PREHARVEST MANIPULATION OF RIND PIGMENTS OF *CITRUS* SPP.

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Master of Science in Agriculture at the University of Stellenbosch.

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DECLARATION

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

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Signature Date

SUMMARY

Rind colour is one of the main cosmetic preferences consumers use when purchasing citrus (*Citrus* spp.) fruit. To enhance the cosmetic quality of citrus fruit, attempts were made to stimulate preharvest chlorophyll degradation and carotenoid biosynthesis to obtain a deeper, more uniform, orange rind colour in early-maturing citrus cultivars. As part of a larger study to stimulate rind colour enhancement, an initial study was conducted on 'Eureka' lemon [*C. limon* (L.) Burm. f.] nursery trees to determine the concentration of various gibberellin biosynthesis inhibitors required to obtain a biological response in citrus trees, as measured by vegetative growth. Thereafter, different concentrations of prohexadione-calcium (ProCa; Regalis®) were applied at various stages of fruit development on early-maturing citrus cultivars to establish the concentration and timing of ProCa required to improve rind colour by enhancing chlorophyll degradation and carotenoid biosynthesis. In addition, a search to enhance rind colour development of early-maturing citrus cultivars was conducted by screening various nutritional, hormonal and possible physiological stress-inducer products and some combination treatments thereof.

Multiple applications of gibberellin biosynthesis inhibitors on 'Eureka' lemon nursery trees significantly reduced internode length and hence vegetative growth. Regalis® applied at 4 to 8 $g \cdot L^{-1}$ and Sunny® (uniconazole) applied at 10 to 20 $mL \cdot L^{-1}$ had the greatest effect in reducing internode length, and were therefore identified as potential candidates for further field studies to test their effect on rind colour enhancement of citrus fruit.

The late, double applications (6 plus 3 weeks before anticipated harvest) of ProCa applied at 400 $mg \cdot L^{-1}$ consistently improved rind colour of all *Citrus* spp. tested. However, these effects

were more pronounced after harvest, as ethylene degreening and cold-storage stimulated additional chlorophyll degradation, unmasking the carotenoids, resulting in overall better coloured fruit. In most instances in this study, ProCa stimulated chlorophyll degradation allowing the underlying carotenoids to be expressed. Therefore, the improvement of rind colour of citrus fruit following the application of a gibberellin biosynthesis inhibitor (400 $mg \cdot L^{-1}$ ProCa applied 6 plus 3 weeks before harvest) supports the hypothesis that there may be a relationship between vegetative vigour and rind colour development of citrus fruit.

Preharvest applications of boric acid, Thiovit® (elemental sulphur), ammonium thiosulphate (ATS) and half the recommended rate of Ethrel® (48% ethephon) in combination with Thiovit® and ATS stimulated chlorophyll degradation in both orange- and yellow-rinded fruit, and ColourUp® (neutralised calcium carbonate) and Figaron® (ethyclozate) stimulated chlorophyll degradation only in orange-rinded fruit. Boric acid and the Thiovit®-ATS-Ethrel® combination treatment stimulated carotenoid biosynthesis in orange-rinded fruit, thereby improving the carotenoid to chlorophyll ratio. The screening of chemical products which stimulate chlorophyll degradation in combination with chemical products which stimulate carotenoid biosynthesis warrants further evaluation.

Worldwide, research on rind colour improvement has received attention for several decades, particularly during the 1980s. Yet, rind colour still remains a problem at the beginning of certain seasons. In the present study, the approach to improving rind colour was to manipulate rind pigments through the reduction of vegetative vigour, which was hypothesised to be an antagonist of chloro-chromoplast transformation. To this end, the preharvest application of prohexadione-calcium stimulated chlorophyll degradation and carotenoid biosynthesis in citrus fruit rinds. Furthermore, preharvest applications of various chemical products provides

a novel approach to stimulate chlorophyll degradation and carotenoid biosynthesis. Together, the results of this study provide potential commercial treatments that will result in deeper, more uniform orange rind colour, thereby meeting consumer needs.

OPSOMMING

Vooroes manipulasie van skil pigmente van Citrus *spp.*

Skilkleur is een van die hoof kosmetiese voorkeure wat verbruikers in ag neem wanneer sitrusvrugte (*Citrus* spp.) gekoop word. Om die kosmetiese kwaliteit van sitrusvrugte te verbeter, is daar gepoog om voor-oes chlorofildegradasie en karotenoïedbiosintese te stimuleer om ’n dieper, meer egalige oranje skilkleur in vroë rypwordende sitruskultivars te verkry. As deel van ’n groter studie om skilkleurverbetering te stimuleer, is ’n aanvanklike studie uitgevoer op ‘Eureka’ suurlemoen [*C. limon* (L.) Burm. f.] kwekerybome. Hierdie studie bepaal die konsentrasie van verskeie gibberellienbiosinteseinhibeerders benodig om ’n biologiese reaksie in sitrusbome te verkry. Dit word gedoen deur vegetatiewe groei te meet. Daarna is verskillende konsentrasies proheksadioon-kalsium (ProCa; Regalis®) toegedien tydens verskeie stadiums van vrugontwikkeling van vroeë rypwordende sitruskultivars, sodat die konsentrasie asook die tyd van toediening van ProCa, benodig om skilkleur te verbeter, deur die bevordering van chlorofildegradasie en karotenoïedbiosintese vasgestel kon word. Verder is daar gepoog om die skilkleur van vroeë rypwordende sitruskultivars te verbeter deur verskeie voedingstowwe, hormonale en waarskynlik fisiologiese stresuitlokker produkte en kombinasies daarvan te keur.

Meervoudige toedienings van gibberellienbiosinteseinhibeerders op ‘Eureka’ suurlemoen kwekerybome, het internode lengte betekenisvol verkort en gevolglik vegetatiewe groei verminder. Regalis® toegedien teen 4 tot 8 $g \cdot L^{-1}$ en Sunny® (uniconazole) toegedien teen 10 tot 20 $mL \cdot L^{-1}$, het die grootste effek op verkorting van internode lengte gehad, en was daarom geïdentifiseer as potensiële kandidate vir verdere veldstudie, om hul effekte op skilkleurverbetering van sitrusvrugte te toets.

Die laat, dubbele toediening (6 plus 3 weke voor verwagte oes) van ProCa, aangewend teen 400 $mg \cdot L^{-1}$, het gereeld die skilkleur van alle *Citrus* spp. wat getoets is, verbeter. Hierdie effekte was egter duideliker na-oes, want etileenontgroening en koue-opberging stimuleer addisionele chlorofildegradasie en ontmasker die karotenoïedes, wat 'n algehele verbetering in vrugkleur tot gevolg het. In die meeste gevalle het die ProCa chlorofildegradasie gestimuleer, wat dan toegelaat het dat die onderliggende karotenoïedes uitgedruk word. Die verbetering van die skilkleur van sitrusvrugte, ná die toediening van 'n gibberellienbiosinteseinhibeerder (400 $mg \cdot L^{-1}$ ProCa toegedien 6 plus 3 weke voor oes), ondersteun die hipotese dat daar dalk 'n verwantskap tussen die vegetatiewe groeikrag en skilkleurontwikkelling van sitrusvrugte is.

Voor-oes toedienings van borigsuur, Thiovit® (elementele sulfaat), ammoniumtiosulfaat (ATS) en die helfde van aanbevole standaard van Ethrel® (48% ethephon) in kombinasie met Thiovit® en ATS het chlorofildegradasie in oranje- sowel as geel-skilvrugte gestimuleer, en ColourUp® (genutraliseerde kalsium karbonaat) en Figaron® (ethyclozate) het chlorofildegradasie net in oranje-skilvrugte gestimuleer. Borigsuur en die Thiovit®-ATS-Ethrel® kombinasie behandeling het karotinoïedbiosintese in oranje-skilvrugte gestimuleer, en het op die manier die verbetering van die karotinoïed tot chlorofil verhouding bewerkstellig. Die keuring van chemiese produkte wat chlorofildegradasie stimuleer, in kombinasie met chemiese produkte wat karotenoïedbiosintese stimuleer, regverdig verdere evaluasie.

Wêreldwye navorsing op skilkleurverbetering was die afgelope dekades baie belangrik, en het veral in die 1980's aandag geniet. Tog bly skilkleur steeds 'n probleem aan die begin van sekere seisoene. Die benadering wat in die huidige studie gevolg is om skilkleur te verbeter,

was om skilpigmente te manipuleer, deur vegetatiewe groeikrag te verminder, wat veronderstel word om antagonisties tot die chloro-chromoplast transformasie te wees. Ter samevatting, stimuleer die voor-oes toediening van proheksadioon-kalsium chlorofildegradasie asook karotenoïedbiosintese in die skil van sitrusvrugte. Verder voorsien voor-oes toedienings van verskeie chemiese produkte 'n nuwe benadering tot die stimulering van chlorofildegredasie en karotenoïedbiosintese. Die gesamentlike resultate van hierdie studie voorsien potensiële kommersiële behandelings wat 'n dieper, meer egalige oranje skilkleur tot gevolg sal hê. Hierdeur word daar voorsien aan verbruikersbehoeftes.

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TABLE OF CONTENTS

Language and style used in this thesis are in accordance with the requirements of the scientific journals of the *American Society for Horticultural Science*. This thesis presents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

Rind colour is an important cosmetic preference of consumers when purchasing citrus (*Citrus* spp.) fruit. In general, consumers prefer a deep orange-coloured fruit (Krajewski, 1996). As citrus fruit mature, changes in rind colour are due to decreased chlorophyll and increased carotenoid concentrations (Goldschmidt, 1988).

Senescence of chlorophyllous tissue in the flavedo of citrus rind results in the degradation of chlorophyll followed by carotenoid biosynthesis and the transformation of chloroplasts into chromoplasts. Chloro-chromoplast transformation is a major physiological response affected by environmental, nutritional and hormonal factors (Goldschmidt, 1988). Before the onset of carotenoid biosynthesis, carotenoid concentration undergoes a “trough” which marks the formation of intensely coloured chromoplast carotenoids from carotenoids of photosynthetic plastids. This transition coincides with the decline in chlorophyll concentration in the flavedo (Eilati et al., 1969b). Chloroplast-chromoplast transformation in early-maturing sweet orange [*C. sinensis* (L.) Osbeck] and mandarin (*C. reticulata* Blanco) cultivars is often less than ideal because of unsuitable environmental conditions during fruit maturation.

