo-oxidative damage from visible and UV light, exacerbated after the fruit has been 'preconditioned' for this damage by high temperatures.

Rabinowitch *et al.* (1974) reported that in addition to heat, visible light was necessary for development of typical sunburn symptoms in tomatoes. They concluded that sunburn is caused by the concurrent action of two external factors, heat and light. Rabinowitch (1981) later reported that fruit skin temperature thresholds of 38 to 40°C were found to be essential for development of sunburn in cucumbers and peppers, respectively. Light in the visible spectrum was found to be essential, as no bleaching occurred in the dark (Rabinowitch, 1981). He suggested that sunburn occurs when photosynthesis is disturbed by excessive heat, so that light energy is redirected into damaging photodynamic processes (Rabinowitch, 1981). Renquist *et al.* (1987) speculates that solar injury in raspberries is due to a combination of UV irradiation and high temperatures.

Schrader *et al.* (2001) distinguishes between two types of sunburn on apples, 'sunburn necrosis' and 'sunburn browning'. Sunburn necrosis results in the formation of a necrotic spot on the sun-exposed side of the fruit, is independent of irradiation and results from thermal death of cells in the peel when temperatures reach 52 +/- 1°C. Electrolyte leakage increases significantly in peel from apples with necrosis (Schrader *et al.*, 2001). Sunburn browning is a sub lethal event that results in a yellow, bronze, or brown spot on the exposed side of the apple (Schrader *et al.*, 2001). Sunburn browning occurs when the fruit surface temperatures reach 46-49°C in the presence of sunlight (Schrader *et al.*, 2001). Electron microscopy indicates that the organelles are damaged before the visual appearance of sunburn (Andrews & Johnson, 1996) The threshold

temperature required for sunburn browning appears to be cultivar dependent (Schrader *et al.*, 2001). On days when air temperature exceeded 36°C, sunburn damage was confined to the tissue directly under the skin of the fruit in the case of 'Starking', whereas in 'Golden Delicious' it had penetrated slightly deeper into the skin (Bergh *et al.*, 1980). In 'Granny Smith' the damage had penetrated right to the core of the fruit on days when air temperature exceeded 38°C (Bergh *et al.*, 1980). Electrolyte leakage from peel that has sunburn browning does not appear to differ from non-sunburned peel (Schrader *et al.*, 2001).

In the State of Washington it is not uncommon for peel temperatures to be at least 10°C above ambient air temperature, and it can be as much as 16.1°C when conditions are favourable. Therefore sunburn may occur any time air temperature is above 27°C (Schrader *et al.*, 2001).

In a study on cantaloupes in California, Lipton (1970) found that ultraviolet (UV) radiation at levels normally encountered in arid areas are the most common cause of solar injury. In severe cases of solar injury, in which the flesh is injured, excessively high temperatures obviously are a contributing factor, because UV generally does not penetrate deeply into plant tissues. These results also permit the distinction between symptoms of solar injury induced by short-wave and long wave components of solar radiation. These results correlate with the findings of Schrader *et al.* (2001), with regard to the two types of sunburn.

Adegoke *et al.* (1983) similarly found using tomatoes that high air temperatures magnified solar injury due to irradiation. They found that prolonged exposure to air temperatures above 45°C or higher caused injury even in darkness. Sunburn in tomatoes was mainly due to overheating (Adegoke *et al.*, 1983). About 50% of direct solar radiation is in the infrared portion of the spectrum, and the absorption of infrared radiation by tissue water appears to make a particularly important contribution to overheating. Infrared wavelengths were the most injurious, irradiances being equal. Water seems to be the major infrared absorber, and tomato fruit having high water content are relatively susceptible to sunburn (Adegoke *et al.*, 1983).

Electron microscopy of the skin surface of apple fruit indicates that another response to the intense sunlight associated with sunburn is dissipation of the wax cuticle (Andrews & Johnson,

1996). The wax of the cuticle is the fruit's first defence against desiccation, and it may act to reduce damaging components of solar radiation before they are absorbed by the cells of the skin (Andrews & Johnson, 1996). Both electron and light microscopy indicate that the outer cells of the fruit accumulate phenolics and that their cell walls thicken and lignify even before skin colour changes, indicative of sunburn, are visible (Andrews & Johnson, 1996).

1.4.2 The role of antioxidants

Photo-oxidative damage, which has only been studied thoroughly in leaves, results in the generation of free radicals, which are very reactive and destructive atoms because they have one or more unpaired electrons (Andrews & Johnson, 1996). Free radicals within biological tissue disrupt cellular membranes and alter the genetic code, thus compromising structural integrity and cellular functions (Andrews & Johnson, 1996). A biochemical system exists in tissues which recycles different types of protective antioxidants, and scavenges and inactivates destructive free radicals in plants (Andrews & Johnson, 1996; Wünsche *et al.*, 2004). One of the most striking symptoms of sunburn injury is discolouration of that portion of the skin exposed to the midday sun (Andrews & Johnson, 1996). Therefore, sunburn affects pigmentation. Generally reds fade, and yellows/oranges increase as sunburn develops. The yellow/orange pigments appearing in sunburn damaged skin are carotenoids which have antioxidant properties (Andrews & Johnson, 1996).

One of these carotenoids, β -carotene, is a vitamin that functions both in the harvesting of light for photosynthesis and in the scavenging of destructive free radicals (Andrews & Johnson, 1996; Salisbury & Ross, 1992). Higher levels of β -carotene were measured in the skin of 'Fuji' fruits exposed to direct sunlight than in the skin on the shaded side of the same fruit (Andrews & Johnson, 1996). Similarly, Awad *et al.* (2001) found higher levels of anthocyanin and quercetin glycosides in apple fruit in the outer part of the canopy as opposed to fruit in the inner canopy.

One function of the complex group of phenolic compounds present in cells is their antioxidant capability (Andrews & Johnson, 1996). A major antioxidant system in cells is the ascorbate-gluthathione cycle, which features the water soluble compounds ascorbate and glutathione and the enzyme super oxide dismutase (SOD) (Andrews & Johnson, 1996; Wünsche *et al.*, 2004).

Higher levels of ascorbate and SOD activity were measured in the skin of fruits exposed to direct sunlight than in the skin on the shaded side of the same fruit (Andrews & Johnson, 1996). Harris (1975) also found that outside fruit exposed to maximum sunlight contain higher amounts of vitamin C (ascorbic acid) than inside and shaded fruit on the same plant. There were also differences in the levels of these compounds between sunburnt and healthy fruit skin, and between shade-covered and unprotected healthy appearing fruit (Andrews & Johnson, 1996). SOD removes the superoxide anion O_2^- , and this reaction forms hydrogen peroxide (H_2O_2), another toxic compound. H_2O_2 can have two potentially damaging results: it can either inactivate sulphhydryl-containing enzymes, or it can react with remaining superoxide to form the even more toxic hydroxyl radical (Andrews & Johnson, 1996). The glutathione–ascorbate cycle occurs in both the cytosol and the chloroplasts, and appears to function in conjunction with α -tocopherol, a free radical scavenger in the thylakoid membranes (Andrews & Johnson, 1996). Glutathione has been shown to protect membrane liposomes from free radical damage (Hausladen & Alscher, 1993). Ascorbate functions as the terminal electron donor in the processes that scavenge H_2O_2 , the superoxide anion and the hydroxyl radical. In addition to this direct antioxidant function, ascorbate also plays a role in the production of various other antioxidants including α -tocopherol and zeaxanthin (Foyer, 1993).

Another group of antioxidants found in plants, flavonoids, are attracting attention. Flavonoids are ubiquitous plant secondary products of which the best known are the characteristic red, blue, and purple anthocyanin pigments of plant tissues (Winkel-Shirley, 2001). These compounds serve essential functions in plant reproduction by recruiting pollinators and seed dispersers. They are also responsible for the beautiful display of autumn colour in many plant species, which has recently been suggested to protect leaf cells from photo-oxidative damage, thereby enhancing the efficiency of nutrient retrieval during senescence (Field *et al.*, 2001). These flavonoids generally absorb in the 280-315 nm range and are thus capable of acting as UV-B filters (Harborne & Williams, 2000). A way to compare plants which possess and those which lack flavonoid synthesis is to use an inhibitor of phenylpropanoid synthesis, 2-amino-indan-2-phosphoric acid (AIP) at 50 μ M. Treatment of red cabbage seedlings with AIP completely blocks anthocyanin synthesis but levels of sinapyl esters are unchanged. These treated plants were twice as sensitive as controls to UV-B damage, suggesting that the anthocyanins, and any occurring flavonol

glycosides, serve as UV-B screens in young cabbage plants (Gitz *et al.*, 1998). In a study using fruit bagging, Ju (1998) found that 'Delicious' apples only produced anthocyanins in response to light, thereby suggesting a photoprotective function.

The accumulation of anthocyanin pigments in vegetative tissues is a hallmark of plant stress, yet the role that flavonoids play in the stress response is still poorly understood (Winkel-Shirley, 2002). In many cases, these compounds may provide antioxidant activity as part of a general stress response, which may also explain their health-promoting qualities in animals (Winkel-Shirley, 2002). However, there is also evidence that flavonoids may function in plants to screen harmful radiation, bind phytotoxins, and help to regulate the stress response by controlling auxin transport (Winkel-Shirley, 2002). It is speculated that these flavonoids may be responsible for health benefits observed with increased consumption of fruits and vegetables (Prior & Cao, 2000).

1.4.3 Environmental and cultivar factors that influence the incidence of sunburn

It was found that apple cultivars like 'Granny Smith', 'Mutsu', 'Fuji', 'Braeburn' and 'Jonagold' tend to burn more easily than 'Delicious', 'Golden Delicious' or 'Gala'. It is difficult to rate cultivars for their susceptibility to sunburn because it varies so much with tree age, vigour and crop load. Young trees tend to have more sunburnt fruit than older trees because the fruit is more exposed. It was found that temperatures under the peel of apple fruit can be between 10-12°C higher than ambient temperature (Bergh *et al.*, 1980; Kotzé *et al.*, 1988; Parchomchuk & Meheriuk, 1996 Schrader *et al.*, 2001;). 'Golden Delicious', 'Granny Smith', and 'Starking' all showed severe damage when fruit temperatures exceeded 50°C and damage was observed on days when the air temperature exceeded 36°C. In Grabouw in the Western Cape province of South Africa, air temperatures like these were registered from 12:00 to 15:15 on most of the days (Bergh *et al.*, 1980) These findings are confirmed by Schrader *et al.* (2001) who found that that fruit surface temperatures of more than $52 \pm 1^\circ\text{C}$ result in 'necrosis', and surface temperatures between 46-49°C result in 'sunburn browning'. Sunburn is always worse on the north and northwest side of the trees in the southern hemisphere (Van den Ende, 1999) Air temperatures are greatly influenced by wind velocity and only slight air circulation caused differences of more than 4°C (Bergh *et al.*, 1980). On days when the temperature exceeded 35°C, the wind direction

was mainly from the south east at the test site in Grabouw, and the presence of a windbreak on the south-eastern side of the orchard greatly reduced air circulation (Bergh *et al.*, 1980). Fruit temperatures are mainly influenced by the intensity of radiation and by the air circulation (Bergh *et al.*, 1980; Van den Ende, 1999). Fruit size, wind direction, and fruit transpiration are of lesser importance (Van den Ende, 1999).

In a study on pawpaws in Pietermaritzburg, South Africa, Savage & Allan (1997) experimented with pawpaws enclosed in brown paper packets and fruit painted white. On a hot and cloudless day, the northern side fruit temperature maximum was more than 16°C greater than the maximum air temperature and 10°C greater than the southern side fruit temperature maximum. North facing fruit exposed to the sun had larger north-south fruit temperature differences (typically 12°C at noon) compared to shaded fruit enclosed in packets (6°C). Enclosed fruit had lower surface temperature and no surface freckles. The maximum infrared measured temperatures of white painted fruit were lower than those of normal fruit. The highest fruit surface temperatures recorded were 49.2°C for normal (unpainted) and 47.5°C for white painted fruit. Sunburn damage to white painted fruit, did not occur. They concluded that the critical surface temperature must, therefore, be around 48°C.

What can be done to lessen or prevent sunburn? There are many different approaches, including horticultural practices, kaolin sprays, evaporative cooling (EC), shade netting, and ascorbic acid sprays.

1.5. Sunburn management strategies

1.5.1 Horticultural practices

Fruit burn when they are exposed to levels of sunlight to which they were not previously exposed. Thus, branches should not be allowed to bend over and expose fruit that were previously in the shade (Van den Ende, 1999). Also, heavily cropped limbs should be propped or tied up to maintain the original limb position, as limb movement or twisting can be responsible for a lot of serious sunburn (Van den Ende, 1999).

Annual extension growth of shoots should be 20-30 cm and the shoots should be well distributed over the tree to enable most fruit to enjoy filtered light rather than excessive amounts of direct sunlight. Basal dominant varieties like 'Braeburn', 'Fuji', and 'Delicious' tend to have less foliage in the upper part of the tree, leading to severe sunburn in this area. Training and supporting the young trees can avoid this problem (Van den Ende, 1999). With summer pruning, care should be taken that fruit are not overexposed following too much leaf removal (Van den Ende, 1999). Watershoots should be removed throughout the growing season to maintain light penetration and allow fruit to condition (Van den Ende, 1999). Removing watershoots several weeks before harvest exposes previously unconditioned fruit (Van den Ende, 1999). This does not apply to very late varieties like 'Pink Lady' (Van den Ende, 1999).

Careful attention to fruit thinning, especially towards the ends of branches, will prevent branch movement (Van den Ende, 1999). In bi-coloured varieties and red varieties, thinning fruit down to ones and twos per bearing position maximises colour development and can minimise sunburn (Van den Ende, 1999). What seems above all to make fruit on a tree more vulnerable to sunburn is the crop load or the leaf to fruit ratio (Van den Ende, 1999). When there are more fruit and fewer leaves, there are more fruit exposed to the sun. If the fruit is covered by a few leaves at some part of the afternoon, it will most likely not burn. Removal of sunburnt fruit should be delayed as long as possible to minimize additional damage to adjacent fruit (Van den Ende, 1999).

Fruit burns less easily if the trees are irrigated regularly. This is due to the maintenance of the tree's water status and some ability to regulate tissue temperature by transpirational water loss (Van den Ende, 1999).

1.5.2 Kaolin particle film applications

Initially used as a deterrent to pests, kaolin particle films have found application in the treatment of sunburn, due to the reflective qualities of the mineral kaolin. The particle film is applied as a suspension through a spreader, and when the water evaporates a white film remains on the vegetation. Other benefits on apples include reduced heat stress, reduced fruit drop and improved colour in some cultivars (Heacox, 1999).

Particle film has shown potential for reducing sunburn in Chile, but application and formulation initially proved to be a problem. A formulation problem led to poor distribution of the product on the fruit surface and caused a blotchy colour pattern due to poor light penetration in areas where the deposit was too thick (Wilton, 1999).

An improved commercial kaolin particle film formulation (Surround[®] WP) increased leaf carbon assimilation, reduced canopy temperatures and improved colour of apples in the United States and Chile (Glenn *et al.*, 2001). Surround[®] WP reduced leaf carbon assimilation rates of apples in New Zealand (Wünsche *et al.*, 2004) and South Africa (Le Grange *et al.*, 2004), but this effect was not noted at a whole canopy basis (Wünsche *et al.*, 2004). Surround[®] WP reduced leaf temperature and leaf to air vapour pressure differences (VPD) on 'Ruby Red' grapefruit leaves. These reductions in leaf temperature and VPD led to higher stomatal conductance and net CO₂ assimilation rates (Jifon & Syvertsen, 2003). Additionally, yields were higher in trials conducted in the United States (Glenn *et al.*, 2001). Particle film is also effective in lowering fruit temperature and sunburn (Glenn *et al.*, 2002, Wünsche *et al.*, 2004). On pomegranates grown in Spain, Surround[®] WP reduced sunburn from 21.9% on the control fruit to 9.4% on treated fruit (Melgarejo *et al.*, 2003). It was found that particle film is especially reflective to ultraviolet wavelengths, which is probably the mechanism whereby particle film reduces sunburn (Glenn *et al.*, 2002).

Surround[®] WP increased yield in trials conducted in the United States (Glenn *et al.*, 2001). Surround[®] WP reduced fruit weight, red colour, and the incidence of sunburn of 'Fuji' apples in Idaho, but had no effect on fruit weight or red fruit colour of 'Honeycrisp' apples in New York when applied in May and June. However, it reduced fruit weight and red colour when applied

later in the season (Schupp *et al.*, 2002). Furthermore, Surround[®] WP applications resulted in undesirable residues in the calyx and stem-end of harvested fruit that were not satisfactorily removed by brushing on a commercial packing line (Schupp *et al.*, 2002).

1.5.3 Evaporative cooling.

There are three types of cooling that can be achieved with overhead sprinklers: air cooling, hydro-cooling and evaporative cooling (EC) (Evans, 1993; Warner, 1997c). Air cooling is achieved when low rates of water are applied. This cools the fruit by cooling the surrounding air. Hydro-cooling involves applying high rates of water that remove heat out of fruit as the water runs off. In the case of EC, the water applications are pulsed which removes heat from the fruit through the loss of latent heat as the water evaporates from the fruit surface (Evans, 1993; Warner, 1997c). The skin temperatures on exposed cooled fruit are between 2.2°C and 11°C lower than on uncooled fruit (Andrews, 1995; Wünsche *et al.*, 2001).

Reportedly EC provides the most efficient cooling while minimising water use (Evans, 1993; Andrews, 1995), but it requires scientific scheduling of irrigation to avoid waterlogging, especially on heavy soils, whereas leaching of minerals in lighter soils can also be a problem (Evans, 1993). By intent, EC is designed to have high water losses due to evaporation (Evans, 1993), therefore it is not a water conservation measure and the use of EC will definitely require more water throughout the season (Evans, 1993). There is a trade-off between relative levels of sunburn protection and water application rates, where the average rate of 26 L ha⁻¹ may not control sunburn on extremely hot days (Evans, 1993). Consequently, at lower rates the decision must be made to either accept increased sunburn over the entire block, or to cool smaller blocks of more valuable fruit at higher rates (Evans, 1993). If the decision is made to use EC in a smaller area, the piping and pumping system must be designed to handle the increased local flows at required pressures. Droplet sizes should be large enough to penetrate the canopy and wet all crop surfaces. A timer is necessary to pulse the water applications. Systems in windy areas need to be designed for high application rates and shorter intervals between pulses, with bigger droplets and a closer spacing of sprinklers (Evans, 1993).

