

**INCREASING CLASS ONE FRUIT IN ‘GRANNY SMITH’ AND ‘CRIPPS’  
PINK’ APPLE**

**By**

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## DECLARATION

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## SUMMARY

Experiments were conducted to increase the percentage class one 'Granny Smith' and 'Cripps' Pink' apples. 'Granny Smith' is the most widely grown apple cultivar in South Africa, but its profitability is compromised by the high incidence of sunburn, red blush and poor green colour development. 'Cripps' Pink' is a very lucrative cultivar and producers are striving to maximise the production of fruit that qualify for export. Fruit technologists and growers are debating whether it is best to maximise class one fruit in 'Cripps' Pink' by increasing total yield or by increasing fruit quality.

The relationship between 'Granny Smith' canopy position and external fruit quality was investigated. Light exposure, peel temperature, green colour development, sunburn and red blush development was followed for individual fruit from the outer, intermediate and inner canopy. Dark green fruit were exposed to moderate to high light levels (25-50% full sun) during the first half of fruit development, similar to fruit that eventually developed sunburn and red blush. The difference came in during the latter half of fruit development when dark green fruit became shaded (3% full sun). Pale green fruit contained less chlorophyll due to consistent low light levels (2% full sun). Fruit at partially shaded canopy positions had a lower occurrence of sunburn and red blush than outside fruit and better green colour development than fruit from the heavily shaded inner canopy.

Based on these data, pruning strategies and mulching were evaluated to alter canopy vigour and the light environment in such a way that green colour development is promoted and the occurrence of sunburn and red blush is reduced. In an older, vigorous orchard with a dense canopy, pruning was done to increase light distribution for green colour development and to induce more growth on the side of the trees that are prone to sunburn and red blush. Pruning improved green colour development without affecting sunburn or red blush. In a younger, non-vigorous orchard, pruning and mulching were used to invigorate the canopy to increase shading of fruit and thereby decrease sunburn and red blush. However, these treatments were not effective. Further research should focus on the use of shade nets, accompanied by rigorous pruning, to reduce sunburn and red blush while not decreasing green colour.

Five different crop loads were established in an exceptionally high yielding (averaging over 100 ton·ha<sup>1</sup>) 'Cripps' Pink' orchard by first the thinning of clusters, then the removal of small fruit and, finally, the selective removal of fruit from the shaded inner canopy. Treatments had no effect on

fruit quality in the first season. The most severe thinning treatment increased the percentage class one fruit in the second season by increasing the number of fruit with adequate red blush. However, seen cumulatively, the higher crop loads yielded more class one fruit per hectare than the lower crop loads, without affecting reproductive and vegetative development or fruit storability. Producers should strive for the highest crop loads allowed by the fruit size limitations in cultivars that are not prone to alternate bearing.

## OPSOMMING

Eksperimente is uitgevoer om die persentasie uitvoerkwaliteit ‘Granny Smith’ en ‘Cripps Pink’ appels te verhoog. ‘Granny Smith’ maak die grootste deel uit van appel aanplantings in Suid Afrika, maar die winsgewendheid daarvan word beperk deur `n hoë voorkoms van sonbrand, rooi blos en swak groen kleurontwikkeling. ‘Cripps’ Pink’ is `n baie winsgewende kultivar en produsente streef daarna om die persentasie uitvoerkwaliteit vrugte te maksimaliseer. Vrugte tegnoloë en produsente debatteer oor die wenslikheid daarvan om uitvoerkwaliteit vrugte te maksimeer deur totale produksie te verhoog of deur vrugkwaliteit te verbeter.

Die verband tussen ‘Granny Smith’ draposisie in die blaredak en eksterne vrugkwaliteit is ondersoek. Ligvlakke, skiltemperatuur, groen kleurontwikkeling, sonbrand en rooi blos ontwikkeling is deur die loop van die seisoen gevolg vir individuele vrugte aan die buitekant, binnekant en intermediêre posisies binne die blaredak. Daar is gevind dat die donkerste groen vrugte, nes vrugte wat uiteindelik sonbrand en rooi blos ontwikkel het, blootgestel was aan matige tot hoë ligvlakke (25-50% vol son) gedurende die eerste helfte van vrugontwikkeling. Donker groen vrugte is egter oorskadu (3% vol son) tydens die tweede helfte van vrugontwikkeling. Vanweë konstante lae beligting (2% vol son) het binne vrugte min chlorofiel geakkumuleer en daarom is hierdie vrugte lig van kleur. Vrugte in gedeeltelike skadu ontwikkel min sonbrand en rooi blos in vergelyking met buite vrugte en toon beter groen kleurontwikkeling as vrugte in diep skadu binne die boom.

Gegronde op bogenoemde resultate is die gebruik van snoei strategieë en deklae om die groeikrag en die ligomgewing van die boom te modifiseer, ten einde groen kleur ontwikkeling te bevorder en sonbrand en rooi blos te verminder, geëvalueer. In ‘n ouer, groeikragtige boord met ‘n digte blaredak is snoei gebruik om ligverspreiding te verbeter vir groen ontwikkeling en om meer groei te stimuleer aan die buitekant van die boom wat meer geneig is tot sonbrand en rooiblos. Groen kleur is wel verbeter, maar sonbrand en rooi blos is nie geaffekteer nie. In `n jonger, minder groeikragtige boord is ‘n deklaag aangebring en eenjarige lote getop, sodoende groei te stimuleer om sonbrand en rooi blos te verminder deur oorskaduwing van vrugte. Hierdie behandelings was egter nie effektief nie. Toekomstige navorsing moet fokus op die gebruik van skadunette tesame met ‘n nougesette snoei strategie om sonbrand en rooi blos te verminder sonder om groen kleur te verswak.

Vyf verskillende vrugladings is geskep in `n uitermatig produktiewe (gemiddeld meer as 100 ton·ha<sup>-1</sup>) ‘Cripps’ Pink’ boord deur eers vrugtrosse uit te dun, gevolg deur die verwydering van

klein vrugte en, laastens, die selektiewe verwydering van vrugte in die diep skaduwee van die binneste blaredak. Vrugkwaliteit is nie in die eerste seisoen nie deur oeslading geaffekteer nie. Die strafte uitdunbehandeling het wel die persentasie uitvoerbare vrugte in die tweede seisoen verhoog deur die aantal vrugte met voldoende rooi blos te vermeerder. Kumulatief gesien, het die hoër oesladings egter meer klas een vrugte per hektaar opgelewer sonder om die reprodktiewe en vegetatiewe ontwikkeling of die stoorvermoë van vrugte te affekteer. Producente moet strew na die hoogste oesladings wat toegelaat word deur vruggrootte beperkings in kultivars wat nie geneig is tot alternerende drag nie.

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## GENERAL INTRODUCTION

To increase the profitability of their business, fruit growers need to maximize the percentage class one fruit produced per hectare. For example, ‘Cripps’ Pink’ fruit meeting the quality standards to qualify to be marketed under the trademark, ‘Pink Lady’, may increase in value by 100% (Van Rensburg, personal communication). Fruit generally fail to qualify as class one due to the presence of internal and external defects caused by an array of environmental, cultural and physiological factors. Hence, class one fruit can be increased by understanding the causation of these defects and by devising preventive or ameliorating horticultural strategies based on this knowledge.

In South Africa, sunburn and red blush may decrease exportable class one ‘Granny Smith’ (GS) fruit by 35% and 20%, respectively (Griessel, personal communication). Since GS is the most widely grown apple in South Africa (24% of the total area planted) (Deciduous Fruit Producers' Trust, 2008), the economic losses attributable to these defects are considerable. Although not so much a major cull factor, insufficient green colour of GS apples may also incur losses in revenue. To deal with this problem, GS fruit is shipped earlier or later during the Southern hemisphere season when European markets are less saturated with fruit. Alternatively, fruit are shipped to lower value markets, which has a negative effect on net profits achieved by producers (Griessel, personal communication). A perception is increasing among fruit importers that South African GS is less green than GS of competing countries (Griessel, personal communication).

The development of sunburn and red blush is associated with high light environments (Tustin et al., 1988; Warrington et al., 1996) while poor green colour development is associated with low light intensities (Hirst et al., 1990). In order to increase GS class one fruit by decreasing the incidence of sunburn and red blush, and by increasing green colour, it is necessary to have a sound understanding of how the light environment affect apple peel pigmentation. After reviewing chlorophyll and chloroplast metabolism as well as factors involved in green colour development, we initiated experiments to assess the relationship between GS canopy positions and fruit quality under South African conditions. The objective was to determine the light environments associated with the development of sunburn and red blush as well as the development of dark and light green colour. Based on these data, the use of pruning strategies and mulching were assessed to alter the canopy light environment in such a way that green colour development is promoted and the occurrence of sunburn and red blush is reduced.

Apart from increasing fruit quality, fruit growers may also potentially increase class one fruit by increasing the total yield per hectare. However, whether this is a sustainable strategy is still debated among fruit technologists and producers since higher crop loads may decrease fruit quality, primarily by decreasing fruit size (Link, 2000), increase the risk of alternate bearing (Monselise and Goldschmidt, 1982) and compromise long-term tree vigour (Palmer, 1992). Although thinning generally improves fruit quality, it may also increase fruit susceptibility to physiological disorders such as bitter pit and internal breakdown, which are mostly calcium-related disorders (Link, 2000; Sharples, 1968). To contribute some scientific grounding to the argument of higher yield and lower quality versus lower yields and higher quality, an high yielding 'Cripps' Pink' orchard (averaging over 100 ton·ha<sup>-1</sup> over the preceding five seasons) were thinned to different crop loads. Horticultural considerations were taken into account when thinning. Hence, we first thinned clusters to single fruit, followed with increasing severity of thinning by removal of small fruit and fruit from the shaded interior canopy. The effect of crop load on fruit quality, reproductive and vegetative development and storability was assessed.

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# **LITERATURE REVIEW: CHLOROPHYLL AND CHLOROPLAST METABOLISM WITH EMPHASIS ON FACTORS THAT AFFECT GREEN COLOUR OF APPLES.**

## **1. Introduction**

The red, yellow, orange and blue colour of mature fruit plays an important evolutionary role in making fruit more conspicuous to a wide range of seed dispersers (Willson and Whelan, 1990). However, some fruit stay green at maturity (Cipollini and Levey, 1991) supposedly for additional carbon-acquisition (Aschan and Pfanz, 2003). Fruit photosynthesis accounts for 20% of the carbon requirement in black cherry (Bazzaz et al., 1979) and 9% in peach (Pavel and De Jong, 1993), and may even contribute positively to the whole plant carbon budget (Aschan and Pfanz, 2003). This is especially useful in plants with energy costly fruit (Cipollini and Levey, 1991).

The aim of this review is to discuss the main factors, namely light, temperature, nitrogen and maturity that affect chlorophyll levels, and therefore green colour, in fruit in particular. To facilitate the discussion, a background will be provided on the biosynthesis, structure and degradation of the chlorophyll molecule and the chloroplast.

## **2. Chlorophyll**

### **2.1 Introduction**

Chlorophylls are pigments that belong to a class of compounds known as tetrapyrroles and provide plants with their characteristic green colour and the ability to photosynthesize. The green colour is caused by the light absorption spectra of chlorophyll. Chlorophyll predominately absorbs light of wavelengths 400-500 nm and 600-700nm, which is blue and red light, respectively. In between these two wavelengths are the green wavelengths, which chlorophyll are unable to absorb and instead reflect. This does not mean that none of the incoming light in the green spectrum is absorbed, since 80% of the green light is reflected by internal plant tissues, resulting in energy release, an increase in photon wavelength to a lower energy red wavelength, and absorbance by chlorophyll (Sallisbury and Ross, 1992).

Several types of chlorophyll are found in plants. Chlorophyll *a* and *b* predominate in higher plants while other chlorophylls are mostly confined to lower plants such as algae (Meeks, 1974). Chlorophyll *a* and *b* form part of photosystems I (PS I) and II (PS II). These photosystems are

protein complexes, which absorb light maximally at 700 nm (PS I) and 680 nm (PS II). The photosystems are made up of an antenna complex and a reaction center. Light is absorbed by the antennae, which consist of carotenoids and chlorophyll *a* and *b*, and transferred to the reaction center. The reaction center of PS II transfers electrons to PS I, which reduces NADP<sup>+</sup> to NADPH. Chlorophyll *b* has a slightly blue tinge because the absorption spectrum of this pigment is slightly higher than chlorophyll *a* (Sallisbury and Ross, 1992). The pigments of the antennae in combination with proteins form the light harvesting complex (LHC). The chlorophyll *a/b* ratio reveals the ratio of photosystem I to II and the size and composition of the LHC, because chlorophyll *b* is restricted to the LHC (Willows, 2004). A low ratio, for example 2.0-2.8, reveals a relative abundance of LHC associated with PS I and II, which is characteristic of shade adapted leaves. The higher abundance of LHC will ensure more efficient usage of low intensity light for photosynthesis. A ratio of about 3.5-4.9 reveals that there are fewer LHC associated with PS I and II, and this is indicative of adaptation to full sun.

## **2.2 Chemical properties and structure of chlorophyll**

The chlorophyll molecule consists of a 'head' and 'tail' part. The 'head' is an electron dense porphyrin made up of four pyrrole rings that are linked by carbon atoms and is responsible for the absorption of light (Halliwell, 1981). The 'tail' is a phytol esterified to the propionic acid substituent at position 7 of the fourth ring. This phytol tail makes chlorophyll hydrophobic, which helps to anchor the molecule into the thylakoid membrane. Chlorophyll differs from other tetrapyrroles, such as vitamin B12, by the presence of a chelated magnesium ion in its structure. The magnesium ion is found in the 'head' of the molecule (Halliwell, 1981). The structure of isomers chlorophyll *a* and *b* differ from each other at position 3 where chlorophyll *a* has a methyl group whereas chlorophyll *b* has an aldehyde group (Lamikanra et al., 2005).

## **2.3 Biosynthesis of chlorophyll**

Chlorophyll biosynthesis is quite complex, and only a brief overview is necessary for the purposes of this review (see figure 1 for the complete pathway).

Higher plants synthesize four major tetrapyrrole molecules (chlorophyll, haem, sirohaem and phytychromobilin) via a common branched pathway (Reinbothe and Reinbothe, 1996). All these tetrapyrroles are synthesized from eight molecules of the five-carbon compound, aminolevulinic acid (ALA). ALA is synthesized from glutamic acid via the Beale or C5 pathway. The conversion of ALA to protoporphyrin IX is found in animals, bacteria and plants; it is the reactions and enzyme

activities that convert protoporphyrin to chlorophyll that are unique to the chlorophyll biosynthetic pathway within plant chloroplasts (Cornah et al., 2003). The biosynthetic pathway splits into two branches after the formation of protoporphyrin IX; it may be chelated with either  $\text{Fe}^{2+}$ , leading to the formation of a haem product, phytychromobilin, or  $\text{Mg}^{2+}$ , leading to the formation of chlorophyll (Reinbothe and Reinbothe, 1996). Chlorophyll is synthesized in the chloroplast and remains there, while haem is found in all cellular compartments (Reinbothe and Reinbothe, 1996).

One of the primary regulators of tetrapyrrole synthesis is the site of ALA synthesis. ALA synthesis is mainly regulated by feedback inhibition. Increased haem down regulates the activity of glutamyl-tRNA reductase (GluTR), the enzyme necessary for ALA synthesis (Bollivar, 2006). Another regulatory factor is FLU proteins (transcribed from the *FLU* gene), which directly influence (inhibiting) GluTR activity (Bollivar, 2006). The insertion of  $\text{Mg}^{2+}$  by Mg-chelatase into the tetrapyrrole structure is the first committed step towards chlorophyll synthesis, because all the other enzymes prior to this step are shared with the haem biosynthetic pathway (Fig. 1). This is a potential regulation site in chlorophyll synthesis. In the presence of light, the requirement for chlorophyll production is higher and due to the increased competitiveness of Mg-chelatase over ferrochelatase to chelate protoporphyrin IX, haem production is inhibited (Cornah et al., 2003). In the absence of light and if tetrapyrrole production is in excess, protoporphyrin IX is used for haem synthesis, resulting in inhibition of ALA synthesis and a reduction in tetrapyrrole levels. Mg-chelatase has a lower  $K_m$  value for protoporphyrin IX and, therefore, has a higher affinity for the substrate than ferrochelatase. Mg-chelatase activity requires ATP, whereas ATP inhibits ferrochelatase. More ATP is available during a light stimulus, thereby inhibiting ferrochelatase. However, it has been proposed that these two enzymes utilize separate pools of protoporphyrin IX.

### 2.3.1 Light as regulatory factor

Light may regulate chlorophyll synthesis at two points in the biosynthetic pathway, i.e., at the synthesis of ALA and at the reduction of protochlorophyllide (Pchlde). ALA synthesis is regulated through light control over GluTR production. Three genes (*HEMA1-3*) are involved in the synthesis of GluTR. The expression of *HEMA1* is light dependent (McCormac et al., 2001) and allows the synthesis of GluTR and the subsequent synthesis of ALA in the presence of light. NADPH-Pchlde oxidoreductase (POR) is responsible for the reduction of Pchlde to form chlorophyllide in angiosperms and is also regulated by light (Mapleston and Griffiths, 1980). Pchlde, NAPPH and POR form a complex within the chloroplast and after a light stimulus, Pchlde is photoconverted to chlorophyllide and POR is released (Griffiths, 1978). ALA is converted to Pchlde even without a

light stimulus, but Pchl<sub>id</sub> will accumulate without reduction to chlorophyll<sub>id</sub> (Reinbothe and Reinbothe, 1996). Light-independent POR (DPOR) exists in non-flowering plants and algae, thereby allowing the production of chlorophyll in the dark (Willows, 2004). PIF1 proteins are negative regulators of chlorophyll synthesis, but the presence of light-activated phytochrome interferes with the function of PIF1 (Bollivar, 2006). Another two genes that are regulated by light are *CRD1* and *CAO*, which encode a subunit of Mg-protoporphyrin IX monomethyl ester cyclase and chlorophyll<sub>id</sub> *a* oxygenase, respectively (Masuda, 2008).

As mentioned before, Mg-chelatase activity is another major site of regulation. The expression of the two subunits of Mg-chelatase is upregulated by the expression of *ChlI* and *ChlH* (Masuda, 2008). Expression of *ChlH* is stimulated by light and follows a distinct circadian rhythm while expression of *ChlI* is constitutive. Stromal Mg<sup>2+</sup> concentrations also increase in response to light, thereby causing Mg-chelatase activity to increase. GUN4, a porphyrin binding protein, was recently identified (Larkin et al., 2003). It is thought to be involved in intracellular signaling and may also stimulate Mg-chelatase activity by lowering the Mg<sup>2+</sup> concentration needed for full Mg-chelatase activity (Masuda, 2008).

## **2.4 Chlorophyll Degradation**

Chlorophyll degradation may occur due to a hostile environment, during a significant change in the life cycle of the organism, and during certain stages of the life cycle of organs and tissues (Hendry et al., 1987). Examples of life cycle changes accompanied by chlorophyll degradation include seed germination (depending on plant species), flowering, and maturation and separation of fruits and seeds from the parent plant. Maturation of vegetative tissue is an example of chlorophyll degradation within the life cycle. Excessive or prolonged heat, high irradiance and deficiencies in minerals such as nitrogen and iron, are examples of hostile environments that may induce chlorophyll degradation.

Chlorophyll degradation during senescence occurs in order to recycle nutrients such as nitrogen that are tied up in photosynthetic proteins (Willows, 2004). Hortensteiner (2006) argues against the theory that chlorophyll is broken down for access to its structural nitrogen because chlorophyll contributes only 2% to cellular nitrogen. Instead he argues that chlorophyll degradation is a prerequisite for access to chlorophyll-associated proteins and serves as a detoxifying mechanism against the generation of free radicals during the dismantling of the photoapparatus (Hortensteiner, 2006).

Chlorophyll degradation starts with the removal of the phytol tail by chlorophyllase (Chlase) forming chlorophyllide (Chlide) (see Fig 2 for the complete catabolic pathway). Expression of Chlase is constitutive, but hormones known to accelerate leaf senescence or fruit ripening, i.e. methyl jasmonate and ethylene, are known to promote the expression of Chlase (Jacob-Wilk et al., 1993; Tsuchiya et al., 1999). The second step in chlorophyll degradation is the release of the Mg-ion by an unidentified, low-molecular weight metal-chelating substance (Hortensteiner, 2006). After the removal of Mg<sup>2+</sup>, pheophorbide is formed and subsequently converted to a colourless primary fluorescent chlorophyll catabolite (pFCC) (Hortensteiner, 2006). The final product of chlorophyll degradation is nonfluorescent chlorophyll catabolites (NCC) stored in the vacuole (Takamiya et al., 2000).

A second type of chlorophyll degradation starts with the splicing of the Mg 'head' of the chlorophyll molecule by peroxidases. However, it is not certain whether chlorophyll bleaching takes place independently or cooperatively with the Chlase pheophorbide *a*-oxygenase pathway (Takamiya et al., 2000).

### **3. Chloroplast**

#### **3.1 Structure** (based on Barber, 1976)

Chloroplasts are double membrane-enclosed plastids that contain chlorophyll and are the sites for photosynthesis (Tiaz and Zeiger, 1998). The chloroplast consists of three major structural regions: the outer membranes, stroma and the internal membranes. The outer membranes are known as the envelope and consist of two separate membranes, each composed of a lipid bi-layer. The envelope contains a variety of transport systems that play an important role in the transport of metabolites in and out of the chloroplast. The stroma is an amorphous solution that contains the enzyme ribulose-1,5-bisphosphate carboxylase (Rubisco) as major protein component and is the site of the carbon reductions of photosynthesis. The internal membranes are known as the thylakoids. The thylakoids are shaped like flattened sacks enclosing a space, creating a disc-like structure. A stack of thylakoids forms a granum (plural grana). Adjacent grana are connected by non-stacked thylakoids, called stroma lamellae. The chlorophyll is contained in the thylakoid membranes, making it the site for the light reactions of photosynthesis.

### 3.3 Biosynthesis (based on Burgess, 1985)

Chloroplasts develop out of proplastids. The conversion from proplastid to chloroplast proceeds gradually along with cell growth under normal conditions of lighting. The proplastid does not contain any of the complex inner membranes. The inner envelope produces porous membranous tubules into the stroma. Later stages of development see the pores disappearing and the formation of the thylakoid membranes. There are variations on the way chloroplasts are formed. In cereals, for instance, the meristem is situated at the base of the leaf and in the shade. Because the cells are formed in the shade, the proplastid first develops into a highly ordered membranous structure with interconnected tubules, the prolamellar body. As the leaf grows, it becomes more exposed to sunlight and chloroplasts develop. The prolamellar body is characteristic of etioplasts, unpigmented, starch-containing plastids that occur in dark grown plants. Upon the exposure of etioplasts to light, the highly ordered arrangement of the prolamellar body is lost followed by the conversion of protochlorophyllide to chlorophyllide and lastly to chlorophyll. These changes occur rapidly, in more or less 20 minutes. As greening continues, the prolamellar body gives rise to parallel membranes extending into the stroma, giving rise to the formation of the thylakoids. The thylakoids are porous at first, but as the plastid matures, the pores disappear. The conversion from etioplast to chloroplast is to some extent reversible.

All the plastids in the cell originate out of the proplastid. The plastid population of any particular cell corresponds to the activity or state of differentiation of that cell and is directly affected by environmental conditions. There is also a relationship between the cell type and plastid population. The presence of light does not turn all plastids into chloroplasts, as observed in flowers, while roots do not have chloroplasts just because of the lack of sunlight. These observations also suggest that the cell has a measure of genetic control over the plastid population. It is also possible for certain plastids to convert to another, for instance chloroplast to chromoplast and amyloplast to chloroplast. There are normally 7-20 proplastids in the cell of the shoot meristem, but in mature plant cells there are about 50 chloroplasts. Considering that the cell number also increases, it is evident that plastids have a replication process. As the plant cells divide and enlarge, so do the plastids. This is substantiated by the fact that all the chloroplasts are at the same level of maturation (same age), and by the fact that the plastids contain their own DNA. The detail of how chloroplasts divide is still unclear. Chloroplast replication by a type of fission process has been observed in lower plants such as *Spirogyra*. Chloroplast replication is light dependent and it is also stimulated by conditions that stimulate cell expansion

### **3.4 Degradation** (based on Matile et al., 1999)

Chloroplast degradation is a symptom of transition of chloroplasts to gerontoplasts. The term gerontoplast is used because the metabolism of gerontoplasts is different from other plastids because it is catabolic. Gerontoplasts develop and remain throughout leaf senescence although they lose volume and density due to loss of stroma components and thylakoids. The formation of gerontoplasts is under nuclear control. Gerontoplasts retain enough genetic information to support regreening and chloroplast reassembly.

## **4. Factors influencing chlorophyll concentration and chloroplast structure**

Climate, plant hormones and minerals such as nitrogen, iron, magnesium, calcium and zinc may all influence chlorophyll levels and chloroplasts number. Rather than an exhaustive discussion of all these factors, the focus here will be on those factors that are relevant to the research presented in papers 1 and 2, i.e., light, temperature, plant maturity and nitrogen nutrition.

### **4.1 Light**

#### **4.1.1 Effect of light on chlorophyll**

As discussed earlier, light plays an important role in the biosynthesis of chlorophyll. It serves as an important regulatory factor in the chlorophyll biosynthetic pathway. Chlorophyll absorbs light energy to drive the process of photosynthesis. Low light environments may lead to plant stress because it may limit photosynthesis, which would lead to a lack in carbon gain and growth (Lambers et al., 1998). High light intensities may also stress the plant, in that it may damage the photosynthetic apparatus. Leaves adapt to their light environment in an anatomical and morphological way. Leaf thickness (due to two layers of palisade cells), specific leaf weight, tissue density and nitrogen content are highest in full sun leaves, whereas chlorophyll concentration is higher in shade leaves and also concentrated in the upper tissue layers (Bjorkman and Holmgren, 1963; Brand, 1997; Kappel and Flore, 1983; Lichtenthaler et al., 1981; Syvertsen and Smith Jr., 1984). These higher concentrations enhance light harvesting (Syvertsen and Smith Jr., 1984). An increase in leaf chlorophyll concentration enables shade grown-plants to more efficiently capture light and thus maximize photosynthesis under low light conditions (Nemali and van Iersel, 2004). Shade leaves have a lower light saturation point of photosynthesis (Kappel and Flore, 1983) and lower chlorophyll *a/b* ratios (Bjorkman and Holmgren, 1963; Kappel and Flore, 1983; Lichtenthaler et al., 1981). Shade leaves placed in a high light environment initially lose chlorophyll due to

temporary photobleaching, but chlorophyll levels do recover over a period of time (Syvertsen and Smith Jr., 1984) while the chlorophyll *a/b* ratio will increase (Bjorkman and Holmgren, 1963; Lichtenthaler et al., 1981). During excess light, all the photons absorbed by the chlorophyll cannot be used in photochemistry (Lambers et al., 1998). Plants, however, have mechanisms in place to dispose of this excess energy. A particular group of carotenoids are responsible for the dissipation of excess energy.

Carotenoids are red, orange and yellow pigments, embedded in the membranes of the chloroplasts and chromoplasts (Bartley and Scolnik, 1995). Their colour is usually masked by chlorophyll in photosynthetic tissues. Carotenoids fulfil two important functions in the plant. Firstly, they act as accessory light-harvesting pigments and secondly, they perform an essential photoprotective function (Young, 1991). Carotenoids absorb light with wavelengths ranging from 300 – 400 nm, which is not accessible to chlorophyll. Thus, carotenoids extend the light harvesting range. Energy absorbed by the carotenoids is transferred to chlorophyll molecules for photosynthesis. Carotenoids of the xanthophyll cycle (violaxanthin, antheraxanthin and zeaxanthin) are used to protect the photosystems against excess energy. Zeaxanthin absorb the excess energy from chlorophyll and dissipates the energy harmlessly as heat via the xanthophyll cycle (Lambers et al., 1998). The dissipation process can be overwhelmed by excess energy resulting in photoinhibition of photosynthesis (Krause, 1988; Lambers et al., 1998). Photoinhibition reduces the efficiency of photosynthesis by a reduction in the optimal photon yield and the capacity of CO<sub>2</sub> fixation. During photoinhibition, electron transport in the thylakoids is inactivated due to an alteration in the reaction centres of PS II. However, photoinhibition is not permanent and may be repaired in minutes or hours, if the inhibitory excess light does not continue. If the high light conditions persist, photobleaching occurs, which entail the oxidation (destruction) of chlorophyll (Lambers et al., 1998). During photobleaching, excess energy, which is not dissipated by the xanthophyll cycle, may be passed on to oxygen via chlorophyll. This results in the creation of toxic oxygen free radicals such as singlet oxygens, superoxide anions, hydrogen peroxide and the hydroxyl radical, which may damage the chloroplast membrane lipids, proteins and nucleic acids (Knox and Dodge, 1985; Lambers 1998). Carotenoids may protect the plant by either preventing the formation of reactive oxygen species, or, it may scavenge existing reactive oxygen species (Young, 1991).