Previously, various experimental and commercial preharvest techniques to enhance rind colour have been used, e.g. reduction in late N applications (Koo, 1988), increasing within-tree light intensities (Sites and Reitz, 1949), decreasing irrigation before the maturation phase (Huff et al., 1981), ethylene applications after colour break (Purvis, 1980), ethyclozate applications at the start of stage II of fruit development (Kamuro and Hirai, 1981), applications of paclobutrazol before the summer flush (Gilfillan and Lowe, 1985) and

prohexadione-calcium (ProCa) applications at colour break (Barry and Van Wyk, 2004). However, rind colour still remains a problem at the beginning of certain seasons.

Chlorophyll degradation coincides with a decrease in night and soil temperatures to below 13 °C and 12 °C, respectively, during the fruit maturation phase (Young and Erickson, 1961). The possibility exists that cool night and soil temperatures do not stimulate rind colour formation directly, but rather slows vegetative growth which, in turn, is antagonistic to the conversion of chloroplasts to chromoplasts (Goldschmidt, 1988). High endogenous gibberellin concentrations in plants are known to enhance stem elongation (Salisbury and Ross, 1992), and gibberellins are associated with vegetative vigour. Therefore, by moderating vegetative vigour through the use of growth retardants, and thereby reducing invigorating growing conditions, chloroplast-chromoplast transformation could be enhanced (Goldschmidt, 1988).

Prohexadione-calcium (3-oxido-4propionyl-5-oxo-3-cyclohexene-carboxylate) traded as Regalis® and Apogee® is used on pome fruit trees (*Malus* and *Pyrus* spp.) to reduce and control vegetative growth (Miller, 2002; Rademacher, 2001). Prohexadione-calcium acts primarily as a gibberellin biosynthesis inhibitor, especially 3β-hydroxylation of GA_{20} to GA_1 (Nakayama et al., 1992). Costa et al. (2001) found that repeated applications of 100 $mg \cdot L^{-1}$ ProCa significantly reduced shoot growth and increased fruit size in pears (*P. communis* L.).

Furthermore, rind colour of citrus fruit can be enhanced through preharvest applications of various chemical products. A search for such products to enhance rind colour development was subdivided into nutritional, hormonal and physiological stress-inducer products. Of the nutritional products tested, boric acid could act on improving rind colour possibly by

increasing the indole acetic acid (IAA)/cytokinin ratio (Puzina, 2004). ColourUp® (neutralised calcium carbonate) improved rind colour of 'Palmer Navel' orange under South African conditions (Barry, 2005). Carotenol® (hydrocarbon substances) allegedly improved citrus rind colour in Spain, by stimulating chlorophyll degradation (Lida Quimica, 2006). Of the hormonal products tested, ethyclozate, a synthetic auxin, enhanced rind colour of 'Satsuma' mandarin (*C. unshiu* Marc.) in Japan by decreasing chlorophyll concentration and increasing carotenoid concentration (Kamuro and Hirai, 1981; Tominaga and Diato, 1981). It is also known that ethephon (2-chloroethylphosphonic acid) improves rind colour of citrus fruit (El-Otmani et al., 1996; El-Zeftawi and Garrett, 1978). Ammonium thiosulphate (ATS), a desiccant used for fruit thinning in apples (*M. domestica* Borkh.), could trigger endogenous ethylene evolution through the induction of physiological stress, thereby stimulating improved rind colour during the maturation phase of fruit development.

The overall objective of this study was to stimulate preharvest chlorophyll degradation and carotenoid biosynthesis to obtain a deeper, more uniform, orange rind colour. To determine the concentration of various gibberellin biosynthesis inhibitors necessary to get a vegetative growth response in citrus trees, nursery trees were sprayed with various concentrations of these inhibitors. To trigger chloroplast-chromoplast transformation, trees were sprayed with a gibberellin biosynthesis inhibitor, to reduce the "gibberellin load" in the aerial portion of trees, which is antagonistic to rind colour development. Screening of various nutritional, hormonal and possible physiological stress-inducer products was also conducted.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Flavedo anatomy

Citrus fruit is botanically classified as a 'hesperidium' berry, arising from ovary development which consists of approximately ten united carpels positioned around and joined at the floral axis, and surrounded by a tough leathery rind, the pericarp (Bain, 1958; Schneider, 1968).

The flavedo of the citrus rind, or exocarp, occupies the outer tissue layer of fruit, and consists of a cuticle-covered epidermis, a hypodermis and a subepidermal layer (Fig. 2.1) (Spiegel-Roy and Goldschmidt, 1996). Embedded within the compactly arranged parenchyma cells of the subepidermis are schyzolysogenic oil glands containing essential oils. The exocarp seems to be derived from the abaxial surface of the carpel primordia (Schneider, 1968), and resembles a modified leaf. The flavedo, with its epidermal and parenchyma cells, are coloured in citrus rind (Lima and Davies, 1984). During the early stages of fruit development, the flavedo is dark green and photosynthetically active with a small number of stomata (20-40 mm^2). As fruit mature, chlorophyll is gradually degraded and carotenoid rich chromoplasts are formed (Goldschmidt, 1988).

2.1.1 Epidermis

Cuticle covered epidermal cells form a continuous layer on the surface of citrus fruit (Fig. 2.1). These cells, which contain pigmented flavoproteins, are usually tabular in shape because of their relatively small depth. This layer of cells imparts mechanical protection to the fruit and restricts transpiration, but does not contribute significantly to photosynthesis nor to rind

colour (Esau, 1965). Randomly occurring guard cells, accessory cells, and oil gland-cover cells occur among the epidermal cells.

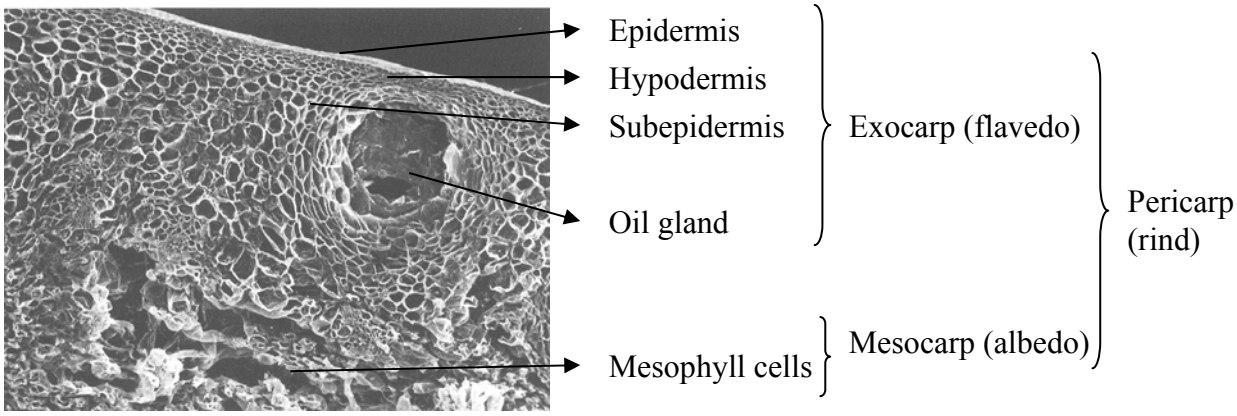


Fig. 2.1. Cross section SEM (x66) photograph of senescent 'Murcott" tangor fruit (Spiegel-Roy and Goldschmidt, 1996).

2.1.2 Hypodermis

The hypodermis is composed of thick-walled parenchyma cells, which increase in size centripetally from the epidermis. Esau (1965) found that hypodermal cells are morphologically and physiologically distinct from the subepidermal cells in the deeper-lying ground tissue (Fig. 2.1). Furthermore, these parenchyma cells contain chloroplasts and later on chromoplasts which give immature fruit their green colour or mature fruit their yellow to orange colour, respectively (Kubo and Hiratsuka, 1999; Spiegel-Roy and Goldschmidt, 1996).

2.1.3 Plastids

Plastids are double membrane-bound organelles that are unusual to plant, fungi and certain bacteria cells (Esau, 1965; Salisbury and Ross, 1992). Plastids constitute an extremely versatile and multifunctional organelle. In green tissue, plastids have developed as chloroplasts, in coloured fruit and flower petals as chromoplasts, and in storage organs as

colourless amyloplasts (Mauseth, 1988). All plastids develop from proplastids, which derived from the unfertilised egg cell, found in plants growing in both light and dark (Esau, 1965; Mauseth, 1988; Salisbury and Ross, 1992)

The coloured plastids, chloroplasts and chromoplasts, are present in most plants, and contain chlorophyll and carotenoids plus other coloured pigments, respectively (Esau, 1965; Salisbury and Ross, 1992). Chloroplast development from proplastids, is triggered by the exposure of the proplastids to light, whereafter enzymes formed or imported from the cytosol to inside the proplastid, give rise to light-absorbing pigments (chlorophyll) (Mauseth, 1988; Taiz and Zeiger, 2002). Chromoplasts can be found in all higher plants (Esau, 1965; Harberlandt, 1965). Rosso (1968), and Spurr and Harris (1968) showed that chromoplasts originate from fully developed chloroplasts. However, Frey-Wyssling and Schwegler (1965) showed that chromoplasts may be differentiated from non-photosynthetic plastids such as amyloplasts. Additionally, Boyer (1989) stated that chromoplasts can develop from undifferentiated proplastids. Conversely, chromoplasts can redifferentiate when subjected to nutritional stress and illumination, for instance citrus regreening (Mayfield and Huff, 1986).

The differentiation of chloroplasts into chromoplasts is necessary for colour expression in the rind of citrus fruit (Spiegel-Roy and Goldschmidt, 1996), as colour expression is the temporal change in chloroplast ultrastructure and metabolism of both chlorophylls and carotenoids (Gross et al., 1983). At colour break, thylakoid degeneration and the transition of chloroplasts to chromoplasts is accompanied by an initial decrease in carotenogenesis and enhanced chlorophyll degradation (Gross et al., 1983). However, the decline in rind chlorophyll takes several months, and the onset of carotenoid accumulation coincides with a disappearance in chlorophyll (Eilati et al., 1969b).