In North Carolina, where heat stress conditions exist, low volume sprinkler irrigation is recommended for apples (Unrath, 1975). The use of overhead sprinklers to evaporatively cool fruit has consistently increased fruit colouration and soluble solids content, and in some years increased fruit size. In addition, overhead cooling has reduced both cork spot and bitter pit disorders in years when these disorders have been a problem (Unrath, 1975).

In South Africa it was found that overhead cooling significantly reduced sunburn on 'Granny Smith' and 'Golden Delicious' apples. However, it suppressed colour formation in 'Starking' apples (Kotzé *et al.*, 1988). In Canada sunburn on 'Jonagold' was reduced under EC by 15.8% in 1991 and 9.4% in 1992 (Parchomchuk & Meheriuk, 1996). Andrews (1995) found that sunburn could be reduced on 'Fuji' apples in Washington by using overhead cooling.

In Spain, overhead cooling of 'Topred Delicious' apples increased red colour and anthocyanin concentration, especially when applied at sunset and at sunrise (Iglesias *et al.*, 2002). Fruit firmness, fruit size and soluble solids concentration were significantly higher in cooled fruits than the control, whereas titratable acidity was not influenced consistently by the EC (Iglesias *et al.*, 2002). Dussi *et al.* (1997) found that EC improved colour and advanced maturity on 'Sensation Red Bartlett' pears, and that these effects were more pronounced in warm seasons compared to cooler seasons.

Reportedly many growers in Washington State (USA) draw water from deep wells and find that it has a high mineral content which forms a calcium carbonate precipitate on the fruit (Warner, 1997b). Precipitates form when water evaporates. Sometimes, when low rates are applied, minerals in the water can affect the leaves. In extreme cases, trees have been defoliated. It is the build-up of salts, possibly sodium, in water that damages the leaves, and there is no chemical treatment to prevent it. The recommendation is leaving the water on for a couple of hours in the evening in an attempt to wash the salts off. Leaf tip and marginal burn become evident when leaf sodium levels reach about 0.2%, but leaf analysis can reveal if high sodium levels are accumulating before the leaves actually burn (Warner, 1997b; Warner, 1997c).

Cooling with water that contains carbonate and bicarbonate can affect the pH of the soil, and high levels of sodium can cause a breakdown of soil structure, which reduces water infiltration (Evans, 1993; Warner, 1997c). A positive side effect of EC is that it increases the humidity in the orchard, reducing evapotranspiration, which in turn reduces the need to irrigate conventionally (Warner, 1997a).

1.5.4 Ascorbic acid sprays

As discussed previously, sunburn is a symptom of photo-oxidative injury. To protect fruit from photo-oxidative injury, active oxygen compounds must be detoxified through a series of oxidation-reduction reactions. Antioxidants and their associated enzymes carry out these reactions. The ascorbate-glutathione cycle is the central antioxidant system for scavenging and detoxifying the active oxygen compounds produced during photo-oxidative injury. This defence cycle includes the antioxidants ascorbic acid and glutathione from their oxidized to their reduced forms, so they can be re-used in the detoxification process. Research indicates that the ascorbate-glutathione cycle is involved in protecting 'Fuji' apples from sunburn, because when ascorbic acid and glutathione are low in the skin, the fruit is more susceptible to sunburn (Johnson, 1999). However, when their levels are augmented, there is a reduction of sunburn (Johnson, 1999). Higher levels of ascorbate and SOD activity were measured in the skin of fruits exposed to direct sunlight than in the skin on the shaded side of the same fruit (Andrews & Johnson, 1996).

In a 'Fuji' apple orchard in Washington State, USA, two and four percent ascorbic acid applications applied three times from mid-August to mid-September reduced the incidence of sunburn from 25% in the control to 14 and 8%, respectively. The 4% spray was mildly phytotoxic. Ascorbic acid did not effectively reduce sunburn in 'Granny Smith'. Perhaps this was due to different conditions in the orchard, and because sunburn in 'Granny Smith' is preceded by photo-bleaching of chlorophyll, which may require augmentation of different photo-oxidative repair mechanisms (Johnson, 1999). The high cost of ascorbic acid sprays has meant that it hasn't been used commercially.

1.5.5 Shading

Shade netting reduces apple fruit temperature (Warner, 1997d). Besides lowering fruit temperature, shade netting reduces wind and increases humidity, reducing the need for irrigation (Warner, 1997d). Because nets reduce the amount of light reaching fruit and leaves, they should be pulled back before harvest to allow fruit to colour. There is some question about the long term effect of shading on yields (Warner, 1997d). Studies in England have shown that yields decline as the amount of intercepted light in the orchard decreases (Warner, 1997d). However, England has a maritime climate, with high cloud cover, and the total intercepted light in a season is much lower than in Washington orchards (Warner, 1997d). In other crops, shade netting has been shown to decrease flower development, increase flower abscission, decrease yields, and decrease soluble solids and starch in fruit (Warner, 1997d).

Shade netting reduces both UV-B and visible radiation reaching the tree (Warner 1997d), the percentage reduction of each depending on the density and colour of the netting. The main reason shade netting reduces sunburn is not because of the light that is filtered out, but because light coming through is diffused and scattered, so that the photons of light are not bearing down on a particular spot on the fruit (Warner, 1997d).

A problem with shade netting is to provide trees and fruit with enough light, but at the same time protect fruit from sunburn (Warner, 1997d). Movable shade netting presents many problems, such as pulling fruit off trees. In areas with snow, the cloth and supporting structures may collapse under the weight of the snow. The returns from orchards with shade covering may not justify the input costs (Warner, 1997d).

The objectives of this thesis are to evaluate evaporative cooling and particle film applications as technologies for the management of sunburn, and to study the effects of high temperatures on apple tissues.

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2. PAPER 1. THE EFFECT OF EVAPORATIVE COOLING ON APPLE AND PEAR FRUIT SURFACE TEMPERATURES AND SUNBURN.

Abstract

Evaporative cooling is a practice used to control sunburn on apples and pears. The effect of evaporative cooling on fruit surface temperatures and sunburn was investigated on 'Cripps' Pink', 'Royal Gala' and 'Fuji' apples as well as 'Forelle' pears in the Western Cape in South Africa. Fruit surface temperatures were measured using an infrared thermometer. Evaporative cooling reduced fruit surface temperatures by 0.6-3.7°C when the fruit were wet. Apple fruit under evaporative cooling treatments appear to have acclimatised and had significantly higher surface temperatures when dry (0.9-4.7°C) than non-cooled fruit. Sunburn was reduced significantly on 'Royal Gala' apples.

Introduction

Sunburn on apples and pears occurs annually in South African orchards and results in large losses of export volumes. It is difficult to accurately determine losses due to sunburn as a large percentage of sunburnt fruit is culled in the orchard. Producers estimate that losses are usually in the region of 10-20%. Sunburn damage in 'Granny Smith' also causes the fruit to change in colour from green to pale yellow during cold storage. This causes an unattractive appearance and further losses after storage could amount to as much as 3.4% (Bergh *et al.*, 1980).

The most obvious symptom of sunburnt fruit is discolouration of the skin exposed to the intense midday sun (Andrews & Johnson, 1996). Schrader *et al.* (2001) describe sunburn as a physiological disorder characterised by a necrotic lesion on the fruit and a browning of the flesh underneath. There are several ways to manage sunburn on fruit, such as shade netting, the application of antioxidant ascorbic acid sprays or reflective particle films, and cooling with overhead sprinklers.

There are three types of cooling that can be achieved with overhead sprinklers: air cooling, hydro-cooling and evaporative cooling (EC) (Evans, 1993; Warner, 1997). Air cooling is achieved when low rates of water are applied (Evans, 1993; Warner, 1997), which cools the fruit by cooling the surrounding air (Evans, 1993; Warner, 1997). Hydro-cooling involves applying high rates of water that take heat out of fruit as the water runs off (Evans, 1993;

Warner, 1997). With EC, the water applications are pulsed and heat is removed from the fruit through the loss of latent heat as the water evaporates from the fruit surface (Evans, 1993; Warner, 1997).

Reportedly EC provides the most efficient cooling while simultaneously minimising water use (Evans, 1993; Warner, 1997). Although mineral deposits on the fruit skin can be a problem, a positive side effect of cooling is that it increases the humidity in the orchard, reducing evapotranspiration, which in turn reduces the need to irrigate conventionally (Evans, 1993; Warner, 1997). EC may slightly decrease crop water usage, but total water usage will be higher where EC is utilised (Evans, 1993). The skin temperatures on exposed cooled fruit are 2.2°C-11°C lower than on uncooled fruit (Andrews, 1995, Wünsche *et al.*, 2001). In South Africa it was found that overhead cooling significantly reduced sunburn on 'Granny Smith' and 'Golden Delicious' apples (Kotzé *et al.*, 1988). However, it suppressed red colour formation in 'Starking' apples (Kotzé *et al.*, 1988). In Canada, sunburn on 'Jonagold' apples was reduced by 15.8% in 1991 and 9.4% in 1992 (Parchomchuk & Meheriuk, 1996). Andrews (1995) found that sunburn could be reduced on 'Fuji' apples in Washington by using overhead cooling. Iglesias *et al.* (2002) found that apples grown under evaporative cooling had improved colour, fruit size and percentage total soluble solids than control fruit, while there was no effect on titrateable acidity. Dussi *et al.* (1997) found that EC improved colour and advanced maturity on 'Sensation Red Bartlett' pears, and that these effects were more pronounced in warm seasons compared to cooler seasons.

The aim of this study was to determine the effect of EC on apple and pear fruit surface temperature as well as sunburn in the Western Cape, South Africa.

Materials and Methods

Plant material and treatments

The 'Forelle' pear (*Pyrus communis* L.) orchard in Stellenbosch (33°56'S, 18°51'E) was planted on Quince A rootstocks in 1998 at a spacing of 4 m x 1.25 m. Pressure compensated DAN 2001 microsprinklers with a 28 L h⁻¹ discharge rate and a wetted radius of 1.5 m were installed in every row in the orchard with a spacing of 2.5 m along a suspended pipe at the top of the tree canopy 3.5 m above the ground. The irrigation rate was ±4 mm h⁻¹ at 200 kPa. During the 2001/2002 season evaporative cooling was activated automatically at a set air temperature and continued at a cycle of 10 minutes on, 20 minutes off until the air

temperature decreased below the set value. The activation temperature was set at 28°C from 06h00 to 18h00 and at 20°C from 18h00 to 21h00. A temperature sensor coupled to the irrigation computer was positioned approximately 1.5 m above the ground in a Gill radiation shield positioned between adjacent trees in the adjacent apple orchard. Normal undertree micro-irrigation was scheduled according to neutron probe readings outside the EC area and was not adjusted for the supplemental water supplied by the EC system.

The trial was designed as a randomised complete block comprising ten blocks in five rows. The two treatments were a control without EC, and an EC treatment started on 4 December 2001. Every third row was selected to allow for two buffer rows. There were three trees per block for each treatment, with five tagged fruit per tree. There were three trees between treatments to act as buffer trees. The control treatments were achieved by plugging sprinklers with stoppers.

The 'Royal Gala' and 'Fuji' apple (*Malus domestica* L. Borkh) orchards at Nooitgedacht farm in the Koue Bokkeveld (33°13'S, 19°20'E) were established in 1996 on M793 rootstocks with a spacing of 4 m x 1.25 m. The overhead sprinklers were spaced at 8 m x 6 m with a wetted radius of 8 m approximately 3.5 m above the ground. The system was activated when the air temperature exceeded 25°C and continued at a cycle of 15 minutes on, 30 minutes off until the air temperature dropped below 25°C. The undertree irrigation was adjusted by means of neutron probe readings to compensate for the altered water usage in the evaporative cooling block.

The 'Cripps' Pink' orchard on Vredelust farm near Villiersdorp (34°14'S, 19°25'E) was grafted onto existing seedling rootstocks in 1999 at a spacing of 4 m x 2 m. The overhead sprinklers were placed in every row 2.5 m apart, approximately 3.5 m above the ground, and operated at 100 kPa at a rate of 25 L h⁻¹. The system was activated when the air temperature exceeded 28°C and continued at a cycle of 20 minutes on, 20 minutes off.

The 'Royal Gala', 'Fuji' and 'Cripps' Pink' trials were non-statistical with fifteen trees in three rows of five trees each in the control block, and fifteen trees in three rows of five trees in the evaporative cooling block.

Measurements

On each tree, five exposed fruit on the exposed aspect of the tree were tagged and the fruit surface temperature was measured using a hand held infra-red thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, CA). Control and EC treatments were measured alternately. Sunburn on the apples was graded at the end of the season on a scale of 1-3 where 1=no sunburn, 2=slight sunburn, and 3=severe sunburn (necrosis). No sunburn measurements were taken on the pears as the fruit were harvested earlier than expected. In the 'Royal Gala' trial the EC system was not active on the days that measurements were taken, thus there is no data to show a cooling effect due to EC, but only data comparing the two treatments when there was no EC running. Sometimes, fruit under EC would not be wetted, due to poor droplet dispersion. A note was made against their temperature values, and they were analysed together with temperature values of times when the EC system was not running.

Statistical Analysis. The data for the pears was analysed using a ANOVA with blocks, while a t-test was used to analyse the apple data (Enterprise Guide release 1.3, SAS Institute Inc. Cary, N.C., U.S.A., 2001). Treatment means were compared by means of an LSD test at the 5% level.

Results

'Cripps' Pink'

EC reduced fruit surface temperatures by 2.9 – 7.0°C (Table 1). There seems to be no correlation between higher ambient temperature and the degree of cooling on this cultivar, or any other cultivar. When the EC system was not running, the fruit in the EC block were 1.9 - 4.7°C warmer than fruit in the control block (Table 2). There were no significant differences in sunburn between treatments (Table 3).

'Fuji'

EC reduced fruit surface temperatures by 1.3 – 3.0°C (Table 4). Dry fruit (when system was not running) in the EC block were 1.7 – 2.4°C warmer than fruit in the control block (Table 5). There were no significant statistical differences in sunburn between treatments (Table 6).

'Royal Gala'

The EC system was not running on any of the dates that measurements were taken. Consistent with the other apple trials' results, dry fruit in the EC block were warmer (1.2 – 1.9°C)

compared to the control fruit on the first two dates, but there was no difference on the third day (Table 7). Sunburn was reduced significantly, with an increase of almost 20% in the 'no sunburn' category (Table 8).

'Forelle'

EC reduced fruit surface temperatures by 2.3 – 6.0°C (Table 9). There were no differences in fruit surface temperatures between treatments on days that the EC was not running (Table 10). Sunburn was not scored on the 'Forelle' pears.

Discussion

Evaporative cooling reduced fruit surface temperatures in all the trials (1.2-7°C). This was probably due to the loss of latent heat as the water evaporated from the fruit surface. These findings are consistent with the findings of Unrath (1975), Andrews (1995) and Wünsche *et al.* (2004), who found reductions of between 2.2-11°C on apples. The variation in temperature reduction could be due to different systems or pulsing cycles. We did not find a correlation between higher ambient temperatures and the degree of cooling.

When apples in the evaporative cooling blocks were not wetted, either due to poor droplet dispersion, or when ambient temperatures were too low for the EC system to be activated, the apple surfaces exhibited higher (1.2-4.7°C) temperatures than the control apples. This phenomenon was not observed on the pears. The higher surface temperatures may be due to acclimatisation resulting in increased heat absorption, or reduced ability to shed heat. The flux density of solar radiation and wind velocity are the two most important environmental parameters determining fruit temperature, while fruit size and the reflective properties of the soil (albedo), wind direction, fruit transpiration and thermal exchange by long-wave radiation are of lower importance (Smart & Sinclair, 1976; Bergh *et al.*, 1980; Van den Ende, 1999). Absorption of radiant heat is affected by the surface characteristics of the object being heated (Cutnell & Johnson, 1995). Wax layers on the fruit may increase the surface reflection of incident radiation (Lambers *et al.*, 1998; Wünsche *et al.*, 2004). It is possible that EC modified the epicuticular wax layers of apples in this study, decreasing their ability to reflect incoming short wave radiation, and resulting in increased radiant heating. It is also possible that the EC improved red fruit colour (an increase in anthocyanins) in this study as found by Iglesias *et al.* (2001) on apples. The presence of anthocyanins increases total light absorption (Merzlyak & Chivkunova, 2000), which would lead to higher surface temperatures.

The acclimatising effect must be considered when an EC system is installed. First, it will shorten the appropriate period between overhead water applications. If the period is too long, the acclimatised fruit may burn more quickly than non-cooled fruit. Wünsche *et al.* (2004) found that EC reduced fruit temperature by up to 8.5°C, but that fruit temperatures could increase by up to 7.5°C between 15 minute cooling cycles. Second, any EC system should adequately and reliably wet a sufficient number of fruit. Droplet dispersion should dependably wet a high percentage of fruit. The EC system should be reliable, because acclimatised fruit could burn at lower ambient temperatures (personal observation).

Sunburn was reduced significantly only on the 'Royal Gala' apples. It appears that 'Royal Gala' was more susceptible to sunburn than 'Cripps' Pink' or 'Fuji' in this trial, as the levels of total sunburn were highest (30%) in 'Royal Gala' compared to 'Cripps' Pink' (20%) or 'Fuji' (16%).

Evaporative cooling is effective in reducing fruit surface temperature and shows potential for managing high sunburn. The acclimatising effect, where fruit in the EC block that are dry exhibit higher temperatures than the control fruit, must be taken into account when designing EC systems.

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Table 1. Wet fruit surface temperature (°C) measured on ‘Cripps’ Pink’ apples under active evaporative cooling treatments in Villiersdorp. Maximum air temperature ranged between 25-34°C. Values are means with standard errors. Data was analysed using a t-test.