In apple fruit, xanthophyll carotenoids are usually up regulated in response to high light intensities (Ma and Cheng, 2004). However, changes in carotenoid concentrations due to high light intensities is cultivar-specific and may increase with cultivars like ‘Fuji’ and ‘Delicious’, but remain unchanged in ‘Granny Smith’ (Felicetti and Schrader, 2009). High temperature in combination with

high light causes photooxidation and photodestruction of chlorophyll in apple peel even though the xanthophylls cycle (carotenoids) and antioxidant systems are up regulated (Chen et al., 2008). Apart from carotenoids, plants also use anthocyanins to trap excess light energy, resulting in the development of red blush on apples (Merzlyak and Chivkunova, 2000). The combination of high light and high temperatures ( $\approx 45\text{ }^{\circ}\text{C}$ ) on fruit peel will destroy chlorophyll molecules, which will lead to the manifestation of sunburn on fruit.

#### **4.1.2. Sunburn: A consequence of high light conditions**

Sunburn is caused by high light and high temperature conditions. Schrader et al. (2008) identified three types of sunburn occurring in apples. The first type (sunburn necrosis) is caused by thermal death of epidermal and sub-epidermal cells when the peel reaches approximately  $52\text{ }^{\circ}\text{C}$  (light not required), which then causes a necrotic spot. The second type (sunburn browning) of sunburn is sub-lethal and results in a yellow, bronze, or brown spot on the fruit when fruit surface temperature reaches  $46\text{ }^{\circ}\text{C}$  to  $49\text{ }^{\circ}\text{C}$  in the presence of sunlight, especially UV-B (Schrader et al., 2003). Sunburn usually occurs from 1230 HR to 1515 HR when the air temperature rises above  $30\text{ }^{\circ}\text{C}$  (Bergh et al., 1980). Fruit surface temperature can exceed  $45\text{ }^{\circ}\text{C}$  if the ambient temperature is higher than  $30\text{ }^{\circ}\text{C}$  and is very depended on environmental factors such as relative humidity, clouds, wind and precipitation, which causes rapid fluctuations of fruit surface temperature. Photooxidative browning is the third type of sunburn and it occurs when fruit peel is suddenly exposed to high light intensity resulting in photobleaching followed by necrosis. It can occur at much lower peel temperatures ( $<30\text{ }^{\circ}\text{C}$ ) in the absence of UV-B radiation and is thought to be due to photooxidative damage (Schrader et al., 2008).

Sunburn is characterized by a decrease in chlorophyll *a* and *b* due to photobleaching and an increase in chlorogenic acid and carotene concentrations, which serves as a possible protection mechanism (Wünsche et al., 2001). Chlorophylls decreased due to sunburn in all the apple cultivars studied by (Felicetti and Schrader, 2009). The decrease in chlorophyll unmasks the carotenoids, thereby leading to the characteristic yellowing of the skin. Dark green fruit are more sensitive to sunburn than red or yellow fruit, because chlorophyll, a photosensitizing pigment, causes the photo-oxidative processes essential for sunburn development (Rabinowitch et al., 1983).

Fruit become more sensitive to sunburn during their development due to a decrease in their photoprotective capacity and ability to quench absorbed light through photosynthesis (Li and Cheng, 2008). Sudden exposure to high light may cause photoinhibition and lead to the up

regulation of the xanthophyll cycle, which would minimize photooxidative stress and contribute to the acclimation to high light (Ma and Cheng, 2004). However, even with the up regulation of the xanthophylls cycle and antioxidant systems, photodestruction will still occur if high temperature and high light persists (Chen et al., 2008).

High light environments such as upper canopy positions give rise to the development of red blush, sunburn and poor green colour due to photodegradation (Tustin et al., 1988; Warrington et al., 1996). According to Bergh et al. (1980), sunburn of apple in South Africa can be reduced by inducing more growth on the exposed northern to north-western sides of trees and by grafting to growth-stimulating, vigorous rootstocks. Sudden exposure of fruit from a low light environment to high irradiance, which can be caused by the bending of branches due to the increasing weight of the developing fruit, will cause the development of red, yellow and orange blush and thus overbearing of fruit is not recommended (Hirst et al., 1990). For the same reason, thinning of sunburned fruit is not recommended as it may expose shaded fruit in the cluster to sunlight (Bergh et al., 1980).

In order to minimize the incidence of sunburn, methods are used that will lower the light levels that fruit are exposed to and reduce peel temperatures. Shade netting may lower the incidence of sunburn to 1% and also decrease red blush development (Smit, 2007), making it the most effective technique. The major drawback of shade netting is that it is also the most expensive method (Smit, 2007). Other techniques to reduce sunburn include evaporative cooling and spray application of particle films. Evaporative cooling entails the wetting of fruit with overhead sprinkles in order to decrease peel temperature (Parchomchuk and Meheriuk, 1996; Unrath and Sneed, 1974). Particle films consisting of white clay minerals, e.g. 'Surround', or natural lipids, e.g. 'Raynox', reflect visible or UV radiation (Glenn et al., 2002). Apart from increased water usage and high installation costs, evaporative cooling may lead to mineral deposits on fruit, over-irrigation, severe sunburn during system malfunctions, and increased pest and disease damage due to higher humidity (Evan, 1993). Evaporative cooling may also increase red blush development (Evan, 1993). Particle film techniques are successful in reducing peel temperatures and reducing sunburn (Glenn et al., 2002; Schupp et al., 2004) and is more affordable than evaporative cooling, but less effective in reducing the occurrence of sunburn (Gindaba and Wand, 2005). Inadequate vigour and water are also likely to cause sunburn (Schrader et al., 2003, 2008).

### **4.1.3 Effect of light on chloroplasts**

Chloroplasts of shade leaves have a higher number of thylakoids per granum, a higher stacking degree of thylakoids and broader grana than sun leaves (Lichtenthaler et al., 1981). Moving shaded chloroplasts to high light results in their destruction causing them to become pale, irregular and partly fragmented (Bjorkman and Holmgren, 1963). Leaf morphological plasticity is a more relevant determinant of foliage adaptation to high irradiance than foliage biochemical adaptation, but in low irradiance the anatomical and biochemical adaptations are similar (Niinemets et al., 1998).

### **4.1.4 Effect of light on apple green colour development**

To determine the effect of low light environments on green colour development in 'Granny Smith' apples, Hirst et al. (1990) covered the fruit with opaque paper bags during various stages of development. Fruit peel became lighter with an increase in the duration and intensity of shading. Green colour loss was dependent on the duration of light exclusion. Further green colour loss occurred on re-exposure of shaded fruit to high light. However, lost green colour could be recovered if the shading was removed during the first half of fruit development. A similar trial suggested that chlorophyll in 'Golden Delicious' apple peel is mainly synthesized at the beginning of the season under a light stimulus (Gorski and Creasy, 1977). Comparing the shaded and exposed sides of on-tree 'Gala' apples, chlorophyll concentrations increased more rapidly and reached a higher maximum in exposed sides (Reay et al., 1998). Overall, 'Granny Smith' tends to become greener with an increase in canopy depth (lower irradiance), with pale fruit occurring in extreme shade conditions caused by the close proximity of leaves, branches and neighbouring fruit (Hirst et al., 1990; Warrington et al., 1996). Light transmission or canopy type does not affect the incidence of pale fruit (Hirst et al., 1990; Warrington et al., 1996). An optimum light level of 37-70% of full sunlight ( $2100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) was suggested for maximum fruit colour and chlorophyll development in 'Granny Smith' (Izso and Larsen, 1990). However, this level of exposure may prove too high under South African conditions, resulting in excessive sunburn and red blush development.

## **4.2 Temperature**

Chlorophyll accumulation is rapid at moderate to high temperatures ( $\sim 28^\circ\text{C}$ ) under various light intensities (McWilliam and Naylor, 1967). Low temperatures ( $16^\circ\text{C}$ ), particularly in combination with high light intensities, inhibit chlorophyll accumulation, because low temperatures increase the susceptibility of chlorophyll to high light damage. Protochlorophyllide synthesis (Virgin, 1955) and

the conversion to chlorophyll are sensitive to low temperatures (McWilliam and Naylor, 1967). Chlorosis is often observed when thermophilic plants are subjected to low temperatures, probably due to the accumulation of photoactive chlorophyll precursors. Heat stress at 45 °C for 8 h depressed chloroplast formation and the effect worsened with longer durations (Adelusi and Lawanson, 1978). The same conditions also depressed the accumulation of chlorophyll in melon seedlings (Onwueme and Lawanson, 1973).

### 4.3 Nitrogen

By estimation, almost 75% of nitrogen (N) in mesophyll cells is located in the chloroplast (Peoples and Dalling, 1988). The integral relationship between N levels and chlorophyll is evident from the use of chlorophyll meters to measure leaf N content (Lee et al., 1999). The majority of leaf N is part of the proteins of the Calvin cycle, e.g. Rubisco, and thylakoids, explaining why N content correlates to photosynthetic capacity (Evans, 1989a). N deficiency reduces chlorophyll formation and decreases chlorophyll density in plant leaves (Thomson and Weier, 1962). The chlorophyll:N ratio is constant regardless of plant N status (Terashima and Evans, 1988).

Citrus seedlings had less chlorophyll per unit leaf area, but a greater chlorophyll *a/b* ratio in N deficient treatments (Bondada and Syvertsen, 2003). Leaf dry mass, thylakoids per granum and total chlorophyll increased, while chlorophyll *a/b* ratio decreased with an increase in applied N. This was attributed to an increase in chlorophyll *b* and not to a decrease in chlorophyll *a*. N deficient spinach leaves contained small chloroplasts with low chlorophyll levels. High N levels resulted in large chloroplasts with well-developed grana and stroma lamellae. The cross sectional area of spinach chloroplasts was larger at higher N levels and/or under lower irradiances (Terashima and Evans, 1988).

There is a very important link between N use efficiency and irradiance. Leaves grown at high irradiance shows greater N efficiency (Terashima and Evans, 1988) and has a higher N content (Evans, 1989b). Depending on the light environment, N can be partitioned, for example, to the electron transport chain and Rubisco activity (as in sun leaves) or to the thylakoids (as in shade leaves) (Evans, 1989a). Leaves adapted to low light environments have a lower electron transport capacity per chlorophyll, but this is compensated for by an increased ratio of chlorophyll to N, whereas N is assigned to electron transport in leaves adapted to high irradiance (Evans, 1989b). At high irradiance, light absorption increased and non-photochemical quenching decreased with an increase in N content. This is due to the improved efficiency of PS II, which reduces the probability of damage through photo-oxidation (Cheng et al., 2000).

Chlorophyll concentrations in 'Fuji' apples increased with an increase in fruit N level (Marsh et al., 1996). Green colour in 'Golden Delicious' apples increased with an increase in leaf N level (Rease and Williams, 1974; Williams and Billingsley, 1974). Urea sprays increased chlorophyll concentration in 'Gala' apples (Reay et al., 1998). Chlorophyll concentration and green colour in mango fruits also correlated positively with an increase in pre-harvest N level (Ngunyeni et al., 2004).

#### **4.4 Maturity**

It appears that chlorophyll synthesis in apple peel is most active during early fruit development (Gorski and Creasy, 1977), and decreases as the fruit matures on and off the tree (Griessel et al., 1992; Knee, 1971; Mussini et al., 1985). Degradation of chlorophyll commences with the onset of fruit ripening due to the action of plant hormones that are linked with ripening such as ethylene and methyl jasmonate. These hormones stimulate chlorophyll breakdown by promoting the expression of Chlase, the key enzyme responsible for chlorophyll breakdown (Jacob-Wilk et al., 1993; Tsuchiya et al., 1999). Mussini et al. (1985) found that total chlorophyll, as well as chlorophyll *a* and *b* concentrations in 'Granny Smith' apple showed a steady decrease during fruit development. Griessel et al. (1992) set an optimum harvest date for 'Granny Smith' apples at approximately 171 days after full bloom, after which chlorophyll decreases rapidly. Chlorophyll concentration on both sides (exposed and shaded) in 'Braeburn' and 'Royal Gala' apples peaked 80-100 days after full bloom (Greer, 2005). Reay et al. (1998) found that the total fruit chlorophyll of 'Gala' apples peaked approximately 70 and 110 days after full bloom in two successive seasons.

Cold storage lowers the rate at which chlorophyll decreases after harvest (Mussini *et al.*, 1985). The decrease in chlorophyll during storage is accompanied by a slight decrease in carotenoid concentration. Chlorophyll degradation progresses in correlation with ripening and senescence, while carotenoids remain stable, resulting in the yellowing of fruit (Mussini *et al.*, 1985). In contrast with this, Knee (1971) found an increase in carotenoid levels of 'Cox's Orange Pippin' apples during on and off tree ripening.

Green colour of 'Granny Smith' apples correlates well with total chlorophyll and chlorophyll *a* concentrations (Griessel et al., 1992). Lightness of fruit skin colour, measured with a colorimeter, decreases linearly with increase in chlorophyll concentration (Lancaster et al., 1997). In 'Golden Delicious' apples, chlorophyll is the most important colour determinant, since yellowing did not

become evident until the chlorophyll concentration was less than 0.15-0.2  $\mu\text{g}/\text{cm}^2$  of the apple skin (Griessel et al., 1992; Knee, 1971).

## 5. Summary

Green tissues owe their colour to the absorbance characteristics of the chlorophyll molecule. Blue and red light are absorbed and used to drive photosynthesis while 20% green light is reflected. Chlorophyll is a tetrapyrrole with a Mg-containing 'head' and a hydrophobic phytol 'tail'. The biosynthesis of chlorophyll is a complex process and light plays an important regulatory role. Chlorophyll is degraded via the chlorophyllase-pheophorbide *a*-oxygenase pathway and degradation usually commences during senescence.

The chloroplast is a chlorophyll containing plant cell organelle. It is made up of three parts: envelope, stroma and thylakoids. The thylakoid is an interconnected membrane and the site of photosynthesis. Chloroplasts are formed from proplastids and develop under genetic control. A light stimulus is often needed. Chloroplasts degrade to gerontoplasts under nuclear control.

Light, temperature, nitrogen and maturity are some of the major factors influencing green colour of chlorophyllous tissues. Light is necessary for the synthesis of chlorophyll and also influences the anatomy and morphology of chloroplasts and the leaves that contain them. However, excessive light may damage the chlorophyll and chloroplasts, manifesting in fruit as sunburn. Excessive high and low temperatures may depress the formation of chloroplasts and synthesis of chlorophyll. Nitrogen content is positively correlated to chlorophyll concentration and N performs an integral role in the functioning of the chloroplast as component of Calvin cycle and thylakoid proteins. As fruit matures, chlorophyll decreases due to ripening-associated hormones that promote chlorophyll catabolic enzymes.

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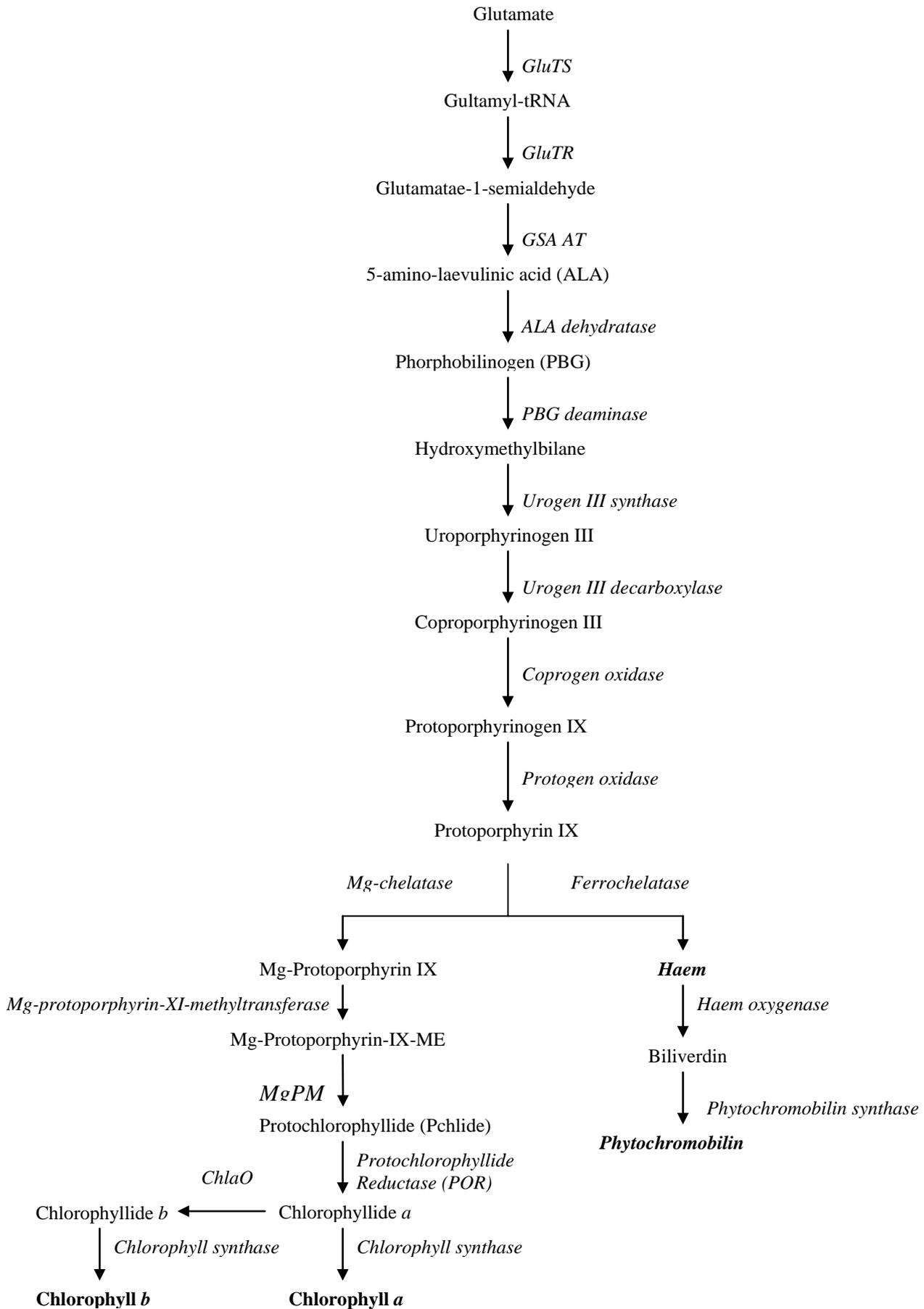


Fig. 1. Tetrapyrrole biosynthesis in higher plants, showing the major end products (boxed) and the responsible enzymes (adapted from Cornah et al. (2003)). Abbreviations: GluTS, glutamyl-tRNA synthetase; GluTR, glutamyl-tRNA reductase; GSA AT, glutamate-1-semialdehyde aminotransferase; MgPME, Mg-protoporphyrin IX monomethyl ester cyclase; ChlaO, chlorophyllide *a* oxidase.

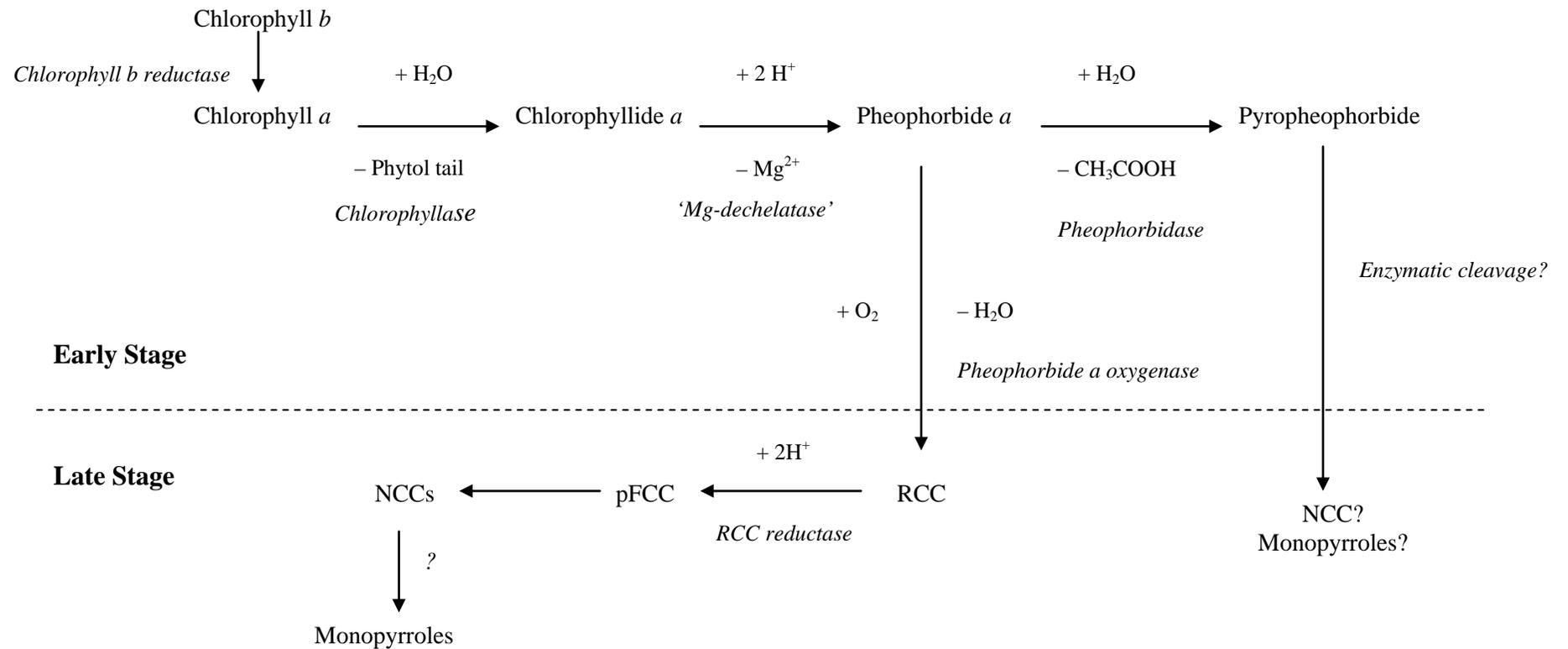


Fig. 2. Chlorophyll degradation pathway in higher plants (adapted from Takayima et al., (2000) and Hortensteiner, (2006)). The pathway is composed of two stages, an early stage before the cleavage reaction of the tetrapyrrole macrocyclic ring and a late stage that includes the cleavage reaction and steps after the reaction. The products in the early stage are green, whereas those in the late stage are colourless. Abbreviations: NCCs, nonfluorescent chlorophyll catabolites; pFCC, primary fluorescent chlorophyll catabolite; RCC, red chlorophyll catabolite.

# SKIN COLOUR AND BLEMISHES IN 'GRANNY SMITH' APPLES IN RELATION TO CANOPY LIGHT ENVIRONMENT

## Abstract

The dark green apple cultivar, Granny Smith (GS), is the most widely grown cultivar in South Africa. However, production of class one quality GS is hampered by the occurrence of sunburn and red blush on the skin caused by the high light intensities and high (sunburn) and low (red blush) temperatures. There are also increasing reports from all markets of South African GS being too light green in colour. This study was conducted to investigate the relationship between canopy position and external fruit quality with the ultimate aim to devise pruning and training strategies to maximize the yield of class one fruit. Light and peel temperature measurements were taken at fully exposed, partially shaded and deeply shaded canopy positions and related to skin colour and the incidence of sunburn and red blush. We hypothesized that fruit from partially shaded canopy positions will be the darkest green in colour while most sunburn and red blush will be found in the outer canopy. During early fruit development (26 DAFB), chlorophyll concentrations were the highest in fruit from higher light environments. Chlorophyll decreased and fruit became lighter green in colour during fruit development. Exposed fruit from the northern side of the row received the most light throughout the season (53% of full sun), had the highest peel temperature (on average 5°C above ambient) and consequently developed sunburn (36% of fruit) and red blush (76% of fruit). Partially shaded fruit from the southern side of the row received approximately 5% of full sunlight and had the highest chlorophyll concentrations and darkest green colour at harvest. Deeply shaded inner canopy fruit received approximately 2% of full sunlight, had low chlorophyll concentrations and were lighter green in colour. The light environments of the 10% darkest green fruit, the 10% lightest green fruit as well as fruit that developed sunburn were compared independent of canopy position. The 10% darkest green fruit received moderately high light levels (25–45% of full sun or 400-700  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) during early fruit development ( $\approx 80$  DAFB), but became progressively shaded (3% of full sun) during the latter half of the season. Fruit that developed sunburn and the lightest green fruit were exposed to high (1300  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and extremely low (50  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) light intensities, respectively, throughout their development. In conclusion, maximum chlorophyll synthesis and dark green skin colour require an open canopy during the first half of fruit development while shading is necessary during the latter half of fruit development to avoid the occurrence

**of sunburn, red blush and green colour loss. The sides of fruit facing the outside of the canopy at partially shaded canopy positions on the southern side of E–W rows meet these requirements.**

## **Introduction**

The green apple cultivar, Granny Smith (GS), has become one of the most well known cultivars in the world since its origin in 1868 (Hampson and Kemp, 2003) and is also the most widely grown cultivar in South Africa making up 24% of the total area planted (Deciduous Fruit Producers' Trust, 2008). Most apple consumers are familiar with the unique and characteristic dark green colour and tart taste of GS. Consequently, GS is set to remain one of the major cultivars in the world (Belrose Inc., 2008). However, the total area planted to GS in South Africa has decreased by almost 15% during the last 15 years due to competition from more lucrative blushed cultivars (Deciduous Fruit Producers' Trust, 2008). Apart from realizing lower returns, the proportion of class one fruit from GS trees is lower compared to other cultivars (Van Rensburg, personal communication). In order to qualify as class one fruit and thereby realize good returns to growers, GS fruit need to be uniformly green and without blemishes such as red blush, sunburn or whitening of the skin. Fruit with sunburn or red blush is downgraded to class three and will not pass for export purposes.

In South Africa, sunburn and red blush may decrease class one fruit by 35% and 20%, respectively (Griessel, personal communication). On arrival at the destination, a further 5% of cartons may be rejected due to sunburn and red blush (Sadie, personal communication). These blemishes occur in high light environments (Tustin et al., 1988; Warrington et al., 1996), where high light in combination with low and high temperatures initiates the synthesis of red anthocyanins and yellow carotenoids, respectively. These pigments serve as light traps to prevent or reduce chlorophyll destruction caused by light stress (Merzlyak and Chivkunova, 2000). Red blush in GS is formed when nights are cool (4 °C), days are mild (20 °C) and fruit are exposed to high levels of UV-B and visible light (Reay, 1999). Schrader et al (2008) defines three types of sunburn, namely sunburn necrosis, sunburn browning and photooxidative browning. Sunburn necrosis is due to thermal death of epidermal and subepidermal cells that leads to formation of a necrotic spot and is caused by extreme heat ( $52 \pm 1$  °C), while sunburn browning is a result of high temperatures (46 °C – 49 °C) in the presence of light. Photooxidative browning occurs when fruit peel is suddenly exposed to high light intensity resulting in photobleaching followed by necrosis. It can occur at much lower peel temperatures (<30 °C) in the absence of UV–B radiation and is thought to be due to photooxidative damage. Chlorophylls decreased due to sunburn in all apple cultivars studied (Felicetti and

Schrader, 2009). However, while carotenoids in red apples generally increased due to sunburn, levels of  $\beta$ -carotene decreased while the xanthophylls violaxanthin and antheraxanthin remained constant in GS (Felicetti and Schrader, 2009). In order to minimize sunburn and red blush, producers use cultural practices such as shade netting, evaporative cooling and pruning techniques that decrease light levels and/or peel temperatures.

In contrast to sunburn and anthocyanin synthesis, peel whitening occurs under extremely low light intensities (Hirst et al., 1990) and may decrease class one fruit in South Africa by approximately 10% (Griessel, personal communication). A perception is increasing among fruit importers that South African GS is less green than GS of competing countries (Griessel, personal communication). In order to deal with this problem, GS fruit is shipped earlier or later during the Southern hemisphere season when European markets are less saturated with fruit. Alternatively, fruit are shipped to lower value markets, which has a negative effect on net profits achieved by producers (Griessel, personal communication). There is currently no coordinated effort to develop cultural practices to address this problem. In order to increase GS class one fruit by decreasing sunburn, red blush and peel whitening, it is necessary to have a sound understanding of green colour development in apple peel and how it is affected by the light environment.