2.2 Chlorophyll

2.2.1 Structure

Synthesis of the chlorophyll molecule takes place in the C5 pathway from the intact carbon skeleton of the amino acid glutamate (Mauzerall, 1977; Reinbothe and Reinbothe, 1996). With a Mg atom occupying the center of the chlorophyll molecule, four pyrrole rings are ligated into a tetrapyrrole ring. Two types of chlorophyll can be classified, one containing a methyl group at position three on the structure (chlorophyll *a*), and the other containing a formyl group instead of a methyl group on position three (chlorophyll *b*) (Figure 2.2). Chlorophyll is a lipophilic molecule which is always associated with intra-cellular membranes, highly insoluble in water (Jones, 1973; Mauzerall, 1977).

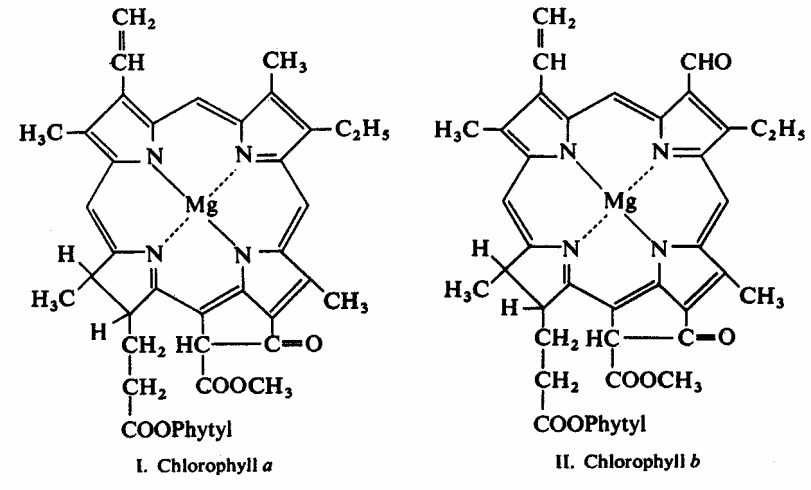


Fig. 2.2. Schematic illustration of chlorophyll *a* and chlorophyll *b* molecules with a Mg atom occupying the centre of the molecule (Jones, 1973).

2.2.2 Location

Chlorophyll molecules are located in the gel-like stroma in thylakoids of chloroplasts (Salisbury and Ross, 1992).

2.2.3 Function

Chlorophyll is actively involved in light absorption and energy transduction during photosynthesis to convert solar energy, or photons, into chemical energy (Mauzerall, 1977; Reinbothe and Reinbothe, 1996). Energy from light is used to oxidise H_2O and form energy-rich adenosine triphosphate (ATP) and nicotineamide adenine dinucleotide phosphate ($NADP^+$) needed by the stroma to convert CO_2 into needed carbohydrates. Chlorophyll molecules capture photons and rapidly transfer the excitation caused to the reaction centre, from where this excitation is transferred to secondary donors and acceptors where excited (light energy) is converted to chemical energy (Mauzerall, 1977).

2.2.4 Degradation

Chlorophyll can be degraded in two pathways, viz. catalysed by chlorophyllase or dechelation of Mg^{2+} into pheophytin, catalysed by Mg-dechelatase. Initially it was believed that the only enzyme capable of cleaving chlorophyll into phytol and Chl-ide was chlorophyllase, the Mg-porphyrin moiety of chlorophyll (Matile et al., 1996). Only recently, because they are colourless Chl-ide breakdown products were identified. Chlorophyll breakdown into phytol, Mg^{2+} and primary cleavage products occurs in three steps, catalyzed by chlorophyllase, Mg-dechelatase and pheophorbide *a* oxygenase. In this process the third step is the most important, where the porphyrin macrocycle is associated with the loss of green colour (Matile et al., 1996).

Chlorophyllase also seems to be regulated at a transcriptional level, with *Chlase1* cDNA gene encoding an active chlorophyllase enzyme which catalyses the dephytylation of chlorophyll. This *Chlase1* gene was obtained from 'Valencia' orange [*Citrus sinensis* (L.) Osbeck] by RT-

PCR using degenerate primers based on the amino acid sequence of the previously purified protein (Jacob-Wilk et al., 1999).

2.3 Carotenoids

2.3.1 Structure

Carotenoids are tetraterpenes consisting of eight isoprenoid units, and are normally yellow, orange and red pigments (Bramley et al., 1993). Carotenoids, which are contained within chromoplasts, are divided into two types, viz. carotenes and xanthophylls. The most abundant carotene found in nature is β-carotene (Fig. 2.3). Carotenes are pure hydrocarbons whereas xanthophylls contain additional oxygen molecules. Both carotenoid types normally consist of 40 C atoms made from eight isoprene units (Salisbury and Ross, 1992). The most recognisable part of all carotenoids is the polyene chain, which can contain up to 15 conjugated double bonds; the length of the chromophore determines the adsorption spectrum of the molecule, and hence the colour. The ability of carotenoids to adsorp light is used experimentally to identify and quantify carotenoid concentrations (Britton, 1985). Carotenoids are not water soluble, but they dissolve readily in alcohols, ether and acetone.

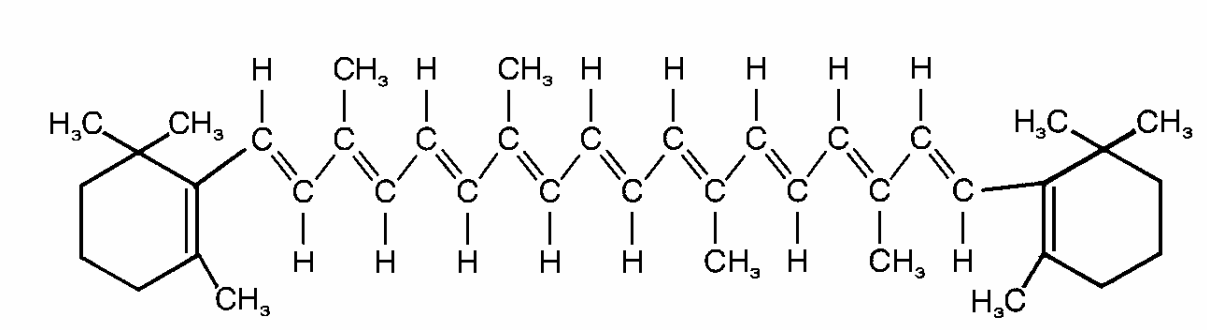


Fig. 2.3. Schematic illustration of β-carotene with an empirical formula of $C_{40}H_{56}$ (Salisbury and Ross, 1992).

2.3.2 Location

Carotenoids are present in chloroplasts of active leaves and fruit, as well as in leaves not active in photosynthesis and in chromoplasts (Goodwin, 1973). In photosynthetically active cells, carotenoids are found in the thylakoid membranes forming part of the photosynthetic pigment protein complex. The chloroplast envelope also contains a small amount of carotenoids as well as the envelope of some amyloplasts (Fishwick and Wright, 1980). Goodwin (1958) also found some carotenoids in plastoglobuli of photosynthetic tissue, while etiolated plants contain carotenoids in their etioplasts.

2.3.3 Function

Except for the attractive colour that carotenoids provide to fruit and flowers, carotenoids aid in indirect seed distribution, photosynthesis and protection of photosynthetic tissue against photosensitised oxidation (Harberlandt, 1965; Stanier and Cohen-Bazire, 1957). All these functions relate to the ability of carotenoids to absorb visible light. In photosynthetic tissue there are mainly two functions of carotenoids, viz. to photosynthesise and to aid in protecting photosynthetic tissue against photosensitised oxidation. Carotenoids also aid in the photoprotection of non-photosynthetic tissue (Goodwin, 1980).

By measuring the enhancement of fluorescence of chlorophyll on illumination of tissue with absorbed wavelengths, the effective participation of carotenoids in photosynthesis has been demonstrated. Carotenoids seem to be present in both photosystem I and II (Goodwin, 1980). Each photosynthetic core complex one (CCI) contains one β-carotene molecule per 40 chlorophyll *a* molecules. The light harvesting complex one (LHCI) on the other hand is associated with lutein, violaxanthin and neoxanthin. The CCII complex is also hosting some β-carotene while the LHCII contains xanthophylls (Lichtenthaler et al., 1982).

Photoprotection was first demonstrated by Stanier and Cohen-Bazire (1957), when they found that *Rhodopseudomonas sphaeroides* lacking carotenoids was killed by different light combinations. In non-photosynthetic tissue, carotenoids protect membranes against photodynamic killing (Goodwin, 1980). In photosynthesising tissue, carotenoids protect chloroplasts from losing the 90S ribosomes, the site where chloroplast proteins are synthesised (Walles, 1972). The carotenoid photoprotection mechanism involves the quenching of singlet oxygen [$^1O_2$] (Goodwin, 1980).

2.3.4 Biosynthesis

Carotenoids share a common early metabolic pathway with other important isoprenoids such as sterols, gibberellins and terpenoid quinones (Fig. 2.4). This pathway starts with the formation of phytoene from the conversion of geranylgeranyl diphosphate (GGDP) (Kleinig, 1989). The head to head condensation of all-*E* GGDP via the cyclopropylcarbinyl diphosphate, prephytoene diphosphate (PPDP) is the first unique step in carotenoid biosynthesis. Depending on the hydrogen atom removal from the PPDP either 15-*Z* or all-*E* phytoene can be formed.

Plant cell extracts are capable of forming phytoene from radioactive precursors such as mevalonic acid (MVA), isopentenyl diphosphate (IDP) and GGDP, but in order to do this a cofactor is needed. This cofactor might be ATP, different pyridines, flavins or a divalent cation such as Mn^{2+} (Clarke et al., 1982). Dogbo et al. (1988) supported the findings that Mn^{2+} is needed, but no other cofactors. A two-step kinetically coupled reaction is catalysed by this enzyme, with inorganic phosphate inhibiting this reaction. Phytoene synthase is located in the stroma of chloroplasts, chromoplasts and amyloplasts, with non-photosynthetic plastids

having the highest activity (Dogbo et al., 1987). Camara (1984) reported that proteins are synthesized on 80S ribosomes prior to post-translational processing and entry into the plastids.