Date	Control	Evaporative cooling	Pr>F
22 February 2002	35.9±0.4a ^z (n=71)	33.0±0.6b (n=50)	0.0233
22 March 2002	34.0±1.0a (n=70)	27.0±1.5b (n=44)	0.0006
16 April 2002	36.4±0.8a (n=50)	32.0±0.8b (n=46)	0.0003

^zFor each date, means with the same letter are not significantly different (LSD, P≤0.05).

Table 2. ‘Cripps’ Pink’ fruit surface temperature (°C) measured on dry fruit under evaporative cooling treatments in Villiersdorp. Maximum air temperature ranged between 25-34°C. Values are means with standard errors. Data was analysed using a t-test.

Date	Control	Evaporative cooling	Pr>F
22 February 2002	31.2±0.7b ^z (n=71)	35.9±0.4a (n=25)	<0.0001
7 March 2002	21.4±0.2b (n=57)	23.3±0.2a (n=51)	<0.0001
22 March 2002	31.4±0.6b (n=68)	34.0±0.9b (n=25)	0.0213

^zFor each date, means with the same letter are not significantly different (LSD, P≤0.05).

Table 3. Percentage sunburn on ‘Cripps’ Pink’ apples under evaporative cooling treatments in Villiersdorp. Values are means with standard errors. Data was analysed using a t-test (n=15).

% Sunburn	Control	Evaporative cooling	Pr>F
No sunburn	80.0±8.3a ^z	88.2±6.5a	0.3840
Slight sunburn	16.7±6.9a	9.1±5.5a	0.3402
Severe sunburn	3.3±2.3a	2.7±2.0a	0.8204

^z For each sunburn category, means with the same letter are not significantly different (LSD, P≤0.05).

Table 4. Wet fruit surface temperatures ($^{\circ}\text{C}$) measured on 'Fuji' apples under active evaporative cooling treatments in Koue Bokkeveld. Maximum air temperature on both days was 31°C . Values are means with standard errors. Data was analysed using a t-test.

Date	Control	Evaporative cooling	Pr>F
2 February 2002	28.5 \pm 0.3a (n=73)	27.2 \pm 0.6a (n=38)	0.0548
22 February 2002	33.4 \pm 0.3a (n=73)	30.4 \pm 0.6b (n=25)	0.0001

^zFor each date, means with the same letter are not significantly different (LSD, $P\leq 0.05$).

Table 5. 'Fuji' fruit surface temperatures ($^{\circ}\text{C}$) measured on dry fruit under evaporative cooling treatments in the Koue Bokkeveld. Maximum air temperature ranged between 27 - 31°C . Values are means with standard errors. Data was analysed using a t-test.

Date	Control	Evaporative cooling	Pr>F
25 January 2002	27.2 \pm 0.3b ^z (n=74)	29.2 \pm 0.3a (n=74)	<0.0001
31 January 2002	26.5 \pm 0.3b (n=74)	28.8 \pm 0.3a (n=74)	<0.0001
2 February 2002	28.5 \pm 0.4b (n=73)	30.9 \pm 0.6a (n=36)	0.0028
22 February 2002	31.8 \pm 0.4b (n=47)	33.5 \pm 0.4a (n=70)	0.0055
3 March 2002	30.5 \pm 0.6b (n=68)	32.5 \pm 0.6a (n=71)	0.0154

^zFor each date, means with the same letter are not significantly different (LSD, $P\leq 0.05$).

Table 6. Percentage sunburn on 'Fuji' apples under evaporative cooling treatments in the Koue Bokkeveld. Values are means with standard errors. Data was analysed using a t-test (n=15).

% Sunburn	Control	Evaporative cooling	Pr>F
No sunburn	84.4 \pm 4.4a ^z	88.4 \pm 4.1a	0.5114
Slight sunburn	13.3 \pm 4.3a	7.5 \pm 3.5a	0.3069
Severe sunburn	2.2 \pm 2.2a	4.0 \pm 2.1a	0.5621

^zFor each sunburn category, means with the same letter are not significantly different (LSD, $P\leq 0.05$).

Table 7. 'Royal Gala' fruit surface temperatures (°C) measured on dry fruit under evaporative cooling treatments in the Koue Bokkeveld. Maximum air temperature ranged between 27-31°C Values are means with standard errors. Data was analysed using a t-test.

Date	Control	Evaporative cooling	Pr>F
25 January 2002	26.7±0.3b ^z (n=74)	27.9±0.3a (n=74)	0.0082
31 January 2002	26.7±0.2b (n=75)	28.6±0.2a (n=74)	<0.0001
2 Febr 2002	29.1±0.4b (n=72)	29.7±0.4a (n=74)	0.2501

^zFor each date, means with the same letter are not significantly different (LSD, P≤0.05).

Table 8. Percentage sunburn on 'Royal Gala' apples under evaporative cooling treatments (EC) in the Koue Bokkeveld. Values are means with standard errors. Data was analysed using a t-test (n=15).

% Sunburn	Control	Evaporative cooling	Pr>F
No sunburn	69.7±6.3b ^z	88.0±5.0a	0.0298
Slight sunburn	27.0±5.4a	12.0±5.0a	0.0507
Severe sunburn	4.7±3.5a	0.0±0a	0.1932

^zFor each sunburn category, means with the same letter are not significantly different (LSD, P≤0.05).

Table 9. Wet fruit surface temperatures ($^{\circ}\text{C}$) measured on 'Forelle' pears under active evaporative cooling treatments in Stellenbosch. Maximum air temperature ranged between $27\text{-}33^{\circ}\text{C}$. Values are means with standard errors. Data was analysed with an ANOVA with blocks.

Date	Control	Evaporative cooling	Pr>F
03 January 2002 (n=8)	30.1 \pm 0.3a ^z	25.9 \pm 0.4b	0.0002
22 January 2002 (n=3)	29.6 \pm 0.4a	27.3 \pm 0.3b	0.0317
23 January 2002 (n=4)	31.0 \pm 0.5a	27.7 \pm 0.5b	0.0338
27 January 2002 (n=5)	31.7 \pm 0.3a	27.1 \pm 0.3b	0.0002
01 February 2002 (n=6)	33.3 \pm 0.6a	27.3 \pm 0.3b	0.0109

^zFor each date, means with the same letter are not significantly different (LSD, $P\leq 0.05$).

Table 10. 'Forelle' fruit surface temperatures ($^{\circ}\text{C}$) measured on dry fruit under evaporative cooling treatments (EC) in Stellenbosch. Maximum air temperature ranged between $27\text{-}33^{\circ}\text{C}$. Values are means with standard errors. Data was analysed using an ANOVA with blocks.

Date	Control	Evaporative cooling	Pr>F
03 January 2002 (n=2)	30.1 \pm 0.9a ^z	30.2 \pm 0.81a	0.6803
22 January 2002 (n=8)	31.3 \pm 1.6a	28.6 \pm 1.7a	0.3294
23 January 2002 (n=7)	32.3 \pm 0.8a	30.5 \pm 3.1a	0.5164
27 January 2002 (n=6)	30.9 \pm 1.1a	30.3 \pm 0.6a	0.4865
01 February 2002 (n=6)	34.5 \pm 1.8a	33.5 \pm 2.9a	0.4885

^zFor each date, means with the same letter are not significantly different (LSD, $P\leq 0.05$).

3. PAPER 2. THE EFFECT OF SURROUND[®] WP PARTICLE FILM ON 'BRAEBURN', 'FUJI' AND 'GRANNY SMITH' APPLE QUALITY.

Abstract

Surround[®] WP is a kaolin-based particle film used in fruit production systems to manage sunburn. The effect of Surround[®] WP on 'Braeburn', 'Fuji' and 'Granny Smith' gas exchange, fruit surface temperature and fruit quality was investigated in the Koue Bokkeveld and Witzenberg regions of the Western Cape, South Africa. Surround[®] WP was applied using either a tower spray rig (four or five applications) or a blower (four applications). Fruit surface temperature was non-significantly lowered by between 0.5°C and 2.4°C. Sunburn on exposed fruit was significantly reduced from 33% to 19% in 'Braeburn', from 44% to 29% in 'Fuji' and from 58% to 29% in 'Granny Smith'. Leaf stomatal conductances and photosynthetic rate were reduced in Surround[®] WP treatments. Four applications of Surround[®] WP with a blower was the most effective treatment on 'Braeburn' apples, increasing total soluble solids significantly compared to tower applications. Four applications with a blower and five applications with a tower spray resulted in increased fruit size in 'Granny Smith' as well as higher firmness and lower percentage starch breakdown. All Surround[®] WP treatments resulted in slightly greener fruit colour in 'Granny Smith'. We conclude that Surround[®] WP is effective in reducing sunburn on apples whilst not adversely affecting other fruit quality parameters.

Introduction

Sunburn on apples and pears results in large losses of export volumes in South African orchards. Producers estimate that losses due to sunburn are usually about 10-20%. Sunburn damage also causes 'Granny Smith' apples to change in colour from green to pale yellow during cold storage. This is unattractive to the overseas market and further losses after storage could amount to as much as 3.4% (Bergh *et al.*, 1980).

The most obvious symptom of sunburnt fruit is discolouration of the skin exposed to the intense midday sun (Andrews & Johnson, 1996). Schrader *et al.* (2001) describes sunburn as a physiological disorder characterised by a necrotic lesion on the fruit and a browning of the flesh underneath. There are several ways to manage sunburn on fruit, such as shade netting,

the application of antioxidant ascorbic acid sprays and reflective particle films, and cooling with overhead sprinklers.

Initially used as a deterrent to pests, kaolin-based particle films have also found application in the management of sunburn, due to the reflective qualities of the mineral kaolin (Heacox, 1999). The particle film is applied as a suspension with a spreader, and when the water evaporates a white film remains on the vegetation (Heacox, 1999). Other benefits on apples include reduced heat stress, reduced fruit drop and improved red colour in some cultivars (Heacox, 1999).

Kaolin-based particle film has shown potential for reducing sunburn in Chile, but application and formulation proved to be a problem which led to poor distribution of the product on the fruit surface and caused a blotchy colour pattern due to poor light penetration in areas where the deposit was too thick (Wilton, 1999). An improved formulation (Surround[®] WP) did, however, increase leaf carbon assimilation, reduce canopy temperatures, and improve the colour of apples in the United States and Chile (Glenn *et al.*, 2001, 2003). A study in New Zealand showed that Surround[®] WP reduced leaf carbon assimilation rates on apples, but this effect was not noted at a whole canopy level (Wünsche *et al.*, 2004). Surround[®] WP also reduced leaf temperature and leaf to air vapour pressure differences (VPD) on 'Ruby Red' grapefruit leaves (Jifon & Syvertsen, 2003). These reductions in leaf temperature and VPD led to higher stomatal conductance and net CO₂ assimilation rates (Jifon & Syvertsen, 2003).

Additionally, particle film increased yield of apples in trials conducted in the United States (Glenn *et al.*, 2001). Particle film has also been shown to be effective in lowering fruit temperature and sunburn on apples and pomegranates (Glenn *et al.*, 2002; Melgarejo *et al.*, 2003; Wünsche *et al.*, 2004). Surround[®] WP reduced fruit weight, red colour, and the incidence of sunburn of 'Fuji' apples in Idaho, but had no effect on fruit weight or red fruit colour of 'Honeycrisp' apples in New York when applied in May and June (Schupp *et al.*, 2002). Surround[®] WP did, however, reduce fruit weight and red colour when applied later (Schupp *et al.*, 2002). While effective for reducing sunburn, Surround[®] WP was ineffective for increasing red fruit colour of apples in this study (Schupp *et al.*, 2002).

This paper reports on results of trials to determine the viability of kaolin applications, specifically Surround[®] WP, in the management of sunburn on apples under South African conditions with its high temperatures and levels of irradiance.

Materials and methods

Plant material

The trials were carried out on the farms Vastrap in the Witzenberg Valley and Tandfontein in the Koue Bokkeveld area near Ceres in the Western Cape, South Africa (33°12'S; 19°19'E). This is a winter rainfall region with an annual rainfall of about 830 mm. Three apple cultivars (*Malus domestica* L. Borkh) were evaluated: 'Braeburn', 'Fuji', and 'Granny Smith'. 'Braeburn' and 'Fuji' were used to evaluate the effect of Surround[®] WP on late season blush cultivars. 'Granny Smith' was selected to determine the effects of Surround[®] WP on a green cultivar in an orchard particularly prone to sunburn damage.

The 'Braeburn' trees on M793 rootstocks were planted at Vastrap in 1996. The trees were planted with a spacing of 4 m x 1 m with 'Hillierie' cross pollinators. The 'Granny Smith' trees were planted on seedling rootstocks at Vastrap in 1964 with a spacing of 6 m x 6 m. The 'Fuji' trees on M793 rootstocks were planted in 1995 with a spacing of 4 m x 1 m with 'Hillierie' cross pollinators. The 'Braeburn' and 'Fuji' trees were trained to a central leader. All the trees were part of mature, full-bearing orchards. Full bloom for 'Granny Smith' and 'Braeburn' occurred on 19 September 2002 while full bloom for 'Fuji' was on 28 September 2002.

The standard, commercial pest and disease control measures, as well as irrigation, fertilisation and thinning programmes were maintained during the trials in all three cultivars.

Experimental design and treatments

The trials were designed as randomised complete blocks with eight replications. Each replication consisted of five trees with at least two buffer trees between adjacent treatments. Four treatments were applied on 'Braeburn' and 'Granny Smith': an unsprayed control, four sprays of Surround[®] WP with a commercial tower spray rig, four sprays of Surround[®] WP with a commercial blower spray rig, and five sprays of Surround[®] WP with a tower rig. For 'Fuji' we planned to apply three treatments, an unsprayed control, four sprays with a blower,

and five sprays with a blower. The harvest date was a week earlier than expected and therefore the fifth application was cancelled. The data for the two Surround[®] WP treatments were pooled and analysed together. In each case the initial application (4 December 2002) consisted of 5.5 kg 100 L⁻¹ water applied at a rate of 1000 L ha⁻¹ on 'Granny Smith' (3.6 L tree⁻¹), on 'Braeburn' (0.8 L tree⁻¹) and on 'Fuji' (0.4 L tree⁻¹)

A surfactant (Breakthru) was added to the mixture at 50 mL 100 L⁻¹. A follow-up application was carried out seven days later (11 December 2002) at 3 kg 100 L⁻¹ of water. On 'Braeburn' and 'Granny Smith' further applications were carried out on 10 January 2003, 24 January 2003 and 3 March 2003 at 3 kg 100 L⁻¹. On 'Fuji' further applications were carried out on 13 January 2003 and 24 January 2003. The blower spray appeared to give better coverage of Surround[®] WP than the tower sprays on both 'Braeburn' and 'Granny Smith'.

Pre-harvest measurements

In each replication 25 fruit were tagged. Fruit diameter was measured at 77, 105, 144 and 168 days after full bloom (DAFB) on the 'Braeburn' and 'Granny Smith' trees and at 69, 105, 133 and 159 DAFB on the 'Fuji' trees. Net CO₂ assimilation rates and stomatal conductances were measured with a LI-6400 Photosynthesis System (Li-Cor, Lincoln, NE, USA) on 'Fuji'. Two leaves per tree on eight control trees and two leaves per tree on eight treated trees were measured on 25 and 26 February 2003 at a CO₂ concentration of 380 μmol mol⁻¹ and ambient leaf to air vapour pressure deficit of 3.5-5.7 kPa. Ambient temperature on 25 and 26 February 2003 during the measurements was about 36°C and 38°C, respectively. Incident solar radiation was tracked with a LI-190-SA external quantum sensor and the same radiation was supplied with an internal light source (LI-6400-02B, Li-Cor, Lincoln, NE, USA). Only readings taken above light saturation (>1000 μmol m⁻² s⁻¹ photosynthetic photon flux density) were used in the analysis.

Fruit surface temperatures were measured on 27 January 2003 on 'Granny Smith' apples, and on 24 and 26 February 2003 on 'Fuji'. The 'Granny Smith' measurements were taken in three blocks with two treatments (control and Surround[®] WP) and 25 fruit per treatment. The 'Fuji' measurements were taken in four blocks with two treatments (control and Surround[®] WP) and 25 fruit per treatment. In all cases a hand held infra-red thermometer (Raynger MX4,

Raytek Corporation, Santa Cruz, CA) was used. Air temperature was measured in a control and a treated tree using TinyTag data loggers (Gemini Data Loggers Ltd., Chichester, UK).

Harvest measurements

Sun-exposed fruit from the 'Braeburn' and 'Fuji' trees were harvested on 13 March 2003, while 'Granny Smith' was harvested on 19 March 2003. These fruit were picked at random and were not the tagged fruit used in the growth measurements. Twenty fruit per replication were brought to the laboratory and the following parameters were determined: a) fruit diameter; b) fresh fruit mass; c) flesh firmness on both cheeks using a penetrometer; d) percentage total soluble solids (TSS) using a refractometer; e) percentage starch breakdown using the iodine test and the URS pome fruit chart for starch conversion; f) colour using the Deciduous Fruit Board chart A38 for 'Granny Smith' (chart values 0.5-5.0, where 0.5 = dark green and 5 = yellow), chart A44 for 'Braeburn' (chart values 1-8, with 1=red) and chart A45 (chart values 1-12, with 1= red) for 'Fuji'. Sunburn was graded on a scale of 1-3 where 1=no sunburn, 2=slight sunburn and 3= severe sunburn (necrosis).

Statistical analysis

Data was analysed using a one-way ANOVA with blocks for the fruit quality measurements and a t-test for the photosynthetic and stomatal conductance measurements (Enterprise Guide VI, release 1.3 SAS Institute, Cary, NC, USA, 2001). Treatment means were compared using the LSD test at the 5% level.

Results

Microclimate

There were no differences in canopy air temperature between sprayed and unsprayed 'Braeburn' trees (representative data for one day shown in Fig. 1). Fruit surface temperatures were reduced by 0.5°C-2.4°C on 'Fuji' and 'Granny Smith', but not significantly (Table 1).