In apple peel, chlorophyll is synthesized at the beginning of the season under a light stimulus (Gorski and Creasy, 1977). After the initial increase during early fruit development, chlorophyll levels begin to decrease from 40 days after flowering (Reay et al., 1998). Ripening fruit gradually change from green to yellow as the chl/car ratio decrease due to relatively faster degradation of chlorophyll (Griessel et al., 1992; Knee, 1971; Mussini et al., 1985). Fruit from different positions in the tree canopy may differ in green colour in accordance with the highly variant light levels that they are exposed to, ranging from high ( $\pm 2000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) to low ( $\pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). Light transmission generally decreases with canopy depth (Warrington et al., 1996). The high light environment of the upper canopy induces the development of red blush, sunburn yellowing and bleaching of chlorophyll (Tustin et al., 1988; Warrington et al., 1996). Overall, GS tends to become greener with an increase in canopy depth, with pale green fruit only found in conditions of intense shade such as where a fruit touches another fruit, a branch or a leaf (Warrington et al., 1996). An optimum light level of 37-70% of full sunlight ( $2100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) was suggested for maximum chlorophyll levels and dark green colour of GS (Izso and Larsen, 1990).

The main objective of this research was to relate GS fruit position in the canopy and the respective light environments to external fruit quality in terms of skin colour, sunburn and red blush

development. The skin colour of fruit at different canopy positions was measured at regular intervals from fruit set until harvest and related to light level and peel temperature. Our hypothesis was that fruit from partially shaded canopy positions exposed to high levels of diffuse light but with limited exposure to direct sunlight would show less shading-related whitening as well as less high radiation-related yellowing and red blush development during fruit development. Results obtained would provide a basis from which to reassess the current planting systems used for GS and to devise pruning strategies for the improving fruit colour and increasing the percentage class one fruit in existing GS orchards.

## **Materials and methods**

Trials were conducted in the Mediterranean-type climate Grabouw (Latitude: 34°8'S; Longitude: 19°3'E) and Vyeboom (Latitude: 34°1'S; Longitude: 19°3'E) regions of the Western Cape province of South Africa.

### **2006/2007.**

*Trial layout and light measurements.* Fruit was selected ca. 1.5 m from the ground, halfway between the outer canopy and trunk on both sides of the row as well as next to the trunk, on three randomly selected trees in each of four mature N–S row orientated GS orchards representative of GS in the Grabouw region. One fruit was selected per position. Light levels experienced at each canopy position were assessed hourly from 0800<sub>HR</sub> to 1800<sub>HR</sub> from 18 Feb. to 31 Mar. 2007 with a quantum sensor attached to a light meter (LI-250, LI-COR, Lincoln, NEB, USA) held horizontally next to each fruit with the sensor facing upwards. Ambient light levels were measured in the middle of the working row.

*Fruit quality at different canopy positions.* Twelve fruit were sampled on 6 and 7 Apr. 2007 (during the commercial picking window  $\pm 170$  DAFB) from four randomly selected trees in the same orchards mentioned above. Fruit were sampled on both sides of the row from the outer canopy as well as halfway between the outer canopy and the trunk. In addition, 25 fruit were sampled from the inner canopy next to the trunk. Samples were assessed for green colour intensity on the greenest side of fruit using a colour chart (Set A 38, Deciduous Fruit Board, South Africa), the occurrence of red blush and sunburn, and chlorophyll concentration of the peel. Hue angle ( $H^\circ$ ), chroma (C) and lightness (L) values of both the greenest and least green sides of the fruit were determined with a Minolta chromameter (Model CR-400, Minolta Co. Ltd., Tokyo, Japan). A 7 cm long and 2 cm

wide lint of skin was peeled from both the greenest and least green sides of fruit. The flesh was scraped off the peel using a teaspoon where after the peel was frozen in liquid nitrogen, freeze dried, milled and kept at -40 °C until pigment analysis.

*Pigment analysis.* Chlorophylls and carotenoids were extracted from 0.1 g freeze-dried material with 4 ml 100% acetone and constantly stirred for 24 h at 4 °C. After centrifugation for 15 min at 10000 x g, the supernatant was decanted and the pellet re-extracted with 4 ml 100% acetone and centrifuged as described above. The supernatant was added to the first. After filtration through 0.45 µm filters (Millex-HV, Millipore Corporation, Milford, MA, USA), pigments were quantified by spectrophotometry (Cary 50 Bio, Varian Australia (PTY) Ltd., Melbourne, Australia), measuring absorbance at 470, 645 and 670 nm. The extinction coefficients of Lichtenthaler (1987) were used to calculate chlorophyll and carotenoid concentrations, which were then expressed in µg g<sup>-1</sup> dry weight of peel.

#### **2007/2008.**

*Trial layout.* Twenty uniform trees were selected in a vigorous orchard on seedling rootstock. Trees were planted in 1983 in an E–W row direction and trained to a free standing central leader form. One fruit was tagged shortly after fruit set at each of five canopy positions, i.e., fully exposed outer canopy on the northern (A) and southern (E) periphery of trees, partially shaded canopy positions on the northern (B) and southern (D) sides of trees, and the shaded inner canopy next to the trunk (C).

*Light, temperature and colour measurements.* Light levels directly above each fruit, peel temperature and peel colour were measured approximately every two weeks on cloudless days from 10 Nov. 2007 (27 DAFB) to 30 Mar. 2008 five times daily at two hour intervals from 0800 HR to 1800 HR. The light sensor was always directed at the sun. Peel temperature was measured using a high performance infrared thermometer (Rayner MX4, Raytek Corporation, Santa Cruz, CA, USA) aimed at the position on the fruit facing the current position of the sun. Fruit color was measured with a Minolta chromameter (Model CR-400, Minolta Co. Ltd., Tokyo, Japan) on the side of the fruit receiving the most sun.

*Fruit quality.* Ten representative fruit were sampled on 9 November 2007 and 14 April 2008 (commercial harvest) from each position on each of the twenty selected trees. Colour was measured on the greenest side of fruit harvested on 9 November with a chromameter and the whole fruit was peeled using a knife for chlorophyll analysis. Green colour intensity, red blush incidence, sunburn

incidence, and chlorophyll concentration of the peel were determined for fruit harvested at maturity. Chlorophyll concentrations were determined as in 2006/07.

## **Results**

### **2006/2007**

Fruit from inner canopy positions received less light than fruit from partially shaded intermediate positions (Fig. 1). A significantly higher proportion of fruit from outside canopy positions developed sunburn and red blush (Table 1). Only 34 to 37% of fruit from the outer canopy did not develop either sunburn or red blush. Note that red blush and sunburn occurred concurrently on a large percentage of fruit. There was no significant difference between the eastern and western sides of rows in the incidence of sunburn and red blush (Table 1). Hardly any fruit (1%) from the inner canopy developed sunburn and no fruit developed red blush while 2 to 6% of fruit from partially shaded canopy positions developed sunburn or red blush (Table 1). According to colour charts, outside fruit were the least green, while inner and intermediate canopy fruit were similar in colour (Table 1). Lower lightness values and higher chlorophyll concentrations also reveal the darker green colour of fruit from intermediate and inner canopy positions (Table 2). However, the least green sides of inner canopy fruit were lighter in colour than the least green sides of outer canopy fruit (Table 2). There were no significant differences between the eastern and western sides of rows in terms of colour and chlorophyll concentration (Table 2).

### **2007/2008.**

Maximum sunlight was relatively constant throughout the season and peaked between 1000 HR and 1400 HR during the day (Fig 2 & 3). Exposed fruit on the northern and southern sides of the E-W row received the most and second most sunlight (53% and 31% of full sunlight, respectively) during an average day and throughout the season followed by fruit from intermediate positions on the northern side of rows (12% of full sunlight) (Fig 2, 3 & 4). Fruit from the inner canopy and intermediate positions on the southern side of the row received very little sunlight throughout the day (2% and 5%, respectively) and during the season. The light intensity received by exposed fruit on the southern side of the row decreased with approximately 60% from the beginning of Jan. to the end of Mar (Fig. 3). Light exposure of fruit from intermediate positions on the northern side of rows increased by approximately 10% of full sunlight from the beginning of Feb. (Fig. 3).

Peel temperatures peaked between 1200 HR and 1600 HR (Fig. 5). Exposed fruit on the northern side of the row had the highest peel temperature throughout the season (Fig. 5, 6 & 7), approximately 5 °C higher on average than the average ambient air temperature (Fig. 6 & 7). The peel temperature of exposed northern fruit reached a maximum of 42 °C, 7 °C above the air temperature, on 20 Feb (data not shown). Exposed fruit on the southern side of rows and fruit from intermediate positions on the northern side of rows had slightly higher ( $\approx 2$  °C) peel temperatures than ambient (Fig. 6). Fruit from the inner canopy and intermediate positions on the southern side of rows did not differ in temperature from ambient (Fig. 6).

At the beginning of the season (9 Nov. 2007), exposed fruit had the lowest lightness values and hue angles (Table 3 & Fig. 8, 9), as well as the highest chlorophyll and carotenoid concentrations and lowest chl/car ratios (Table 3). Fruit from intermediate canopy positions on the northern and southern sides of the row had similar lightness values (Table 3), with fruit on the southern side of the tree having a slightly higher hue angle, chlorophyll and carotenoid concentrations (Table 3). Inner canopy fruit had the highest lightness values and the highest hue angles, as well as low chlorophyll and carotenoid concentrations (Table 3).

Fruit at all canopy positions became lighter in peel colour as the season progressed (Fig. 8). Exposed northern fruit were the lightest in colour at harvest, followed by inner canopy fruit, northern intermediate fruit, exposed southern fruit and southern intermediate fruit (Table 4). This corresponds with the visual assessment of colour by chart (Table 4). Hue angle at all positions increased initially during fruit development, where after it remained constant until shortly before harvest when fruit on the northern side of trees showed a decrease in hue while exposed southern fruit increased in hue (Fig 9). The difference in lightness and hue angle between the on-tree measurements (Fig. 8 & 9) and the sampled measurements (Table 5) is because on-tree measurements were not taken on the side of the fruit with the best colour, but rather on the side facing the sun.

There was little difference in hue angle between treatments at harvest (Table 5). Southern intermediate fruit were the darkest and greenest in colour with the highest chlorophyll and carotenoid concentrations and chl/car ratios (Tables 5 & 6). However, these fruit differed most in colour and chlorophyll concentration between their best and worst coloured sides, indicating a less uniform colour over the fruit as a whole (Tables 5 & 6). Southern exposed fruit followed southern intermediate fruit in terms of colour, chlorophyll and carotenoid concentration and chl/car ratio, and also differed much in colour between the best and worst coloured sides of the fruit (Tables 5 & 6).

Exposed northern fruit were the lightest in colour, with respect to both the best and worst coloured sides of the fruit, compared to fruit at other canopy positions (Table 5). These fruit had the lowest chlorophyll and carotenoid concentrations as well as the lowest chl/car ratios (Table 6). However, the difference in colour between the best and worst coloured sides of exposed northern fruit was less compared to fruit at the southern and inner canopy positions (Table 5). Northern intermediate fruit had the most uniform colour with respect to their best and worst green sides (Table 5). These fruit were darker green on their best side compared to exposed northern and inner canopy fruit, but had similar chlorophyll and carotenoid concentrations as inner canopy fruit on their best coloured side (Tables 5 & 6). Northern intermediate fruit were darker green on their worst side compared to fruit at other canopy positions (Table 5). Inner canopy fruit showed a large difference in colour between their best and worst sides despite similar chlorophyll concentrations (Tables 5 & 6). Chlorophyll and carotenoid concentrations of the best sides of exposed southern, intermediate northern and inner canopy fruit were comparable (Table 6). Inner and exposed southern fruit had similar chlorophyll concentrations in the worst side of the fruit, with inner fruit being more uniform in terms of chlorophyll concentrations. On the best and worst coloured sides, chl/car ratios of intermediate northern and exposed southern fruit were similar, while inner and southern intermediate fruit had similar values (Table 6).

The percentage fruit without sunburn or red blush was considerably higher in shaded regions of the canopy, while a low percentage of exposed fruit on the northern side of the row followed by exposed fruit on the southern side of the row was marketable due to the prevalence of sunburn and red blush (Table 4). A large proportion of exposed northern fruit developed sunburn and red blush while inner canopy and southern intermediate fruit did not develop any sunburn and developed little red blush (Table 4). Exposed southern fruit showed little sunburn, but developed considerable red blush. Northern intermediate fruit developed little red blush and sunburn. Sunburn was first observed on 27 Dec. shortly after ambient air temperatures first rose above 35 °C (Fig. 10). Thereafter the percentage of fruit with sunburn steadily increased until harvest with temperatures above 30 and 35 °C occurring at regular intervals during fruit development (Fig 10).

The 10% lightest and 10% darkest green fruit as well as fruit that developed sunburn were compared with regard to the light levels that they received during fruit development (Fig. 11). The darkest green fruit received moderate light (ca. 35% of ambient light) during Nov. and Dec. where after they became progressively shaded and received very little light (ca. 5% of ambient light) in the two months before harvest. In contrast, fruit that developed sunburn were exposed to high light levels ( $\approx$  73% of ambient light) throughout their development, but especially during the two months

before harvest (ca. 79% of ambient light). The lightest green fruit received very little light (ca. 3% of ambient light) throughout their development.

## Discussion

As expected (Warrington et al., 1996), light intensity decreased with increasing depth in the canopy. The western and eastern sides of N-S rows received comparable light level while the northern side of E-W rows received higher light intensities than the southern side. This is due to the maximum zenith angle of 79° that the sun reaches on 21 Dec. at this latitude, causing more sun light to reach the northern side of the tree, especially during midday (Bergh et al., 1980). Exposed fruit on the southern side and fruit inside the canopy on the northern side of E-W rows received similar light levels during the latter half of fruit development after the exposed southern fruit received considerably higher light levels during the first half of the season. This might be due to changes in the position of the fruit during the course of the season as branches bend under the weight of fruit (Hirst et al., 1990). The zenith angle of the sun decreases from 79° on 22 Dec. to 56° at the end of March thereby increasing shading on the southern sides of trees. Both bending of branches and the inclination of the sun may also affect light distribution within the canopy (Bergh et al., 1980) The different light levels that fruit are exposed to at different canopy positions also results in differences in fruit peel temperature.

Fruit temperature is a function of radiation intensity and air circulation (Bergh et al., 1980). Bright sunlight and low wind velocity can raise fruit (grape berries) temperature by 10–15°C above air temperature (Smart and Sinclair, 1976). Bergh et al. (1980), Chen et al. (2008) and Parchomchuk & Meheriuk (1987) measured apple peel temperatures in excess of 50°C on occasions when air temperatures exceeded 30°C. According to Schrader et al. (2003), sunburn occurs when fruit peel temperature exceeds 45°C in the presence of light, which may occur when air temperature exceeds 30°C. High temperature in combination with high light causes photooxidation and photodestruction of chlorophyll in apple peel even though the xanthophyll cycle (carotenoids) and antioxidant systems are up regulated (Chen et al., 2008). Sunburn was first observed in late December on fruit from the northern periphery of trees. In agreement with Bergh et al. (1980), these fruit received the most sunlight, especially during the warmest part of the day (1200 HR to 1600 HR) due to the sun's inclination and consequently had the highest peel temperature. Also due to the sun's inclination, sunburn was considerably less on the southern side of E-W rows while partially shaded fruit on the northern side also developed some sunburn. Sunburn only occurred in the northern upper canopy in

New Zealand with percentages ranging from 5 to 18% of the total crop (Hirst et al., 1990; Warrington et al., 1996).

Sensitivity to sunburn increases as fruit develops due to a decrease in photoprotective ability and in photosynthesis (Li and Cheng, 2008). This may explain why some northern intermediate fruit eventually also developed sunburn even though these fruit were exposed to relatively low light intensities. In contrast, exposed southern fruit received much lower light levels than exposed northern fruit after 26 Jan. until harvest and developed much less sunburn. Due to a combination of the lower inclination of the sun and the bending of branches under the weight of fruit, the light intensity and peel temperature that northern intermediate fruit were exposed to increased during the last two months of fruit development while the light exposure of southern outer canopy fruit decreased. The sudden exposure of shaded fruit to high light intensities carries a high sunburn risk (Wünsche et al., 2001). This was evident in the 2006/2007 season when sunburn occurred even in the inner (most shaded) canopy. Sudden exposure to high light may cause photoinhibition and lead to the up regulation of the xanthophyll cycle, which would minimize photooxidative stress and contribute to the acclimation to high light (Ma and Cheng, 2004). Sunburn results when the photooxidative stress exceeds the photoprotective capacity. Shade leaves that were exposed to a high light environment experienced an initial decrease in chlorophyll because of temporary photobleaching, although they do recover after several weeks (Bjorkman and Holmgren, 1963; Lichtenthaler et al., 1981). When moved into high light, chloroplast destruction occurs, resulting in pale, irregular and partly fragmented chloroplasts (Bjorkman and Holmgren, 1963). The high incidence of sunburn in our results appears to be due the unrelenting exposure to high light throughout fruit development often concurrent with high peel temperatures.

Red blush developed mostly in fruit from the outer canopy and is caused by the synthesis of anthocyanins in response to high irradiance when cold nights are followed by mild, clear days (Curry, 1997; Reay, 1999). Warrington et al (1996) established that 40% of full sunlight, which is considerably higher than required for red cultivars, is needed for red blush development in GS under New Zealand conditions. Although leaf shading considerably decreased the occurrence of red blush, it was still evident on fruit from the inner canopy in 2006/2007. It is important to note that even inner canopy fruit receives occasional sun flecks during the day, creating the opportunity for the development of red blush and sunburn. According to Hirst et al (1990), red blush in GS commonly occurs when previously shaded fruit are exposed to high light intensities (Hirst et al., 1990). Previously shaded peel has a much greater potential to accumulate anthocyanins (Reay and Lancaster, 2001). Anthocyanins may form during the ripening of red cultivars and during early fruit

development, even in cultivars that are not inclined to form anthocyanins during ripening (Saure, 1990). Early red blush dissipates over the course of the season (Curry, 1997). The occurrence of early red blush is the reason why GS fruit from the outer canopy initially had low hue angles.

Hue angles stayed more or less the same throughout the season except in exposed northern fruit where it decreased due to sunburn and photodegradation of chlorophyll. Hue angle did tend to be higher in low light environments, indicating that fruit were greener in colour. The ratio of carotenoids to chlorophylls was higher in fruit more exposed to light. The lightest and least green fruit were found in the two extreme light environments, namely the deeply shaded interior canopy and the exposed northern periphery of the tree. Results from 2007/2008 revealed that partially shaded fruit were darker and greener in colour than fruit from the inner canopy. Tustin et al (1988) reported that green colour is negatively correlated with percentage light transmission into the canopy, with white blemishes only occurring on fruit that are subjected to profound shading, such as caused by the close proximity of branches, leaves or neighboring fruit (Hirst et al., 1990; Warrington et al., 1996). Hirst et al (1990) and Warrington et al (1996) also found the lightest and least green fruit in areas exposed to the highest irradiance.

At the beginning of the season, the darkest green fruit with the highest chlorophyll concentrations were found in the most exposed canopy positions. This is because chlorophyll synthesis during early fruit development requires light (Gorski and Creasy, 1977). The decline of chlorophyll during fruit development has been well documented (Griessel et al., 1992; Knee, 1971; Mussini et al., 1985; Reay et al., 1998). During fruit development, chlorophyll concentrations gradually decrease due to reduced synthesis and dilution as the fruit grows with the consequence that fruit gradually becomes lighter in colour.

Interestingly, the 10% darkest green fruit at harvest experienced comparable light levels during early development to fruit that developed sunburn. The difference came in during the last two months of fruit development when dark green fruit became shaded whereas fruit that developed sunburn remained exposed to high sunlight. Fruit that received very low light levels (3% of ambient) throughout their development were pale in colour due to lower chlorophyll synthesis. Izso and Larsen (1990) estimated 37–70% full sun ( $2100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) as the optimum light level for green colour development in GS. These estimates would be too high for South African conditions, as indicated by our results. Light appears to be necessary during early fruit development for adequate green colour development, while continuous high light levels and the high peel temperatures associated with it, particularly during the second half of fruit development, result in

sunburn. Fruit also become more sensitive to sunburn during fruit development due to a decrease in their photoprotective capacity and ability to quench absorbed light through photosynthesis (Li and Cheng, 2008).

Our 2006/2007 assessment of light levels in relation to fruit peel characteristics was performed in N–S rows while in 2007/2008 an E–W orchard was used. New apple orchards in South Africa are typically planted in an N–S row direction to maximize light interception. N–S rows generally intercept more light if the hedgerows are low, while E–W hedgerows intercept more light at high latitudes with tall trees and late maturing cultivars (Palmer, 1989). The efficiency of N–S hedgerows to intercept light changes little over the season, while E–W rows intercept more light in late summer than mid summer (Jackson and Palmer, 1972). However, an E–W row direction is generally not recommended for apple because of poor light distribution resulting in lower yields and poor red colour development (Middleton and McWaters, 2001). The deficiencies of E–W row direction become more pronounced with an increase in tree size and canopy density (Lombard and Westwood, 1977).

Maximizing light interception may be to the detriment of external fruit quality in GS due to the positive correlation between light levels and the incidence of sunburn and red blush. Consequently, the standard orchard practices used for red and blushed cultivars, and for green cultivars that are less susceptible to sunburn, may not be ideal for GS. Total light interception of N–S and E–W rows is comparable, but light interception is more evenly spread between the E and W sides of N–S rows (De Jong and Doyle, 1985; Jackson and Palmer, 1972). Sunlight is mainly intercepted on the exposed northern side of E–W rows, which increases sunburn while the southern side is shaded from high light (Middleton and McWaters, 2001). We did not find any significant difference in the incidence of red blush and sunburn between the E and W sides of N–S rows, whereas sunburn was significantly more on the N side of E–W rows. We considered that sunburn and red blush may be reduced over the entire tree in E–W rows. However, Warrington et al. (1996) found that the upper canopy of GS in E–W rows had a higher light transmission and 10% more orange/red blush compared to the upper canopy of N–S rows. The E–W orchard had less whitening of the skin, but the overall colour was slightly lighter. In pear, sunburn was slightly higher in N–S row directions (De Jong and Doyle, 1985).

Dense and rounded canopies such as achieved in low density plantings of open vase trees may increase the proportion of green GS fruit, but are less efficient in terms of production. It is also necessary to bear in mind that sufficient light (25–50% of full sun, according to our results) is

needed in the first half of the season for optimum green colour development. Apart from colour development, light is also needed to ensure sufficient flower bud development for an optimum return crop (Jackson, 1980). Hence, as suggested by Hirst et al. (1990), an open-textured but leafy environment creating a filtered light environment throughout the season is recommended for GS. Summer pruning is an effective way to increase canopy light transmission. It is usually used in red cultivars to improve red colour development, but may be a potentially risky method to improve green colour in GS because it may also increase sunburn (Miller, 1982) and red blush development (Ma and Cheng, 2004). However, if performed during early fruit development (4 to 8 weeks after full bloom), the potential green colour development will be maximized. Early summer pruning could also stimulate more regrowth later in the season to provide shade during the latter part of fruit development (Ferree et al., 1984; Miller, 1982) when the risk of sunburn is highest. (Li and Cheng, 2008).

Shade netting is the most effective way to reduce the incidence of sunburn. It may reduce fruit peel temperature by 5 to 9 °C and reduce sunburn and red blush to less than one percent of total fruit (Smit, 2007; Gindaba & Wand, 2005). However, the increased shading together with increased vigor of shaded trees may increase the percentage pale green fruit. Rigorous pruning, vigor control and the use of dwarfing rootstocks to ensure an open canopy for maximum light distribution could negate this problem. In addition, pale green fruit is a minor concern compared to sunburn and red blush. The only drawback to shade netting is the high overhead costs, but this may be overcome by high yields of class one GS fruit.

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Table 1. Green colour, incidence of sunburn and red blush in ‘Granny Smith’ apples sampled from different canopy positions on 2 Mar. 2007. The values are means  $\pm$  SE ( $n = 16$ ).

Canopy position	Green colour (chart) <sup>z</sup>	Sunburn (%) <sup>y</sup>	Red blush (%) <sup>y</sup>	Green fruit (%) <sup>x</sup>
Outside (East)	3.4 $\pm$ 0.1	46.1 $\pm$ 6.0	25.6 $\pm$ 7.2	33.8 $\pm$ 6.3
Intermediate (East)	3.2 $\pm$ 0.1	11.4 $\pm$ 5.9	1.9 $\pm$ 1.1	86.7 $\pm$ 6.1
Inner	3.2 $\pm$ 0.1	1.0 $\pm$ 0.8	0.0	98.9 $\pm$ 0.8
Intermediate (West)	3.1 $\pm$ 0.1	5.2 $\pm$ 2.2	2.0 $\pm$ 0.9	92.9 $\pm$ 2.1
Outside (West)	3.3 $\pm$ 0.1	46.6 $\pm$ 3.4	18.4 $\pm$ 5.0	37.3 $\pm$ 5.8

<sup>z</sup>chart values 1 – 12 where 1 = green; 12 = pale green/yellow

<sup>y</sup> the same fruit may have sunburn and red blush

<sup>x</sup> without any sunburn and/or red blush

Table 2. Peel lightness, hue angles and chlorophyll concentrations of the best and worst coloured sides of ‘Granny Smith’ apples sampled from different canopy positions on 2 Mar. 2007. The values are means  $\pm$  SE ( $n = 16$ ).

Position	Best side			Worst side		
	L value	Hue (°)	Chlorophyll concn ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	L value	Hue (°)	Chlorophyll concn ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)
Outside (East)	58.0 $\pm$ 0.3	117.1 $\pm$ 0.2	711 $\pm$ 21	60.8 $\pm$ 0.3	115.8 $\pm$ 0.2	586 $\pm$ 18
Intermediate (East)	57.0 $\pm$ 0.3	117.6 $\pm$ 0.1	820 $\pm$ 21	61.0 $\pm$ 0.3	116.6 $\pm$ 0.1	734 $\pm$ 27
Inner	56.4 $\pm$ 0.3	118.1 $\pm$ 0.1	879 $\pm$ 27	61.6 $\pm$ 0.3	116.8 $\pm$ 0.1	747 $\pm$ 21
Intermediate (West)	56.1 $\pm$ 0.3	117.8 $\pm$ 0.2	831 $\pm$ 27	60.4 $\pm$ 0.3	116.9 $\pm$ 0.2	752 $\pm$ 24
Outside (West)	57.4 $\pm$ 0.3	117.2 $\pm$ 0.2	757 $\pm$ 22	60.3 $\pm$ 0.3	115.8 $\pm$ 0.2	599 $\pm$ 23

Table 3. Peel lightness and hue angles as well as plastid pigment concentrations of ‘Granny Smith’ apples sampled from different canopy positions on 9 Nov. 2007 (26 DAFB). The values are means  $\pm$  SE ( $n = 20$ ).

Canopy position	L value	Hue (°)	Chlorophyll concn ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	Carotenoid concn ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	Chlorophyll/carotenoid ratio
A. Outside (North)	42.9 $\pm$ 0.7	101.6 $\pm$ 1.8	1352 $\pm$ 29	234 $\pm$ 4	5.8 $\pm$ 0.1
B. Intermediate (North)	49.4 $\pm$ 0.4	115.9 $\pm$ 0.9	1284 $\pm$ 30	208 $\pm$ 5	6.2 $\pm$ 0.1
C. Inner	51.4 $\pm$ 0.2	118.1 $\pm$ 0.1	1221 $\pm$ 34	213 $\pm$ 16	6.1 $\pm$ 0.3
D. Intermediate (South)	49.3 $\pm$ 0.3	117.5 $\pm$ 0.3	1352 $\pm$ 28	220 $\pm$ 4	6.1 $\pm$ 0.1
E. Outside (South)	42.7 $\pm$ 0.5	102.7 $\pm$ 1.7	1380 $\pm$ 29	238 $\pm$ 5	5.8 $\pm$ 0.1

Table 4. Green colour, incidence of sunburn, red blush and fruit without these blemishes in ‘Granny Smith’ apples sampled from different canopy positions at commercial harvest on 14 April 2008. The values are means  $\pm$  SE ( $n = 20$ ).