Phytoene is then dehydrogenised, forming double bonds, converting colourless phytoene into yellow, orange and red carotenoids. Significant differences in carotenoid composition are found between plant species, but β-carotene and lutein seems to be in the order of 25% and 45%, respectively, in most plants (Goodwin and Britton, 1988). β-Cryptoxanthin, 9-*Z*-Violaxanthin, β-citraurin together with phytofluene and ζ-carotene are also known to accumulate in citrus rind (Baldwin, 1993; Oberholster et al., 2001).

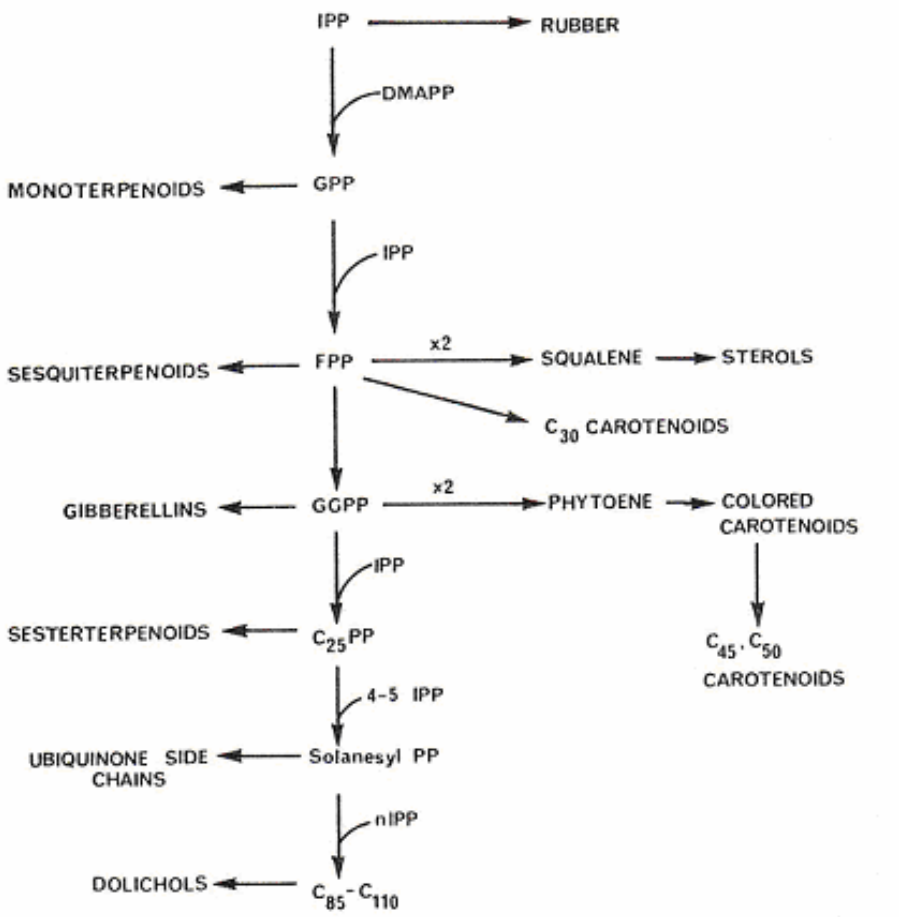


Fig. 2.4. Schematic illustration of the isoprenoid biosynthesis pathway (Bramley et al., 1993).

Lois et al. (2000) concluded that carotenoid biosynthesis is controlled by gene expression with an increased concentration of 1-deoxy-D-xylulose 5-phosphate during fruit maturation. Phytoene synthase also increased during the maturation process (Giuliano et al., 1993). In-Jung et al. (2001) suggested that the expression of *Chx*, a carotenogenesis regulatory enzyme, does not contribute to changes of carotenoid biosynthesis in ripening fruit at the transcriptional level. Stroma of chromoplasts, chloroplasts and amyloplasts are known to host this process of phytoene synthase (Dogbo et al., 1987). Thomas and Jen (1975) reported that carotenoid biosynthesis was stimulated by red light but inhibited by far red light.

2.4 Fruit morphology and development

Bain (1958) subdivided citrus fruit development into stages I, II and III (Fig. 2.5). These stages seem appropriate for most citrus types although the actual times and duration of development may vary according to the different climatic conditions and cultivars. Stage I of fruit development is the cell division stage, stage II is characterised by fruit growth due to cell expansion, and stage III is the fruit maturation stage, including rind colour development (Bain, 1958).

During stage III of fruit development, chlorophyll content in the rind of citrus fruit decreases due to senescence of chlorophyllous tissue in the flavedo resulting in the transformation of chloroplasts into chromoplasts (Fig. 2.6) (Goldschmidt, 1988). Before the onset of carotenoid accumulation, carotenoid concentration undergoes a “trough” which marks the formation of intensely coloured chromoplast carotenoids from carotenoids of photosynthetic plastids. This change in carotenoid concentration coincides with the decline in flavedo chlorophyll (Eilati et al., 1969b).

At a given time, fruit on the same tree are not at the same stage of maturity or colour development, viz. inside fruit being green to yellow, partly shaded fruit being yellow and full sunlight exposed fruit being orange (Sites and Reitz, 1949, 1950). Farin et al. (1983) quantified the amount of specific carotenoids present during various stages of fruit maturation of 'Michal' mandarin (*C. reticulata* L.). They found carotenoids, viz. lutein, *trans*-violaxanthin, *cis*-violaxanthin, *trans*-neoxanthin and α-carotene being in highest percentages in green fruit, *β*-citraurin, lutein, *trans*-violaxanthin and *cis*-violaxanthin were the highest at colour break, and *β*-citraurin and *cis*-violaxanthin were the highest in mature fruit. Oberholster et al. (2001) also reported (9*Z*)-violaxanthin and *β*-citraurin when they analysed fully coloured 'Valencia' orange fruit.

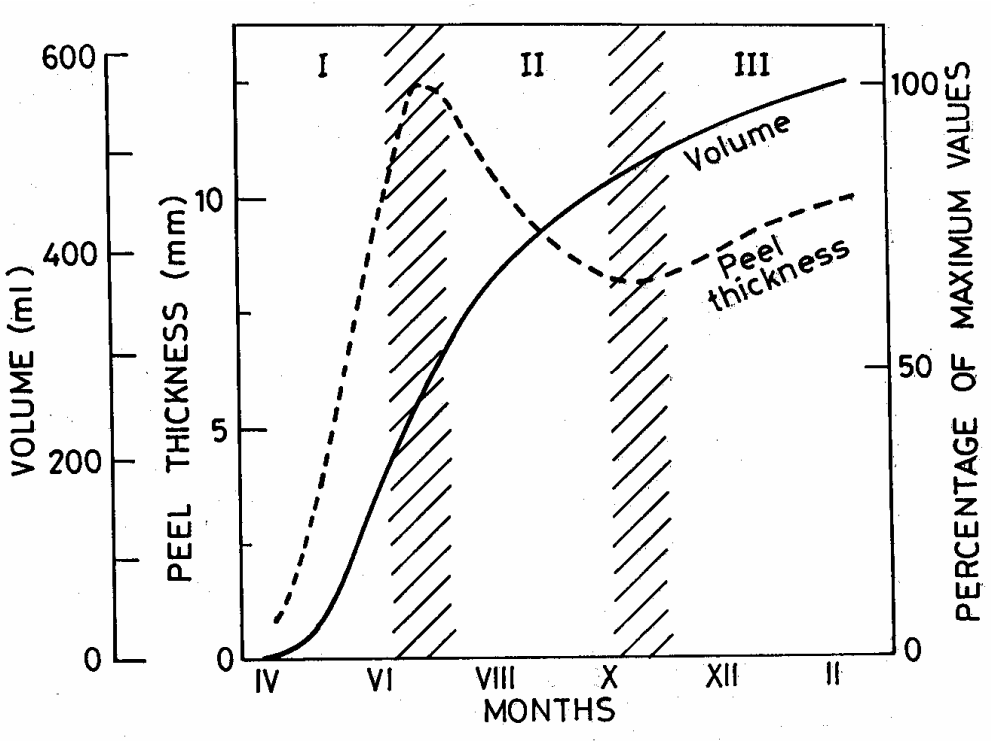


Fig. 2.5. Fruit growth and development of 'Valencia' orange. Stages I, II and III refer to the three developmental stages of citrus according to Bain (1958), adapted by Spiegel-Roy and Goldschmidt (1996) to northern hemisphere countries.

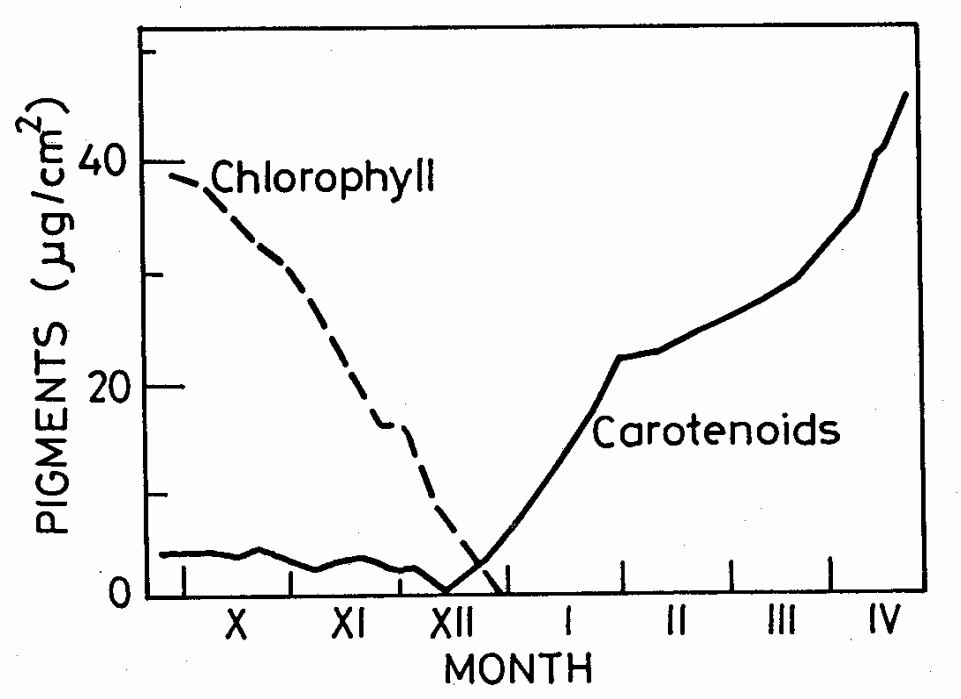


Fig. 2.6. Carotenoid changes during maturation of 'Shamouti' orange in Israel (Spiegel-Roy and Goldschmidt, 1996).