Gas exchange

Surround[®] WP significantly reduced light saturated photosynthetic rates in 'Fuji' on both measurement dates, as well as stomatal conductance on the second measurement date (Table 1).

Fruit growth

Fruit growth was not affected by Surround[®] WP (Figs 2, 3 and 4) until a week or two before harvest when the last measurements were taken. ‘Granny Smith’ fruit were, however, slightly heavier at harvest when Surround[®] WP was applied using a blower but the statistical result was not significant when the F-statistic is considered (Table 2, Fig. 2). Surround[®] WP did not have a meaningful effect on fruit size of ‘Fuji’ (Table 3, Fig. 3) or ‘Braeburn’ (Table 4, Fig. 4).

Fruit quality

Surround[®] WP treatments slightly improved green colour on ‘Granny Smith’ apples (Table 2). Firmness of ‘Granny Smith’ apples was slightly higher when Surround[®] WP was applied five times with a tower compared to four applications with a tower or no applications (Table 2). There were no effects on TSS of ‘Granny Smith’ apples, but starch breakdown was retarded when Surround[®] WP was applied four times with a blower or five times with a tower compared to four applications with a tower spray or the unsprayed control (Table 2). Surround[®] WP had no effect on harvest quality of ‘Fuji’ apples (Table 3). TSS of ‘Braeburn’ was lower by 0.9-1.1% when Surround[®] WP was applied with a tower spray rig as opposed to a blower, but the unsprayed control did not differ significantly from the treatments (Table 4). Starch breakdown seemed to be slightly retarded when Surround[®] WP was applied five times with a tower spray rig compared to four applications with a blower, but the result was not statistically significant when the F-statistic is considered (Table 4).

All three cultivars had a lower incidence of sunburn when Surround[®] WP was applied (Tables 5, 6 and 7). ‘Granny Smith’ (Table 5) had the best results and total sunburn was reduced from 58% to about 22%. Severe sunburn (brown necrotic tissue) was almost eliminated. In ‘Braeburn’ sunburn was reduced from 33% to about 20% (Table 6), and in ‘Fuji’ there was a significant reduction in total sunburn from 44% to 29%. Severe sunburn, however, was not eliminated (possibly this had already occurred prior to the first Surround[®] WP application). It should be pointed out that fruit were sampled from exposed positions on the outside of the canopy, which resulted in high sunburn percentages compared to farm statistics based on the total crop.

Discussion

Similar to results of Le Grange *et al.*, (2004), we found that Surround[®] WP significantly reduced sunburn on exposed fruit. Absorption of radiant heat is affected by the surface characteristics of the object being heated (Cutnell & Johnson, 1995) and Surround[®] WP is highly reflective to ultraviolet as well as visible wavelengths (Glenn *et al.*, 2002). Rabinowitch *et al.* (1974) showed that visible light as well as heat are necessary for the development of sunburn in tomatoes. Andrews & Johnson (1996) postulate that sunburn injury is caused directly by photo-oxidative damage from visible and UV light, exacerbated after the fruit has been 'preconditioned' for this damage by high temperatures. Unlike Glenn *et al.* (2002), Melgarejo *et al.* (2003) and Wünsche *et al.* (2004) who measured reductions of up to 7°C in Surround[®] WP treated fruit compared to uncoated fruit, no significant effect on fruit surface temperatures was found in this study. We did, however, notice a trend towards lower fruit surface temperatures. Therefore, the reduction of sunburn by particle film is probably due to both a reduction in temperature as well as an increase in reflection of ultraviolet and visible light.

Slight reductions in photosynthetic rate were found in 'Fuji' on both measurement dates, accompanied by, and probably due to reductions in stomatal conductance. Other researchers have reported a reduction in single leaf carbon assimilation due to reduced light absorption, but found no effect on whole canopy carbon assimilation (Warner, 2001; Wünsche *et al.*, 2004). It is thought that increased reflection inside the canopy compensates for the lower carbon assimilation. Wünsche *et al.* (2004) did not, however, find any reduction in stomatal conductance or transpiration, and concluded that increased light reflection reduces leaf surface temperature, thereby maintaining high stomatal conductance under high ambient temperatures. The same argument of heat stress reduction has been put forward to explain increases in conductance and assimilation rate found in other studies (Glenn *et al.*, 2003; Jifon & Syvertsen, 2003). In the Wünsche *et al.* (2004) study, Surround[®] WP was applied at 5% with a single nozzle handgun sprayer, which generally results in better coverage than that achieved with blower or tower sprays, and thus possibly greater reductions in fruit as well as leaf surface temperature. Since no significant reduction in fruit surface temperature was found in this study, in spite of visually adequate coverage, it is likely that Surround[®] WP did not reduce leaf temperatures to a large degree either. Under these conditions, the increased

reflectance of the treated leaves probably resulted in lower light absorption and thus lower demand for CO₂, which would then lower stomatal conductance. Le Grange *et al.* (2004) also found a reduction in carbon assimilation and stomatal conductance in Surround[®] WP treated ‘Cripps’ Pink’ apple trees in South Africa on one of two measurement days.

Applying Surround[®] WP four times with a blower resulted in slightly heavier fruit in ‘Granny Smith’ compared to the control, but the results were not significant when the F-statistic is considered. Le Grange (2001) also found no effect on ‘Granny Smith’ fruit mass. Glenn *et al.* (2001; 2003) found an improvement in fruit size and/or yield due to particle film applications, accompanied by, and probably due to increased carbon assimilation due to lower leaf temperatures. We, however, observed a slight reduction in photosynthetic rate in Surround[®] WP treated leaves. Schupp *et al.* (2002) found that Surround[®] WP reduced fruit weight on ‘Fuji’ and ‘Honeycrisp’ apples. They proposed that the increases in fruit mass in Glenn’s studies could be due to the fact that the measurements were taken in a hot environment with trees showing signs of heat stress. In such conditions, Schupp *et al.* (2002) argued, high temperatures would limit carbon assimilation, whereas in the cooler conditions in which their study was conducted the increased reflection due to Surround[®] WP would be limiting. Another explanation could be that Surround[®] WP reduced fruit temperature, which would lower respiration in the fruit. However, Bergh (1990) and Calderon-Zavala *et al.* (2004) found that final fruit size was not affected by temperature after about 20 DAFB.

All applications of Surround[®] WP slightly improved colour in ‘Granny Smith’. Glenn *et al.* (2001) also found an improvement in colour on ‘Red Chief’ apples in Santiago, which they attributed in part to reduced fruit surface temperature. Schupp *et al.* (2002) found a reduction in red fruit colour on ‘Fuji’ and ‘Honeycrisp’ apples which they attributed to increased reflection. The improvement in green colour on ‘Granny Smith’ apples in our trial could be due to a reduction in fruit surface temperature, as high temperatures have been associated with chlorophyll breakdown (Ma & Cheng, 2003). Also, the improvement in green colour could be due to the reduction of sunburn-associated discolouration. Unlike Le Grange (2001), we found no effect on ‘Fuji’ colour, which is probably due to the lighter covering of Surround[®] WP achieved with the blower compared to the hand-held spray boom used in their study. Applying Surround[®] WP four times with a blower or five times with a tower resulted in lower levels of starch breakdown in ‘Granny Smith’ compared to four applications with a

tower and the unsprayed control. However, the differences were small and the effect on firmness was erratic. Neither Schupp *et al.* (2002) nor Glenn *et al.* (2001) found any meaningful differences in firmness on apples due to Surround® WP.

In conclusion, Surround® WP was effective in reducing sunburn on apples without adversely affecting other fruit quality parameters at harvest. If reliable and affordable methods of removing Surround® WP deposits before packing can be found, our recommendation would be that Surround® WP be applied at least five times during the season with a spray device which ensures the best coverage on a particular orchard.

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Table 1. Fruit surface temperature (°C) of ‘Granny Smith’ and ‘Fuji’ and light-saturated photosynthetic rate and stomatal conductance of ‘Fuji’, measured on trees sprayed with Surround® WP particle film on clear days in January and February 2003.

	Control	Surround® WP	Pr>F
<u>‘Granny Smith’</u>			
Fruit surface temperature (°C) 27 Jan	39.4 a ^z	37.0 a	0.6172
<u>‘Fuji’</u>			
Fruit surface temperature (°C) 24 Feb	32.5a	32.0a	0.4908
Fruit surface temperature (°C) 26 Feb	41.7a	39.9a	0.2600
Photosynthetic rate 25 Feb ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	15.9 a	14.2 b	0.0282
Stomatal conductance 25 Feb ($\text{mol m}^{-2} \text{s}^{-1}$)	0.206 a	0.175 a	0.1061
Photosynthetic rate 26 Feb ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	15.3a	13.7b	0.0339
Stomatal conductance 26 Feb ($\text{mol m}^{-2} \text{s}^{-1}$)	0.177a	0.131b	0.0065

^z Means in a row with the same letter are not significantly different (LSD $P \leq 0.05$).

Table 2. The effect of Surround[®] WP particle film applications, sprayed with a tower sprayer (four or five times) or a blower (four times) in the 2002/2003 season at Vastrap (Koue Bokkeveld), on harvest quality of 'Granny Smith' apples.

	Control	Tower x 4	Tower x 5	Blower x 4	Pr>F
Mass (g)	181.9b ^y	188.6ab	190.5ab	198.7a	0.0791
Diameter (mm)	70.2ab	69.1b	74.9a	75.3a	0.0584
Colour (chart ^z)	4.4a	4.1b	4.1b	4.0b	0.0168
Firmness (kg)	7.8bc	7.7c	8.0a	7.9ab	0.0018
TSS (%)	10.9a	10.8a	11.0a	11.0a	0.2034
Starch (% breakdown)	31.3a	30.7a	24.2b	22.5b	0.0050

^y Means in a row with the same letter are not significantly different (LSD $P \leq 0.05$).

^z Values 1-12 with 1=dark green

Table 3. The effect of four Surround[®] WP applications with a blower on harvest quality of 'Fuji' apples in the 2002/2003 season at Tandfontein (Koue Bokkeveld).

	Control	Blower x 4	Pr>F
Mass (g)	170.4a ^y	173.4a	0.0756
Diameter (mm)	72.9a	73.2a	0.1139
Colour (chart ^z)	7.0a	6.8a	0.1237
Firmness (kg)	8.4a	8.5a	0.1217
TSS (%)	15.3a	15.6a	0.1763
Starch (% breakdown)	33.4a	35.2a	0.2239

^y Means in a row with the same letter are not significantly different (LSD $P \leq 0.05$).

^z Values 1-12 with 1=red

Table 4. The effect of Surround[®] WP particle film applications, sprayed with a tower sprayer (four or five times) or a blower (four times) in the 2002/2003 season at Vastrap (Witzenberg Valley), on harvest quality of 'Braeburn' apples.

	Control	Tower x 4	Tower x 5	Blower x 4	Pr>F
Mass (g)	177.8a ^y	182.9a	177.8a	181.2a	0.5952
Diameter (mm)	71.9a	72.8a	71.8a	72.6a	0.4203
Colour (chart ^z)	2.7a	2.8a	2.8a	3.0a	0.2890
Firmness (kg)	10.1a	9.9a	10.1a	10.0a	0.7582
TSS (%)	12.0ab	11.3b	11.5b	12.4a	0.0489
Starch (% breakdown)	14.1ab	14.6ab	11.0b	15.9a	0.0677

^y Means in a row with the same letter are not significantly different (LSD $P \leq 0.05$).

^z Values 1-8 with 1=red

Table 5. The effect of Surround[®] WP particle film applications, sprayed with a tower sprayer (four or five times) or a blower (four times) in the 2002/2003 season at Vastrap (Witzenberg Valley), on sunburn of exposed 'Granny Smith' apples.

	Control	Tower x4	Tower x5	Blower x4	Pr>F
No sunburn (%)	41.9b	78.1a	78.1a	76.9a	<0.0001
Slight sunburn (%)	50.6a	21.9b	21.3b	23.1b	0.0003
Severe sunburn (%)	7.5a	0.0b	0.6b	0.0b	0.0003

^z Means in a row with the same letter are not significantly different (LSD $P \leq 0.05$).

Table 6. The effect of Surround[®] WP particle film applications, sprayed with a tower sprayer (four or five times) or a blower (four times) in the 2002/2003 season at Vastrap (Witzenberg Valley) on sunburn of exposed 'Braeburn' apples.

	Control	Tower x4	Tower x5	Blower x4	Pr>F
No sunburn (%)	67.0b ^z	78.4ab	80.6a	81.3a	0.0790
Slight sunburn (%)	33.0a	21.6ab	19.4b	18.7b	0.0790
Severe sunburn (%)	0.0a	0.0a	0.0a	0.0a	-

^zMeans in a row with the same letter are not significantly different (LSD $P \leq 0.05$).

Table 7. Percentage sunburn of exposed 'Fuji' apples in response to four applications of Surround[®] WP particle film, sprayed with a blower sprayer on Tandfontein in the Koue Bokkeveld.

	Control	Blower x4	Pr>F
No sunburn (%)	55.6b	70.1a	0.0450
Slight sunburn (%)	36.9a	25.9a	0.3234
Severe sunburn (%)	7.5a	3.4a	0.6104

^zMeans in a row with the same letter are not significantly different (LSD $P \leq 0.05$).

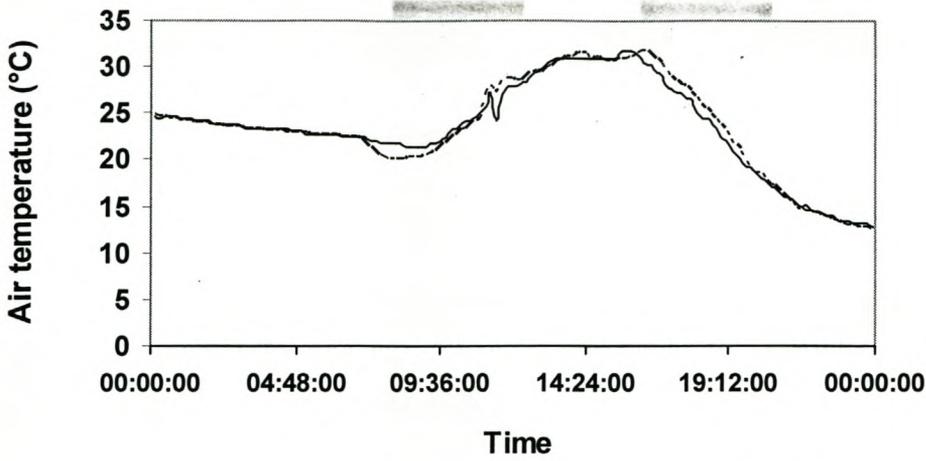


Figure 1. Air temperature within 'Braeburn' apple trees sprayed with Surround WP or unsprayed (control) from midnight until midnight on 24 January 2003. Information on the identity of each data set was lost.

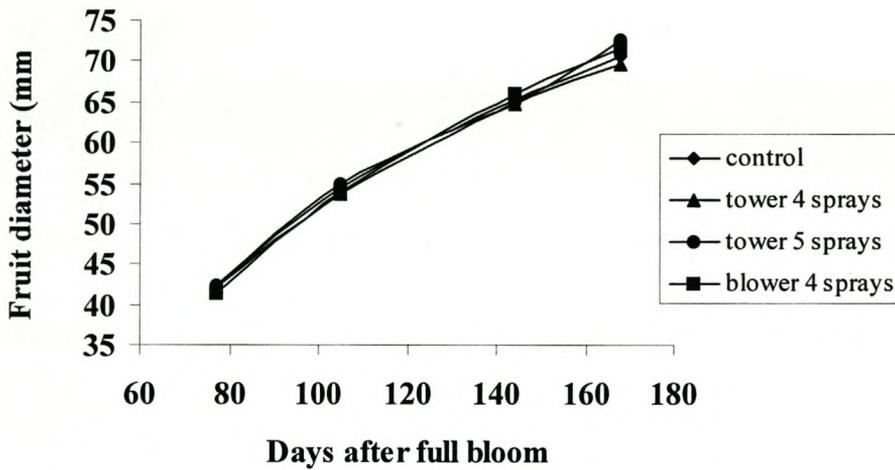


Figure 2. Fruit diameter of 'Granny Smith' apples in response to applications of Surround WP particle film, sprayed with either a tower sprayer (4 or 5 times) or a blower (4 times) throughout the 2002/2003 season at Vastrap in the Witzenberg Valley.

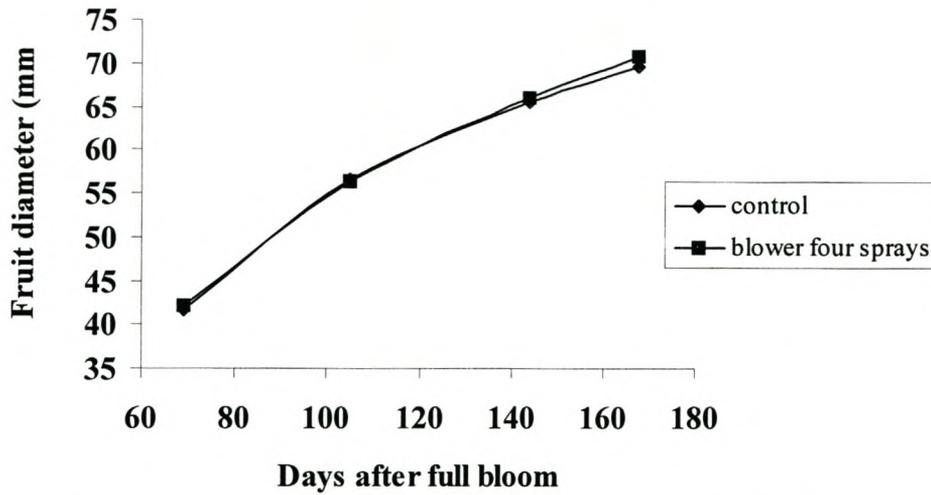


Figure 3. Fruit diameter of 'Fuji' apples in response to applications of Surround WP particle film, sprayed four times with a blower sprayer throughout the 2002/2003 season at Tandfontein in the Koue Bokkeveld.