Position	Green colour (chart) <sup>z</sup>	Sunburn (%) <sup>y</sup>	Red blush (%) <sup>y</sup>	Unblemished fruit (%) <sup>x</sup>
A. Outside (North)	3.4 $\pm$ 0.2	36.0 $\pm$ 2.9	75.5 $\pm$ 4.3	22 $\pm$ 5.5
B. Intermediate (North)	2.6 $\pm$ 0.1	1.5 $\pm$ 0.8	14.7 $\pm$ 3.2	85 $\pm$ 3.2
C. Inner	3.0 $\pm$ 0.1	0.0	4.2 $\pm$ 2.1	96 $\pm$ 2.0
D. Intermediate (South)	2.1 $\pm$ 0.1	0.0	5.6 $\pm$ 1.6	94 $\pm$ 1.6
E. Outside (South)	2.5 $\pm$ 0.1	4.0 $\pm$ 1.1	61.4 $\pm$ 5.1	39 $\pm$ 4.9

<sup>z</sup> chart values 1 – 12 where 1 = green; 12 = pale green/yellow

<sup>y</sup> the same fruit may have sunburn and red blush

<sup>x</sup> without any sunburn and/or red blush

Table 5. Peel lightness and hue angles of the best and worst coloured sides of ‘Granny Smith’ apples sampled from different canopy positions at commercial harvest on 14 Apr. 2008. The values are means  $\pm$  SE ( $n = 20$ ).

Position	Best side		Worst side	
	L value	Hue (°)	L value	Hue (°)
A. Outside (North)	62.4 $\pm$ 0.5	115.6 $\pm$ 0.3	65.7 $\pm$ 0.4	114.6 $\pm$ 0.2
B. Intermediate (North)	60.4 $\pm$ 0.3	116.7 $\pm$ 0.1	62.4 $\pm$ 0.0	115.6 $\pm$ 0.0
C. Inner	61.1 $\pm$ 0.2	116.9 $\pm$ 0.1	66.3 $\pm$ 0.3	115.4 $\pm$ 0.2
D. Intermediate (South)	58.0 $\pm$ 0.3	117.6 $\pm$ 0.1	64.4 $\pm$ 0.4	116.0 $\pm$ 0.1
E. Outside (South)	59.6 $\pm$ 0.4	116.4 $\pm$ 0.1	64.7 $\pm$ 0.3	115.3 $\pm$ 0.1

Table 6. Chlorophyll and carotenoid concentrations ( $\mu\text{g}\cdot\text{g}^{-1}$  dry weight) of the best and worst coloured sides of ‘Granny Smith’ apples sampled from different canopy positions at commercial harvest on 14 April 2008. The values are means  $\pm$  SE ( $n = 20$ ).

Position	Best side			Worst side		
	Chlorophyll concn ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	Carotenoid concn ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	Chl/car ratio	Chlorophyll concn ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	Carotenoid concn ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	Chl/car ratio
A. Outside (North)	379 $\pm$ 13	78 $\pm$ 2	4.9 $\pm$ 0.1	307 $\pm$ 9	66 $\pm$ 2	4.7 $\pm$ 0.1
B. Intermediate (North)	447 $\pm$ 11	87 $\pm$ 2	5.1 $\pm$ 0.1	400 $\pm$ 8	77 $\pm$ 2	5.2 $\pm$ 0.1
C. Inner	454 $\pm$ 27	81 $\pm$ 7	5.6 $\pm$ 0.1	435 $\pm$ 13	78 $\pm$ 2	5.6 $\pm$ 0.1
D. Intermediate (South)	619 $\pm$ 18	110 $\pm$ 4	5.6 $\pm$ 0.1	490 $\pm$ 13	87 $\pm$ 2	5.6 $\pm$ 0.1
E. Outside (South)	479 $\pm$ 15	91 $\pm$ 4	5.3 $\pm$ 0.1	440 $\pm$ 10	87 $\pm$ 4	5.1 $\pm$ 0.1

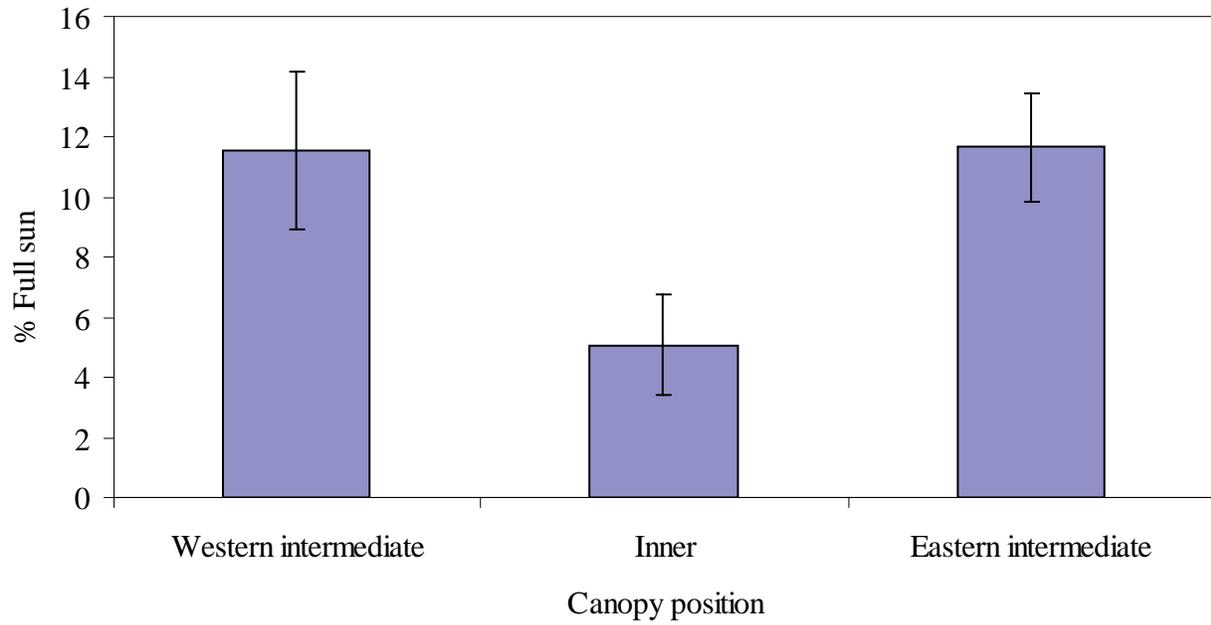


Fig. 1. Percentage of full sun received by GS fruit from middle and intermediate canopy positions on an average day during the 2006/2007 season. Values are means  $\pm$  SE ( $n = 12$ ).

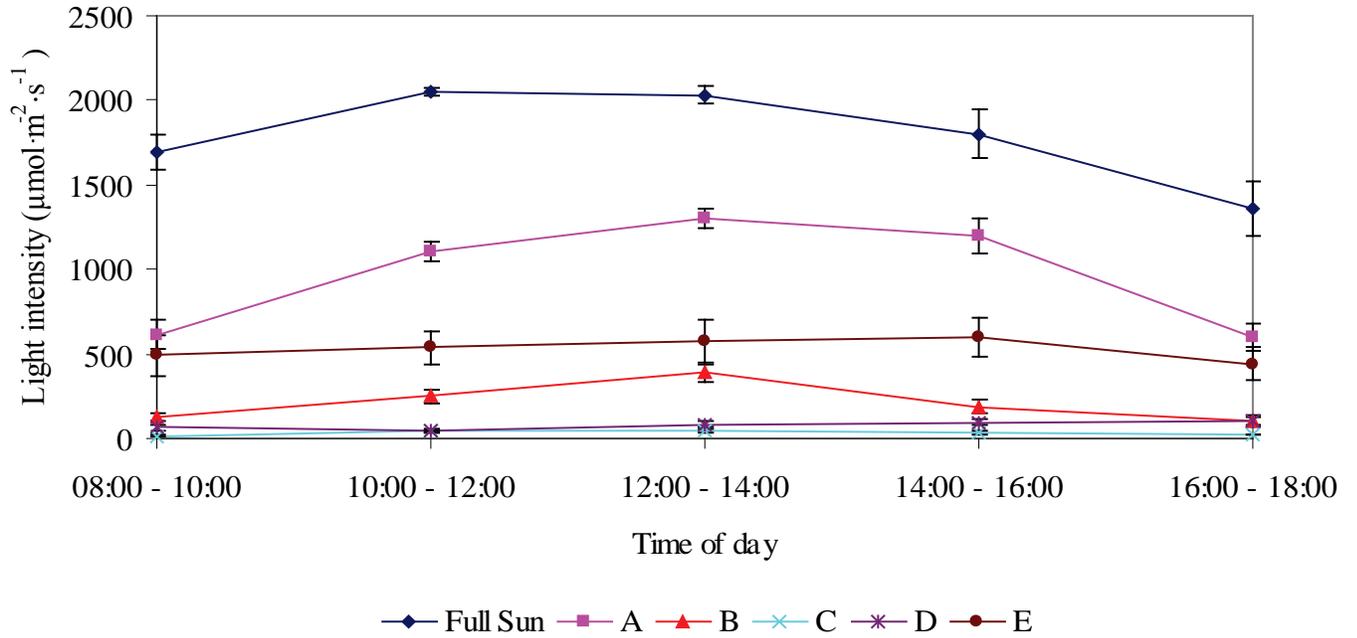


Fig. 2. Average daily light intensity received by GS fruit from different canopy positions during the 2007/2008 season. A and B are the outside and intermediate canopy positions on the northern side of trees. C is the inner position. D and E are the intermediate and outside positions on the southern side of trees. Values are means  $\pm$  SE ( $n = 20$ ).

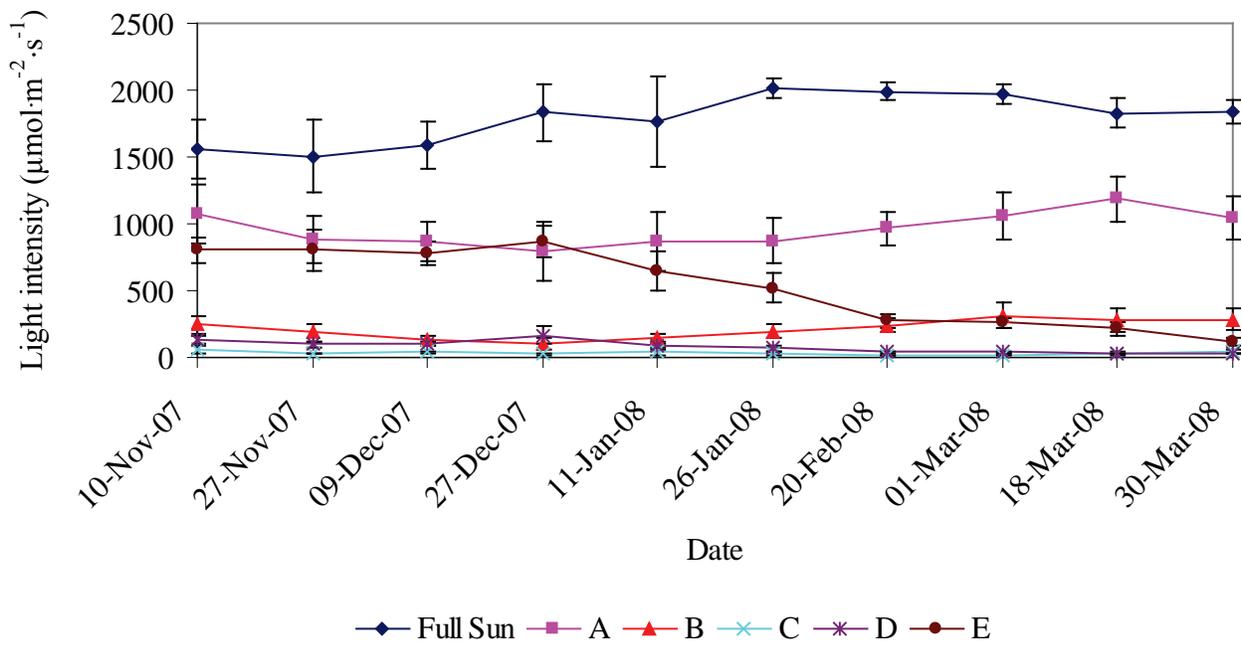


Fig. 3. Average light intensity received by GS fruit from different canopy positions during the 2007/2008 season. A and B are the respective outside and intermediate canopy positions on the northern side of trees. C is the inner position. D and E are the respective intermediate and outside positions on the southern side of trees. Values are means  $\pm$  SE ( $n = 20$ ).

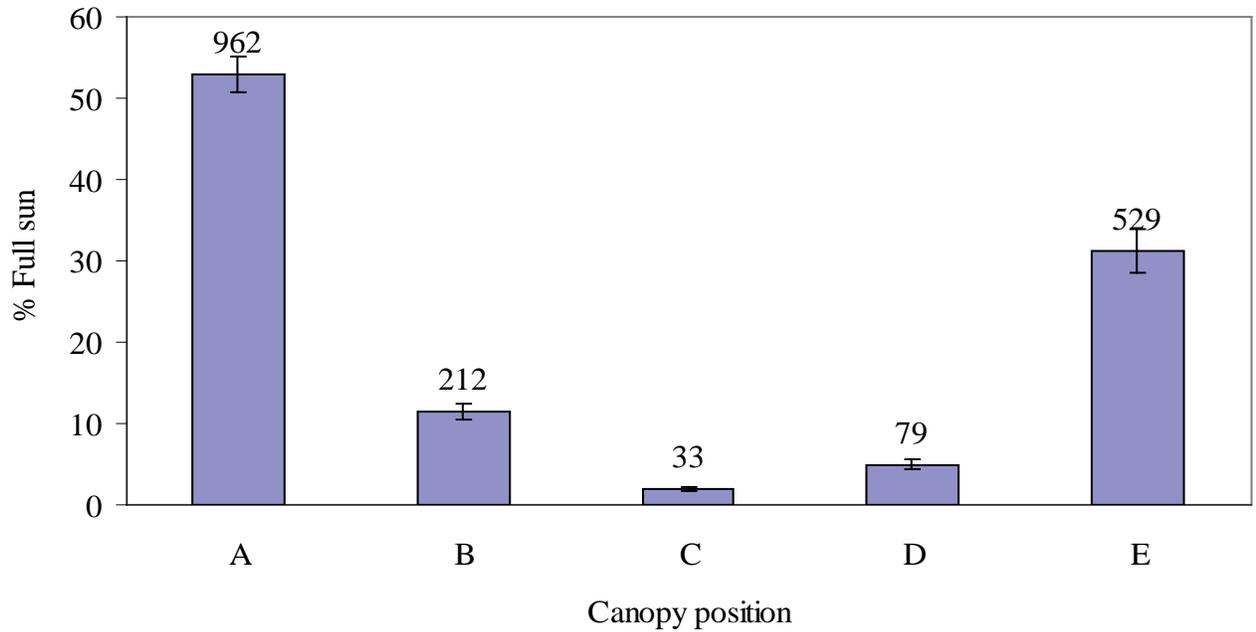


Fig. 4. Percentage of full sun and average light intensity ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) (above columns) received by GS fruit from different canopy positions during the course of an average day during the 2007/2008 season. A and B are the outside and intermediate canopy positions on the northern side of trees. C is the inner position. D and E are the intermediate and outside positions on the southern side of trees. Values are means  $\pm$  SE ( $n = 20$ ).

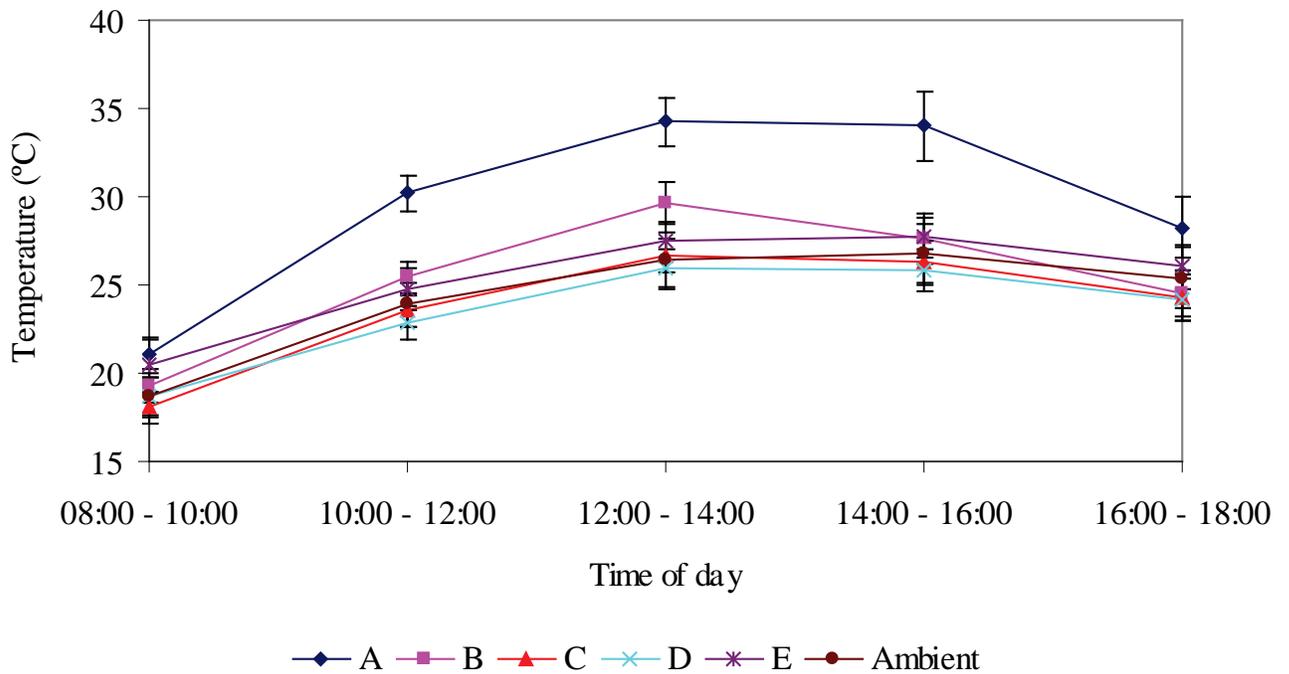


Fig. 5. Average daily peel in GS and ambient temperature during the 2007/2008 season. A and B are the outside and intermediate canopy positions on the northern side of trees. C is the inner position. D and E are the intermediate and outside positions on the southern side of trees. Values are means  $\pm$  SE ( $n = 20$ ).

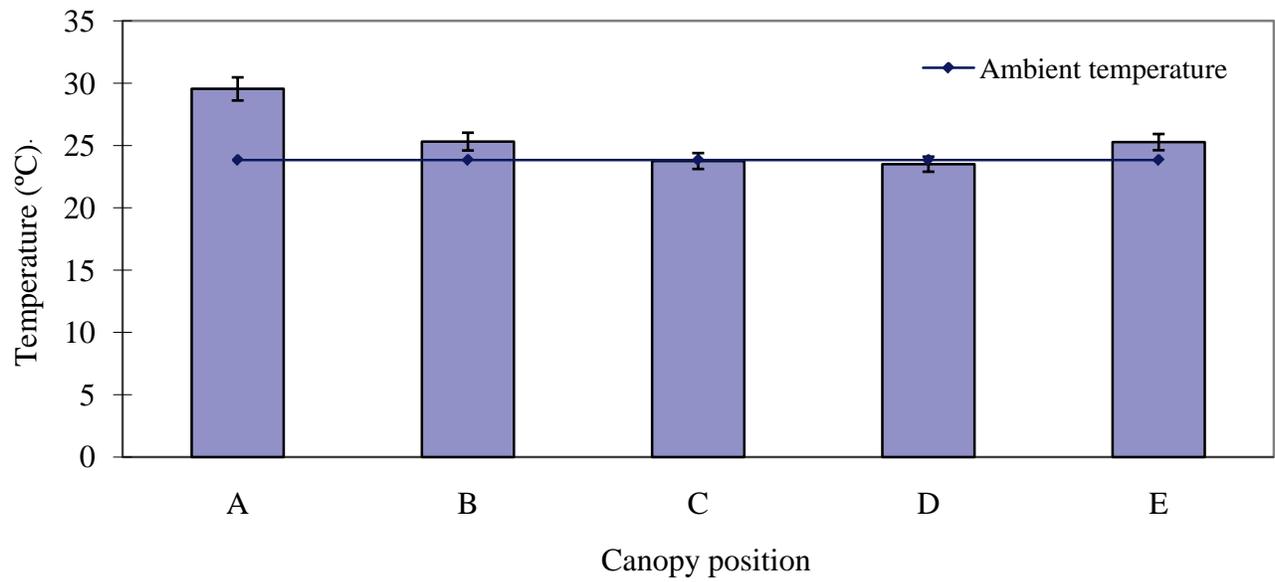


Fig. 6. Ambient temperature and average peel temperature of GS fruit from different canopy positions during the 2007/2008 season. A and B are the outside and intermediate canopy positions on the northern side of trees. C is the inner position. D and E are the intermediate and outside positions on the southern side of trees. Values are means  $\pm$  SE ( $n = 20$ ).

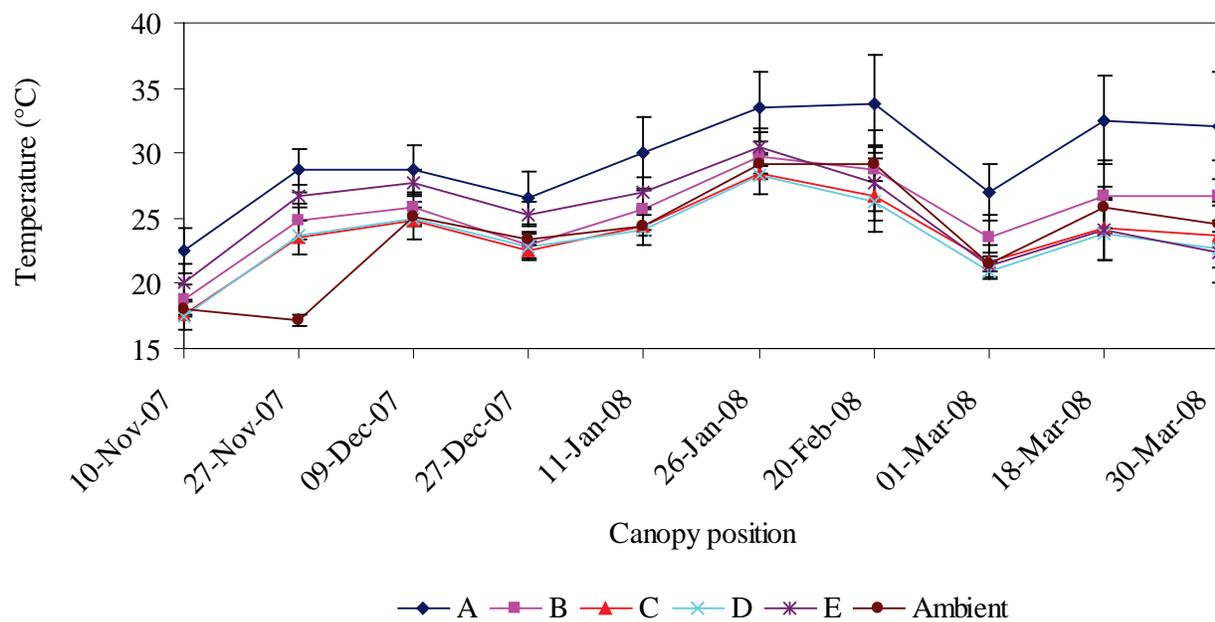


Fig. 7. Ambient temperature and average peel temperature of fruit from different canopy positions during the 2007/2008 season. A and B are the outside and intermediate canopy positions on the northern side of trees. C is the inner position. D and E are the intermediate and outside positions on the southern side of trees. Values are means  $\pm$  SE ( $n = 20$ ).

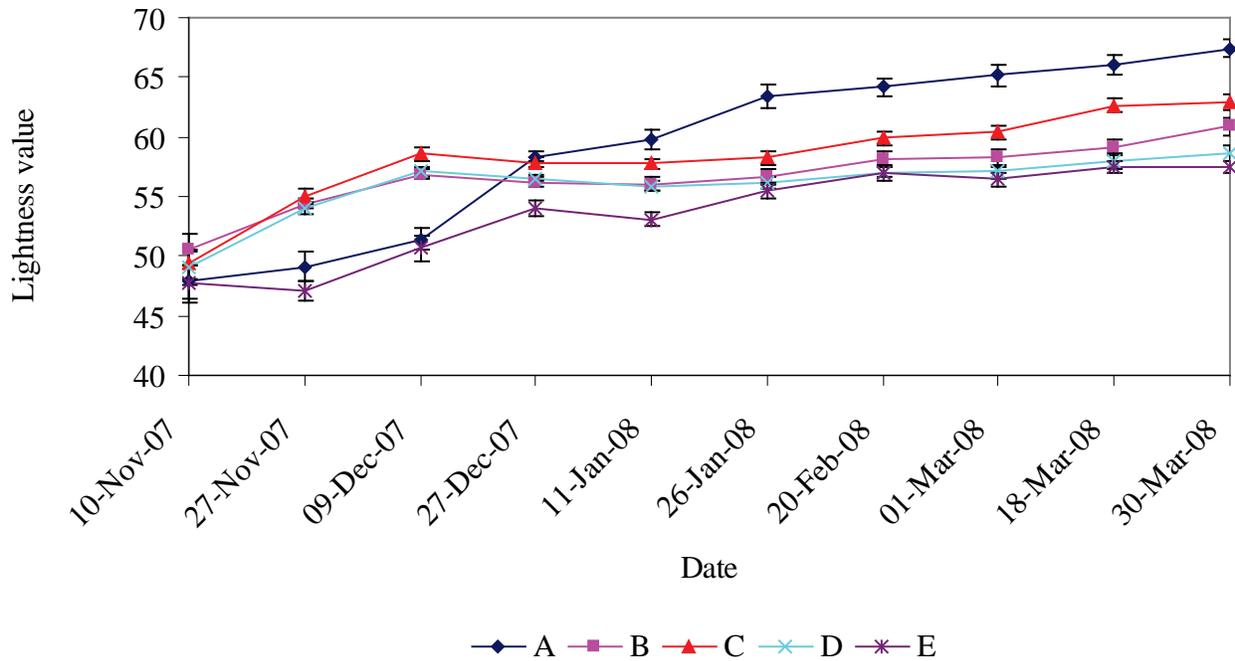


Fig. 8. Lightness of fruit from different canopy positions during the 2007/2008 season. A and B are the outside and intermediate canopy positions on the northern side of trees. C is the inner position. D and E are the intermediate and outside positions on the southern side of trees. Values are means  $\pm$  SE ( $n = 20$ ).

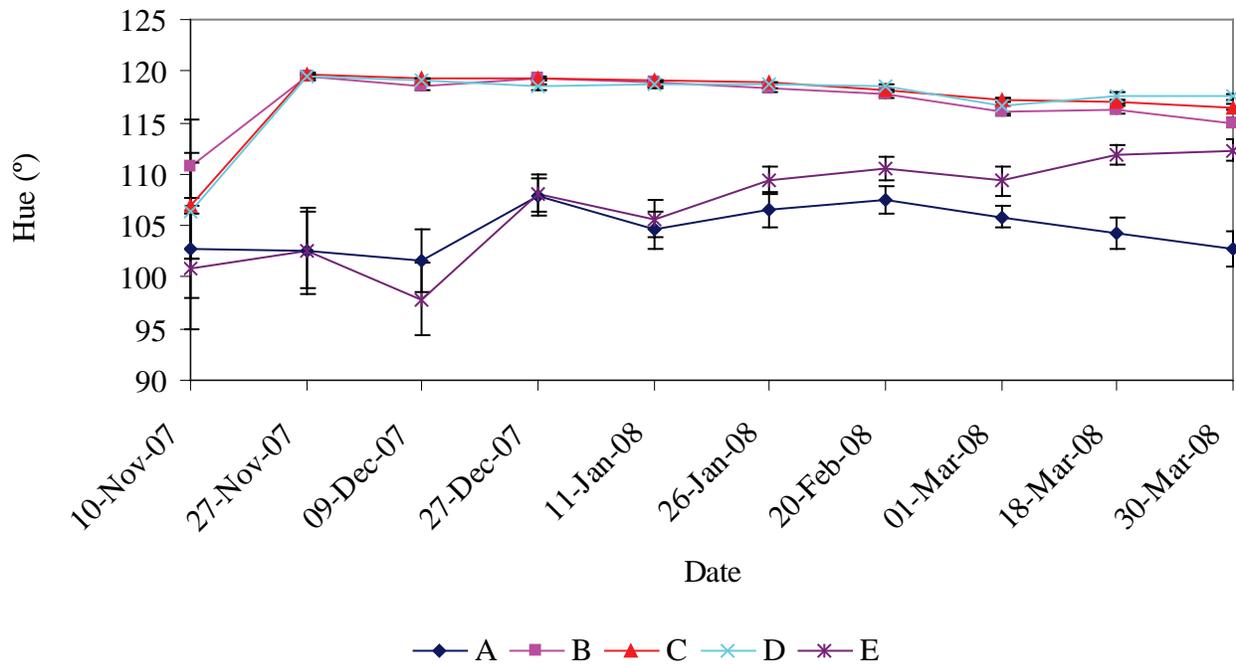


Fig. 9. Hue angle of GS fruit from different canopy positions during the 2007/2008 season. A and B are the outside and intermediate canopy positions on the northern side of trees. C is the inner position. D and E are the intermediate and outside positions on the southern side of trees. Values are means  $\pm$  SE ( $n = 20$ ).