2.5 Factors affecting carotenoid biosynthesis

Chloro-chromoplast transformation is a major physiological response affected by various environmental, nutritional and hormonal factors. Citrus rind colour formation is inhibited when generally high root temperatures stimulate root formation which, in turn, stimulates the formation of root hormones (gibberellins and cytokinins) which inhibit chlorophyll degradation and fruit senescence. This root activity also allows the uptake and transport of nitrogenous compounds to the fruit, also inhibiting chlorophyll degradation (Fig. 2.7) (Goldschmidt, 1988). Various factors affect carotenoid biosynthesis, including environmental, nutritional and hormonal, viz. temperature, carbohydrates, ethylene and to some extent auxins and vegetative growth inhibitors. These factors are reviewed in this section and summarised in Table 2.3 at the end of this section.

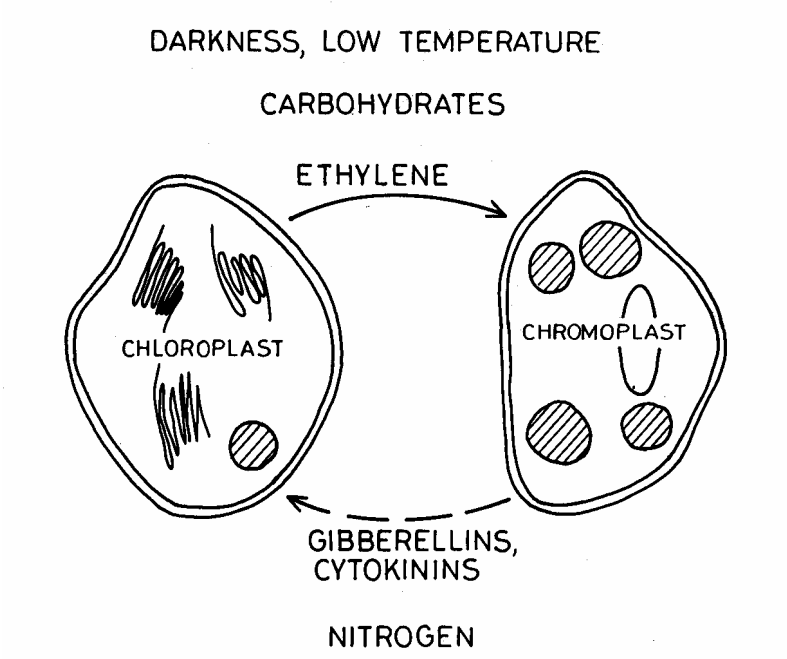


Fig. 2.7. Factors affecting chloroplast-chromoplast transformation (Spiegel-Roy and Goldschmidt, 1996).

2.5.1 Genetic

2.5.1.1 Scion selection

Genetics plays an overriding role in the composition of different carotenoids found in the flavedo of citrus fruit. Cultivars commercially cultivated in South Africa with a yellow flavedo are grapefruit (*C. paradisi* Macf.), pummelo (*C. grandis* Osbeck), lemon [*C. limon* (L.) Burm. f.] and lime (*C. aurantifolia* Christm.), and all originated from tropical areas (Scora, 1975). In 'Marsh' grapefruit, the colourless carotenoids phytoene and phytofluene contribute up to 74% of the total carotenoid observed at the ultraviolet wavelength (400-325 nm). This accumulation of the colourless carotenoids is a result of genetic blockages hindering further dehydrogenation steps leading to coloured carotenoids. In 'Star Ruby' grapefruit, however, this blockage has been overcome, and production of lycopene and β-carotene is possible (Yokoyama and Keithley, 1991).

Orange-coloured citrus cultivars are sweet orange [*C. sinensis* (L.) Osbeck], sour orange (*C. aurantium* L.) and mandarin (*C. reticulata* Blanco). These *Citrus* spp. originated in subtropical regions (Scora, 1975). These orange-rinded *Citrus* spp. contain relatively large amounts of complex carotenoid mixtures, including cryptoxanthin and β-citraurin, which are present in relatively small amounts, but have a high tinctorial value (Lee and Castle, 2001; Molnar and Szabolcs, 1980).

2.5.1.2 Rootstock selection

Rootstocks can be classified into three groups, viz. invigorating, moderately invigorating and non-invigorating rootstocks (CRI, 1995). Rough lemon (*C. jambhiri* Lush) and 'Volkameriana' lemon (*C. volkameriana* Ten. and Pasq.) are invigorating rootstocks whereas 'Carizzo' and 'Troyer' citranges (*C. sinensis* x *Poncirus trifoliata* L. Raf.) and 'Swingle' citrumelo (*C. paradisi* x *P. trifoliata*) are classified as moderately invigorating rootstocks (Saunt, 2000). The only true dwarfing rootstock is 'Flying Dragon', probably a strain of trifoliate orange (*P. trifoliata*), or possibly a hybrid of unknown parentage, results in trees not higher than 2 m after 9 years of growth (CRI, 1995; Saunt, 2000).

Rootstock vigour affects rind colour development of the fruit of scions budded onto the rootstock. For example, fruit from scions budded on rough lemon rootstock had medium-late colour development, whereas 'Troyer' citrange rootstock resulted in 8 to 10 days earlier rind colour development compared with rough lemon, and scions on 'Swingle' citrumelo have delayed rind colour development (CRI, 1995). Young vigorously growing roots, beside other organs, are a major site for the biosynthesis of gibberellins and cytokinins, which are antagonistic to rind colour development, and are subsequently transported to the aerial portion of the tree via the xylem (Saidha et al., 1983). Vigorous rootstocks also have a higher

hydraulic conductivity, allowing more water and mineral nutrients, e.g. N, to be transported to leaves and fruit (Syvertsen, 1981).

By combining these findings the hypothesis can be drawn that factors promoting invigorating conditions lead to poor rind colour development of fruit, and factors promoting non-invigorating conditions lead to acceptable rind colour development of citrus fruit.

2.5.2 Environmental

2.5.2.1 Tree age

Young trees tend to be more vigorous than old, mature trees. Differences in vegetative vigour is thought to be the main reason why young trees have poorer colour development compared with older less vigorous trees (Krajewski, 1997). Rind colour development is also adversely affected by vegetative growth flushes during stage III of fruit development. Such flushes are more common in young trees of vigorous rootstock-scion combinations (Krajewski, 1997).

2.5.2.2 Soil type

Citrus trees can be planted in a wide range of soil types. Soil types with a high clay content also have a higher ‘cation exchange capacity’ and higher ‘soil water capacity’ than sandy soils, and have the ability to supply N and other essential nutrients until late in the season (Stassen et al., 1999). Excess supply of N can result in too high N concentrations in the tree causing excessive late vegetative growth resulting in poor rind colour development (Reitz and Koo, 1960).

2.5.2.3 Weather conditions

Prevailing weather conditions during fruit development is the primary factor affecting citrus fruit quality. Citrus is mainly produced at latitudes of 20 to 40° north and south of the equator with the subtropics producing fruit with a better rind quality compared with the tropics (Reuther, 1988). Rind colour development is largely dependent on weather conditions, principally temperature, during stage III of fruit development (Caprio, 1956).

2.5.2.3.1 Temperature

Citrus production regions where the average temperature remains high all year (e.g. lowland tropical regions) produce fruit with a higher chlorophyll content (greener fruit), compared to production regions where the air and soil temperatures drop below 13 ºC during autumn (Caprio, 1956). Stearns and Young (1942) claimed that there is no sudden change in rind colour, and that colour formation is rather an acceleration in the change from dark-green to yellow-green colour. This acceleration, however, coincides with a cold period such as the occurrence of a cold front.

Chlorophyll degradation coincides with a drop in night air temperature to below 13 ºC during the maturation phase. This chlorophyll degradation, however, depends on cultivar and the duration of low temperature. Additionally, Young and Erickson (1961) found that rind colour development occurs faster when soil temperatures drop below 12 ºC, and this drop in soil temperature aided in chlorophyll degradation. However, no correlation was found between carotenoid biosynthesis and soil temperature (Coggins et al., 1981), although trends were seen to support the findings of Young and Erickson (1961). Young and Erickson (1961) stipulated that night temperatures below 7 ºC in combination with day temperatures below 20 ºC stimulated xanthophyll accumulation, but no effect was observed on carotene concentration. Meredith and Young (1969) found that ‘Redblush’ grapefruit and ‘Ruby blood’ sweet orange

needed high day/night temperatures (35/30 ºC) for lycopene formation and low day/night temperature (16/5 ºC) for carotenoid formation, respectively. Low temperatures also resulted in increased carotenoid concentrations in 'Redblush' grapefruit. Coggins et al. (1981) reported that when 'Frost Valencia' orange was exposed to low (20/15 ºC) and high (30/15 ºC) day/night air temperatures, the low temperatures resulted in fruit containing higher carotenoid concentrations compared with the high temperatures.