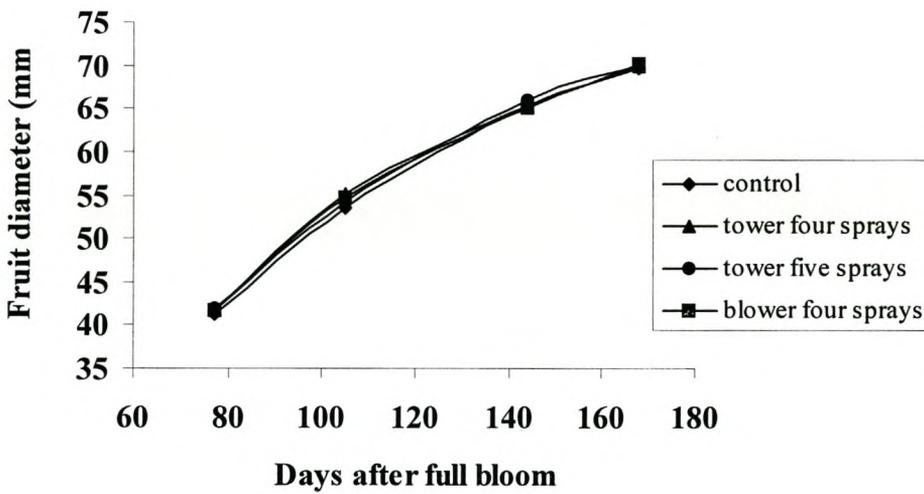


Figure 4. Fruit diameter of 'Braeburn' apples in response to applications of Surround WP particle film, sprayed with either a tower sprayer (4 or 5 times) or a blower (4 times) throughout the 2002/2003 season at Vastrap in the Witzenberg Valley.

4. PAPER 3. THE EFFECT OF HIGH TEMPERATURE ON FV/FM OF SUN-EXPOSED AND SHADED APPLE SURFACES

Abstract

The effect of fruit surface temperatures (24°C, 30°C, 33°-35°C, 40°-43°C and 48°-49°C) for increasing periods of time (two, four, six or eight hours) on maximum quantum yield of fluorescence (Fv/Fm) was measured on exposed and shaded sides of detached 'Cripps' Pink' and 'Royal Gala' apples. Measurements were taken directly after removal from the chamber as well as 12 hours later to gauge whether there was any recovery. Permanent damage to PSII occurred between 48°-49°C irrespective of duration, with permanent damage possible at 42°-43°C when exposure exceeded six hours. No meaningful differences in Fv/Fm were found between shaded and exposed sides of apple fruit after exposure to high temperature. Duration of exposure was important in lowering PSII efficiency in 'Cripps' Pink' but less so in 'Royal Gala'. 'Cripps' Pink' apples harvested later in the season appeared to be able to recover from heat stress better than apples harvested earlier in the season. The results are discussed in relation to known mechanisms of heat resistance.

Introduction

Fruit manifest solar injury when complementary protective mechanisms existing within the fruit skin are overcome by a combination of excess ultraviolet, visible, and/or thermal solar radiation (Andrews & Johnson, 1996). It is postulated that sunburn injury is caused directly by photo oxidative damage from visible and ultraviolet (UV) light, exacerbated after the fruit has been 'preconditioned' for this damage by high temperatures (Andrews & Johnson, 1996). Rabinowitch *et al.* (1974) reported that in addition to heat, visible light was necessary for development of typical sunburn symptoms in tomatoes. They concluded that sunburn is caused by the concurrent action of two external factors, heat and light. Rabinowitch (1981) later reported that fruit skin temperature thresholds of 38°C to 40°C were found to be essential for development of sunburn in cucumbers and peppers, respectively. Light in the visible spectrum was found to be essential, as no bleaching occurred in the dark (Rabinowitch, 1981). He suggested that sunburn occurs when photosynthesis is disturbed by excessive heat, so that light energy is redirected into damaging photodynamic processes (Rabinowitch, 1981). Schrader *et al.* (2001) distinguishes between two

types of sunburn on apples: 'sunburn necrosis' and 'sunburn browning'. Sunburn necrosis results in the formation of a necrotic spot on the sun-exposed side of the fruit, is independent of irradiation and results from thermal death of cells in the peel when temperatures reach $52 \pm 1^\circ\text{C}$. Electrolyte leakage increases significantly in peel from apples with necrosis (Schrader *et al.*, 2001). The second type is sunburn browning, a sub-lethal event that results in a yellow, bronze, or brown spot on the exposed side of the apple (Schrader *et al.*, 2001). Sunburn browning occurs when the fruit surface temperature reaches $46^\circ\text{--}49^\circ\text{C}$ in the presence of sunlight (Schrader *et al.*, 2001). The threshold temperature required for sunburn browning appears to be cultivar dependent (Schrader *et al.*, 2001).

It is known that apples exposed to sunlight accumulate higher levels of anthocyanins than shaded fruit (Andrews & Johnson, 1996; Awad *et al.*, 2001, Ma & Cheng, 2003) which may be a protective function against photo-oxidative damage. Severest damage occurs on fruit which becomes suddenly exposed to direct sun (Wilton, 1999) whereas fruit which developed in exposed positions are less susceptible. Woolf & Laing (1996) found that pretreating avocados with hot water (38°C for one hour) reduced subsequent external browning after exposure to 50°C . This suggests that fruit have a way of developing resistance to the effects of intense sunlight and high temperatures.

Chlorophyll *a* fluorescence measurements are applied to various fields of plant physiology (Krause & Weiss, 1984; Maxwell & Johnson, 2000). When a chlorophyll molecule absorbs a photon of light an electron is excited from a ground state to a higher orbital (Salisbury & Ross, 1992). This energy can be used for photochemical charge separation, or dissipated as heat, or emitted via fluorescence (Salisbury & Ross, 1992; Maxwell & Johnson, 2000), with a peak at 682 nm, and a broad shoulder at about 740 nm (Krause & Weiss, 1984). The maximum quantum efficiency of Photosystem II (PSII) is given by the measurement F_v/F_m , which in healthy leaves is close to 0.8, irrespectively of the plant species studied (Fracheboud, 2004). 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, which are apparently the most heat sensitive part of the photosynthetic mechanism (Krause & Santarius, 1975; Weiss & Berry, 1988). This fact makes the measurement of fluorescence a handy tool for

studying the effects of heat stress on apple surface tissues. If we understand more about how apples react to high temperatures we will be able to formulate more effective strategies to combat sunburn. The purpose of this paper is to study the effects of high temperature on maximum quantum yield of fluorescence (F_v/F_m) on the sun-exposed and shaded sides of 'Cripps' Pink' and 'Royal Gala' apples in order to establish temperature thresholds for sunburn necrosis.

Materials and Methods

Fruit from apple trees (*Malus domestica* L. Borkh) was obtained from Welgevallen experimental farm in Stellenbosch (33°56'S, 18°51'E). The 'Royal Gala' and 'Cripps' Pink' trees on M793 rootstocks were planted in 1998 at a spacing of 4m x 1.5 m. The row orientation for both cultivars is north-east by south-west. 60 'Royal Gala' apples were picked on 30 January 2002 a few days before harvest, and 60 'Cripps' Pink' apples were picked on 7 February 2002 and 25 March 2002 (harvest 9 April 2002) as well as 25 March 2003 (harvest 7 April 2003). 20 fruit per row for three rows were picked from the south-eastern aspect of the trees and 20 fruit per row for three rows were picked from the more exposed north-western aspect. The fruit were cut in half and placed face down in a shallow tray filled with water. The fruit halves used were the exposed side of an exposed fruit from the north-western aspect, the accompanying shaded side of the same exposed fruit, and one half of a shaded fruit from the south-eastern aspect. The fruit were then placed in the dark in growth chambers or ovens at air temperatures of 24°C, 32°C, 37°C, 48°C and 57°C, as measured with Tiny Tag data loggers (Gemini Data Loggers Ltd, Chichester, UK), for two, four, six or eight hours. For every combination of time exposed to the heat treatment, fruit side and temperature there were three replications.

Maximum quantum yield of fluorescence (F_v/F_m) was measured using a pulse modulated fluorometer (FMS2, Hansatech Instruments Ltd., Norfolk, England), and fruit surface temperature was measured using a hand held infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, CA) immediately after fruit removal at the above time intervals. Fluorescence was again measured 12 hours after the heat treatments, at room temperature (20°C), to determine possible recovery. Actual fruit surface temperatures measured directly after removal from the chambers are shown in Table 1. These were fairly consistent between the experiments,

only the highest temperature treatment differed noticeably, being about 5°C lower on 25 March 2002 than on the other dates owing to poor temperature control on this oven.

Data was analysed using a three-way ANOVA and Tukey's studentised range (HSD) was calculated at a 5% level to compare means (Enterprise Guide VI, release 1.3 SAS Institute, Cary, NC, USA, 2001).

Results

'Cripps' Pink'

7 February 2002. There was significant interaction ($P < 0.0068$) between duration of exposure and temperature (Table 2). The temperatures 23°C and 30°C did not affect Fv/Fm regardless of the duration (Fig. 1a). Exposure to 35°C caused a decrease of Fv/Fm to around 0.7 after four hours of exposure after which Fv/Fm stabilised at this level (Fig. 1a). Fv/Fm decreased to around 0.6 after two hours of exposure to 42°C after which it steadily declined with every two hour interval reaching a minimum of 0.4 after eight hours of exposure (Fig. 1a). Two hours of exposure to 48°C caused Fv/Fm to decrease to 0.4, with a rapid decline in Fv/Fm in the next two hours to 0.2, after which the rate of decline slowed reaching 0.1 after eight hours (Fig. 1a).

There was significant interaction between the fruit side exposed and temperature ($P > 0.0121$, Table 2). The three kinds of sides exhibited similar Fv/Fm values of between 0.8 and 0.7 at 23°C and 30°C, after which the rate of decline was slower on the shaded fruit than on the exposed fruit (both sides) (Fig. 1c). An increase in duration of exposure resulted in rather constant levels of Fv/Fm on the different fruit sides (Fig. 1c), with no significant interaction between these factors (Table 2).

8 February 2002 (Recovery). There was significant three-way interaction (Table 3). Fruit subjected to 23°C, 30°C and 35°C exhibited full recovery on the day following the heat stress treatments (Fig. 2a). The 42°C treatments showed higher Fv/Fm values (Fig. 4) for every time interval measured compared to the day before but not full recovery (Fig. 1a). Fruit exposed to 48°C for two hours had recovered well to around 0.75 (Fig. 2a). However, Fv/Fm values for the 48°C treatments lasting four, six and eight hours were all below 0.1 (Fig. 2a), indicating

irreversible tissue damage. Even though the factor 'side' appeared to have significant effects, the differences were very small (Fig. 2b, 2c).

25 March 2002. There was significant interaction between duration of exposure and the temperature of the heat treatments ($P_{r>F}$ 0.0004, Table 4). Similar to the results of 7 February 2002, no damage to the photosystem (F_v/F_m of around 0.8) was measured on fruit exposed to 24°C, 30°C, 33°C and 41°C for all exposure periods (Fig. 3a). The interaction between fruit side and treatment temperature was significant ($P_{r>F}$ 0.0300, Table 4). The relationship between fruit side and F_v/F_m was, however, not clear, due to fluctuating values of F_v/F_m (Fig. 3b). There was no significant interaction between duration of exposure and fruit side (Table 4).

26 March 2002 (recovery). There was significant interaction between duration of exposure and temperature of the heat treatment ($P_{r>F}$ 0.0029; Table 5). F_v/F_m values showed full recovery in most cases, with only the 43°C treatment measuring below 0.8, and then only after exposure longer than six hours (Fig. 4a). The interaction between duration of exposure and fruit side was significant ($P_{r>F}$ 0.0029, Table 5), however, the different fruit sides exhibited recovery F_v/F_m values of around 0.8 for all the heat treatments (Fig. 4b), so that any differences are not regarded as meaningful. There was no significant interaction between duration of exposure and fruit side (Fig. 4c).

25 March 2003. There was significant three-way interaction between the factors (Table 6). No damage to the photosystem was measured for temperature treatments $\leq 30^\circ\text{C}$ (Fig. 5a). The 34°C treatment also did not exhibit F_v/F_m values indicative of photosystem damage until after six hours, when it decreased to 0.7 (Fig. 5a). The 41°C treatment returned F_v/F_m values of about 0.65 until exposure exceeded four hours, after which it steadily declined to 0.4 after eight hours (Fig. 5a). Very low F_v/F_m values (< 0.2) were measured for the 48°C after all exposure times (Fig. 5a).

The shaded side of exposed fruit, as well as shaded fruit showed no damage to photosystems for temperatures $\leq 34^\circ\text{C}$, with no damage on shaded fruit even at 41°C (Fig. 5b). At temperatures below 34°C, the exposed side of exposed fruit returned lower values (Fig. 5b). When temperature

increased above 34°C, Fv/Fm declined steadily on both sides of exposed fruit (Fig 5b). The different sides of the fruit did not appear to respond differently to increasing duration of exposure (Table 6, Fig. 5c).

26 March 2003 (recovery). There was significant interaction between duration of exposure and chamber temperature ($P < 0.0001$, Table 7). The temperature treatments $\leq 34^\circ\text{C}$ exhibited no damage to the photosystems with Fv/Fm values of approximately 0.8 (Fig. 6a), similar to the previous day's measurements (Fig. 6a). The 41°C treatment recovered to values slightly lower than 0.8 although still in the range considered healthy (Fig. 6a). Fv/Fm values for the 48°C treatment were still below 0.1 (Fig. 6a), except when duration of exposure exceeded six hours, when Fv/Fm values were 0.3 (Fig. 6a). There was significant interaction ($P = 0.0034$) between fruit side and chamber temperature (Table 7). Fv/Fm values for the three fruit sides indicated no permanent damage to the photosystems (0.8) until the temperature exceeded 41°C (Fig. 6b). At 48°C shaded sides of exposed fruit and shaded fruit had Fv/Fm values of < 0.1 , whereas exposed sides remained slightly higher at about 0.2 (Fig. 6b). There was no interaction between fruit side and duration of exposure (Table 7, Fig. 6c).

'Royal Gala'

30 January 2002. There was significant interaction between duration of exposure and treatment temperature ($P = 0.0041$, Table 8). Regardless of duration of exposure, fruit exposed to the 24°C, 30°C and 35°C treatments exhibited Fv/Fm values of about 0.8 (Fig. 7a). Exposure to 40°C reduced Fv/Fm values to fractionally below 0.8 for the first six hours of exposure, but then they decreased to below 0.7 after eight hours (Fig. 7a). Exposure to 49°C yielded very low Fv/Fm values of below 0.1 throughout the exposure range (Fig. 7a).

There was no interaction between fruit side and treatment temperature ($P = 0.1326$, Table 8, Fig 7b), or between fruit side and duration of exposure ($P = 0.5134$, Table 8, Fig. 7c).

31 January 2002 (recovery). There was significant interaction between duration of exposure and treatment temperature ($P = 0.0115$, Table 9). The 40°C and 48°C treatments were the only treatments where Fv/Fm did not recover to above 0.8, but in the case of the 40°C treatment the

only value slightly lower than 0.8 occurred after eight hours (Fig. 8a). There was no significant interaction between side and chamber temperature ($P > F$ 0.0974, Table 9, Fig. 8b). There was also no significant interaction between duration of exposure and side ($P > F$ 0.2728, Table 9, Fig. 8c).

Discussion

Throughout the trials, increasing temperature was the main cause of reduction in PSII efficiencies. Heat treatments lower than or equal to 35°C did not lower PSII operating efficiency on either 'Cripps' Pink' or 'Royal Gala' apples as measured by dark adapted Fv/Fm. Exposure to 40°-41°C significantly reduced Fv/Fm in both cultivars, but Fv/Fm fully recovered to 0.8 overnight. Exposure to temperatures in the 42°-43°C range significantly reduced Fv/Fm values in 'Cripps' Pink', with those apples harvested on 25 March 2002 exhibiting higher levels of recovery than those harvested on 7 February 2002, especially when duration of exposure exceeded six hours. These treatments did not experience full recovery. Temperatures of 48°-49°C strongly and permanently reduced PSII efficiency in both cultivars, with no recovery by the following day except after only two hours of exposure in younger 'Cripps' Pink'. The 'Cripps' Pink apples picked a few days before harvest (25 March) exhibited higher Fv/Fm values in the 42°-43°C temperature range, as well as higher levels of recovery, than apples harvested earlier (7 February 2002). Recovery was also high in mature apples during 2003 after exposure to 41°C, although the immediate effects on Fv/Fm were more pronounced than during 2002 at this temperature. It appears that the 'Cripps' Pink' apples acclimatise to high temperatures during the season.

Organisms ranging from bacteria to humans respond to high temperatures by synthesising a new set of proteins, the heat shock proteins (HSP's) (Salisbury & Ross, 1992; Larcher, 1995). Proteins with a molecular mass of 15-42 kDa are designated small heat shock proteins (sHSP's) and due to the rapidity with which they can be expressed they seem to be important in helping plants adapt quickly to changing conditions (De Rocher *et al.*, 1991; Waters *et al.*, 1996). The role played by HSP's in heat tolerance is apparently that of stabilising chromatin structures and membranes and promoting repair mechanisms (Larcher, 1995). 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 (Salisbury & Ross, 1992). Al-Niemi & Stout (2002) found that sHSP's as well as other HSP's were expressed after exposure to 40°C for two hours in the perennial grass *Dichanthelium lanuginosum*, and that these elevated levels of sHSP's and HSP's persisted for 5-7 days after the heat treatment. Takeda *et al.* (2003) found that HSP's were expressed at 45°C in strawberries.