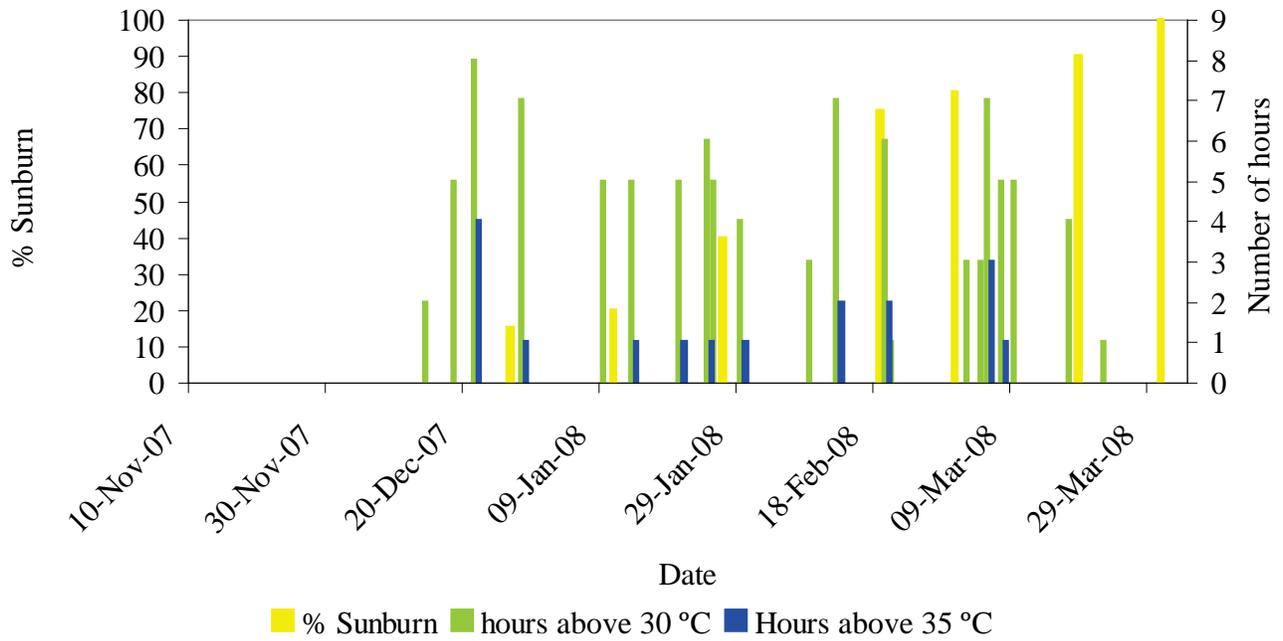


Fig. 10. Sunburn development in GS fruit in relation to the amount of hours above 30 °C and 35 °C, during the course of the 2007/2008 season.

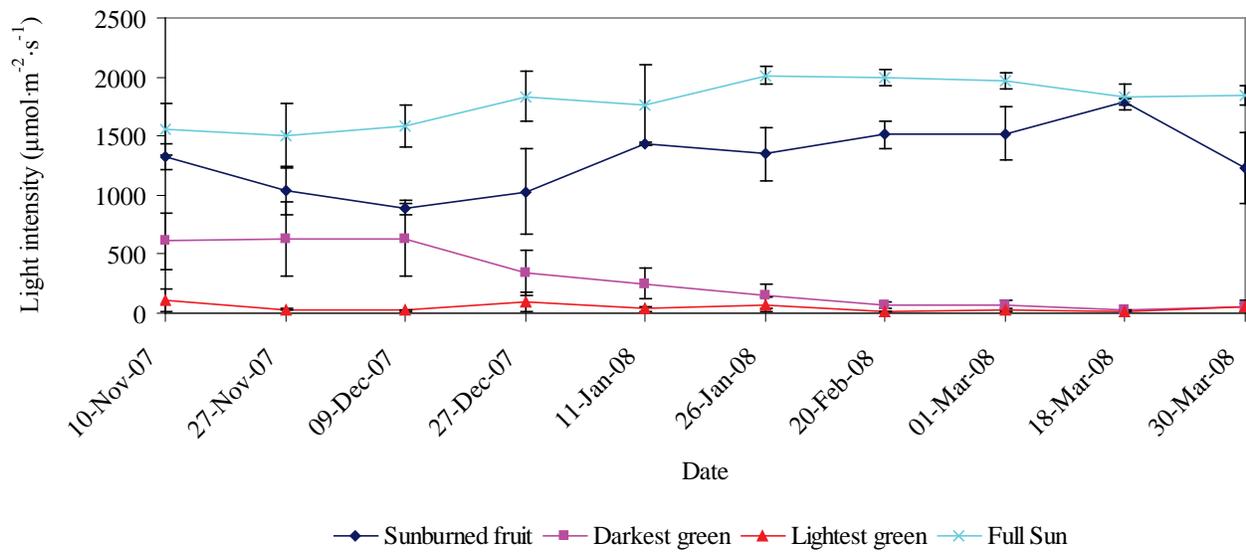


Fig. 11. Light levels received during the 2007/2008 season by the 10% darkest green and 10% lightest green ‘Granny Smith’ fruit at harvest as well as fruit that developed sunburn. Values are means  $\pm$  SE ( $n = 4$ ).

# **THE EFFECT OF PRUNING AND MULCHING PRACTICES ON EXTERNAL QUALITY OF 'GRANNY SMITH' APPLES**

## **Abstract**

The dark green, 'Granny Smith' (GS), is the most widely grown apple cultivar in South Africa. However, production of class one quality GS is hampered by the high incidence of sunburn and red blush on fruit skin caused by the high light intensities and high temperatures of South African summers. There are also increasing reports from export markets of South African GS being too light in colour. Older orchards were typically planted at low density and trees are large with round canopies on seedling rootstocks. The dense, shaded interior canopies of these trees tend to be unproductive with poor fruit colour. Newer orchards are planted at high density to smaller trees with open canopies on clonal rootstocks. Losses to sunburn and red blush are high in these plantings due to the relatively greater surface area of trees exposed to high light. The aim of the research reported here was to evaluate the use of pruning strategies to improve green colour and reduce sunburn and red blush. In an older vigorous orchard with a dense canopy, pruning was done in winter or summer to increase light distribution for green colour development and to induce more growth on the side of the trees that are prone to sunburn and red blush development. In the younger, non-vigorous orchard, pruning and mulching were used to invigorate the canopy to increase shading of fruit and thereby decrease sunburn and red blush. Pruning increased light levels inside the canopy of vigorous trees and this led to improved green colour development without affecting sunburn or red blush. However, yield decreased significantly with severity of pruning. Mulching and pruning did not affect green colour, sunburn or red blush development. While the contrast between the commercial pruning, that served as control, and the pruning treatment was probably not enough to markedly affect growth, it may take more than one season of mulching to attain a beneficial effect on growth. This study reveals that pruning is effective in increasing canopy light distribution thereby improving green colour, but it does not seem to be effective in reducing sunburn and red blush development.

## Introduction

Granny Smith (GS) is the most widely grown apple cultivar in South Africa, making up 24% of the total area planted (Deciduous Fruit Producers Trust, 2008). However, the area planted to GS has decreased by almost 15% during the last 15 years due to competition from more lucrative blushed cultivars (Deciduous Fruit Producers Trust, 2008). There are few new plantings and most of the production (83%) is from old (>15 years) orchards (Deciduous Fruit Producers Trust, 2008). Apart from realizing lower returns, the proportion of class one quality fruit from GS trees is lower compared to other cultivars, primarily due to the cosmetic skin deficiencies, sunburn, red blush and skin whitening. These deficiencies are due to extreme high (sunburn and red blush) and low light exposure (skin whitening) that alters the pigment composition and concentration in the peel (Hirst et al., 1990).

The characteristic dark green colour of GS apple peel is due to high chlorophyll levels. Low chlorophyll synthesis during early fruit development in the low light environment of the inner canopy, or where fruit touch each other or a limb (Hirst, 1990; Paper 1), give rise to pale green fruit. This may not necessarily reduce class one fruit, but less lucrative markets with lower standards for green colour have to be targeted (Griessel, personal communication). Yellow carotenoids are unmasked when chlorophyll is degraded due to high light intensities (Felicetti and Schrader, 2009) while red anthocyanins accumulate when nights are cool (4 °C), days are mild (20 °C) and fruit are exposed to high levels of UV-B and visible light (Reay, 1999). Sunburn necrosis, due to thermal death of epidermal and subepidermal cells, is caused by extreme heat ( $52 \pm 1$  °C), while sunburn browning is a result of high temperatures (46 °C – 49 °C) in the presence of light (Schrader et al., 2008). Photooxidative browning occurs at much lower peel temperatures (<30 °C) in the absence of UV-B radiation when peel is suddenly exposed to high light intensity resulting in photobleaching followed by necrosis. Sunburn and red blush, induced by the high light intensities of South African summers, are the main factors that decrease the percentage class one GS fruit.

In order to minimize the incidence of sunburn and red blush, producers rely on methods that lower the light levels that fruit are exposed to and reduce peel temperatures. Shade netting may lower the incidence of sunburn to 1% and also decrease red blush development (Smit, 2007), making it the most effective technique. The major drawback of shade netting is that it is also the most expensive method (Smit, 2007) and, therefore, not an option that most farmers would consider for the relatively low

income GS. Other techniques to reduce sunburn include evaporative cooling and spray application of particle films. Evaporative cooling entails the wetting of fruit with overhead sprinkles in order to decrease peel temperature (Parchomchuk and Meheriuk, 1996; Unrath and Sneed, 1974). Particle films consisting of white clay minerals, e.g. 'Surround', or natural lipids, e.g. 'Raynox', reflect visible or UV radiation (Glenn et al., 2002). Apart from increased water usage and high installation costs, evaporative cooling may lead to mineral deposits on fruit, over-irrigation, severe sunburn during system malfunctions, and increased pest and disease damage due to higher humidity (Evans, 1993). Evaporative cooling may also increase red blush development (Evans, 1993). Particle film techniques are successful in reducing peel temperatures and reducing sunburn (Glenn et al., 2002; Schupp et al., 2004) and is more affordable than evaporative cooling, but is less effective in reducing the occurrence of sunburn (Gindaba and Wand, 2005).

Many older GS orchards in South Africa were planted at low densities (4.5 x 2.5 m) on vigorous seedling rootstock. Such orchards typically have voluminous, round canopies with a low area to volume ratio and low light transmission into the canopy (Heinicke, 1963; Looney, 1968). Since relatively fewer fruit are carried at exposed positions, the incidence of sunburn and red blush may be lower (Bergh et al., 1980). However, a very dense canopy may also decrease the chlorophyll content and increase the incidence of pale fruit due to excessive shading (Hirst et al., 1990; Paper 1). Jackson (1970) found that the main cropping zone received a minimum of 35% full sun, while the more shaded areas produced relatively few fruits. This led to the rule of thumb that the effective penetration depth of light into unrestricted apple canopies is approximately 1 m (Jackson, 1970). Currently, clonal rootstocks are used exclusively as rootstock when establishing new GS orchards due to the higher yield efficiency attained. These plantings are at higher density with thin, open canopies with a high area to volume ratio and are likely to be more susceptible to sunburn and red blush development. Hence, it seems that both older, vigorous GS orchards with poor light distribution and more modern orchards on semi-dwarfing rootstocks and with high light exposure may produce unsatisfactory fruit quality. The challenge faced is how to reduce sunburn and red blush in the outer canopy without also decreasing fruit production and chlorophyll levels in the inner canopy (Paper 1).

Dormant pruning can be used to decrease excess vigorous growth (Marini and Barden, 1982), but may also be used to induce more growth and thereby increase shading on the side of the tree receiving the most light during the warmest parts of the day (Bergh et al., 1980). It may be possible to decrease the incidence of poor green fruit in dense canopies by improving canopy light distribution during early

fruit development. Light is necessary for chlorophyll synthesis at the beginning of fruit development (Gorski and Creasy, 1977) while shading during the second half of fruit development reduces the occurrence of sunburn and red blush (Paper 1). Fruit increase in susceptibility to sunburn (Li and Cheng, 2008) and ability to form red blush (Saure, 1990) during development. Summer pruning is usually performed during the second half of fruit development to promote red colour development in red cultivars by increasing the light distribution within the canopy (Ystaas, 1992). Increased light exposure of previously shaded GS may increase sunburn and red blush development (Paper 1; Reay & Lancaster 2001; Wünsche et al., 2001). Early summer pruning may prove advantageous in GS because of providing sufficient light at the beginning of fruit development for chlorophyll synthesis (Gorski and Creasy, 1977) and flower initiation (Jackson, 1980) while increasing vegetative regrowth and shading later in the season (Ferree et al., 1984; Miller, 1982) when day temperatures and sunburn risk are higher (Paper 1). Early summer pruning may acclimatize fruit to higher light thereby decreasing the potential for photodegradation later in the season when fruit are more sensitive to high light (Li and Cheng, 2008; Schrader et al., 2008).

An old vigorous GS orchard on seedling rootstock and a younger less vigorous orchard on MM109 rootstock were selected as representative of GS in the Grabouw production region. The more vigorous orchard was pruned in summer in order to improve light distribution and thereby possibly green colour development, as well as in winter in order to decrease the exposure of outer canopy fruit to light and thereby decreasing sunburn and red blush development. Pruning and mulching were used to induce more growth and increase leaf water potential in the less vigorous orchard in order to reduce sunburn. The effect of the mulching and pruning treatments on yield and fruit quality was assessed.

## **Materials and methods**

Trials were conducted in two GS orchards in the Grabouw region (Latitude: 34°8'S; Longitude: 19°3'E) of the Western Cape province of South Africa.

### ***Experiment 1.***

GS on seedling rootstock were planted in 1983 in an E–W row direction at a spacing of 4.25 x 2.25 m and trained to the central leader form. Tree height, row width and canopy width was 3 m, 3.5 m, and

2.5 m, respectively. The orchard yielded 58 ton·ha<sup>-1</sup> in 2007. After orchard cull of 50%, 75% of the remaining fruit were class one with sunburn (12%) and red blush (5%) as the main quality defects.

*Trial layout.* Trees were selected for uniformity in vigor and subjected to four pruning treatments. Control trees (Fig. 1a) were lightly pruned, removing only vigorous upright one-year old shoots “water shoots” on 28 August 2007. Winter (17 August 2007) and summer (6 November 2007) (Fig. 1b) pruning treatments entailed the removal of water shoots and heavy scaffold branches (Fig. 2a) competing with the central leader, simplifying branches, heading one-year-old shoots in the outer canopy and removing all growth on scaffold branches within 40 cm of the main trunk, opening up a “light chimney”) (Fig. 2b). For trees of the fourth treatment, winter pruning was followed up by opening up the light chimney in summer (6 November 2007). Treatments were randomized in 9 blocks with 3 trees per plot.

*Fruit quality at harvest.* Fruit harvested from each tree on the commercial harvest dates of 11 and 14 April 2008 ( $\pm$  180 DAFB) were weighed and 12 fruit from both sides of the tree were sampled randomly. The remaining fruit were pooled per treatment for grading at the Two-a-Day pack house in Grabouw to assess whether sampling was representative. Samples were assessed for green colour intensity on the greenest side of fruit using a colour chart (Set A 38, Deciduous Fruit Board, South Africa), the occurrence of red blush and sunburn, and chlorophyll concentration of the peel. Hue angle (H°) and lightness (L) values of both the greenest and least green sides of the fruit were determined with a Minolta chromameter (Model CR-400, Minolta Co. Ltd., Tokyo, Japan). A 7-cm long and 2-cm wide lint of skin was peeled from both the greenest and least green sides of fruit. The flesh was scraped off the peel using a teaspoon where after the peel was frozen in liquid nitrogen, freeze dried, milled and kept at -40 °C until pigment analysis.

*Pigment analysis.* Chlorophylls and carotenoids were extracted from 0.1 g freeze-dried material with 4 ml 100% acetone and constantly stirred for 24 h at 4 °C. After centrifugation for 15 min at 10000 x g, the supernatant was decanted and the pellet re-extracted with 4 ml 100% acetone and centrifuged as described above. The supernatant was added to the first. After filtration through 0.45 µm filters (Millex-HV, Millipore Corporation, Milford, MA, USA), pigments were quantified by spectrophotometry (Cary 50 Bio, Varian Australia (PTY) Ltd., Melbourne, Australia), measuring

absorbance at 470, 645 and 670 nm. The extinction coefficients of Lichtenthaler (1987) were used to calculate chlorophyll and carotenoid concentrations, which were then expressed in  $\mu\text{g}^{-1}$  dry weight of peel.

*Light distribution.* Canopy light levels were measured on 12 December 2007 using a ceptometer (AccuPAR LP-80, Decagon Devices, Inc., Pullman, WA, USA) with a probe length of 86.5 cm and eight light sensors spread evenly along the probe. Measurements were taken approximately 1.5 m from the ground, five times during the day in two-hour intervals from 0800 HR to 1800 HR.

*Statistical analysis.* Data were analysed using the General Linear Models (GLM) procedure of SAS Enterprise Guide 3.0 (SAS Institute Inc., 2004, Cary, NC, USA). Trunk circumference measured on 24 October 2007 was used as covariate for analysis of fruit quality results and treatment means adjusted where applicable.

## ***Experiment 2.***

GS trees on MM109 rootstock were planted in 1998 at a spacing of 4.0 x 1.5 m and a NE–SW row direction and trained to the central leader form. Tree height, row width and canopy width is 3 m, 1 m and 1.5 m, respectively. The orchard yielded 54  $\text{ton}\cdot\text{ha}^{-1}$  in 2007. After orchard cull of 65%, 73% of the remaining fruit were class one with sunburn (9%) and red blush (3%) as the main quality deficiencies.

*Trial layout.* Trees were selected for uniformity in vigor. Control trees were pruned on 28 August 2007 according to commercial practices, which entailed the removal of water shoots and heavy scaffold branches competing with the central leader, simplifying branches, heading one-year-old shoots in the outer canopy and removing all growth on scaffold branches within 20 cm of the central leader (opening up the chimney). The light pruning treatment consisted of removal of waters shoots, simplifying branches, heading of all one-year-old shoots and opening of the chimney to 20 cm from the central leader. The mulching treatment entailed pruning as for the control treatment and the application of 10 L compost around the tree, covering an area of approximately 0.3  $\text{m}^2$  on each side of the tree. Soil around the trees was loosened with a shovel before the application of the compost. Straw was placed over the compost, creating a layer approximately 15 cm thick. Straw mulch was applied 18

September 2007 and again on 29 September and 1 November 2007. Light winter pruning and mulching were combined in a fourth treatment. Treatments were randomized in 9 blocks with 3 trees per plot.

*Fruit quality at harvest and pigment analysis.* Fruit were harvested on 9 and 10 April 2008 and quality and pigments assessed as in experiment one.

*Leaf water potential.* Water potential of trees was measured on 28 and 29 March 2008 at 1300 HR using a pressure bomb (Model 670, PMS Instrument Co., Albany, OR, USA). One leaf were cut per tree and immediately secured in the pressure bomb. Pressure was increased and the measurement was taken when the first bubbles appeared from the cut end of the leaf.

*Fruit peel temperature.* Fruit peel temperature of three fully exposed fruit per tree on the western side of the row was measured using a high performance infrared thermometer (Rayner MX4, Raytek Corporation, Santa Cruz, CA, USA) between 1200 HR and 1400 HR on 3 March 2008, which was a cloudless day with max temp of 33°C.

*Statistical analysis.* Data were analysed using the General Linear Models (GLM) procedure of SAS Enterprise Guide 3.0 (SAS Institute Inc., 2004, Cary, NC, USA). Trunk circumference measured on 24 October 2007 was used as covariate for fruit quality results. Treatment means were adjusted where the covariate was significant ( $P < 0.05$ ).

## **Results**

### *Experiment 1.*

Canopy light levels decreased with increasing canopy depth (Fig. 3). In general, pruning treatments increased light levels within the canopy, especially on the northern side of trees. Summer pruning was the most effective treatment increasing light levels at all canopy positions on the northern side of trees compared to other treatments. All the pruning treatments increased light levels 90 cm from the trunk on the southern side of trees compared to the control. Winter pruning with follow-up significantly increased light levels 180 cm and 90 cm from the trunk on the northern side of trees compared to

winter pruning alone (Fig. 3).

All three pruning treatments improved fruit green colour and chlorophyll concentrations on the northern side of trees compared to the control, but were generally less effective or ineffective on the southern sides of trees (Table 1, 2 & 3). According to colour chart and averaged over the tree, summer pruning increased fruit green colour compared to the control and winter pruning while winter pruning with follow-up in summer also improved green colour compared to the control (Table 1). Fruit from pruning treatments had higher chlorophyll concentrations and were greener (lower hue angle) as well as darker in colour (lower L value) compared to control fruit on their best sides on the northern sides of trees. The worst green sides of northern borne fruit of pruning treatments were also significantly greener and, except for winter pruning, also had significantly higher chlorophyll concentrations compared to the control treatment (Table 2 & 3). The best colored sides of northern fruit of summer pruned trees were darker in green colour compared to fruit from other treatments. Summer pruning also increased the darkness and greenness of the worst sides of fruit on both sides of the tree compared to other treatments (Table 2). Summer pruning increased chlorophyll concentrations of the worst sides of fruit compared to other treatments except for winter pruning with follow up in the northern canopy (Table 3). Winter pruning with follow-up increased chlorophyll concentrations of the worst sides of fruit in the northern canopy compared to the control (Table 3). In general, chlorophyll concentrations were higher in northern canopy fruit in pruning treatment compared to control trees, while only summer pruning increased chlorophyll concentrations in southern canopy fruit (Table 3). The better fruit colour on the southern compared to the northern sides of trees is apparent. Fruit from the summer pruning treatment differed less in colour and chlorophyll concentrations between their best and worst sides compared to fruit of other treatments (Table 2 and 3).

No significant differences were found between treatments in the incidence of red blush, sunburn and the percentage fruit without any of these blemishes (Table 4). The northern sides of trees showed a higher incidence of red blush and sunburn than the southern sides of trees. Pruning did seem to slightly increase the occurrence of red blush on the southern sides of trees thereby decreasing the percentage fruit without blemishes, but the difference was not statistically significant (Table 4). Sample grader results concurred with the sampling on the absence of a treatment effect on the incidence of sunburn and red blush (Table 5). Many fruit developed both sunburn and a red blush. These fruit were first scored for sunburn during sample grading resulting in a lower value for red blush compared to data recorded on samples when the same fruit would be scored for both sunburn and red blush. The

percentage fruit with insufficient green colour was very low (<1.5 %) for all treatments (Table 5).

The proportion class one, two and three fruit were comparable between treatments (Table 5). Control trees had the highest yield of class one fruit per hectare due to the considerable reduction in yield per tree induced by the pruning treatments. In addition, summer pruning decreased yield compared to winter pruning with or without follow-up light management in summer.

### *Experiment 2.*

The more severe control pruning treatment did tend to increase the green colour of fruit on the western sides of rows compared to light pruning treatments (Table 6, colorimeter data not presented). The effect was only significant for the best coloured sides of fruit.

Light pruning decreased the percentage sunburn on the eastern side of trees and also decreased chlorophyll concentrations of the best coloured sides of fruit on the western sides of rows compared to the control treatment (Table 7 and 8). Light pruning also seemed to increase the percentage red blush and sunburn on the western sides of rows, but the effect was not significant ( $p=0.0998$  and  $p=0.1232$  for red blush and sunburn, respectively). There were no differences between the two sides of the row (Table 8). Summed over the entire tree, there was no significant difference in sunburn percentages and in the percentage fruit without sunburn and red blush (Table 8).

Light pruning increased yield to  $36 \text{ ton}\cdot\text{ha}^{-1}$  compared to the control ( $22 \text{ ton ha}^{-1}$ ) (Table 9). Hence, although treatments did not differ with regard to fruit quality (Table 9), light pruning increased the yield of class one fruit. There were no differences between treatments in leaf water potential and peel temperature (data not presented).

## **Discussion**

Different pruning techniques were performed to improve light distribution in the dense interior canopy of a vigorous GS orchard in order to improve the green colour of fruit. This orchard was planted in 1985 on seedling rootstock, and is representative of most of the older GS orchards in South Africa. Most GS orchards (83%) in South Africa are older than 15 years and 56% of the total orchards are older than 25 years (Deciduous Fruit Producers Trust, 2008). All the pruning treatments, i.e., severe

winter pruning with or without follow-up light management in summer, and severe summer pruning, improved green colour of GS apples compared to the dense control canopy. The improvement in colour was due to increased light penetration (measured in March) into the canopy, particularly into the northern canopy during early fruit development when most chlorophyll is synthesized (Gorski and Creasy, 1977). Another reason for creating canopies with better light distribution is to decrease the considerable colour difference between the best and worst coloured sides of GS fruit (Paper 1). This difference in colour is undesirable because of the colour variation that it may bring about in class one cartons. Only the severe summer pruning treatment, which had the biggest effect on light levels, were to some extent effective in increasing the evenness of colour development on the best and worst coloured sides of the fruit.

As a possible negative effect of a higher light environment, the incidence of red blush and sunburn was expected to increase. However, this was not the case, possibly due to increased regrowth after winter and relatively early summer pruning, which provided shade during the latter part of the season (Ferree et al., 1984; Miller, 1982). Heading of shoots may reduce yield (Kim et al., 2000; Marini et al., 1993), which may have contributed to the decrease in crop load that was observed. The lower fruit load and shortening of shoots could potentially decrease the bending of limbs under fruit weight, thereby decreasing sunburn and counteracting the effect of increased light. Exposure of previously shaded fruit to high light intensities strongly induces sunburn (Li and Cheng, 2008; Schrader et al., 2008). Bergh et al. (1980) and Hirst et al (1990) also caution against over cropping to avoid the bending of branches and exposure of shaded fruit to high light. The more exposed light environment brought about by rigorous pruning may also reduce the chances of fruit being suddenly exposed to high levels of light, thereby reducing sunburn (Schrader et al., 2008). In comparing GS canopies with different light environments, Warrington et al. (1996) found that sunburn in New Zealand was not necessarily higher in canopies with higher light transmission and that sunburn predominantly occurred in the northern upper region of the different canopies where light transmission was the highest. The occurrence of red blush also did not increase linearly with an increase in light transmission. A threshold of over 40% light transmission was needed to stimulate red blush development.

Yield decreased considerably with pruning severity. The effect on yield can be attributed to the removal of scaffold branches and heading of all one-year-old shoots in the outer canopy. Summer pruning was particularly harsh as indicated by the increased canopy light levels compared to other treatments. It is possible that the beneficial effect of pruning treatments on fruit colour could have been

attained without the removal of scaffold branches, which was primarily done to redress shortcomings in tree structure. In hindsight, scaffolds should have been retained for a proper comparison of the effect of light management pruning treatments on fruit quality. A high proportion of GS fruit from the high light environment of the outer canopy is lost to either sunburn or red blush development. Hence, all one-year-old shoots in the outer canopy were headed to stimulate new shoot growth and increase shading of fruit borne at these exposed positions. As can be seen from Fig. 3, heading apparently did not increase growth and shading in the outer canopy. It does not seem possible to increase light distribution in the inner canopy whilst at the same time increasing shade in the outer canopy.

Newer GS plantings are typically at higher density with small, thin canopies with a low volume to area ratio. Previous data (Paper 1) suggest that these orchards should have a higher incidence of sunburn and red blush due to the higher proportion of fruit exposed to high light levels. Growth-stimulating heading cuts and very little shoot thinning were used to increase shading in an N-S planted, 11-year-old orchard on MM109 rootstock, representative of newer GS plantings in South Africa. Thinning cuts are useful in ensuring increased yield and improved fruit colour, due to improved canopy light incidence (Kim et al., 2000; Stiles, 1984). Heading cuts stimulate growth on the remaining part of the shoot, which may lead to an increase in extension shoot growth (Kim et al., 2000) and is usually used for invigorating old or weak branches (Stiles, 1984). However, heading cuts were also made as part of the control treatment, thereby decreasing the contrast between the treatments. This is probably the reason for the absence of pruning treatment effect on green colour, sunburn or red blush. However, the less rigorous shoot thinning did increase yield by nearly 30%.