2.5.2.3.2 Light

Carotenoid development in citrus rinds coincide with a decrease in chlorophyll concentration during the fruit maturation stage (Miller et al., 1940). Lewis et al. (1964) concluded that high light intensities are needed for chlorophyll and carotenoid formation. Sites and Reitz (1949) found that fruit on the outside of trees, which were exposed to high light intensities, had more intensely coloured fruit. Similarly De Vries and Bester (1996) reported that fruit in tree tops were significantly more coloured than fruit at the bottom of trees. Boswell et al. (1982) also demonstrated that fruit produced in widely spaced orchards coloured faster and more intensely, possibly due to exposure to higher light intensities. Morales and Davies (2000) also improved rind colour of fruit when they increased the light intensity of 'Orlando' tangelo (*C. paradisi* Macf. x *C. reticulata* Blanco) by pruning. They found that fruit harvested from the bottom of trees had a higher hue angle (73.3 °) compared with fruit from the upper portion of the tree canopy (65.5 °), concluding that the exposed fruit were better coloured. Relative light intensity (RLI) had little effect on rind colour when the RLI was in the region of 40 to 100%, but when the RLI decreased below 40% rind colour decreased drastically (Iwagaki and Kudo, 1977). Additionally, Lewis and Coggins (1964) demonstrated that fruit exposed to 5% of the normal RLI had 42% less carotenoids compared to fruit exposed to normal RLI.

2.5.2.3.3 Water

Water as a factor affecting rind colour is manifested as rainfall or irrigation. Koo and Reese (1977) found that well-irrigated citrus orchards produced fruit higher in chlorophyll and lower in carotenoids than under-irrigated orchards. Huff et al. (1981) also found that trickle irrigation resulted in greener fruit when compared with fruit from flood irrigated trees, which could be caused by better N uptake by roots from trees under trickle irrigation. Peng and Rabe (1996b) showed that normal irrigation, which allowed soil water tension to reach -30 kPa, resulted in greener fruit compared with deficit irrigation, which allowed soil water tension to reach -70 kPa. Norman et al. (1990) concluded that water stress during the fruit maturation stage does not influence the carotenoid content of fruit.

2.5.3 Nutritional

The principal nutrient elements used to fertilise citrus trees with are N, P and K, with N affecting both production and internal (juice quality) and external (rind thickness, texture and rind colour) fruit quality. Other macronutrients and micronutrients do not seem to play any significant role in rind colour development, except when they are severely deficient or in excess (Koo, 1988).

2.5.3.1 *Nitrogen*

Excess N (> 160 kg/ha/annum) delayed rind colour formation, increasing the amount of green fruit at harvest from 18.3% to 31.8% (Koo, 1988). Sala et al. (1992) reported that cuttings of 'Navelina Navel' orange irrigated with ammoniacal-nitric nutritive solution resulted in more orange-yellow fruit than cuttings irrigated with a nitric nutritive solution. Even nitric solutions enriched with Ca produced better rind colour than nitric solutions alone. Sala et al. (1992) also suggested that rind colour of fruit at harvest was better the earlier N fertiliser was applied

during the season. Ammonium-nitric solution not only resulted in better rind colour, but also increased the sugar content in the flavedo of fruit. Collado et al. (1996) found that 'Navelina Navel' orange cuttings irrigated with an ammonium-nitrate solution resulted in flavedo with higher protein concentrations, than when cuttings were irrigated with a pure nitric solution. This high protein concentration coincides with a more orange-yellow rind colour. Collado et al. (1996) also suggested that the amount, concentration and timing of these N sprays did not influence the protein concentration nor rind colour development. However, earlier work by Reitz and Koo (1960) suggested that high N concentrations in leaf analysis (2.5%) contribute to high incidences of green fruit at maturity. In contrast, Reuther and Smith (1952) stated that N did not have a large effect on rind colour development, and that high K application rate (1.36 kg/tree) had the biggest effect, especially when applied in combination with high N applications.

CRI (1995) suggested optimal leaf analysis standards for different citrus cultivar groups (Table 2.1). Managing trees according to these standards would reduce the chance of poor rind colour development due to excess or deficiencies of essential nutrients.

2.5.3.2 *Phosphorus*

Phosphorus is highly mobile in citrus trees, forming part of nucleoproteins and phospholipids, and P is also actively involved in energy transfer in leaves and fruit (Chapman, 1968). Koo (1988) reported that excess P may result in an increased percentage of green fruit at harvest. This green fruit was because of delayed colour break, resulting in fruit not being able to reach their full orange colour at physiological maturity (Smith et al., 1963).

Table 2.1. Optimal leaf macro-nutrient norms for 6-month-old leaves on fruiting terminals for different citrus cultivar groups (CRI, 1995).

Cultivar group	**%N**	**%P**	**%K**
Valencia	2.10-2.30	0.11-0.14	0.90-1.80
Navel	2.40-2.60	0.11-0.14	0.70-1.10
Grapefruit	2.30-2.50	0.10-0.14	0.80-1.00
Lemon	2.30-2.60	0.11-0.14	0.80-1.20

2.5.3.3 *Potassium*

Excess K applications early in the season may result in greener fruit at harvest compared with low K applications (Koo, 1988). This K effect was more pronounced when N was also applied at high concentrations. Tree plots exposed to high N/low K levels seemed to develop a redder shade of orange than high N/high K plots (Koo, 1988; Reuther and Smith, 1952). Reitz and Koo (1960) reported the same results as high K applications reduced the amount of first grade fruit, due to inadequate rind colour development, in a year with environmental conditions favouring rind colour development.

2.5.3.4 *Micronutrients*

Koo (1988) studied the effect of micronutrients on rind colour of 'Pineapple Navel' orange and found that Mn, Zn, Cu and B had a small negative effect on rind colour development and Fe applications promoted rind colour development of fruit (Table 2.2).

Table 2.2 Micronutrient effect on percentage of green fruit at harvest (Koo, 1988).

Manganese		**Zinc**		**Copper**		**Boron**		**Iron**	
+	-	+	-	+	-	+	-	+	-
26.7%	23.7%	25.4%	22.7%	25.4%	16.2%	25.4%	19.8%	13.1%	21.3%

2.5.3.5 *Flavedo sugar content*

Analysis done by Mitcham and McDonald (1993) indicated that arabinosyl and galactosyl were the most abundant cell wall neutral sugar residues followed by xylosyl, mannosyl, glucosyl and fucosyl in 'Marsh' grapefruit flavedo cells. These residues were reduced when fruit were treated with GA_3, 3 months prior to harvest, resulting in greener fruit.

Huff (1983) found that regreening of flavedo segments, placed on an agar medium, was prevented when the agar medium had a high (150 mM) concentration of sucrose. Nitrate seems to overcome this effect of sucrose and promoted regreening of flavedo rind segments when placed on agar. This might be because nitrate reduced the endogenous sugar level in cells and stimulated the production of amino acids. High soluble sugar concentrations in the flavedo of fruit, seem to be correlated with low chlorophyll concentrations in the flavedo as the season progresses, as low soluble sugars and high chlorophyll concentrations are present during stage I and stage II and high soluble sugars and low chlorophyll concentrations are present during stage III. Additionally, Huff (1984) noticed that during regreening of 'Valencia' orange fruit sugar concentrations in the flavedo of fruit decreased with subsequent increases in chlorophyll concentrations.

Iglesias et al. (2001) proposed that sucrose might play a role in internal ethylene biosynthesis of 'Satsuma' mandarin (*C. unshiu* Marc.) fruit, thereby affecting rind colour development. Iglesias et al. (2001) also reported that by removing leaves close to the fruit, this treatment subsequently reduced the buildup of sucrose and reduced N in the flavedo. When sucrose was supplemented either *in vivo* or *in vitro* to the fruit it stimulated rind colour development. This rind colour development was unaffected by ethylene, but was delayed by GA_3 applications.

2.5.4 Hormonal

2.5.4.1 *Abscisic acid (ABA)*

Sesquiterpenoid growth regulators, or ABA, biosynthesis occur predominantly via the metabolism of epoxy-carotenoids (Parry et al., 1990). Norman (1991) (added to this) by discovering that ABA biosynthesis increased with a change in β-carotene concentration. Cowan and Richardson (1993) concurred with the findings of Norman (1991) in that ABA is formed from all-*trans*-β-carotene. Afitlhile et al. (1993) also demonstrated that ABA levels increased concomitantly with a decrease in all-*trans*-violaxanthin and 9'-*cis*-neoaxanthin at an apparent relationship of 1:1. Parry and Horgan (1991) also reported that xanthophyll neoxanthin is used in ABA biosynthesis. Chayet et al. (1973) found that isomeric aldehydes 2,6-*trans*-*trans*-farnesal and 2-*cis*-6-*trans*-farnesal can be intermediates in the biosynthesis of ABA. Parry (1993) indicated that there are two main routes for ABA biosynthesis, viz. the direct C_{15} pathway, where a C_{15} precursor such as farnesyl pyrophosphate is converted to ABA and an indirect C_{40} pathway where carotenoids like violaxanthin are cleaved to yield a C_{15} ABA precursor. Cowan and Richardson (1993) also indicated that ABA is a byproduct of the mevalonic acid pathway with 1',4'-*trans*-abscisic acid forming from R-[2-^{14}C]-mevalonic acid. Mevalonate incorporation into ABA is less in intact chloroplasts than in chloroplast preparations (Milborrow, 1974).

Harris and Dugger (1986) as well as Richardson and Cowan (1995) demonstrated that the ABA concentration in the flavedo of the citrus rinds increased with normal rind colour development and was at its highest at colour break. They also found that in late maturing non-Navel orange cultivars, viz. Midknight Valencia and Moss Seedless, ABA concentration was substantially higher than in early-maturing cultivars and only at rind colour break did the

ABA concentrations decrease. These results suggest that ABA might contribute to the retardation of colour development in these cultivars. Their studies indicated that the development of the bright orange colour was in association with a decline in β,β-carotenoid levels, an increase of violaxanthin and the formation of xanthophyll acyl esters. Rodrigo et al. (2003) mentioned that ABA amounts present in the fruit could affect the type of carotenoids formed in citrus fruit rinds. Aung et al. (1991) found that free ABA levels in citrus fruit increased progressively during fruit development and maturation in contrast to conjugated ABA, which decreased as fruit develop. Goldschmidt et al. (1973) established that this ratio of free- to -conjugated ABA was in the order of 10:1 in mature citrus fruit. Rasmussen (1974, 1975) found that ABA in fruit stimulated the formation of ethylene later in the season. ABA also seems to accelerate chlorophyll degradation and anthocyanin synthesis (Wang et al., 2005). In comparison, Brisker et al. (1976) found that ethylene influenced ABA concentration in citrus flavedo. This ABA concentration was also influenced by the cytokinin benzyladenine (BA) by delaying ethylene formation in fruit.