The temperature range at which HSP's are expressed, as well as their persistence, could explain our results, that temperatures in the range of 41°-43°C caused a significant reduction in Fv/Fm that recovered 12 hours later, except on some occasions where duration of exposure exceeded six hours. It is possible that the expression of HSP's reach a peak before six hours and that these proteins are unable to limit further damage. Woolf & Laing (1996) found that pretreating avocados with hot water (38°C for one hour) reduced subsequent external browning after exposure to 50°C. However, they found little correlation between the effect of hot water pre-treatment and Fv/Fm ratio. Paull & Jung Chen (1990) likewise found that exposing papayas to 42°C for 4 hours or 38-42°C for one hour followed by three hours at 22°C resulted in the development of thermotolerance to the otherwise injurious heat treatment of 49°C for 70 minutes. Paull & Jung Chen (1990) found that polypeptides with molecular weights of 17, 18, and 70 kDa showed marked increases after two hours at 38°C. Even though Woolf & Laing (1996) found little correlation between the effect of hot water pre-treatment and Fv/Fm on avocados, the reduction in external browning following hot water pre-treatment, as well as the development of thermotolerance following heat pre-treatment reported by Paull & Jung Chen (1990) in papayas could explain our findings on 'Cripps' Pink' where more mature fruit are apparently better equipped to recover from high temperatures. It is well known that fruit surface temperature can be 10°C-16°C higher than ambient (Schrader *et al.*, 2001). In the Western Cape the period between February and April is often associated with high temperatures. We harvested 'Cripps' Pink' apples on 7 February as well as on 25 March 2002. The apples picked on 25 March 2002 showed higher levels of recovery of Fv/Fm compared to the fruit harvested on 7 February.

In a study on 'Fuji', 'Jonagold' and 'Gala' apples, Ritenour *et al.* (2001) found that apples exposed to direct sunlight accumulated higher levels of sHSP's than apples grown in shaded parts of the canopy. It is possible that the seven week period between the two trials exposed the fruit to

higher temperatures, resulting in fruit surface temperatures in excess of 40°C more frequently, prompting the fruit to express HSP's that would in turn stabilise the membranes allowing them to recover. It appears that the 48°-49°C treatments were too extreme for the heat shock proteins to continue having a protective function. Larcher (1995) distinguishes between two types of heat resistance in eukaryotes, heat sensitive species and relatively heat resistant species. Heat sensitive species are injured at 30°-40°C, or at the most 45°. Relatively heat resistant eukaryotes are plants from sunny and dry localities and are as a rule, able to acquire hardiness to heat and survive heating of 50°-60°C for half an hour.

Another factor which may explain acclimation in more mature fruit could be changes in membrane structure. Raison *et al.* (1980) found that chlorophyll fluorescence yield increased at about 43°C for *Nerium oleander* grown at 20°/15°C, but that fluorescence yield increased only at 53°C for *Nerium oleander* grown at 45°/32°C. Upon investigating the membrane structure, Raison *et al.* (1980) found that the membranes of plants grown at 20°C exhibited the same physical properties at 43°C that the membranes of plants grown at 45°C exhibited at 53°C. From this data it is concluded that the thermal stability of photosynthetic membranes are related to their physical properties.

Contrary to the findings of Ma & Cheng (2003) who reported that the exposed side of apples exhibited lower Fv/Fm values than the shaded side, no meaningful differences on Fv/Fm of sun-exposed and shaded sides of the fruit were found in this study. In a study on mangoes, Hetherington (1997) found lower PSII efficiency on green fruit than on purple fruit, suggesting that anthocyanins are effective in protecting mangoes against photoinhibition. However, only the effects of high temperature were investigated in our study, and not high temperature in conjunction with high light levels. It is likely that anthocyanins play a role in protecting plant tissues against excess light, but do not play a role in heat resistance. Unfortunately we did not measure colour or determine the levels of anthocyanins in this study.

Temperature was the overriding factor in lowering PSII efficiency, but this also depended on duration of exposure, and permanent damage to PSII occurred between 48°-49°C after two hours, with permanent damage possible at the 41°-43°C temperature range when exposure exceeded six

hours. Duration of exposure was important in lowering PSII efficiency on 'Cripps' Pink', but less so on 'Royal Gala'.

The findings that apples are able to recover from heat treatments in the range 40°-43°C when exposure doesn't exceed six hours, could have implications in the management of sunburn, for example, the determination of threshold temperature for the activation of evaporative cooling. However, it should be noted that these heat treatments occurred in the absence of light, which is also a requirement for the development of sunburn browning (Schrader *et al.*, 2001). Further experiments are required to evaluate the effect of these temperatures in conjunction with various light intensities, in order to understand the individual and interactive roles of these two factors in the development of heat stress and sunburn in apples. Ideally, this should also be investigated *in situ* since picked fruit may show differential sensitivity compared to fruit with an intact xylem water supply.

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Table 1. Fruit surface temperatures measured immediately after removal from heat chamber.

Experiment	Temperature treatment 1	Temperature treatment 2	Temperature treatment 3	Temperature treatment 4	Temperature treatment 5
'Royal Gala' 30 Jan 2002	24°C	30°C	35°C	40°C	49°C
'Cripps' Pink' 7 Feb 2002	23°C	30°C	35°C	42°C	48°C
'Cripps' Pink' 25 Mar 2002	24°C	30°C	33°C	41°C	43°C
'Cripps' Pink' 25 Mar 2003	24°C	30°C	34°C	41°C	48°C

Table 2. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), fruit side (side) and chamber temperature (temp) for the ‘Cripp’s Pink’ PSII efficiency trial on 7 February 2002. Refer to Fig. 1.

	Degrees of freedom	F-value	Pr>F
Model	59	8.18	<.0001
Time	3	4.59	0.0040
Side	2	7.23	0.0011
Temp	4	95.6	<.0001
Time*Side	6	1.82	0.1000
Time*Temp	12	2.45	0.0068
Side*Temp	8	2.59	0.0121
Time*Side*Temp	24	0.44	0.9892

Table 3. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), fruit side (side) and chamber temperature (temp) for the ‘Cripp’s Pink’ PSII efficiency recovery trial on 8 February 2002. Refer to Fig. 2.

	Degrees of freedom	F-value	Pr>F
Model	59	124.3	<.0001
Time	3	152.2	<.0001
Side	2	4.35	0.0149
Temp	4	1330.4	<.0001
Time*Side	6	2.41	0.0310
Time*Temp	12	118.9	<.0001
Side*Temp	8	4.74	<.0001
Time*Side*Temp	24	2.90	<.0001

Table 4. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), fruit side (side) and chamber temperature (temp) for the 'Cripp's Pink' PSII efficiency trial on 25 March 2002. Refer to Fig. 3.

	Degrees of freedom	F-value	Pr>F
Model	59	4.4	<.0001
Time	3	2.8	0.0442
Side	2	7.34	0.0010
Temp	4	41.3	<.0001
Time*Side	6	0.45	0.8434
Time*Temp	12	3.28	0.0004
Side*Temp	8	3.23	0.0300
Time*Side*Temp	24	0.46	0.9840

Table 5. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), fruit side (side) and chamber temperature (temp) for the 'Cripp's Pink' PSII efficiency recovery trial on 26 March 2002. Refer to Fig. 4.

	Degrees of freedom	F-value	Pr>F
Model	59	3.37	<.0001
Time	3	1.36	0.0442
Side	2	21.8	0.0010
Temp	4	14.5	<.0001
Time*Side	6	2.00	0.0704
Time*Temp	12	2.80	0.0029
Side*Temp	8	3.15	0.0029
Time*Side*Temp	24	0.93	0.5670

Table 6. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), fruit side (side) and chamber temperature (temp) for the 'Cripp's Pink' PSII efficiency trial on 25 March 2003. Refer to Fig. 5.

	Degrees of freedom	F-value	Pr>F
Model	59	37.8	<.0001
Time	3	3.88	0.0109
Side	2	1.88	0.1564
Temp	4	515.0	<.0001
Time*Side	6	1.40	0.2208
Time*Temp	12	5.21	<.0001
Side*Temp	8	5.33	<.0001
Time*Side*Temp	24	1.80	<.0001

Table 7. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), fruit side (side) and chamber temperature (temp) for the 'Cripp's Pink' PSII efficiency recovery trial on 26 March 2003. Refer to Fig. 6.

	Degrees of freedom	F-value	Pr>F
Model	59	39.6	<.0001
Time	3	0.70	0.5532
Side	2	0.05	0.9533
Temp	4	549.1	<.0001
Time*Side	6	0.64	0.6948
Time*Temp	12	6.11	<.0001
Side*Temp	8	3.08	0.0034
Time*Side*Temp	24	1.52	0.0749

Table 8. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), fruit side (side) and chamber temperature (temp) for the 'Royal Gala' PSII efficiency trial on 30 January 2002. Refer to Fig. 7.

	Degrees of freedom	F-value	Pr>F
Model	59	50.5	<.0001
Time	3	1.29	0.2804
Side	2	2.88	0.0600
Temp	4	724.5	<.0001
Time*Side	6	0.88	0.5143
Time*Temp	12	2.61	0.0041
Side*Temp	8	1.60	0.1326
Time*Side*Temp	24	0.97	0.5044

Table 9. Abbreviated ANOVA table for the analysis of effects of duration of exposure (time), fruit side (side) and chamber temperature (temp) for the 'Royal Gala' PSII efficiency recovery trial on 31 January 2002. Refer to Fig. 8.

	Degrees of freedom	F-value	Pr>F
Model	59	214.5	<.0001
Time	3	2.91	0.0374
Side	2	0.04	0.9639
Temp	4	3142.9	<.0001
Time*Side	6	1.28	0.2728
Time*Temp	12	2.29	0.0115
Side*Temp	8	1.73	0.0974
Time*Side*Temp	24	1.10	0.3522

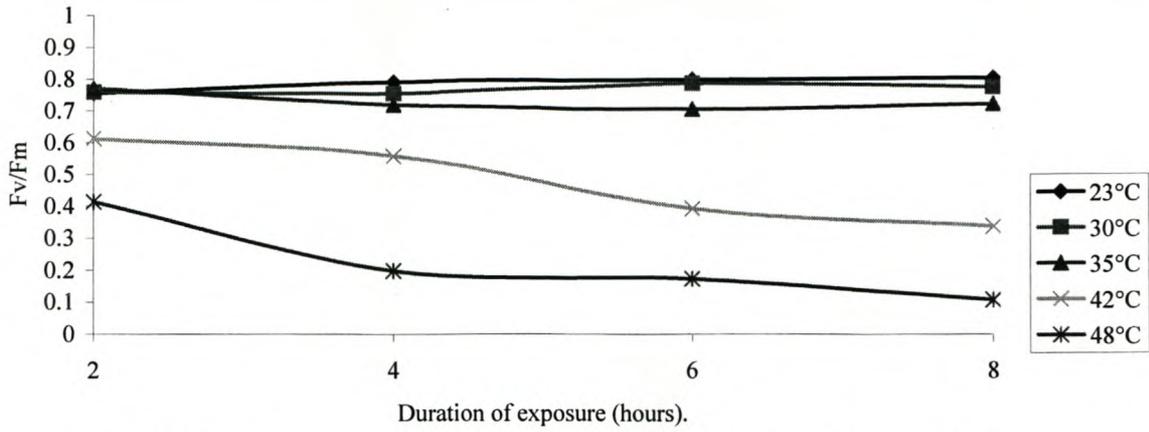


Figure 1a. The effect of duration of exposure to a range of temperatures on Fv/Fm on 'Cripp's Pink' apples on 7 February 2002.

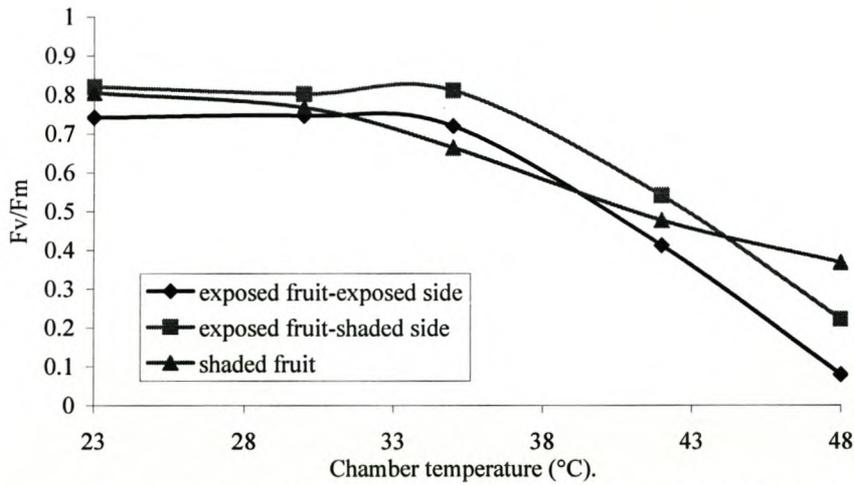


Figure 1b. Fv/Fm on 'Cripp's Pink' fruit grown in different light environments in response to increasing temperature (7 February 2002).

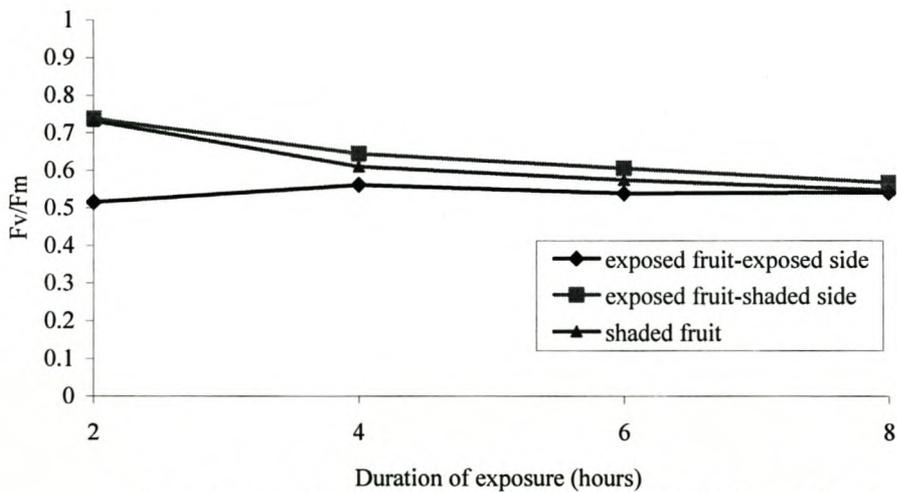


Figure 1c. The effect of duration of exposure on Fv/Fm of 'Cripp's Pink' apples grown in different light environments measured on 7 February 2002.

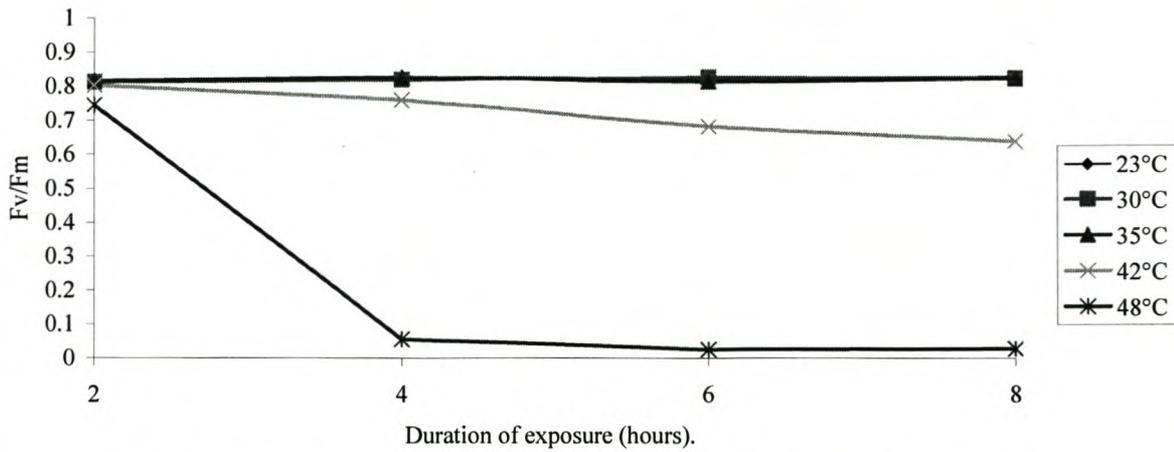


Figure 2a. Recovery of Fv/Fm (8 February 2002) on 'Cripp's Pink' apples that were exposed to different temperatures for increasing amounts of time on 7 February 2002.

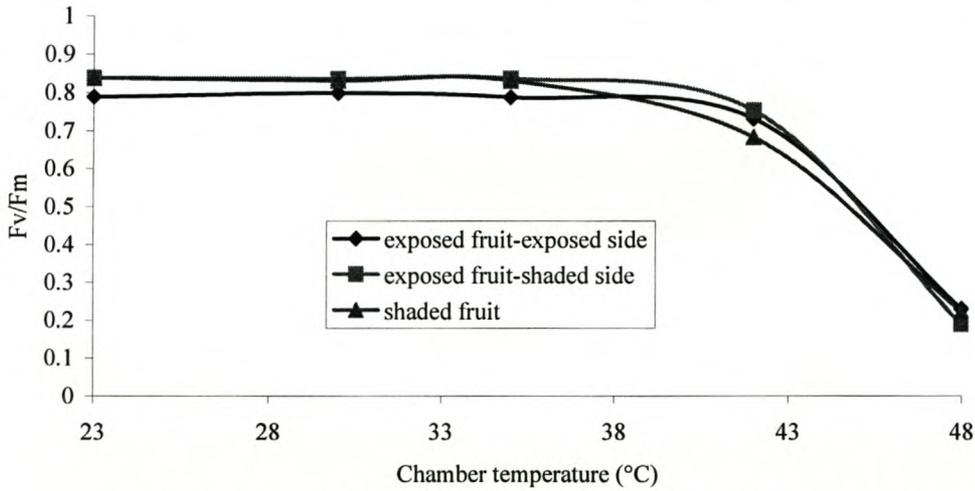


Figure 2b. Recovery (8 February 2002) of Fv/Fm on 'Cripp's Pink' apples grown in different light environments exposed to increasing temperatures on 7 February 2002.