Mulching was used with the aim to improve the root and water uptake environment by potentially increasing microbial activity, soil structure, soil moisture, reducing weeds and providing additional nutrition (Bubán et al., 1996; Mathews et al., 2002; Pinamonti, 1998; Schultz and Matthews, 1988; Smith et al., 2000). These improvements may stimulate tree growth, providing shade for fruit in a relatively small canopy. Mulching may also increase leaf water potentials (Pinamonti, 1998) because of higher soil moisture, thereby purportedly reducing peel temperature and sunburn (Schrader, 2003). In a newly established 'Delia' apple orchard, a variety of mulches (including straw, pine bark, polypropylene and manure) increased average shoot length and shoot number by 12-22% and 6-22%, respectively (Bubán et al., 1996; Mathews et al., 2002). The increase in shoot growth was accompanied by an increase in trunk circumference. Mulching may also potentially improve the soil moisture and mineral status, which may increase leaf nitrogen levels and leaf water potential (Smith et al., 2000).

Green colour in apple is positively correlated with leaf nitrogen levels (Rease and Williams, 1974; Williams and Billingsley, 1974). According to our results, mulching did not affect chlorophyll levels, leaf water potential and the incidence of sunburn. However, mulching may only become effective in improving tree growth after a number of seasons.

Mulching newly established ‘Giles’ pecan and ‘Gala’ apple orchards only increased tree growth (as measured as an increase in trunk cross-sectional area) during the third year of application (Autio et al., 1991; Smith et al., 2000). Straw mulch did not increase TCSA during the first year of planting in ‘Empire’ and ‘Jonagold’ apples, but became effective during later seasons (Merwin and Stiles, 1994). Leaf nitrogen levels positively correlated with mulching, but not yet after the first year of application in ‘Giles’ pecan orchards (Smith et al., 2000). Increasing soil moisture may not necessarily decrease the occurrence of sunburn as partial rootzone drying experiments have revealed (Caspari et al., 2004).

In conclusion, it appears that pruning, especially during summer, can be used to improve light distribution and, thereby, green colour in vigorous, low-density GS orchards without markedly increasing the incidence of sunburn and red blush. However, it seems less possible to simultaneously increase outer canopy shading, and thereby decrease the incidence of sunburn and red blush. We suggest that shade netting should be considered to reduce sunburn and red blush in GS. However, GS is not a very lucrative cultivar and further research is necessary to ascertain whether expensive infrastructure such as shade netting will be cost effective, especially in the older low-density orchards that make up the majority of GS plantings in South Africa. The mulching treatment most likely was of too short duration to affect tree growth and thereby the incidence of sunburn. It may form part of an integrated strategy to manage tree vigour and decrease sunburn.

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Table 1. Effect of different pruning strategies on colour chart values (1–12 where 1 = green; 12 = yellow) and yield of ‘Granny Smith’ apples sampled from both sides of a vigorous orchard with an E–W row direction during the 2007/2008 season. Means, adjusted for trunk circumference where applicable, were separated by LSD (5%).

Treatment	Green colour (North)	Green colour (South)	Avg green colour
Control	3.1 a <sup>z</sup>	2.7 <sup>ns</sup>	2.9 a
Winter	2.8 b	2.6	2.7 ab
Winter with follow-up	2.7 b	2.5	2.6 bc
Summer	2.6 b	2.3	2.4 c
Pr>F			
Trunk circum.	-	0.0139	0.0104
Treatment	0.0001	0.0608	0.0036

<sup>z</sup> Treatments with different letters differ significantly at P<0.05.

<sup>ns</sup> Not significant.

Table 2. Lightness values (L) and hue angles of the best and worst coloured sides of ‘Granny Smith’ apples sampled from both sides of a vigorous orchard with an E–W row direction during the 2007/2008 season. Means were separated by LSD (5%).

Treatment	Best side				Worst side			
	North		South		North		South	
	L value	Hue (°)	L value	Hue (°)	L value	Hue (°)	L value	Hue (°)
Control	62.61 a <sup>z</sup>	115.73 b	60.34 a	116.38 <sup>ns</sup>	66.18 a	114.82 c	65.14 a	115.24 b
Winter	61.09 b	116.22 a	60.18 a	116.31	64.95 b	115.25 b	64.78 a	115.27 b
Winter with follow-up	61.18 b	116.32 a	59.94 a	116.40	64.50 b	115.22 b	64.82 a	115.32 b
Summer	59.62 c	116.44 a	58.57 b	116.63	62.11 c	115.57 a	61.70 b	115.83 a
Pr>F	<0.0001	0.0003	0.0017	0.1933	<0.0001	0.0001	<0.0001	0.0002

<sup>z</sup>Treatments with different letters differ significantly at P<0.05.

<sup>ns</sup>Not significant.

Table 3. Effect of pruning on chlorophyll concentration in the best and worst coloured sides of ‘Granny Smith’ apples sampled from both sides of a vigorous orchard with an E–W row direction during the 2007/2008 season. Means were separated by LSD (5%).

Treatment	Chlorophyll concn ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)					
	North			South		
	Best	Poor	Total	Best	Poor	Total
Control	370 b <sup>z</sup>	351 c	360 b	478 <sup>ns</sup>	370 c	424 b
Winter	424 a	373 bc	399 a	458	397 bc	427 b
Winter with follow-up	415 a	385 ab	400 a	487	398 b	443 b
Summer	434 a	407 a	421 a	491	464 a	478 a
Pr>F	0.0056	0.0095	0.0021	0.1211	0.0095	0.0004

<sup>z</sup>Treatments with different letters differ significantly at  $P<0.05$ .

<sup>ns</sup>Not significant.

Table 4. Effect of pruning treatments on the incidence of sunburn and red blush, and the percentage unblemished fruit in ‘Granny Smith’ apples sampled from a both sides of a vigorous orchard with an E–W row direction during the 2007/2008 season. There were no significant differences between means at LSD (5%).

Treatment	Sunburn % <sup>z</sup>			Red blush % <sup>z</sup>			Unblemished fruit % <sup>y</sup>		
	North	South	Total	North	South	Total	North	South	Total
Control	21	9	15	54	42	48	40	55	47
Winter	19	11	15	54	50	52	41	48	44
Winter with follow-up	17	12	14	53	49	51	42	49	45
Summer	23	10	16	57	52	54	40	46	42
Pr>F	0.3563	0.8616	0.8402	0.8609	0.3492	0.5443	0.9521	0.4190	0.5186

<sup>z</sup> the same fruit may have sunburn and red blush

<sup>y</sup> without any sunburn and/or red blush

Table 5. Effect of pruning treatments on yield, packout distribution (with ton·ha<sup>-1</sup> in brackets) and cull of ‘Granny Smith’ apples from a vigorous orchard with an E–W row direction during the 2007/2008 season based on sample grader data. Treatment means for yield were separated by LSD (5%).

Treatment	Yield (ton·ha <sup>-1</sup> )	Class1 %	Class2 %	Class3 %	Pink blush %	Poor green %	Sunburn %
Control	73.6 a <sup>z</sup>	75 (55)	2 (2)	23 (17)	4.4	1.0	20
Winter	44.3 b	74 (33)	2 (1)	24 (11)	3.4	0.2	22
Winter with follow-up	45.4 b	75 (44)	2 (1)	23 (11)	4.7	1.5	19
Summer	27.7 c	78 (22)	1 (0.4)	21 (6)	4.2	1.0	17
Pr>F	<0.0001						

<sup>z</sup>Treatments with different letters differ significantly at P<0.05.

Table 6. Effect of different pruning strategies on colour chart values (1–12 where 1 = green; 12 = yellow) of ‘Granny Smith’ apples sampled from both sides of a high density orchard with a NE–SW row direction the 2007/2008 season. There were no significant differences between means at LSD (5%).

Treatment	Green colour (East)	Green colour (West)	Avg Green colour
Control	2.6	2.5	2.6
Light winter pruning	2.8	2.8	2.8
Mulch	2.9	2.9	2.9
Light winter pruning and mulch	2.9	2.9	2.9
Pr>F	0.2318	0.0707	0.0821

Table 7. Effect of pruning on chlorophyll concentration from the best and worst coloured sides ‘Granny Smith’ apples sampled from both sides of a high density orchard with a NE–SW row direction during the 2007/2008 season. Means were separated by LSD (5%).

Treatment	Chlorophyll concn ( $\mu\text{g}\cdot\text{g}^{-1}$ dry weight)					
	Best side			Worst side		
	East	West	Total	East	West	Total
Control	460 <sup>ns</sup>	483 a <sup>z</sup>	472 <sup>ns</sup>	389 <sup>ns</sup>	397 <sup>ns</sup>	393 <sup>ns</sup>
Light winter pruning	448	438 b	443	357	383	370
Mulch	443	456 ab	448	362	378	369
Light winter pruning and mulch	450	426 b	439	383	362	372
Pr>F	0.8724	0.0472	0.2306	0.5207	0.4263	0.5710

<sup>z</sup> Treatments with different letters differ significantly at  $P < 0.05$ .

<sup>ns</sup> Not significant.

Table 8. Effect of pruning treatments on the incidence of sunburn and red blush, and on the percentage fruit without these blemishes in 'Granny Smith' sampled from both sides of a high density orchard with a NE–SW row direction during the 2007/2008 season. Means were separated by LSD (5%).

Treatment	Sunburn % <sup>z</sup>			Red blush % <sup>z</sup>			Unblemished fruit % <sup>y</sup>		
	East	West	Total	East	West	Total	East	West	Total
Control	30 a <sup>x</sup>	23 <sup>ns</sup>	27 <sup>ns</sup>	49 <sup>ns</sup>	44 <sup>ns</sup>	46 <sup>ns</sup>	43 <sup>ns</sup>	48 <sup>ns</sup>	46 <sup>ns</sup>
Light winter pruning	21 b	31	26	43	53	48	49	39	44
Mulch	24 ab	28	26	42	42	42	54	52	53
Light winter pruning and mulch	20 b	33	27	41	54	48	53	38	47
Pr>F	0.0175	0.1232	0.9876	0.3406	0.0998	0.3915	0.1063	0.0816	0.0840

<sup>z</sup> the same fruit may have sunburn and red blush

<sup>y</sup> without any sunburn and/or red blush

<sup>x</sup> Treatments with different letters differ significantly at P<0.05.

<sup>ns</sup> Not significant.

Table 9. Effect of pruning treatments on yield, packout distribution (with ton·ha<sup>-1</sup> in brackets) and cull of ‘Granny Smith’ apples from a less vigorous orchard with a NE–SW row direction during the 2007/2008 season based on sample grader data. Treatment means for yield were separated by LSD (5%).

Treatment	Yield (ton·ha <sup>-1</sup> )	Class1 %	Class2 %	Class3 %	Red blush %	Poor green %	Sunburn %
Control	22 b <sup>z</sup>	68 (14)	2 (1)	31 (8)	4.3	0.9	27
Light winter pruning	36 a	65 (26)	2 (1)	34 (9)	4.0	1.3	30
Mulch	31 ab	62 (19)	3 (1)	35 (11)	4.4	2.7	31
Light winter pruning and mulch	26 ab	71 (18)	2 (1)	27 (7)	5.1	1.7	22
Pr>F	0.0452						

<sup>z</sup>Treatments with different letters differ significantly at P<0.05.



a)



b)

Fig. 1. a) Control pruning treatment and b) summer pruning treatment practiced in a vigorous 'Granny Smith' orchard on seedling rootstock planted, in 1983 at a spacing of 4.25 x 2.25 m.

a)



b)



Fig. 2. a) Removal of heavy scaffold branches competing with the central leader and b) removal through thinning cuts on scaffold branches within 40 cm of the main trunk (aka chimney clearance) in a vigorous ‘Granny Smith’ orchard on seedling rootstock planted, in 1983 at a spacing of 4.25 x 2.25 m.

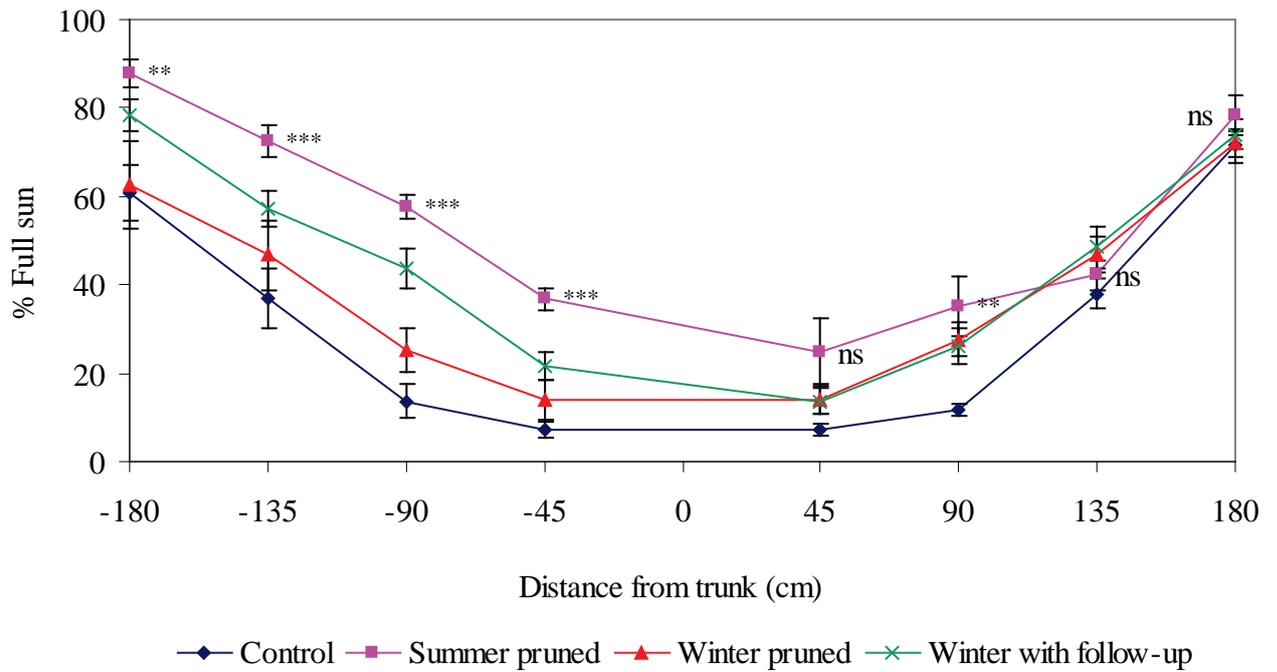


Fig. 3. Effect of different pruning strategies on percentage full sun penetration from the outer canopy on the northern sides of trees (-180 cm) to the outer canopy on the southern side of trees (180 cm), 1.5 m from the ground in a vigorous orchard with an E–W row direction on 12 December 2007. Means adjusted are separated by LSD (5%). ns = non-significant \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

# EFFECT OF CROP LOAD ON 'CRIPPS' PINK' FRUIT QUALITY AND PRODUCTIVITY OVER TWO SEASONS

## Abstract

Crop load has a significant effect on fruit quality and future productivity of apple orchards. The optimum fruit load balances yield and quality to achieve the highest possible profit over the life span of the orchard. The objective of this research was to determine the effect of crop load on 'Cripps' Pink' fruit quality and long-term productivity. Ten-year-old 'Cripps' Pink' apple trees on M793 rootstock were hand thinned in the 2006/2007 and 2007/2008 seasons to establish five crop levels. The control treatment was thinned to either 2 to 3 or 1 to 2 fruits per cluster, according to the commercial thinning practice for the respective seasons. Treatments two to five entailed further thinning to one fruit per cluster in the lower half (< 1.8 m) of the tree (2), one fruit per cluster throughout the tree (3), single fruit and further selective removal of small fruit (4), and thinning to single fruit, with selective removal of small fruit and fruit from the inner canopy (5). Fruit were sampled at each of three commercial harvests to assess external and internal fruit quality, as well as the fruit storability (2007/2008 only) after three months of cold storage at -0.5 °C and one week of shelf life at 15 °C. Vegetative and reproductive development of the trees was also assessed. The different levels of thinning gave rise to yields ranging from 99 to 138 ton·ha<sup>-1</sup> in 2006/2007 and 97 to 136 ton·ha<sup>-1</sup> in 2007/2008. Average fruit diameter, mass and titratable acidity (TA) increased linearly with fruit per trunk cross-sectional area (TCSA). Fruit firmness and total soluble solids (TSS) increased with decreasing yield efficiency, but the effect was small and not of commercial significance. The highest thinning severity advanced the average harvest date of fruit, but this effect was apparently due to increased blush development and not to advanced fruit maturity. The improvement of red colour was due to the selective thinning of fruit from the shaded interior canopy and did not seem to relate to fruit number. The incidence of sunburn was not affected by thinning level. There were no significant differences in vegetative and reproductive development or in the storability of fruit. Although the percentage class one fruit increased with increased thinning (significant only in 2007/2008 when fruit size was generally small and colour development poor), more tons of class one fruit was obtained per hectare at lower thinning levels. Hence, the increase in fruit quality at lower crop load and in response to

**selective removal of small and interior canopy fruit did not compensate for the higher class one yield at the higher crop level. Since the extremely high yields attained at the highest crop load did not induce alternation and also did not have an adverse effect on tree vigour, it would appear that producers should aim to achieve maximum yields at the minimum permissible class one fruit quality. However, the outcome of this study may be different for cultivars that are more prone to alternate bearing, at stricter quality standards or in orchards with a low (<50 ton·ha<sup>-1</sup>) maximum yield potential.**

## **Introduction**

Cripps' Pink (CP) is the second most grown blushed apple cultivar in South Africa after Royal Gala, and makes up 7% of the total area planted (Deciduous Fruit Producers Trust, 2008). CP is a high value product due to its novel and attractive pink-red blush that develops towards harvest, especially on the sun-exposed side of fruit. However, to be marketed under the 'Pink Lady' (PL) brand name and thereby realize the high incomes, more than 40% of the fruit surface needs to be covered by a blush with adequate intensity. If the blush covers less area and is less intense, fruit may only be marketed as CP. PL apples were worth approximately 70% more per ton in 2008 than CP (South African 'Pink Lady' Association, 2009). Red and pink colour in apples is due to the synthesis of anthocyanins induced by high light intensity (Arakawa et al., 1985), in combination with low night temperatures and moderate day temperatures (Curry, 1997; Reay, 1999). This response may be modified or even negated by excessive day temperatures (>30 °C) (Iglesias et al., 2002; Reay, 1999). Excessive day temperatures occur regularly during South African summers leading to insufficient red colour development and, in combination with high light levels, may cause sunburn (Schrader et al., 2003). Sunburn may reduce class one fruit in CP by about 5 to 10% (M. Pretorius, personal communication). Due to the substantial difference in value between CP and PL, it is imperative to produce PL category fruit. This requires the rigorous management of cultural practices, such as fruit thinning that affect the external quality of the fruit.

Thinning of apple trees entails the chemical induction of abscission followed up, if necessary, with the supplementary mechanical removal of fruit by hand to decrease the crop load in order to increase fruit quality and to prevent alternate bearing (Link, 2000). Different fruit quality components are affected by different levels of thinning. More severe thinning (to ca 30 t·ha<sup>-1</sup>) affects fruit size, colour, firmness, soluble solids content and acidity while less

severe thinning (to ca 80 t·ha<sup>-1</sup>) decreases fruit susceptibility to physiological disorders, especially during storage, due to a more balanced calcium/potassium ratio (Link, 2000). Generally, lower crop loads increase fruit size and firmness, soluble solids concentration and acidity while maturity is advanced (Awad et al., 2001a; Link, 2000; Palmer et al., 1997; Sharples, 1968; Wünsche and Palmer, 2000; Wünsche and Ferguson, 2005). Palmer et al. (1997) found a linear increase in the area covered with red blush in 'Braeburn' with increasing severity of thinning, ranging from 35% blush coverage at high yields (30 t·ha<sup>-1</sup>), to 55% blush coverage at lower yields (6 t·ha<sup>-1</sup>). Awad et al. (2001a) also found 14% higher blush coverage in 'Elstar' apples at low crop loads (35 t·ha<sup>-1</sup>) compared to moderate (50 t·ha<sup>-1</sup>) and high crop loads (70 t·ha<sup>-1</sup>) although the increase in anthocyanin levels was not statistically significant. Sunburn did tend to be slightly higher in 'Fuji' apples at lower crop loads, but the effect was not statistically significant, which led to the conclusion that sunburn is unaffected by crop load (Wünsche et al., 2004). Although thinning generally improves fruit quality, it may also increase fruit susceptibility to physiological disorders such as bitter pit, which are mostly calcium-related disorders (Link, 2000; Sharples, 1968) and internal breakdown. Atkinson et al. (1995) found that fruit from thinned trees showed an increase in mineral nutrient concentration, with the notable exception of calcium, while Johnson (1992) found a decrease in fruit calcium concentrations at lower crop loads. Apart from fruit quality, thinning may also affect vegetative and reproductive development.

It is widely recognized that heavy crop loads reduce reproductive development and are the main cause of alternate bearing (Monselise and Goldschmidt, 1982). The inhibitory effect of high fruit numbers on flower initiation is due to the high amount of gibberellins produced by the seeds of young fruit (Buban and Faust, 1982). Thinning provides a means of overcoming alternate bearing. Previous research has shown that the thinning of trees in the previous season increased the number of blossom clusters and also increased fruit set in 'Cox Orange Pippin' trees (Sharples, 1968). In addition to the negative effect on reproductive development, high crop loads also reduce vegetative development as evident from a decrease in dry matter partitioning to new shoot, secondary vascular and root growth (Palmer, 1992). In contrast, vegetative growth increases, because of greater availability of assimilates in light cropping trees (Wünsche et al., 2005). A low crop load also alters sink activity, with carbohydrates partitioned to alternative sinks such as shoot growth (Wünsche and Palmer, 2000). In extreme cases, a heavy crop load may result in tree collapse. Trees of Murcott, an extremely fruitful tangerine cultivar, may wilt and start to defoliate due to carbohydrate depletion caused by an excessive crop load in the previous season (Smith, 1976). Thinning during the on-year

prevents these symptoms. CP is not prone to alternate bearing or poor fruit set but exceptionally heavy crops on young or vigorous trees can lead to poor return bloom (Hurndall, 2003; Wilton and Hornblow, 2004).

Ultimately, the producer needs to find a compromise between the quality and quantity of fruit that suits his unique farming environment (Link, 2000). The main objective of this research was to determine the effect of fruit number on fruit quality and reproductive and vegetative development in a high yielding CP orchard in contribution to the ongoing debate among South African producers as to whether they should strive for lower, but sustainable yields of better quality fruit, or for maximum yield at minimum permissible quality. Taking into account horticultural considerations, trees were hand thinned to five crop loads and the effect on fruit size, colour, sunburn incidence, maturity, internal quality and storability assessed. Fruit were classed according to size, colour and the presence of external blemishes such as sunburn, and the yield of PL and CP fruit calculated in  $t \cdot ha^{-1}$ .

## **Materials and methods**

The study was conducted in the Mediterranean-type climate Vyeboom region (Latitude:  $34^{\circ}4'S$ ; Longitude:  $19^{\circ}6'E$ ) of the Western Cape province of South Africa in a high-yielding CP apple orchard, averaging  $110 t \cdot ha^{-1}$  per year since 2004. Trees on M793 rootstock were planted in 1996 on a slope of  $30^{\circ}$  W at a spacing of 4 x 1.5 m and trained as free standing central leaders. The row direction is NE-SW. Trees are approximately 4 m high with permanent horizontal scaffold branches of 1.5 m long radiating from the central leader at intervals of 30 cm. A light summer pruning strategy is followed in the orchard to ensure optimum light distribution for red colour development and consists of removing vigorous upright shoots and shortening older bearing branches to fruit bearing positions. Fruit set and flower initiation were maximized by girdling trees during full bloom and ~4 weeks after full bloom, respectively.

### **2006/2007.**

*Trial layout.* Trees were selected for uniformity in vigor and hand thinned to five fruit loads on 29 November 2006 (48 days after full bloom) with the thinning treatments randomized in 12 blocks with one tree per plot. No chemical thinning was applied. The control treatment was thinned to 2 to 3 fruit per cluster (1), according to the commercial thinning practice of the producer. Treatments two to five entailed further thinning to respectively one fruit per cluster

in the lower half (< 1.8 m) of the tree (2); one fruit per cluster throughout the tree (3); thinning to single fruit and removal of small fruit (< 29 mm) (4), and thinning to single fruit with removal of small fruit as well as fruit from the inner canopy (5). Thinned fruit were counted. In designing the respective thinning treatments, we took into account that red colour development is generally worst in the lower and inner canopy and is also decreased by shading by other fruit in the cluster (Awad et al., 2000). Since our objective was to decrease down classing of fruit due to poor red colour and small size, we preferentially thinned small fruit and fruit in shaded positions.

*Yield, fruit quality and maturity at harvest.* Fruit were sampled on three commercial harvest dates, 18 April, 2 and 7 May 2007. Fruit harvested from each tree were weighed and 25 fruit randomly sampled for assessment of fruit quality and determining average fruit mass. Remaining fruit were pooled per treatment for grading at the Two-a-Day pack house in Grabouw to ascertain whether samples were representative. The estimated number of fruit per tree was determined by dividing the yield per tree by average fruit mass. Yield per hectare was determined by multiplying the mass of fruit from each tree with the number of trees per hectare.

Fruit quality and maturity parameters assessed included fruit diameter, foreground (red) colour, anthocyanin concentration of the peel, background (green to yellow) colour, sunburn incidence, flesh firmness, percentage starch conversion, total soluble solids (TSS), and titratable acidity (TA). The extent and intensity of red colour were assessed using a colour chart (PL colour chart, TopFruit (PTY) Ltd., Simondium, South Africa). A Minolta chromameter (Model CR-400, Minolta Co. Ltd., Tokyo, Japan) was used to measure the hue angle ( $H^\circ$ ), chroma (C) and lightness (L) at the reddest position on the fruit. The background colour was assessed using a colour chart (Colour chart for pears and apples, Unifruco Research Services (PTY) Ltd., Belville, South Africa). Sunburn incidence was noted when the severity was more than 2 on the Pink Lady sunburn chart (PL sunburn chart, TopFruit (PTY) Ltd., Simondium, South Africa). Flesh firmness was measured on opposite peeled sides of the fruit using a GÜSS fruit texture analyser with an 11 mm tip (GÜSS Manufacturing (PTY) Ltd, Strand, South Africa) and fruit diameter was measured with an electronic calliper. A 7 cm-long lint of skin was removed from the reddest part of the fruit using a potato peeler. The flesh was scraped off the peel after which the peel was frozen and ground in liquid nitrogen and kept at  $-40^\circ\text{C}$  until pigment analysis. Fruit were cut in half and one half used to assess starch conversion by applying 1% iodine solution to the cut surface and determining the

percentage starch conversion using a chart (Starch conversion for pome fruit, Unifruco Research Services (PTY) Ltd., Belville, South Africa). The remaining halves of apples were juiced to determine TSS using a refractometer (PR32, ATAGO Co. Ltd., Tokyo, Japan) and TA (expressed as percentage malic acid) by titrating NaOH (0.1 mol.L<sup>-1</sup>) to the juice with a Metrohm 760 Sample Changer (Metrohm Ltd., Herisau, Switzerland).

*Pigment analysis.* Anthocyanins were extracted from 2 g fresh ground peel with 5 ml of extraction solvent (5% 3M hydrochloric acid, 95% methanol) and constantly stirred for 1 h at 4 °C. After centrifugation for 10 min at 10000 x g, the supernatant was decanted and the pellet re-extracted with 5 ml extraction solvent and centrifuged as described above. The supernatant was added to the first. After filtration through 0.45 µm filters (Millex-HV, Millipore Corporation, Milford, MA, USA), pigments were quantified by spectrophotometry (Cary 50 Bio, Varian Australia (PTY) Ltd., Melbourne, Australia), measuring absorbance at 530 and 653 nm. The reading at 530 nm was corrected for the presence of chlorophyll by subtracting 24% of absorbance at 653 nm (Murray and Hackett, 1991). Anthocyanin concentrations were determined using idaein chloride (cyanidin-3-galactoside) (Carl Roth, Karlsruhe, Germany) to obtain a standard curve. Anthocyanins were expressed as µgg<sup>-1</sup> fresh weight of peel.

*Vegetative and reproductive development.* A scaffold branch, representative of the tree, was selected to assess vegetative and reproductive development. One-year-old shoot growth, branch circumference (2 cm from the trunk) and trunk circumference (10 cm above the ground) were measured on 11 September 2007. Flowers, flower clusters and mixed and vegetative buds were counted at full bloom on 12 October 2007. Fruit were counted on 16 November 2007 to determine fruit set. Spur leaves were sampled on 5 May 2008 from 5 spurs on a branch to determine spur leaf area using a portable area meter (LI-3000A, LI-COR, Inc., Lincoln, NEB, USA).