2.5.4.2 *Auxins*

Ethyclozate, ethyl 5-chloro-1H-3-indazolylacetate, an auxin plant growth regulator with different characteristics to 1-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic (2,4-D) enhanced rind colour in ‘Satsuma’ mandarin by decreasing chlorophyll concentration and increasing carotenoid concentration (Kamuro and Hirai, 1981; Tominaga and Diato, 1981). Two sprays applied 90 and 105 days after full bloom had the best effect on colour enhancement with no difference in rind colour between the two concentrations (67 and 200 $mg \cdot L^{-1}$) used (Iwahori et al., 1986). Cooper and Henry (1968) suggested that ethyclozate enhanced rind colour development through the stimulation of ethylene biosynthesis.

2.5.4.3 *Cytokinins*

Cytokinins are derived from adenine, and are known to promote cell division in plant callus cultures. Cytokinins play an important role in root-canopy relations, thereby negatively affecting senescence processes and fruit colour development (Eilati et al., 1969a). Benzyladenine (BA) significantly delayed chlorophyll degradation in 'Feizixiao' mandarin and inhibited anthocyanin biosynthesis (Wang et al., 2005). Eilati et al. (1969a) demonstrated that BA significantly delayed rind colour formation of citrus fruit. This observation was expected as cytokinins preserve chlorophyll in detached leaves. BA also delays fruit abscission and increases the amount of regreening in 'Valencia' oranges (Cooper and Henry, 1968). Eilati et al. (1969b) reported that BA did not influence carotenoid accumulation during the onset of fruit maturation. In contrast, Garcia-Luis et al. (1986) demonstrated that cytokinins reduced carotenoid accumulation in citrus rind and can be used as a maturation retardant.

Sakoda et al. (1991) showed that 3-methoxy-4-methylthio-2-piperithione (raphanusanin) strongly inhibited cytokinin activity of tassel flower (*Amaranthus caudatus* L.) at concentrations of 10^{-5} M. Whether the "anti-cytokinin" activity of raphanusanins could affect rind colour of citrus is unknown.

2.5.4.4 *Ethylene*

Normally fruit do not produce ethylene until the onset of ripening. During ripening of climacteric fruit, concentrations of this endogenous gas rise dramatically from almost undetectable amounts to about 0.1 to 1 $\mu L \cdot L^{-1}$ in the intercellular air spaces between cells (Tucker and Grierson, 1987). In contrast, non-climacteric fruit synthesise little ethylene and are not induced to ripen by it, as is the case with citrus. However, when Goldschmidt et al.

(1993) applied ethylene antagonists, viz. 2,5-norbornadiene (NBD) and silver nitrate, to citrus fruit, they established that ethylene does significantly increase rind colour formation and chlorophyll degradation. Apelbaum et al. (1976) suggested that endogenous ethylene may not be the primary colour change inducer in detached 'Shamouti' oranges. Ethylene does however stimulate chloroplast structure changes as well as increase chlorophyllase activity in cells (Purvis, 1980; Shimokawa et al., 1978).

El-Zeftawi and Garret (1978) dipped individual fruit on the tree with 2-chloroethyl-phosphonic acid (ethephon) at a concentration of 350 $mg \cdot L^{-1}$ and subsequently increased carotenoid concentration. When ethephon was applied at 480 $mg \cdot L^{-1}$ on trees, it improved rind colour development, but caused leaf abscission, possibly due to increased respiration caused by ethephon (El-Zeftawi and Garret, 1978). The extent of this colour development and leaf abscission were, however, cultivar and climate dependent (Protopapadakis and Manseka, 1992). El-Otmani et al. (1996) increased the export yield by 14% when ethephon was applied at concentrations of 240 and 480 $mg \cdot L^{-1}$ on 'Clementine' mandarin, but leaf abscission was also a problem. Pons et al. (1992) reported that ethephon applications of 200 $mg \cdot L^{-1}$ 20 to 25 days before colour break on mature 'Oroval Clementine' and 'Marisol Clementine' mandarin trees resulted in increased rind colour development and allowed 15 days earlier harvest, leaf abscission was, however, again one of the major drawbacks of this treatment. Young and Jahn (1972), however, stated earlier that the best rind colour improvement was obtained when ethephon was applied at concentrations of 100 to 300 $mg \cdot L^{-1}$ after colour break.

2.5.4.5 *MCP and AVG*

Pozo and Burns (2000) demonstrated that when 1-methylcyclopropane (1-MCP) was applied on calamondin (*C. madurensis* Lour.) trees it reduced the amount of leaf drop caused by the

application of ethephon. The application of aminoethoxyvinylglycine (AVG), however, intensified the amount of leaf drop which may be related to ACC synthase activity stimulation in the leaves. Porat et al. (2001) agreed with Pozo and Burns (2000) that 1-MCP decreased abscission of fruit and leaves caused by ethylene treatments, and they also suggested that 1-MCP does not influence green rind colour retention in 'Oroblanco' pummelo-grapefruit hybrid (*C. grandis* Osbeck x *C. parasisi* Macf.) as GA does. Gonzalez and Lovatt (2004) reported the same results only with AVG on 'Washington Navel' orange, in that AVG reduced ethylene activity in the fruit without having an effect on rind colour retention. However, Porat et al. (1999) showed that 1-MCP might be used as an ethylene degreening inhibitor of 'Shamouti' orange fruit at a concentration of 50-100 $nL \cdot L^{-1}$.

2.5.4.6 *Gibberellic acid*

Gibberellins were first classified as a plant hormone in the 1930s. Since then more than 80 different gibberellins have been classified. All gibberellins are derivatives of the ent-gibberallane skeleton. Gibberellins are acidic, and are thus named gibberellic acid (GA). All gibberellins have either 19 or 20 C atoms normally grouped in four or five ring structures (Sponsel, 1987). Gibberellins are isoprenoid compounds synthesised from acetyl coenzyme A in the mevalonic acid pathway with geranylgeranyl pyrophosphate (GGDP) serving as the C donor for all gibberellins. GGDP is then converted to copalylpyrophosphate, with a two ring system; kaurene which has a four ring system is then formed from the latter (Fig. 2.8). Kaurene is then further oxidized into kaurenol, kaurenal and kaurenoic acid in the endoplasmic reticulum (ER). The aldehyde of GA_{12} is the first compound with a true gibberellane ring system, containing 20 C atoms. From this aldehyde arise both 19- and 20-C gibberellins, most likely also in the ER (Salisbury and Ross, 1992).

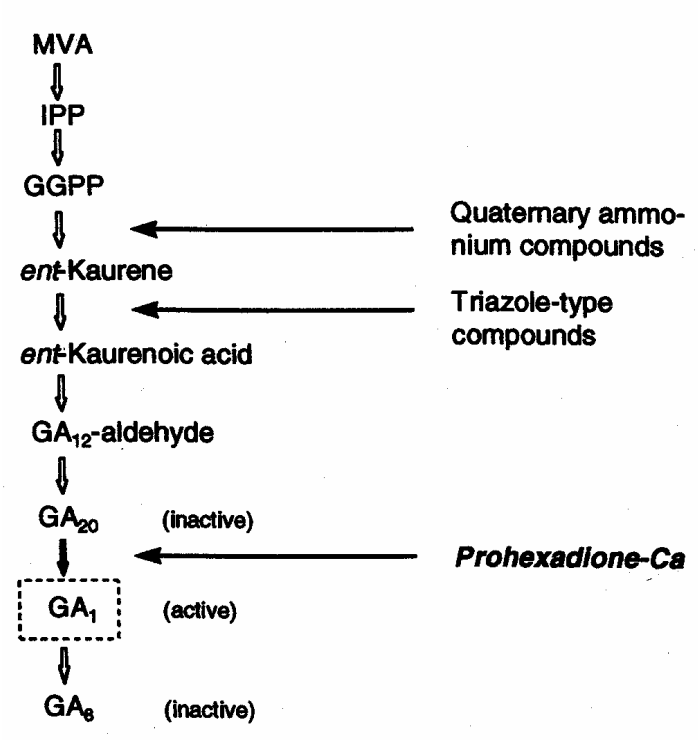


Fig. 2.8. Pathway illustrating the biosynthesis of gibberellins and the mode of action of prohexadione-calcium (Evans et al., 1999).

Talon et al. (1990) reported GA_{19}, GA_{20}, GA_{29}, GA_1, GA_8, GA_3 and iso-GA_3 to be the most abundant GAs in 'Satsuma' mandarin ovaries at anthesis. They also found that only GA_3, GA_{29} and detectable levels of GA_8 were present in 'Clementine' mandarin. Their studies concluded that GA activity during anthesis was a lot higher in 'Satsuma' mandarin than 'Clementine' mandarin.

Goldschmidt (1988) suggested that high GA levels in fruit during maturation delayed chloro-chromoplast transformation. Coggins and Lewis (1962) demonstrated that high levels of GA in fruit increased the retransformation of chromoplasts to chloroplasts, resulting in regreening of late hanging 'Valencia' orange fruit. Thomson et al. (1967) also reported that GA is needed for regreening to occur.

GA_3 seems to delay chloroplast to chromoplast transformation of citrus fruit rinds (Thomson et al., 1967). When Coggins and Hield (1962) applied potassium gibberellate (KGA) during the season, chlorophyll degradation was retarded resulting in greener fruit at maturation. This retardation in chlorophyll degradation was more pronounced when KGA was applied closer to rind colour break of citrus fruit. Gilfillan et al. (1974) supported the findings of Coggins and Hield (1962) in that GA applied at colour break resulted in unacceptably green fruit at harvest. Garcia-Luis et al. (1992) found the same results and added that the 10-day period between the onset of chlorophyll degradation and the onset of carotenoid accumulation was important for rind colour development. The severity of GA_3 effects on rind colour was however linear to the concentration of GA_3 applied (Coggins and Henning, 1988). The delay of colour formation due to GA_3 applications also strongly depended on environmental conditions (primarily temperature), and complete chlorophyll loss after GA applications may take up to 2 months during the maturation stage (Coggins, 1981). GA-treated fruit also ended up with lower carotenoid concentration after full colour development resulting in paler coloured fruit (Lewis and Coggins, 1964; Rasmussen, 1973).