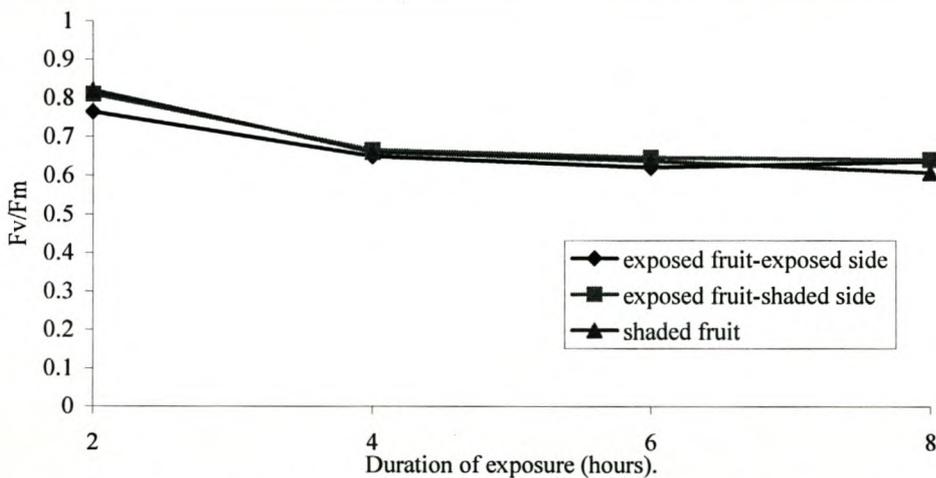


Figure 2c. Recovery of Fv/Fm (8 February 2002) on 'Cripp's Pink' fruit grown in different light environments exposed to heat stress treatments for increasing amounts of time on 7 February 2002.

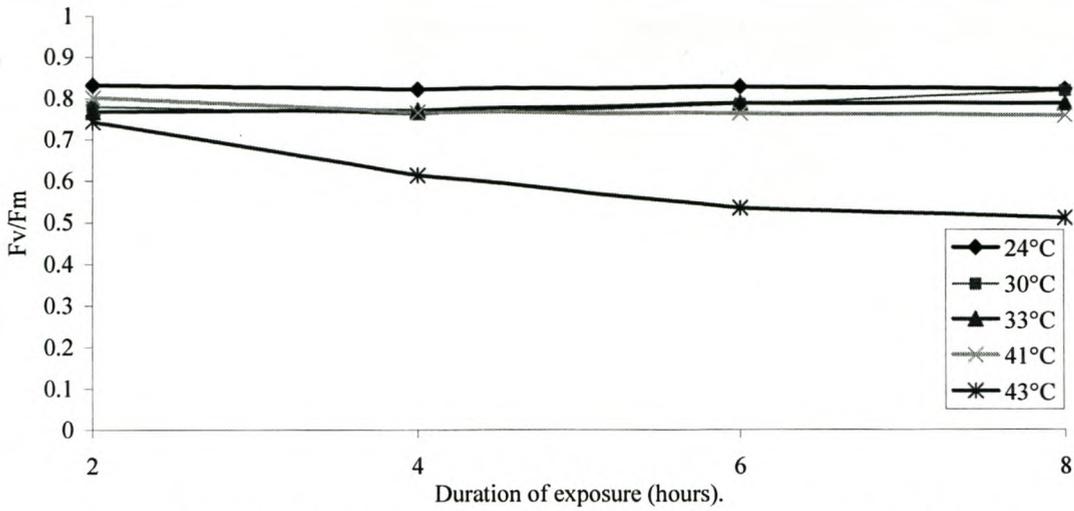


Figure 3a. The effect of duration of exposure to a range of temperatures on Fv/Fm on 'Cripp's Pink' apples on 25 March 2002.

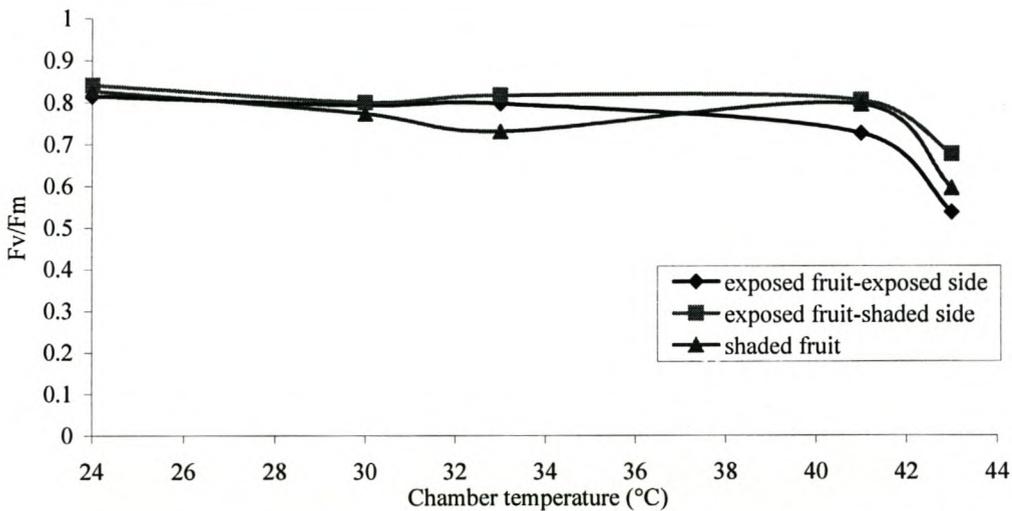


Figure 3b. The effect of increasing temperature on Fv/Fm on 'Cripp's Pink' fruit grown under different light environments on 25 March 2002.

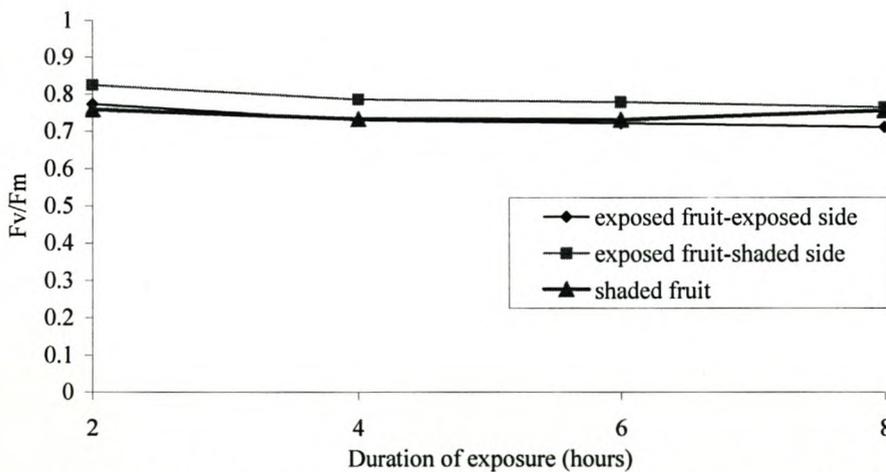


Figure 3c. The effect of duration of exposure to heat stress treatments on Fv/Fm of 'Cripp's Pink' apples grown in different light environments measured on 25 March 2002.

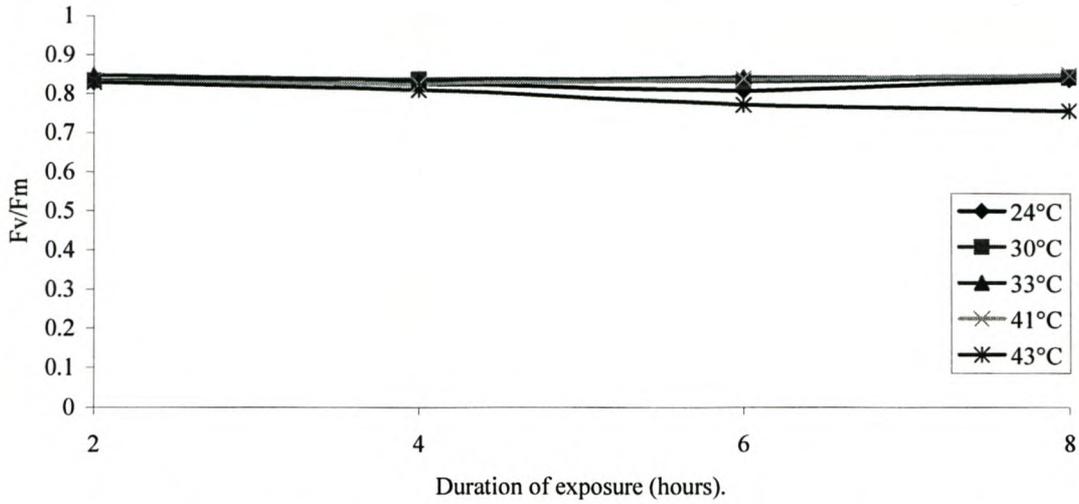


Figure 4a. Recovery (26 March 2002) of Fv/Fm after exposure to a range of temperatures on Fv/Fm of 'Cripp's Pink' apples on 25 March 2002.

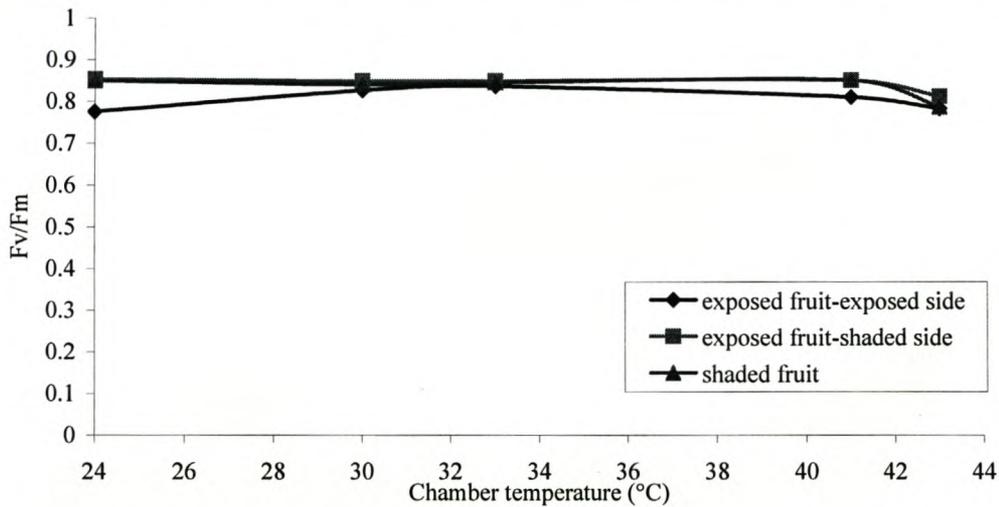


Figure 4b. Recovery (26 March 2002) of Fm/Fv of 'Cripp's Pink' apples grown under different light environments exposed to increasing temperatures on 25 March 2002.

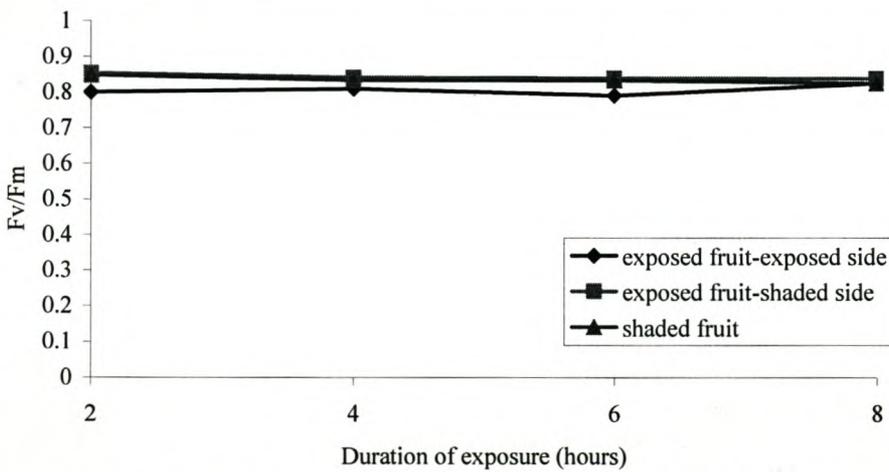


Figure 4c. Recovery (26 March 2002) of Fv/Fm on 'Cripp's Pink' apples grown in different light environments after exposure to heat stress treatments for increasing periods of time on 25 March 2002.

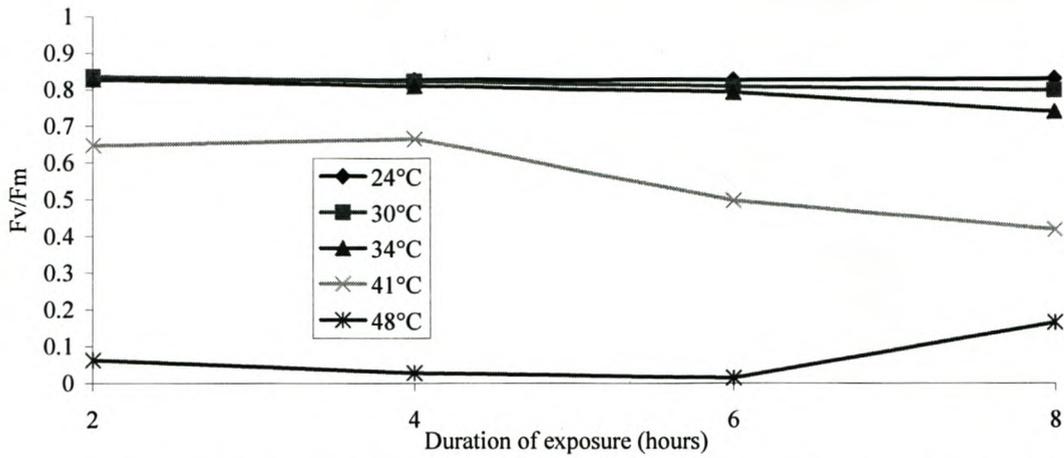


Figure 5a. The effect of duration of exposure to a range temperatures on Fv/Fm on 'Cripp's Pink' apples on 25 March 2003.

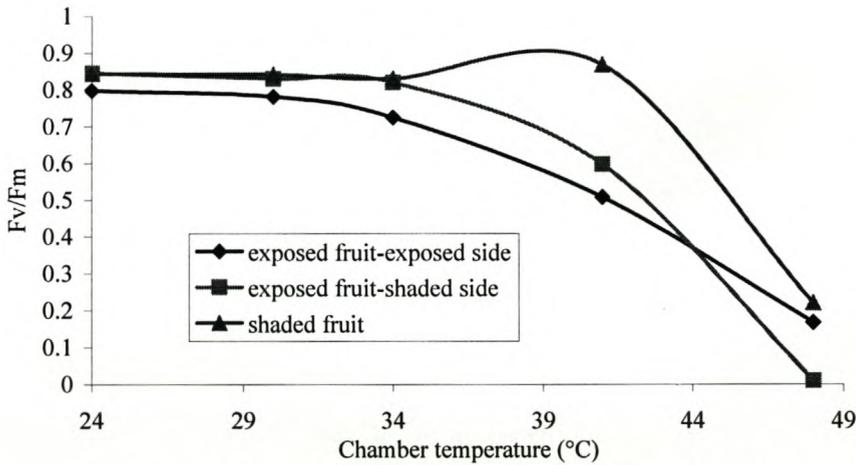


Figure 5b. The effect of increasing temperature on Fv/Fm of 'Cripp's Pink' apples grown in different light environments measured on 25 March 2003.

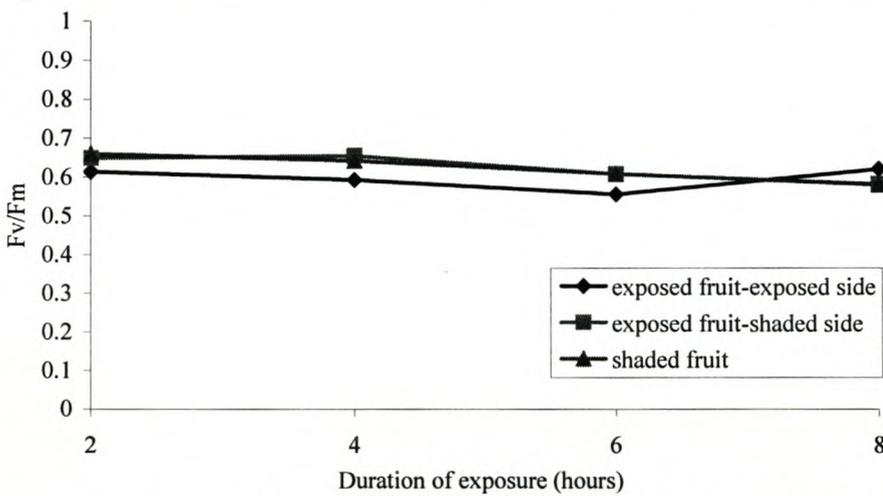


Figure 5c. The effect of duration of exposure to heat stress on Fv/Fm of 'Cripp's Pink' fruit grown under different light environments on 25 March 2003.

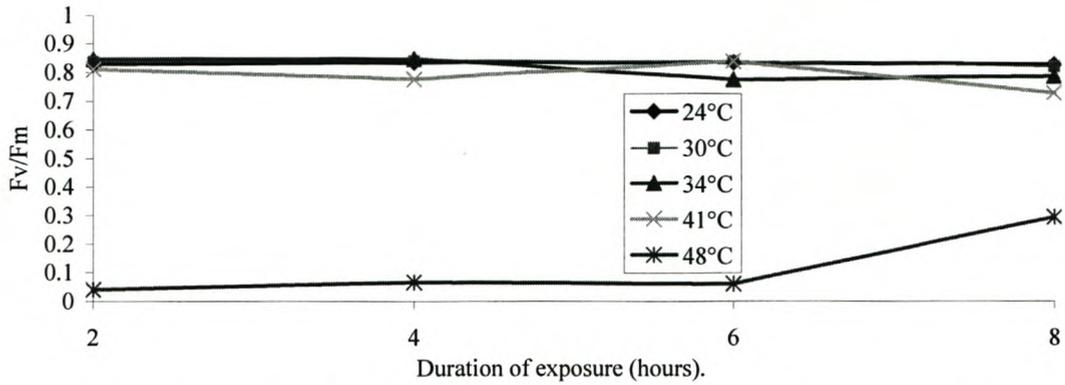


Figure 6a. Recovery of Fv/Fm (26 March 2003) on 'Cripp's Pink' apples that were exposed to different temperatures for increasing amounts of time on 25 March 2003.