### **2007/2008.**

*Trial layout.* The treatments of the previous season were repeated on the same trees on 4 December 2007, 50 days after full bloom. Control trees were thinned to 1/2 fruit per cluster, the commercial thinning practice followed by the producer. Beforehand, trees were chemically thinned by the producer using Maxcel<sup>®</sup> (6-benzyladenine, 1.9% w/w, Philagro South Africa, Pty Ltd.) at 2.5 ml·L<sup>-1</sup> applied on 1 November 2007.

*Yield, fruit quality and maturity at harvest.* Fruit was sampled on three commercial harvest dates, 17 and 28 April and 8 May 2008, and fruit quality, maturity and pigments assessed as in 2006/2007.

*Vegetative and reproductive development.* The same scaffold branches selected in 2006/2007 were used to assess vegetative and reproductive development. One-year-old shoot growth, branch circumference and trunk circumference were measured on 5 September 2008. Flowers, flower clusters and reproductive and vegetative buds were counted at full bloom on 21 October 2008. Fruit was counted on 18 November 2008 to determine fruit set.

*Storage potential.* Storability was evaluated after the 2007/2008 season by randomly selecting 40 fruit per tree at the second harvest date (28 April). After storage at -0.5 °C for three months in regular atmosphere, 20 fruit of each sample were evaluated with regard to background colour, red colour (chroma meter), flesh firmness, TSS, TA and greasiness. All these parameters were evaluated as mentioned earlier. Greasiness was assessed by a trained technician on a scale of 1 to 4 (4 being very greasy). The remaining 20 fruit were kept at 15 °C for a further 7 days after which the same variables as after storage were measured in order to assess shelf life.

*Statistical analysis.* Data were analysed using the General Linear Models (GLM) procedure of SAS Enterprise Guide 3.0 (SAS Institute Inc., 2004, Cary, NC, USA). Trunk circumference was used as covariate for fruit quality data, while the branch circumference was used as covariate for shoot growth, flowering, fruit set and reproductive and vegetative bud count data. Treatment means were adjusted where the covariate was significant ( $P < 0.05$ ). The yield efficiency ( $\text{kg} \cdot \text{cm}^{-2}$  trunk cross-sectional area) of each thinning treatment was calculated and used to calculate coefficients for fitting linear and quadratic orthogonal contrasts to the data, except for the number of thinned fruit, yield and number of fruit at harvest. The number of thinned fruit was used to calculate coefficients for fitting linear and quadratic contrasts for yield and number of fruit at harvest. Regression analysis of SAS Enterprise Guide 3.0 (SAS Institute Inc., 2004, Cary, NC, USA) was used to examine the relationship between fruit quality and fruit number at harvest. After an initial analysis, the ten trees with the largest as well as the ten trees with the smallest trunk circumferences were excluded from a second regression analysis. This was done since fruit quality characteristics in these inherently vigorous and weak trees apparently related better to tree vigour than to fruit number per TCSA.

## Results

### 2006/2007.

The number of thinned fruit increased linearly with an increase in the severity of the thinning treatment resulting in three distinct crop levels in terms of the number of fruit per tree (Table 1). Treatments one and two had more fruit per tree at harvest than other treatments while treatments three and four had more fruit than treatment five. At 139 ton ha<sup>-1</sup>, treatment two had a higher yield than treatments three (120 ton ha<sup>-1</sup>) and five (99 ton ha<sup>-1</sup>), but not treatments one (137 ton ha<sup>-1</sup>) and four (126 ha<sup>-1</sup>). Treatments one, three and four had a higher yield than treatment five. Fruit size increased linearly with a decrease in fruit cm<sup>-2</sup> TCSA. Fruit of treatments one to three weighed less than fruit of treatments four and five. Treatments four and five fruit had a larger diameter than fruit of other treatments while treatment three fruit were larger in diameter than fruit of treatment one (Table 1). The number of leaves per fruit increased linearly with a decrease in fruit cm<sup>-2</sup> TCSA. Treatment five had more leaves per fruit than treatments one, two and four (Table 1) while treatment three had more leaves per fruit than treatment one.

A higher percentage of treatment five fruit were harvested on the first harvest date and a lower percentage harvested on the third harvest date compared to other treatments, except for treatment four (Fig. 1a). A higher percentage treatment three and four fruit were harvested on the first date compared to treatment one fruit, while a lower percentage treatment three and four fruit were harvested on the last harvest date compared to treatment one fruit.

Fruit firmness, TSS and TA increased linearly with decreasing fruit cm<sup>-2</sup> TCSA (Table 2). Treatment five fruit were firmer than fruit of treatments one, three and four while treatment two fruit were firmer than fruit of treatment four. The greatest difference in firmness was 0.39 kg between treatments five and four. Treatment five fruit were significantly higher in TSS and TA compared to the other treatments, which all had similar TSS levels. Treatment three fruit had higher TA than fruit of treatment one. The TSS to TA ratio decreased linearly with a decrease in fruit cm<sup>-2</sup> TCSA (Table 2). Treatment one had a significantly higher TSS/TA ratio than treatments three and five. Treatments two and four also had higher ratios than treatment five. Starch conversion was not affected by any of the treatments. Differences in background colour between treatments were statistically significant, but very small.

No significant differences were found between treatments for lightness (L value) and hue angle (Table 3). Chroma values increased linearly with decreasing fruit  $\text{cm}^{-2}$  TCSA. Treatment five fruit had significantly higher chroma than the rest of the treatments while treatment four fruit had a higher chroma than fruit of treatment one. Unfortunately, peel of treatment three was lost due to a malfunctioning freezer. No significant differences in anthocyanin concentration were found between the other treatments.

Regression analysis did not reveal significant trends between the number of fruit  $\text{cm}^{-2}$  TCSA and most fruit quality parameters, except for fruit mass, fruit diameter and TA. These parameters decreased linearly with an increase in fruit  $\text{cm}^{-2}$  TCSA, but the regressions were not very strong (Fig. 2a, b & c).

Treatments did not differ significantly with regard to the percentage sunburn and poor red colour (Table 4). Industry has two specifications for undersize fruit. Depending on the market, fruit in excess of 55 or 60 mm in diameter may qualify as PL. The percentage undersized fruit below both 55 and 60 mm decreased linearly with decreasing fruit per  $\text{cm}^2$  TCSA, but differences between treatments were much larger and only significant at <60 mm due to a considerable increase in undersized fruit at higher crop loads. Hence, treatments five, four and three had a lower percentage undersized fruit (<60 mm) than treatments one and two. Percentage class one fruit was similar when the size limit was 55 mm. However, when the minimum size limit was increased to 60 mm, percentage class one fruit were considerably lower in treatments one and two, whereas class one fruit in treatments three to five decreased only slightly. Tons of fist class fruit per ha were significantly higher in treatments one to four, while treatment five yielded the least amount of class one fruit per hectare at a size limit of 55 mm or 60 mm.

Sample grader results generally showed the same trends between treatments as found with sampling, although the incidence of poor colour and small fruit were scored higher and the incidence of sunburn scored lower (Table 5). This may suggest that sampling was preferentially done from the outer canopy. In spite of the higher percentage class one fruit of the more severe thinning treatments, less severe thinning treatments still yielded substantially more class one tons per hectare (Table 6).

There were no significant differences between treatments with regard to shoot growth, flower numbers, reproductive and vegetative bud development, and fruit set (data not presented).

## 2007/2008.

The number of thinned fruit increased linearly with increasing severity of thinning (Table 7). However, the severity of thinning did not differ significantly between treatments five and four, treatments four and three and treatments two and one. Treatments one and two had more fruit per tree at harvest than other treatments while treatments three and four had more fruit than treatment five. At 136 ton ha<sup>-1</sup>, treatment two had a higher yield than other treatments except for treatment one (134 ton ha<sup>-1</sup>). Treatment one had a higher yield than treatments three (108 ton ha<sup>-1</sup>) and five (97 ton ha<sup>-1</sup>) while treatment four (120 ha<sup>-1</sup>) yielded more than treatment five. Fruit size increased linearly with decreasing fruit per cm<sup>2</sup> TCSA (Table 7). Treatment five had the heaviest fruit, followed by treatments three and four, with treatment one and two having the lightest fruit. Fruit of treatments five, four and three were larger than those of treatments one and two (Table 7). Leaves per fruit increased linearly with decreasing fruit per cm<sup>2</sup> TCSA, even though there were no significant differences between treatments (Table 7).

A higher percentage of treatment five fruit were harvested on the first harvest date and a lower percentage harvested on the third harvest date compared to other treatments (Fig. 1b). A higher percentage treatment three fruit were harvested on the first date compared to treatment one, while a lower percentage treatment three and four fruit were harvested on the last harvest date compared to treatment one.

Fruit firmness, background colour, TSS and TA increased linearly and TSS/TA decreased linearly with decreasing fruit per cm<sup>2</sup> TCSA (Table 8). The differences in TSS between treatments were, however, practically and commercially insignificant and will not be discussed. Treatment five had firmer fruit compared to all other treatments while treatment three fruit were firmer than fruit of treatment one. The difference between the firmest (treatment five) and least firm (treatment one) fruit was only 0.3 kg. Background colour was slightly more yellow in treatments three and five compared to treatments one and two, but as with TSS, differences were small and not of horticultural significance. Treatment five fruit had the highest TA, followed by treatments four and three, with treatments one and two having the lowest TA. Treatments five, four and three had a lower TSS/TA ratio than treatments one and two. Treatment five fruit had a significantly lower percentage starch conversion compared to fruit of the other treatments.

No significant differences were found between treatments in lightness (L value) and chroma (Table 9). However, as in 2006/2007, the chroma of fruit increased slightly with a decrease in fruit per cm<sup>2</sup> TCSA. Treatment five fruit were significantly redder than fruit of treatments one, three and four, but differences in anthocyanin concentrations between treatments were not statistically significant (Table 9). Treatment four anthocyanins could not be determined due to loss of sample caused by a malfunctioning freezer.

Regression analysis did not reveal significant trends between the number of fruit per cm<sup>2</sup> TCSA and most fruit quality parameters, except for fruit size (fruit mass and diameter) and percentage malic acid that both decreased linearly with an increase in fruit per cm<sup>2</sup> TCSA (Fig. 3a, b & c).

Treatments did not differ significantly with regard to percentage sunburn (Table 10). The percentage poor red fruit was considerably less for treatment five ( $p=0.0543$ ). Undersized fruit (<55 mm) did not differ significantly between treatments, but did increase linearly with an increase in fruit per cm<sup>2</sup> TCSA (Table 10 and Fig. 3a). At <60 mm, the percentage undersized fruit was considerably higher and increased linearly with increasing fruit per cm<sup>2</sup> TCSA (Table 10). Treatment five had less undersized fruit (3%) than treatments one and two (~9%) at <60 mm. The percentage class one fruit (size >55 mm and >60 mm) increased significantly and linearly with a decrease in fruit per cm<sup>2</sup> TCSA (Table 10). The percentage class one fruit of treatment five was between 5.4 and 10.4% higher than that of other treatments (Table 10). After increasing the undersize limit to 60 mm, first class fruit were slightly less for all the treatments, but with the same trend as with the 55 mm size limit. Total class one tons per hectare did not differ between treatments, even when the size limit was increased to 60 mm. The cumulative yield of class one fruit (ton·ha<sup>-1</sup>) over the two seasons increased linearly with an increase in fruit per cm<sup>2</sup> TCSA for both fruit size limits. However, the difference between treatments was only significant at the 55 mm limit with treatment five having a lower cumulative yield of class one fruit than other treatments and treatment two also yielding more class one fruit than treatment three.

Sample grader results generally gave the same trends between treatments as found with the samples taken in the orchard although, as in 2006/2007, the incidence of poor colour and small fruit were scored higher and the incidence of sunburn scored lower (Table 11). In spite of the higher percentage class one fruit of the more severe thinning treatments, less severe thinning treatments still yielded substantially more class one tons per hectare (Table 12).

There were no consistent differences in vegetative and reproductive development between treatments (data not presented). There was also no difference in storage potential between the treatments and no internal browning was found after three months of regular atmosphere storage (data not presented).

## Discussion

An orchard yielding  $>100 \text{ t}\cdot\text{ha}^{-1}$  in three consecutive years was selected to assess the effects of high fruitfulness on fruit quality and to determine whether these high yields are sustainable in terms of biennial bearing and overall tree health. A lower crop load may ensure regular yields at higher fruit quality (Link, 2000).

Thinning trials normally entail removing a certain amount of fruit per branch or trunk cross-sectional area. Our experiment differed from this method in that we formulated thinning methods that took into account horticultural considerations. Hence, we preferentially removed small and interior fruit that could be expected not to meet first class quality standards at harvest. Our premise was that additional thinning should always be aimed at increasing the yield of first class fruit and attaining sufficient return bloom. Regression analysis reveals that fruit number per trunk cross-sectional area (TCSA) influenced only fruit size (mass and diameter) and TA significantly, which means that the differences between the treatments for the other quality parameters (e.g. fruit colour) were mainly due to the way in which thinning was performed. Thinning treatments were successful in creating different cropping levels. An unexpected result was that treatment four, consistently over the two seasons that the experiment was conducted, had a higher yield than treatment three despite more severe thinning. The reason for this is uncertain, except that we noted that treatment four had by chance some exceptionally large trees in which yield and fruit quality parameters seemed to have been less responsive to fruit load per TCSA than in smaller trees. Generally, yield efficiency decreases as rootstock vigor increases while yield increases as trunk circumference area increases. However, after a certain TCSA (depending on the rootstock and cultivar), yield does not increase, revealing a curvilinear relationship (Barrit et al., 1997).

The negative effect of increasing fruit numbers on fruit size was quite apparent. Fruit size increased with 13 g and 3.1 mm, and 17 g and 2.2 mm in the respective seasons with decrease in cropping level from 700 to 450 fruit and 770 to 490 fruit per tree. However, this was

accompanied by a yield decrease of 38 and 39 ton·ha<sup>-1</sup> in 2006/2007 and 2007/2008, respectively. The beneficial effect of a lower yield on fruit size has been thoroughly documented. The increase in fruit size due to thinning is most likely due to an increase in cell division as no correlation was found between cell size and fruit size (Bergh, 1985; Denne, 1960; Goffinet et al., 1995; Pearson and Robertson, 1953). Thinning needs to be done during the first six to seven weeks after bloom when cell division occurs (Denne, 1960), in order to allow remaining fruits to continue cell division under less competition (Goffinet et al., 1995). For logistic reasons, our thinning was performed at seven and eight weeks after full bloom, which is considered as a rather late stage to still influence cell division. Commercial hand thinning is generally conducted even later. The time of thinning may also influence its effect on flower bud formation, vegetative development, fruit size and maturity (Denne, 1960; Goffinet et al., 1995; McArtney et al., 1996; Quinlan and Preston, 1968; Tromp, 2000). Generally, the earlier thinning is done, the more pronounced the effects will be.

In general, maturity indicators did not reveal an advance in maturity with increased thinning. In fact, the small increase in the firmness and lower starch breakdown of fruit of the most severe thinning treatment at harvest suggest that these fruit were less mature at harvest. This is contradictory to most literature on the effect of fruit load on maturity (Awad et al., 2001a; Daugaard and Grausland, 1999; Johnson, 1992; Palmer et al., 1997; Quinlan and Preston, 1968; Wünsche and Palmer, 2000; Wünsche et al., 2005). Differences in fruit maturity (background colour) were more pronounced when thinned earlier (Quinlan and Preston, 1968). Despite a more yellow background colour, Awad et al. (2001a) also did not find a difference in starch conversion at different crop loads. Smaller fruit are normally firmer than larger fruit (Blanpied et al., 1978). However, this is not always the case when fruit are sampled from trees that are under different crop loads, as in our case. Larger fruit from lower crop loads will have a higher cell number in the cortical tissue and increased cell turgor (Wünsche et al., 2005), which would lead to an increase in fruit dry matter and thus fruit firmness (Wünsche and Palmer, 2000; Johnson, 1992). The harvest distribution is not a function of maturity because CP is harvested according to red colour development. Red fruit would be picked first and the rest of the fruit would be left on the tree in order to develop red colour.

In order to be classified as class one fruit and to qualify for marketing under the PL trade mark, more than 40% of the fruit surface needs to be covered by a solid red blush of adequate intensity (Hurndall, 2003). Class 2 and 3 fruit (respectively) have red blush coverage of 10% to 40% and less than 10%. These fruit may only be marketed as CP, which has a considerably

lower value than PL. Fruit thinning increased both the percentage of fruit with good blush as well as the average blush coverage of individual fruit. Fruit from the inner canopy are less able to synthesize anthocyanins and develop red colour due to the low light intensity at this canopy environment (Awad et al., 2001b). Red colour could be improved in lower cropping treatments, because of an increased pool of sugars (Awad et al., 2001a). Sugar is necessary for anthocyanin synthesis and an increase in sugar availability could lead to increased anthocyanin formation. However, the amount of sugars and colour development are not always correlated (Uota, 1952). Awad et al. (2001a) conclude that crop load does not influence anthocyanin formation because carbohydrate supply might not be a limiting regulatory factor between trees with a low and high crop load. Palmer et al. (1997) found that the percentage surface area coverage with red blush increased from 35% to 55% in 'Braeburn' apples as yield decreased from 30 to 6 t·ha<sup>-1</sup>. Percentage surface area covered by red blush may be higher in lower yielding trees due to less shading caused by neighbouring fruit in a cluster (Awad et al., 2001a). According to our results, there was no correlation between red colour and fruit number. The improvement in fruit colour of the most severe thinning treatment can be attributed to the selective thinning of shaded interior canopy fruit.

High light environments in combination with high temperatures cause sunburn (Paper 1; Schrader, 2003). However, anthocyanin synthesis is also dependent on high light levels (Saure, 1990). In order for the fruit to develop sufficient red colour, it has to be borne in high light environments, which may also increase the incidence of sunburn. In agreement with Wünsche et al. (2004), the incidence of sunburn was unaffected by crop load, even though preferential thinning in the interior canopy would be expected to increase the proportion of fruit with sunburn. Sunburn may be lower in low cropping trees due to an increase in leaves per fruit, which would supply shade for the fruit (Van den Ende, 1999). As the leaf to fruit ratio increases with severity of thinning (Sharples, 1968), the lower than expected sunburn incidence of treatment five may be due to increased shading of outer canopy fruit and reduced bending of branches due to less fruit per branch (Hirst, P.M 1990).

The results of the sample grader supply us with more comprehensive data, as well as a means to recognise sampling bias. Undersized fruit was underestimated by our sampling, sunburn was over estimated and red blush was more or less similar the first season but over estimated in the second season. However, the trends between treatments regarding these culling factors were similar. The percentage of fruit with poor red colour was considerably higher ( $\pm 20\%$ ) in the 2007/2008 season according to our scoring standards. A higher L value, hue angle and

lower anthocyanin concentration also reflected the weaker red colour development of the second season. It is possible that the packhouse might have adjusted their standards to accommodate the lower colour development of the 2007/2008 season. The greater incidence of sunburn in our samples may be due to biased sampling with more fruit sampled from the outer canopy. However, it is also difficult to recognise less severe sunburn on CP with its strong red over colour. Hence, the total incidence of sunburn may be underestimated at the packhouse where assessment may be less rigorous compared to the lab, even though the same standards were used to assess sunburn. The percentage undersized fruit was very low ranging from 0.1% to 1.7%, which reflects the lenient size specification (55 mm). After increasing the size limit to 60 mm, it became more noticeable that less severe thinning treatments had a higher occurrence of undersize fruit. Culling factors such as undersized fruit, poor red colour and sunburn decrease the proportion of class one fruit. Percentage class one fruit were similar in the first season for the different thinning treatments, but increased significantly with thinning severity in the second season, characterised by generally smaller fruit size and less red colour development. Despite a 15% decrease in the proportion of class one fruit on high yielding trees, these trees still produced 13 to 18 tons more class one fruit per hectare than lower yielding trees. We found a linear trend for class one fruit per hectare in the 2006/2007 season, but no trend in the following season. Cumulatively over the two seasons, less severe thinning also yielded more class one fruit per hectare. Not taking into account management and production costs, aiming for maximum yields at the minimum permissible fruit quality seem to be more profitable to the producer than aiming for maximum fruit quality but at lower yields.

Thinning treatments did not have a significant effect on vegetative and reproductive development in either season. Atkinson et al. (1995) did not find any affect of crop load on shoot length in 'Cox's Orange Pippin' apples, while McArtney et al. (1996) found no significant difference in mean shoot length with later thinning in 'Royal Gala' and 'Braeburn' apples although the total number of shoots were less at higher yields. Forshey and Marmo (1985) also found that deblossoming increased shoot number but not total shoot length in 'McIntosh' apple. Generally though, vegetative development is promoted by lower cropping, because of a shift in sink activity (Erf and Proctor, 1987; Forshey and Marmo, 1985; Inglese et al., 2001; Lakatos, 2004; Palmer et al., 1997; Wünsche et al., 2005). In peach, the reduction in vegetative growth due to cropping depended on the vigour of the rootstock, with trees on a vigorous rootstock showing a smaller reduction in growth, because vigorous rootstocks have more carbohydrate reserves available to support fruit growth (Inglese et al., 2001). Our

thinning may have been performed too late to affect shoot growth. Thinning later than two weeks after full bloom did not affect shoot growth in ‘Sunset’ apples, whereas earlier thinning increased shoot count but not length (Quinlan and Preston, 1968).

Spur foliage area was measured for the first season of the trial, but no significant difference was found between treatments (data not shown). Although foliage area generally decreases at high cropping levels (Lakatos, 2004), Palmer (1992) did not find any significant effect of crop load on spur leaf area in ‘Crispin’ apple. Our thinning might have been done too late in the season to have an effect, because leaf area decreased from thinning at full bloom to four weeks after full bloom, with no further reduction in leaf area with later thinning (McArtney et al., 1996).

Fruit number has an inhibitory effect on flower bud production and return bloom, due to an increase in seeds that produce gibberillic acid (GA), which inhibits floral initiation (Tromp, 2000). Apart from GA, polar auxin transport also positively correlates with the number of seeds per fruit and acts as a secondary messenger to GA during the inhibition of flower initiation (Bangerth, K.F 2008). For example, ‘Cox Orange Pippin’ apple trees thinned the previous season, carried more blossom trusses and had a higher fruit set than trees without thinning (Sharples, 1968). In apparent agreement with Wilton and Hornblow (2004), our data indicated that CP does not seem to be prone to alternate bearing. Wilton and Hornblow (2004) do mention that exceptionally high crop loads on young or vigorous trees may result in alternate bearing in CP. A possible reason for its regular cropping is that CP bearing units may balance vegetative and reproductive growth by reducing the number of growing buds through an extinction mechanism as found for Type III and IV apple ideotypes (Lauri et al., 1995). Heavy spur bearing cultivars with strong vegetative and erect scaffold branches are more prone to alternate bearing than Type III and IV ideotype cultivars with semi-vigorous, pendulant bearing units with an ability for bourse over bourse production and extinction of non fruit bearing spurs (Lauri et al., 1995).

Post storage fruit quality was unaffected by crop load. Fruit also did not develop symptoms of physiological storage disorders, such as internal browning, one of the post harvest disorders affecting CP (Gualanduzzi et al., 2005). Thinning tends to increase the susceptibility of fruit to physiological disorders (Johnson, 1992) by altering the mineral composition of the fruit. Thinning increases P, K and dry matter, while decreasing Ca concentrations (Johnson, 1992; Johnson, 1994). However, thinning does not seem to affect the incidence of internal browning

(Brown et al., 2003). Harvesting when fruit are over mature, in order to obtain a high percentage red fruit is another major cause of internal browning (Brown et al., 2005). Hence, it would have been advisable to also store fruit of the final harvest date to assess the susceptibility of these more mature fruit to internal browning. Our fruit was not stored in a controlled atmosphere (CA), which may explain why internal browning did not occur. CP is more prone to develop internal browning when stored in CA (De Castro Hernandez et al., 2005). The storage period may also have been too short to notice internal browning, because there were no delays or interruptions in the cold chain of sampled fruit as would be found in commercial cold chains. A longer storage period, i.e., 4-6 months, might have compensated for this.

To conclude, despite an 10% increase of percentage first class fruit in response to selective thinning, heavy cropping trees yielded considerably more first class fruit per hectare, without affecting the storability of the fruit or the vegetative and reproductive development of the trees. Hence, aiming for a higher crop load at minimum permissible fruit quality would appear to be more profitable to the producer. It seems to be easier to maximize class one fruit in CP by increasing yield than by increasing fruit quality by thinning. We have to caution that different results may be obtained for Type I and II cultivars that are more prone to alternation and if more mature fruit from the final harvest date were stored in CA. Furthermore, all fruit above the minimum size do not generate the same income and this has to be taken into account in the financial analysis that this data is still to undergo. The cost of production may also play a greater role in CP orchards with a much lower maximum yield potential or if quality standards were to become more stringent. In addition, thinning carries a cost and the severity of thinning will affect the cost of pruning, harvesting, fruit transport, sorting and storage. In a high profit cultivar such as CP, these cost factors may be of lesser importance in influencing profitability. It may, however, play a bigger role in the profitability of less lucrative cultivars and should be kept in the debate over yield versus fruit quality.

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Table 1. Effect of fruit thinning intensity on yield and fruit size in ‘Cripps’ Pink’ apple during the 2006/2007 season. Means adjusted for trunk circumference are separated by LSD (5%).

Thinning treatment	Number of thinned fruit	Estimated fruit number at harvest	Total yield (ton ha <sup>-1</sup> )	Avg. fruit mass (g)	Avg. fruit diameter (mm)	Leaves per fruit
1. Control	0 e <sup>z</sup>	700 a	137 ab	135 b	66.8 c	13.9 c
2. Single - <1.8 m	70 d	710 a	139 a	137 b	67.2 bc	14.7 bc
3. Single - whole tree	150 c	580 b	120 b	140 b	67.9 b	18.7 ab
4. Single and small	240 b	590 b	126 ab	146 a	69.0 a	17.0 bc
5. Single, small and inside	320 a	450 c	99 c	148 a	69.3 a	20.8 a
Pr>F						
Trunk circumference	<0.0001	<0.0001	<0.0001	0.0177	0.0032	0.0015
Treatment	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	0.0067
Crop load linear	–	<0.0001	<0.0001	<0.0001	<0.0001	0.0003
Crop load quadratic	–	0.2529	0.2372	0.8492	0.5672	0.7295

<sup>z</sup>Means with different letters differ significantly at P<0.05.

Table 2. Effect of fruit thinning intensity on fruit quality in ‘Cripps’ Pink’ during the 2006/2007 season. Means adjusted for trunk circumference are separated by LSD (5%).

Thinning treatment	Firmness (kg)	TSS (° brix)	Acidity %	TSS/Acidity	Background colour (charts)	Starch conversion %
1. Control	8.35 bc <sup>z</sup>	13.9 b	0.51 c	27.5 a	3.49 b	58 <sup>ns</sup>
2. Single - <1.8 m	8.53 ab	14.1 b	0.53 bc	26.8 ab	3.57 ab	57
3. Single - whole tree	8.42 bc	14.2 b	0.55 b	26.0 bc	3.53 b	61
4. Single and small	8.25 c	14.1 b	0.53 bc	26.6 ab	3.64 a	63
5. Single, small and inside	8.64 a	14.7 a	0.58 a	25.3 c	3.57 ab	58
Pr>F						
Trunk circumference	0.0092	0.0376	–	–	–	–
Treatment	0.0007	0.0014	<0.0001	0.0042	0.0327	0.3735
Crop load linear	0.0065	0.0003	<0.0001	0.0003	0.3871	0.4721
Crop load quadratic	0.0589	0.0245	0.0314	0.3508	0.4510	0.0638

<sup>z</sup>Means with different letters differ significantly at P<0.05.

<sup>ns</sup>Not significant.

Table 3. Effect of fruit thinning intensity on peel lightness (L), chroma value and hue angle at the reddest position on the fruit, as well as anthocyanin concentration in 'Cripps' Pink' apple peel during the 2006/2007 season. Means adjusted for trunk circumference are separated by LSD (5%).

Thinning treatment	L value	Chroma value	Hue (°)	Anthocyanin concn ( $\mu\text{g}\cdot\text{g}^{-1}$ FW)
1. Control	49.0 <sup>ns</sup>	43.2 c <sup>z</sup>	34.1 <sup>ns</sup>	310 <sup>ns</sup>
2. Single - <1.8 m	48.5	43.1 c	32.7	343
3. Single - whole tree	48.7	43.6 bc	33.0	–
4. Single and small	49.1	44.0 b	32.8	259
5. Single, small and inside	47.8	45.0 a	31.5	292
Pr>F				
Trunk circumference	–	0.0396	0.0800	–
Treatment	0.0870	<0.0001	0.1648	0.0944
Crop load linear	0.1057	<0.0001	0.0729	0.0760
Crop load quadratic	0.0236	0.1284	0.2411	0.1459

<sup>z</sup>Means with different letters differ significantly at  $P<0.05$ .

<sup>ns</sup>Not significant.

Table 4. Effect of fruit thinning intensity on fruit quality disorders and class 1 % in ‘Cripps’ Pink’ during the 2006/2007 season. Means adjusted for trunk circumference are separated by LSD (5%).

Thinning treatment	% Poor red fruit <sup>y</sup>	% Sunburn incidence <sup>y</sup>	% Undersized fruit (<55 mm) <sup>y</sup>	Class 1% at >55 mm	Class 1 at >55 mm (ton·ha <sup>-1</sup> )	% Undersized fruit (<60 mm)	Class 1% >60 mm	Class 1 at >60 mm (ton·ha <sup>-1</sup> )
1. Control	25 <sup>ns</sup>	19 <sup>ns</sup>	0.7 <sup>ns</sup>	56 <sup>ns</sup>	76 a <sup>z</sup>	8.7 a <sup>z</sup>	52 <sup>ns</sup>	70 a <sup>z</sup>
2. Single - <1.8 m	20	20	0.6	61	84 a	7.3 a	58	80 a
3. Single - whole tree	24	16	0.1	62	74 a	3.5 b	61	73 a
4. Single and small	24	16	0.2	61	76 a	3.0 b	59	74 a
5. Single, small and inside	21	21	0.2	61	57 b	1.7 b	60	57 b
PR>F								
Trunk circumference	–	–	–	–	<0.0001	–	–	<0.0001
Treatment	0.1262	0.3245	0.1045	0.6581	0.0126	0.0004	0.1629	0.0318
Crop load linear	0.2560	0.9612	0.0351	0.4927	0.0009	<0.0001	0.0841	0.0059
Crop load quadratic	0.0310	0.0357	0.1979	0.8431	0.3259	0.4121	0.7283	0.2768

<sup>z</sup> Means with different letters differ significantly at P<0.05.

<sup>y</sup> Undersized fruit may also be scored for poor red colour and sunburn.

<sup>ns</sup> Not significant.

Table 5. Effect of fruit thinning intensity on fruit size and fruit quality disorders in 'Cripps' Pink' during the 2006/2007 season based on sample grader data.

Thinning treatment	Avg. fruit mass (g)	% Undersized fruit (<55 mm)	% Poor red fruit	% Sunburn incidence
1. Control	127	5	32	6
2. Single - <1.8 m	130	4	28	5
3. Single - whole tree	136	2	28	6
4. Single and small	142	2	35	6
5. Single, small and inside	143	2	21	9

Table 6. Effect of fruit thinning intensity on fruit quality distribution, with ton·ha<sup>-1</sup> in brackets, in ‘Cripps’ Pink’ during the 2006/2007 season based on sample grader data.

Thinning treatment	Class1 %	Class2 %	Class3 %
1. Control	61 (83)	33 (45)	7 (9)
2. Single - <1.8 m	65 (90)	29 (41)	6 (8)
3. Single - whole tree	65 (78)	31 (37)	4 (5)
4. Single and small	60 (75)	37 (47)	3 (4)
5. Single, small and inside	71 (70)	26 (26)	3 (3)

Table 7. Effect of fruit thinning intensity on yield and fruit size in ‘Cripps’ Pink’ apple during the 2007/2008 season. Means adjusted for trunk circumference are separated by LSD (5%).

Thinning treatment	Number of thinned fruit	Estimated fruit number at harvest	Total yield (ton·ha <sup>-1</sup> )	Avg. fruit mass (g)	Avg. fruit diameter (mm)	Leaves per fruit
1. Control	434 c <sup>z</sup>	773 a	134 ab	117 c	64.1 b	15 <sup>ns</sup>
2. Single - <1.8 m	494 c	771 a	136 a	119 c	64.2 b	19
3. Single - whole tree	650 b	571 b	108 cd	128 b	65.3 a	21
4. Single and small	757 ab	621 b	120 bc	129 b	65.7 a	27
5. Single, small and inside	808 a	487 c	97 d	134 a	66.3 a	30
Pr>F						
Trunk circumference	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	–
Treatment	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1494
Crop load linear	–	<0.0001	<0.0001	<0.0001	<0.0001	0.0375
Crop load quadratic	–	0.7067	0.9540	0.3093	0.5802	0.8602

<sup>z</sup>Means with different letters differ significantly at P<0.05.

<sup>ns</sup>Not significant.

Table 8. Effect of fruit thinning intensity on fruit quality in ‘Cripps’ Pink’ apple during the 2007/2008 season. Means adjusted for trunk circumference are separated by LSD (5%).

Thinning treatment	Firmness (kg)	TSS (° brix)	Acidity (%)	TSS/Acidity	Background colour (chart)	Starch conversion (%)
1. Control	8.01 c <sup>z</sup>	12.6 bc	0.40 c	32.0 a	2.77 b	36 a
2. Single - <1.8 m	8.03 bc	12.5 c	0.41 c	31.1 a	2.79 b	34 a
3. Single - whole tree	8.15 b	12.8 ab	0.44 b	29.0 b	2.89 a	35 a
4. Single and small	8.06 bc	12.7 abc	0.44 b	29.1 b	2.84 ab	35 a
5. Single, small and inside	8.32 a	12.9 a	0.47 a	27.9 b	2.93 a	25 b
Pr>F						
Trunk circumference	0.0239	<0.0001	–	0.0121	0.0186	–
Treatment	0.0002	0.0081	<0.0001	<0.0001	0.0074	0.0052
Crop load linear	<0.0001	0.0003	<0.0001	<0.0001	0.0004	0.0019
Crop load quadratic	0.0949	0.6506	0.5099	0.5375	0.8838	0.0194

<sup>z</sup>Means with different letters differ significantly at P<0.05.

Table 9. Effect of fruit thinning intensity on peel lightness (L), chroma value and hue angle at the reddest position on the fruit, as well as anthocyanin concentration in the peel in 'Cripps' Pink' apple during the 2007/2008 season. Means adjusted for trunk circumference are separated by LSD (5%).

Thinning treatment	L value	Chroma	Hue (°)	Anthocyanin concn ( $\mu\text{g}\cdot\text{g}^{-1}$ FW)
1. Control	51.6 <sup>ns</sup>	37.1 <sup>ns</sup>	40.7 a <sup>z</sup>	221 <sup>ns</sup>
2. Single - <1.8 m	51.0	37.5	38.2 ab	242
3. Single - whole tree	51.0	37.7	39.0 a	225
4. Single and small	51.5	37.6	39.5 a	–
5. Single, small and inside	46.4	38.3	33.7 b	254
Pr>F				
Trunk circumference	–	0.0004	–	0.0211
Treatment	0.2587	0.2630	0.0351	0.2105
Crop load linear	0.0683	0.0371	0.0177	0.0728
Crop load quadratic	0.1652	0.3858	0.0329	0.3705

<sup>z</sup>Means with different letters differ significantly at  $P < 0.05$ .

<sup>ns</sup>Not significant.

Table 10. Effect of fruit thinning intensity on fruit quality disorders and class 1 % in ‘Cripps’ Pink’ during the 2007/2008 season. Means adjusted for trunk circumference are separated by LSD (5%).

Thinning treatment	% Poor red fruit <sup>y</sup>	Sunburn incidence (%) <sup>y</sup>	% Undersized fruit (<55 mm) <sup>y</sup>	Class 1 % at >55 mm	Class 1 at >55 mm (ton·ha <sup>-1</sup> )	Total class 1 yield of both seasons (>55 mm)	Undersize % (<60 mm)	Class 1% >60 mm	Class 1 at >60 mm (ton·ha <sup>-1</sup> )	Total class 1 yield of both seasons (>60 mm)
1. Control	49 <sup>ns</sup>	14 <sup>ns</sup>	0.6 <sup>z</sup>	37 b	46	123 ab	9.6 a	33 b	43	113
2. Single - <1.8 m	45	16	0.0	40 b	54	138 a	9.2 a	37 b	50	130
3. Single - whole tree	48	12	0.5	41 b	43	118 bc	5.8 ab	38 b	40	114
4. Single and small	48	10	0.10	41 b	48	125 ab	5.7 ab	38 b	48	120
5. Single, small and inside	40	12	0.0	49 a	46	102 c	3.0 b	48 a	45	101
Pr>F										
Trunk circumference	<0.0001	–	–	0.0010	0.0076	0.0002	<0.0001	–	0.0009	<0.0001
Treatment	0.0534	0.1542	0.2892	0.0107	0.2603	0.0166	0.0145	0.0008	0.3450	0.0743
Crop load linear	0.0884	0.0650	0.6172	0.0016	0.1046	0.0011	0.0008	0.0001	0.3935	0.0138
Crop load quadratic	0.0124	0.1254	0.2539	0.1440	0.2394	0.7550	0.9369	0.0427	0.1659	0.7991

<sup>z</sup>Means with different letters differ significantly at P<0.05.

<sup>y</sup> Undersized fruit may also be scored for poor red colour and sunburn.

<sup>ns</sup>Not significant

Table 11. Effect of fruit thinning intensity on fruit size and fruit quality disorders in ‘Cripps’ Pink’ during the 2007/2008 season based on sample grader data.

Thinning treatment	Avg. fruit mass (g)	% Undersized fruit	% Poor red fruit	% Sunburn incidence
1. Control	112	5	22	6
2. Single - <1.8 m	113	5	19	7
3. Single - whole tree	120	3	21	6
4. Single and small	123	3	20	5
5. Single, small and inside	126	1	16	6

Table 12. Effect of fruit thinning intensity on fruit quality distribution, with ton·ha<sup>-1</sup> in brackets, in ‘Cripps’ Pink’ during the 2007/2008 season based on sample grader data.

Thinning treatment	Class1 %	Class2 %	Class3 %
1. Control	69 (93)	24 (33)	6 (8)
2. Single - <1.8 m	68 (93)	26 (35)	6 (8)
3. Single - whole tree	70 (76)	25 (27)	4 (5)
4. Single and small	70 (84)	26 (31)	4 (5)
5. Single, small and inside	77 (75)	19 (19)	4 (4)

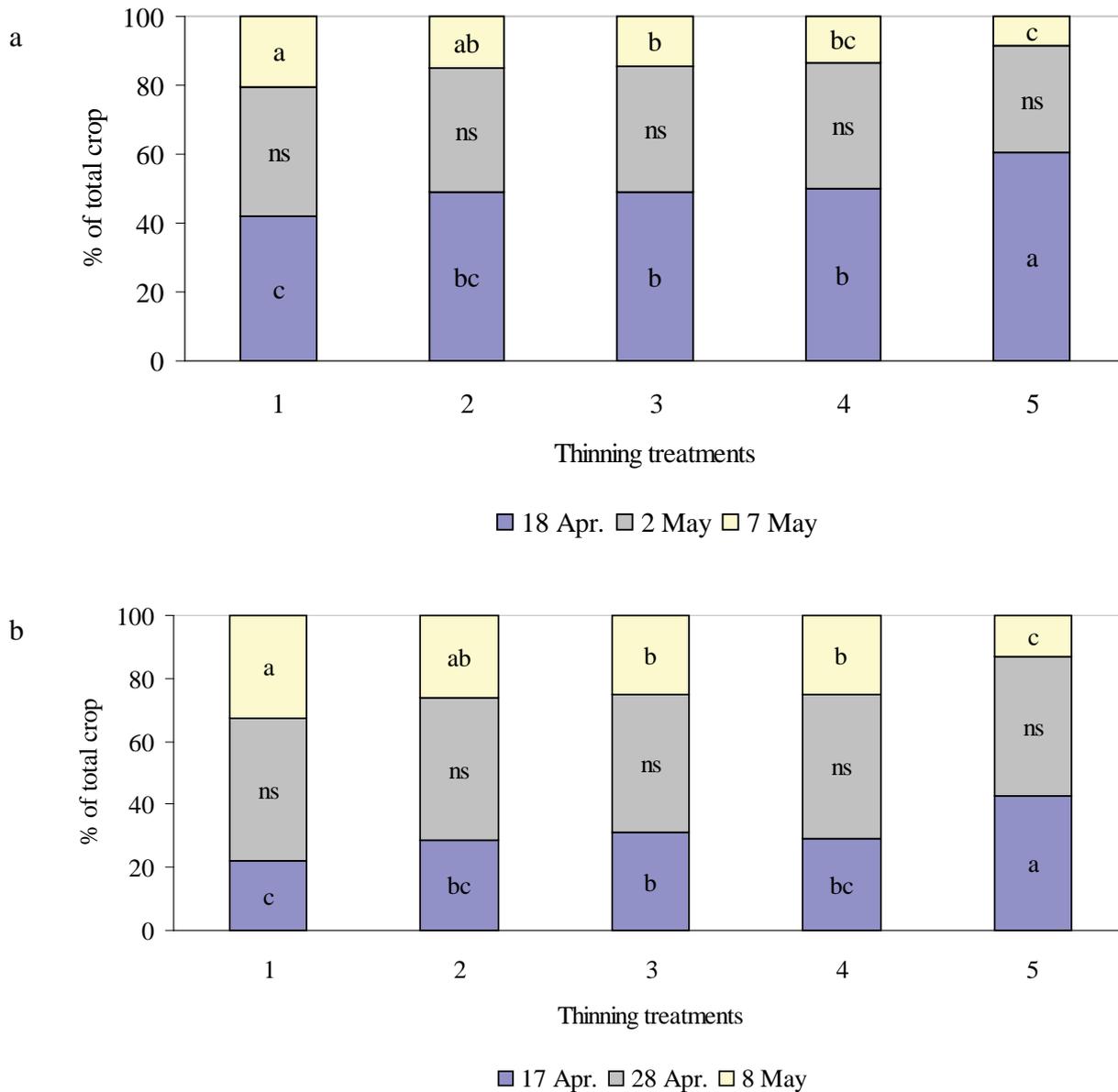


Fig 1. Effect of crop load on harvest distribution of ‘Cripps’ Pink’ apples during the 2006/2007 (a) and 2007/2008 (b) season. Treatment one was thinned to 2 to3 and 1 to 2 fruit per cluster for the respective seasons. Treatments two to five entailed further thinning to respectively one fruit per cluster in the lower half (< 1.8 m) of the tree (2); one fruit per cluster throughout the tree (3); thinning to single fruit and removal of small fruit (< 29 mm) (4), and thinning to single fruit with removal of small fruit as well as fruit from the inner canopy (5). Means adjusted for trunk circumference are separated by LSD (5%).

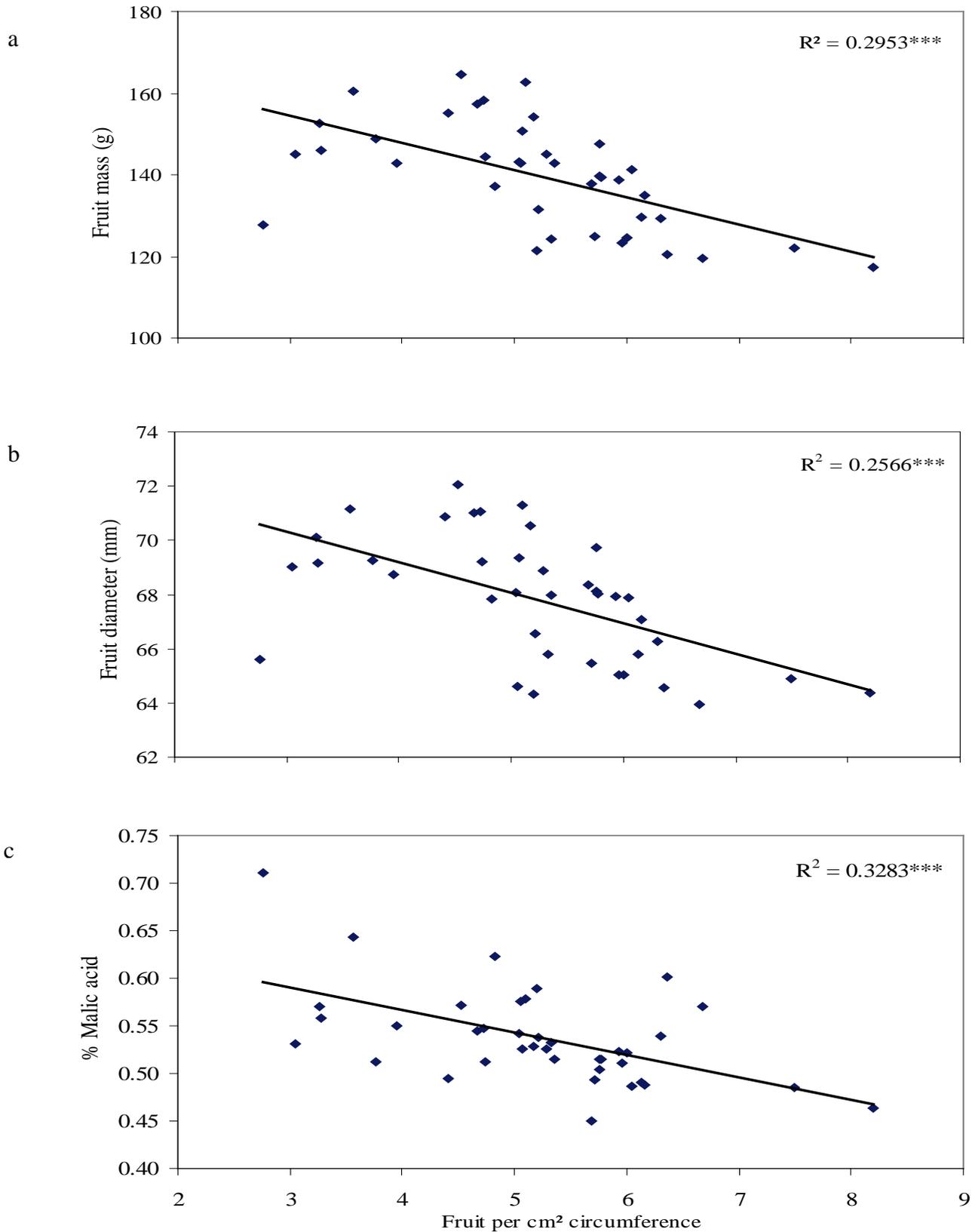


Fig. 2. Effect of fruit number per trunk cross sectional area on fruit diameter (a), mass (b) and percentage malic acid (c) in 'Cripps Pink' apples during the 2007/2008 season. \*\*\* =  $p < 0.0001$ .

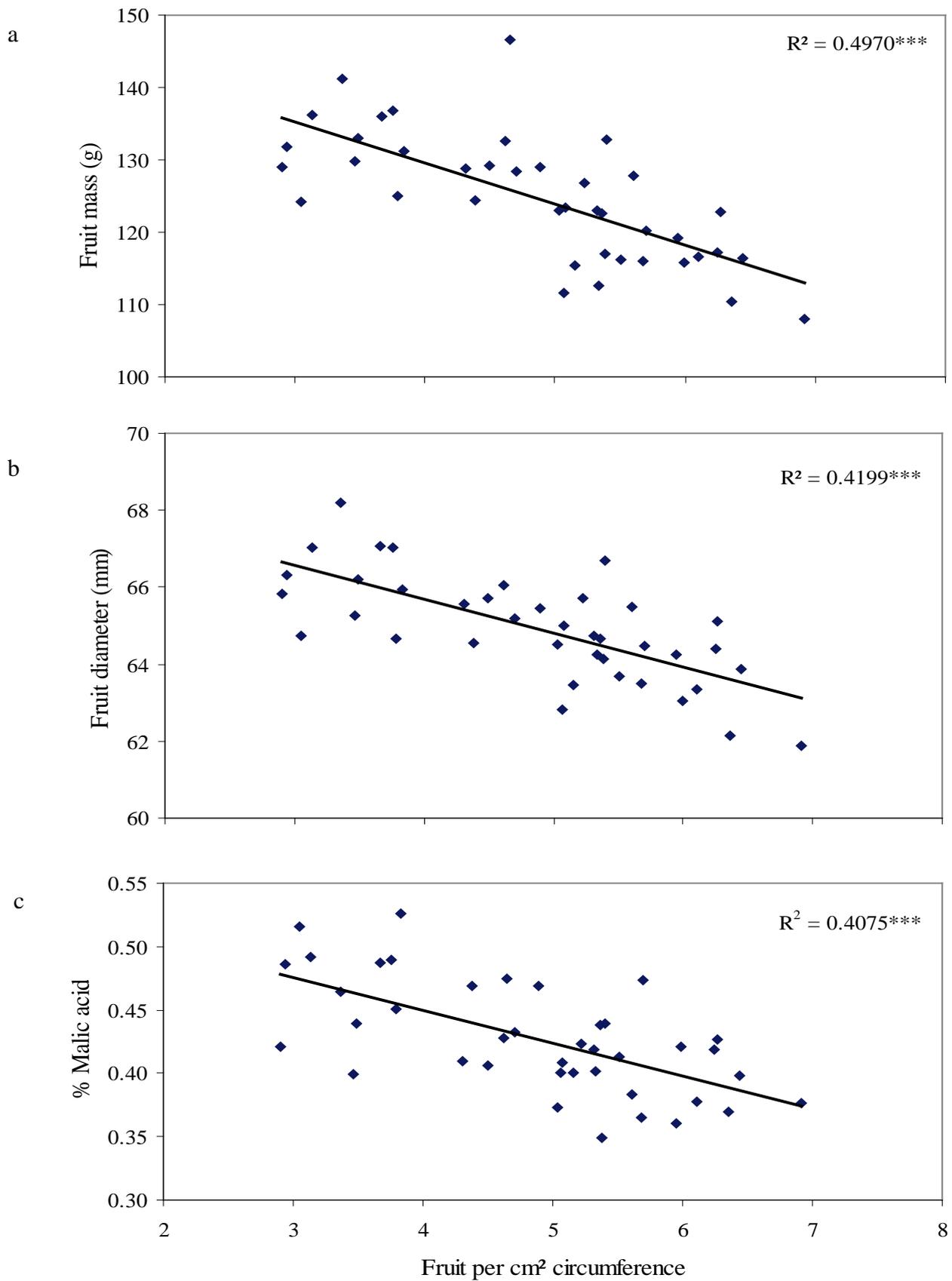


Fig. 3. Effect of fruit number per trunk cross sectional area on fruit diameter (a), mass (b) and percentage malic acid (c) in 'Cripps Pink' apples during the 2007/2008 season. \*\*\* =  $p < 0.0001$ .

## GENERAL DISCUSSION AND CONCLUSION

The profitability of fruit production is largely determined by the per area basis yield of fruit that qualify for export purposes. Improving fruit quality can increase the yield of first class fruit. Whether the yield of class one fruit can also be increased by increasing production is still a contentious issue among fruit producers since high yields may have a detrimental effect on fruit quality. The aim of this research was to gain insight into how to increase the yield of class one quality fruit in 'Granny Smith' and 'Cripps Pink' apple.

'Granny Smith' is the most widely grown apple cultivar in South Africa (24% of area planted), but its prominence is decreasing due to the high incidence of external defects such as sunburn, red blush and inadequate green colour development. Since these defects are related to the light levels and temperatures that fruit are exposed to, the relationship between canopy position and external fruit quality was investigated. Partially shaded canopy positions yielded the highest quality fruit with the best green colour. The outer canopy had the highest incidence of sunburn and red blush, whereas fruit from the shaded inner canopy had poor green colour. The darkest green fruit on the tree were exposed to moderate to high light levels (25-50% of full sun) during their early development, similar to fruit that developed sunburn and red blush. The difference came in during the second half of fruit development when green fruit became shaded (3% of full sun) while fruit that developed sunburn and red blush remained exposed to high light. Pale green fruit had low chlorophyll levels and were exposed to low light levels (2% of full sun) throughout the growing season, emphasizing the importance of exposure to sufficient light levels during early fruit development when chlorophyll synthesis occurs (Gorski and Creasy, 1977).

It appears that maximizing fruit quality in 'Granny Smith' requires the shading of outer canopy bearing positions to avoid sunburn and red blush development while the heavily shaded inner canopy should be opened up, especially during early fruit development, to create a higher light environment suitable for green colour development. This knowledge was put to test in a vigorous, low-density orchard and a non-vigorous, high-density orchard, representative of 'Granny Smith' orchards in the Grabouw region. In the vigorous orchard, heading cuts, to stimulate growth on the outer canopy, and thinning cuts, to increase light penetration in the inner canopy, were done in either winter or summer. The enhanced light distribution within the canopy improved green colour of fruit without affecting the incidence sunburn and red blush. However, we did not succeed in decreasing light levels in the outer canopy and pruning also substantially decreased the yield of

trees due to its severity. All one-year-old shoots in the non-vigorous orchard were headed to promote growth and thereby provide shading for fruit while a straw mulch and compost combination was applied to improve the soil water content and growth, thereby decreasing peel temperatures and sunburn. However, these treatments were not effective in decreasing sunburn and red blush. We unfortunately did not quantify the effect of the treatments on growth and light levels incident on fruit. However, the commercial pruning of the control treatment also entailed some heading, which may have decreased the contrast between treatments. The beneficial effects of mulching are cumulative and may only become evident in subsequent seasons.

Overall, it appears that while pruning can be used to increase light distribution and, thereby, green colour within the canopy, it may not be possible to simultaneously increase shading in the outer canopy. It seems to be more difficult to decrease the incidence of sunburn and red blush through pruning. If found to be cost effective, shade nets may be used to good effect in ‘Granny Smith’ to minimise sunburn and red blush development. However, this will have to be accompanied by rigorous light management to ensure sufficient light distribution within the canopy for green colour development.

‘Cripps’ Pink’ is a high value cultivar, but only if the fruit has adequate fruit quality, i.e., fruit size above 55 mm, at least 40% red blush coverage of adequate intensity and the absence of sunburn. The fruit may then be marketed as ‘Pink Lady’, which could lead to a 100% increase in value (South African ‘Pink Lady’ Association, 2009). To maximize profits, it is necessary to maximise the yield of first class fruit. This can be achieved by improving fruit quality or by increasing the total yield. Whether the latter is an effective strategy is still a contentious issue among fruit producers, since high yields generally have a detrimental effect on fruit quality. Fruit size decreases with an increase in crop load, but the risk of internal disorders such as internal browning and bitter pit increases (Link, 2000). Lower crop loads may also improve fruit colour, firmness, sugar content and acidity (Link, 2000). The effect of crop load on two of the major culling factors, insufficient red colour development and sunburn, has not been thoroughly documented. In order to evaluate how crop load may affect fruit quality and potential income, an exceptionally high yielding ‘Cripps’ Pink’ orchard was thinned to five crop load levels. Since the objective of the additional thinning was to maximise fruit quality, we preferentially removed fruit that would be expected to be of poor quality at harvest. Instead of removing a certain amount of fruit per trunk or branch circumference, increasing severities of thinning entailed the thinning of clusters, then the removal of small fruit and, finally, the selective removal of fruit from the shaded inner canopy.

Yields obtained with the various thinning treatments ranged from 99 to 138 t~~ha~~ ha<sup>-1</sup> in 2006/2007 and 97 to 136 ton·ha<sup>-1</sup> in 2007/2008. Significantly higher class one yield per hectare was attained at the higher crop loads in both seasons. The most severe thinning treatment increased the percentage class one fruit in 2007/2008, mainly due to an increase in the proportion of fruit with adequate red blush colour. Different crop loads did not affect fruit storability or reproductive and vegetative development. The increase in first class yield at the higher crop loads will be more profitable to the producer as long as tree health and future yield are not compromised. It is not certain to what extent the yield could have been increased even further before incurring significant negative effects on growth, production and fruit quality. Although fruit size decreased significantly with an increase in fruit number per tree, the percentage fruit that were too small for export was low and did not have a major effect on the yield of exportable fruit. Hence, the yield potential of this particular orchard seems to be even higher than the exceptional yields already attained. It is necessary to emphasize that these results are orchard specific and that different results may be attained for cultivars that are more prone to alternate bearing or with different quality standards. However, the fundamental idea remains that the highest class one yields in 'Cripps' Pink' are attained at higher crop loads. This is because the gain in yield more than make up for any decrease in fruit quality and because 'Cripps' Pink' does not appear to be prone to alternate bearing at high crop loads. The trial will be repeated for a third season and the cumulative results of the three seasons subjected to a financial assessment.

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