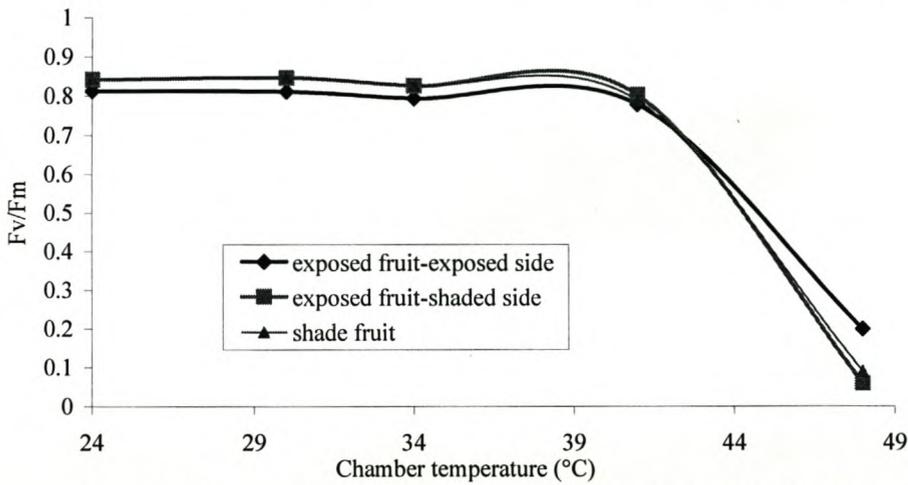


Figure 6b. Recovery of Fv/Fm (26 March 2003) on 'Cripp's Pink' apples grown in different light environments after exposure to increasing temperatures on 25th of March 2003.

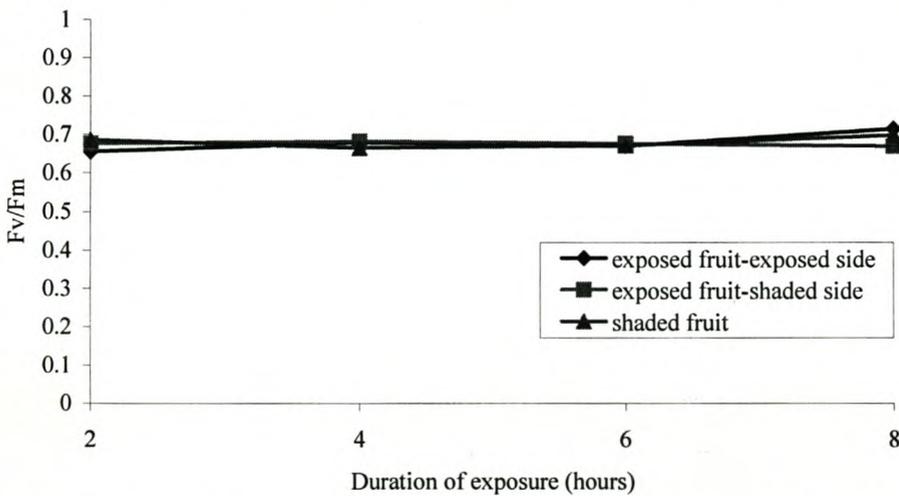
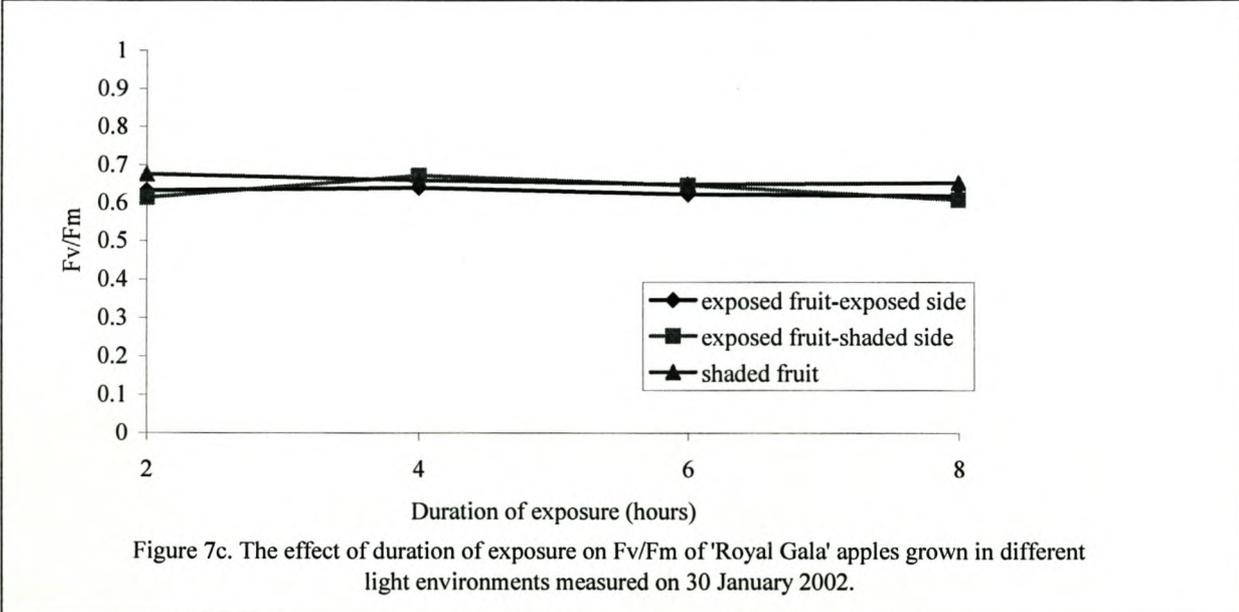
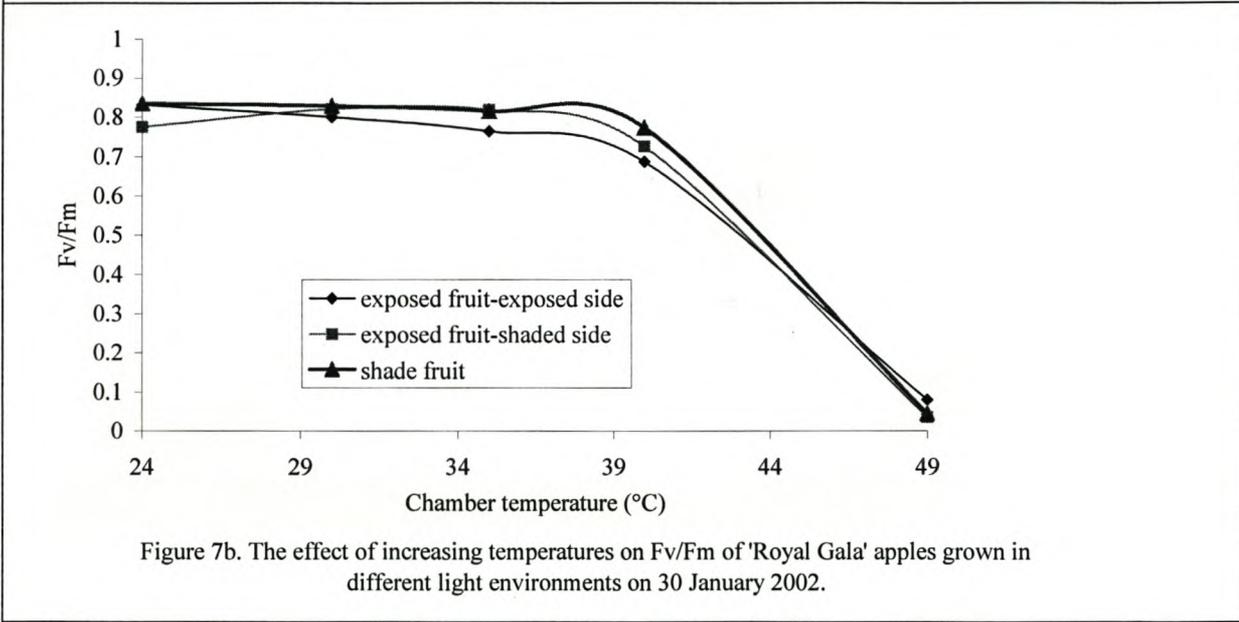
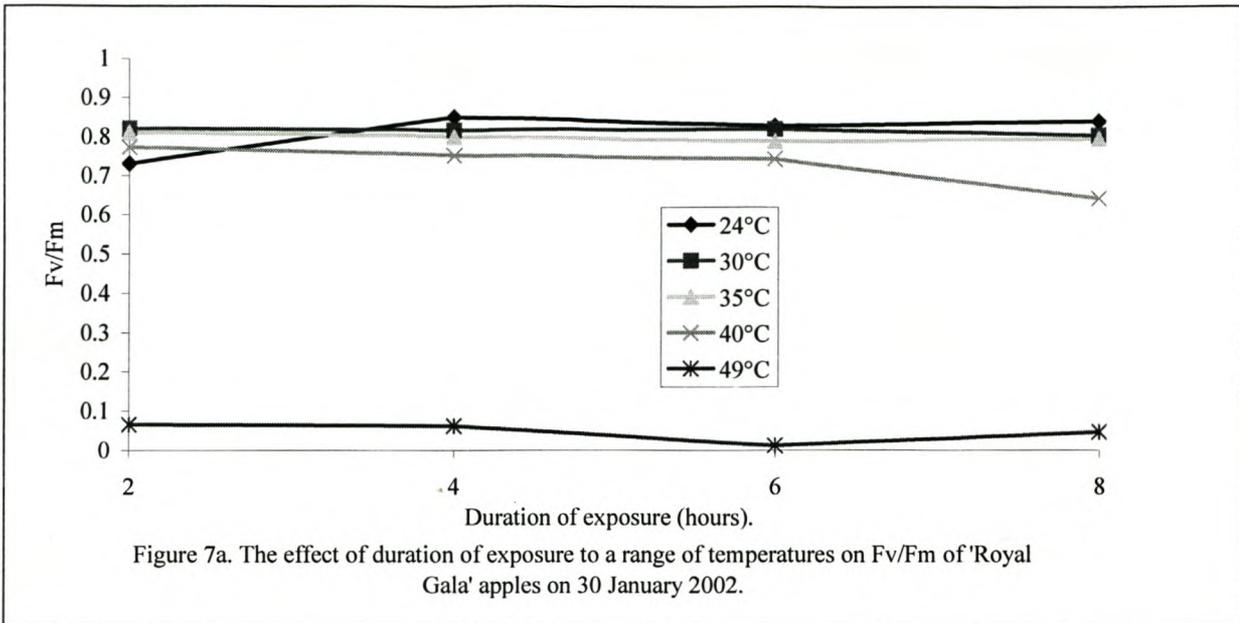


Figure 6c. Recovery of Fv/Fm (26 March 2003) on 'Cripp's Pink' apples grown in different light environments after exposure to increasing periods of heat stress on 25 March 2003.



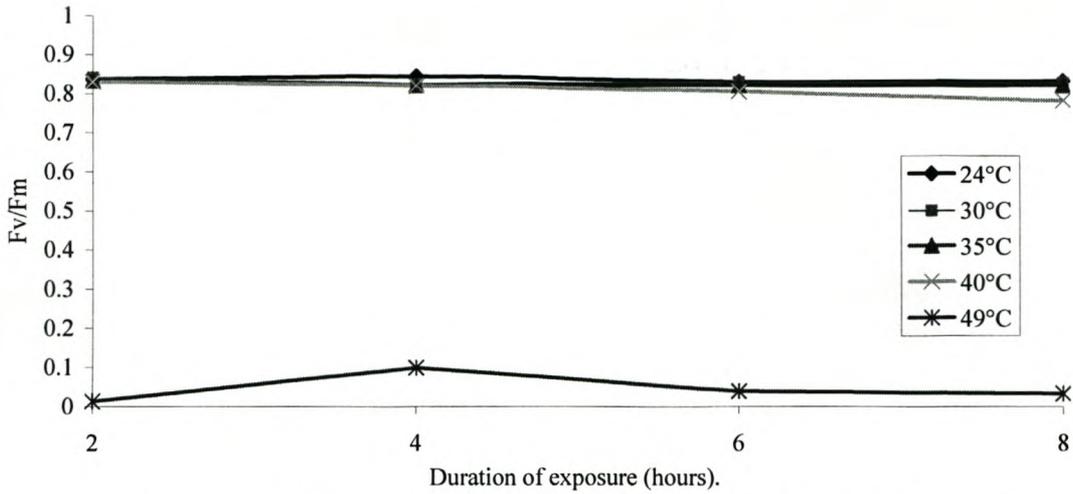


Figure 8a. Recovery of Fv/Fm (31 January) on 'Royal Gala' apples after exposure to a range of temperatures for increasing amounts of time (30 January)

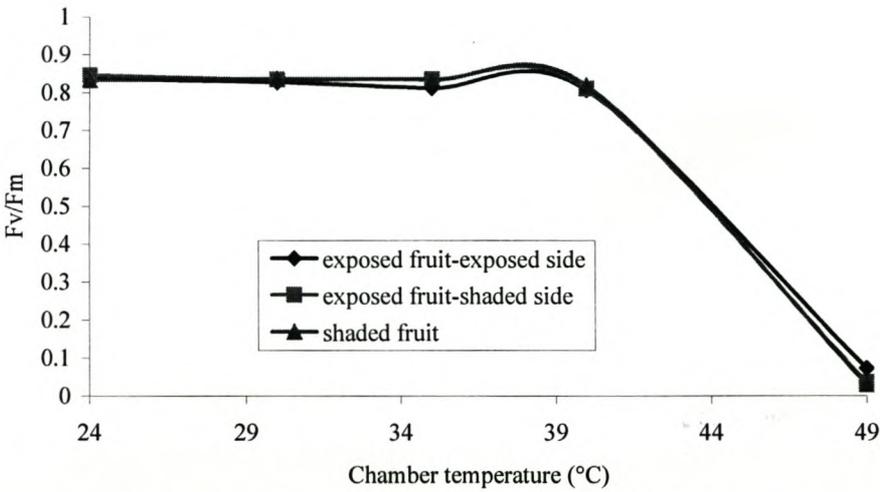


Figure 8b. Recovery (31 January) of Fv/Fm on 'Royal Gala' apples after exposure to increasing temperatures for increasing periods of time on 30 January 2002.

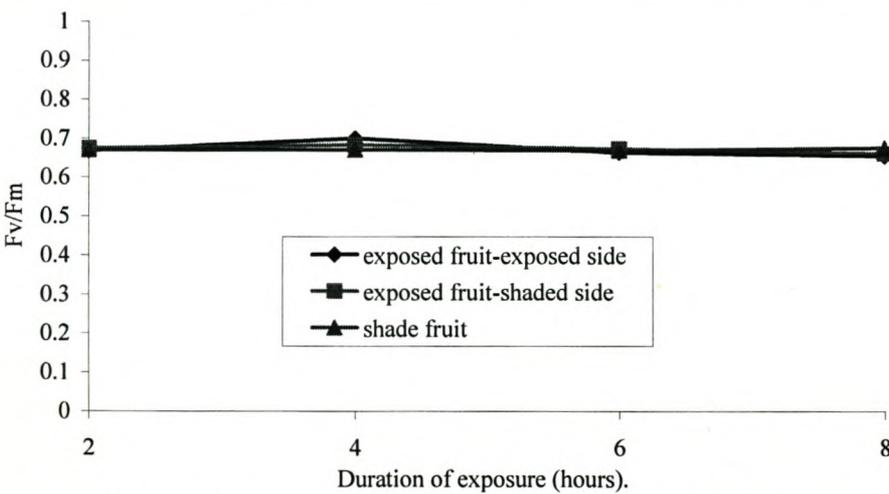


Figure 8c. Recovery of Fv/Fm (31 January 2002) on 'Royal Gala' apples after exposure to heat stress for increasing periods of time (30 January 2002).

5. General conclusion

The effect of evaporative cooling was investigated on 'Cripps' Pink', 'Royal Gala' and 'Fuji' apples as well as 'Forelle' pears in a bid to reduce sunburn. Fruit surface temperatures were significantly reduced on wet fruit. Apple fruit under evaporative cooling treatments appeared to have acclimatised and had significantly higher surface temperatures when dry than non-cooled fruit. Sunburn was reduced significantly on 'Royal Gala' apples. Further research is needed, possibly on the cuticle, to establish the mechanism whereby apple fruit acclimatise to the cooler environment due to evaporative cooling.

The effect of four or five Surround[®] WP applications on shoot growth, gas exchange, fruit surface temperature and fruit quality of 'Braeburn', 'Fuji' and 'Granny Smith' apples was investigated. Surround[®] WP had no significant effect on fruit surface temperature. Sunburn on exposed fruit was significantly reduced on all three apple cultivars. Leaf stomatal conductance and photosynthetic rate were reduced in Surround[®] WP treatments. Four applications of Surround[®] WP with a blower sprayer was the most effective treatment on 'Braeburn' apples, increasing TSS significantly compared to tower applications. Four applications with a blower and five applications with a tower spray resulted in increased fruit size in 'Granny Smith' as well as higher flesh firmness and lower percentage starch breakdown. All Surround[®] WP treatments resulted in slightly better green fruit colour in 'Granny Smith'. More research is needed to establish whether there is a reliable and economically viable method of removing Surround[®] WP from apple fruit for packing as well as the storage ability of such cleaned fruit.

The effect of fruit surface temperatures (24°C, 30°C, 33°-35°C, 40°-43°C and 48°-49°C) for increasing periods of time (two, four, six or eight hours) on maximum quantum yield of fluorescence (F_v/F_m) was measured on exposed and shaded sides of detached 'Cripps' Pink' and 'Royal Gala' apples. Measurements were taken directly after removal from the chamber as well as 12 hours later to gauge whether there was any recovery.

Permanent damage to PSII occurred between 48°-49°C irrespective of duration, with permanent damage possible at 42°-43°C when exposure exceeded six hours. We found no meaningful differences in Fv/Fm between shaded and exposed sides of apple fruit after exposure to high temperature. Duration of exposure was important in lowering PSII efficiency in 'Cripps' Pink' but less so in 'Royal Gala'. 'Cripps' Pink' apples harvested later in the season appeared to be able to recover from heat stress better than apples harvested earlier in the season. Further research is needed on the effect of both high temperatures and high light levels on apple fruit in an orchard to investigate which conditions lead to irreparable damage.