Ferguson et al. (1986) found that this exogenously applied GA to fruit was taken up from 1 hour after application continuing for 8 hours, but when this GA was applied to leaves it was immediately taken up, reaching a maximum 2 hours after application. Translocation of GA from fruit seems to be slower than that of leaves, resulting in higher GA concentrations in the rind of fruit later in the season (Embleton et al., 1973; Garcia-Luis et al., 1985).

2.5.4.7 *Gibberellin biosynthesis inhibitors*

Prohexadione-calcium [(ProCa); BAS-125W (3-oxido-4propionyl-5-oxo-3-cyclohexene-carboxylate)], traded as Regalis® and Apogee® and developed by BASF (Limburgerhof,

Germany), is widely used on pome fruit trees to reduce and control vegetative growth (Miller, 2002). Prohexadione-calcium acts primarily as a gibberellin biosynthesis inhibitor, especially 3β-hydroxylation of GA_{20} to GA_1 (Fig. 2.8) (Nakayama et al., 1992; Rademacher, 2001). Costa et al. (2001) reported that applications of 100 mg·L^{-1} ProCa significantly reduced shoot growth and increased fruit size in pears (*Pyrus communis* L.). Ilias and Rajapakse (2005) found not only a reduction in stem growth but also a loss in flower colour when ProCa was applied on petunia (*Petunia hybrida*) plants. ProCa applied on 'Navelina Navel' orange, 2 weeks before anticipated harvest at a concentration of 100 mg·L^{-1}, improved rind colour development. This application of ProCa aided in chlorophyll degradation and carotenoid biosynthesis (Barry and Van Wyk, 2004).

Other known growth retardants, viz. paclobutrazol and uniconazole, are known to reduce gibberellin levels in plants and subsequently reducing vegetative vigour (Smeirat and Qrunfleh, 1989; Tukey, 1989; Wheaton, 1989). Aron et al. (1985) found that when paclobutrazol was applied at 1 g·L^{-1} on citrus trees just before the onset of the summer flush it reduced shoot length, internode length and the number of shoots developed by 41%, 76% and 44%, respectively. However, both paclobutrazol and uniconazole reduced fruit size, increased fruit number and retarded maturation when applied on 'Rio Red' grapefruit (Fucik and Swietlik, 1990). Gilfillan and Lowe (1985) however demonstrated that paclobutrazol increased 'Satsuma' mandarin rind colour by 1-2 colour rating units. These results were achieved when paclobutrazol was applied at 1 g·L^{-1} after November fruit drop, as well as, January and February. These results suggest that paclobutrazol suppresses the November-December growth flush, which might be more important for fruit colour development than the January-February flush.

2.5.4.8 *2-(4-Chlorophynylthio)-triethylamine hydrochloride (CPTA) and related compounds*

CPTA was reported by Coggins et al. (1970) to influence carotenoid biosynthesis. Their studies indicated that lycopene accumulated in flavedo of 'Marsh' grapefruit. CPTA also caused, to some extent, lycopene accumulation in 'Valencia' and 'Washington Navel' oranges. Yokoyama et al. (1971) found similar results when fruit were treated with CPTA at different stages of maturity during the season. They also reported that β-carotene concentrations decreased when CPTA was applied both pre- and post-harvest. Yokoyama et al. (1972) demonstrated that CPTA did not enhance rind colour, except when CPTA was injected into fruit.

Applications of diethyloctylamine and diethylnonylamine, tertiary amines, caused a 3.5- and 4.6-fold increase in γ-α- and β-carotene, respectively. This increase in cyclic carotenes was much larger than that caused by CPTA (Poling et al., 1975). Poling et al. (1976) also reported that 2-(diethylamino)ethyl p-toluate and 2-(diethylamino)ethyl p-bromobenzoate significantly increased β-carotene in fruit flavedo. This effect is believed to be similar to that of CPTA, in that these compounds act as a derepressor of gene regulating synthesis of enzyme(s) and the inhibition of cyclase(s) of carotenoid biosynthesis (Poling et al., 1973). Subsequent treatments by Poling et al. (1977) indicated that 2-diethylaminoethyl esters of hexanoic and cinnamic acid caused a significantly large increase in β-carotene without additional lycopene formation. 2-diethylaminoethyl hexanoate causes an initial accumulation in lycopene which was converted to β-carotene after 2 days of storage.

2.5.4.9 *Girdling*

Jahn and Young (1972) demonstrated that girdled 'Bearss' lemon trees responded better to preharvest ethylene application, subsequently improving rind colour. This response suggests

that non-girdled trees provide factors which may inhibit fruit from responding to ethylene treatment, or the girdling treatment possibly increase sucrose content in the flavedo. Peng and Rabe (1996a) also reported that girdling 'Miho Wase Satsuma' mandarin trees 2 to 4 weeks after physiological fruit drop resulted in significantly better rind colour at harvest. This response might be because of the reduction in vegetative vigour caused by girdling. However, summer trunk girdling did not influence rind colour development at harvest of 'Delta Valencia' orange fruit (Verreynne, 1999).

Table 2.3. Summary of factors affecting citrus rind colour formation.

Factor	Promotes green fruit	Promotes orange fruit	Reference
Genetic			
Rootstock	Invigorating	Non-invigorating	CRI, 1995
Environmental			
Tree age	Young	Old	Krajewski, 1997
Soil type	Clay	Sandy	CRI, 1995
Weather conditions			
Temperature	Day >30 ºC	Day 20 ºC	Coggins et al., 1981
		Night <13 ºC	Young and Erickson, 1961
		Soil <12 ºC	Young and Erickson, 1961
Light	Low PAR	High PAR	Sites and Reitz, 1949
Water	Excess	Deficit	Peng and Rabe, 1996b
Nutritional			
Nitrogen	Excess	Deficit	Reitz and Koo, 1960
Phosphorus	Excess	Deficit	Koo, 1988
Potassium	Excess	Deficit	Koo, 1988
Micronutrients	Mn, Zn, Co and B	Fe	Koo, 1988
Carbohydrates	Excess	Deficit	Iglesias et al., 2001
Hormonal			
Abscisic acid	High concn.	Low concn.	Richardson and Cowan, 1995
Auxins	Low concn.	High concn.	Kamura and Hirai, 1981
Cytokinins	High concn.	Low concn.	Cooper and Henry, 1968
Ethylene	Low concn.	High concn.	El-Zeftawi and Garret, 1978
MCP and AVG	No effect	No effect	Gonzalez and Lovatt, 2004
ProCa	Early low concn.	High late concn.	Barry and Van Wyk, 2004
CPTA		Seasonal applications	Poling et al., 1976
Girdling		Early applications	Peng and Rabe, 1996a

2.6 Quantification of rind colour

Colour is a matter of individual perception and subjective interpretation by different people, with verbal expression of colour often being difficult. Colour has many attributes, with the retina's trichromacy capability making it possible to observe colour as a function of three variables (Hunt, 1977). The observed colour is dependent on the light intensity, reflectance of light from the object being observed and spectral sensitivity of the human eye. Changing any of these factors may change the colour observed (Voss and Hale, 1998). The human eye is capable in distinguishing among 10 million different colours. Instrumental observation of colour, on the other hand, is limited to the sample presented to the instrument and only light reflected or transmitted from the sample will in effect be measured (Francis, 1980).

2.6.1 Colour rating

In South Africa, citrus rind colour ratings are conducted by comparing fruit to a series of photographs ranging from dark green to intense orange (CRI, 2004a, 2004b, 2004c), where T8 is the rating for dark green rinds, T3 for yellow-orange fruit with a tinge of green, and T1 for fully coloured fruit.

Since the perception of colour is different for each individual, the observed colour in a colour rating chart can be inconsistent as reflectance and intensity of light vary together with different angles of viewing making it difficult to obtain the true rind colour descriptor.

2.6.2 Colorimeter measurements

In the food industry, there are mainly three different mathematical colour solids being used to measure colour, viz. the Commission Internationale d'Eclairage X Y Z, the Judd-Hunter L a b and the Lovibond-Scofield Y R B systems. All three systems are capable of locating a point in a three-dimensional space. Thus, by locating a specific colour, one would locate a specific

point in a colour solid. Colour measurements via one of these instruments are only limited to the ingenuity of the operator and the sample presented (Francis, 1980).

Colour can be quantitatively defined into three dimensions of hue, chroma and lightness. Hue can be calculated from the Hunter *a** and *b** values: $H^* = \tan^{-1} b^*/a^*$, where *a** measures the difference between light reflected from the green and red zones of the colour spectrum, and *b** measures the difference in light reflectance between the yellow and blue zones in the colour spectrum (Jimenez-Cuesta et al., 1981). Hue thus represents an angle between the *x* and *y* axes, depending on the colour. This angle obtained from the previous calculation is a good indication whether the colour is red, green, yellow or blue (Little, 1976). In orange-coloured citrus fruit, a hue angle of at least <80º is considered to be acceptable in most markets (G.H. Barry, personal communication).

By measuring the chroma of the object one is able to determine the pure chromatic colour present in the sample. It is also the difference between neutral and grey of the same lightness values. Chroma can also be calculated from the Hunter values: $C^* = \sqrt{a^2 + b^2}$. As the chroma increases, citrus rind colour becomes more intense (Lancaster et al. 1997). A chroma of at least >60 is considered to be acceptable in most markets (G.H. Barry, personal communication). Lightness of the object is represented by L, which ranges from 0 (black) to 100 (pure white) (Ihl et al., 1994). A lightness of between 65 and 70 is considered to be acceptable in most markets (G.H. Barry, personal communication).

Jimenez-Cuesta et al. (1981) determined a citrus colour index (CCI) to be used when fruit are to be degreened. This CCI is calculated by using the Hunter values: $CCI = 1000\, a^*/_{L.b^*}$. They established that if fruit had a CCI of 7 and over, degreening was not necessary.

2.6.3 Rind pigment analysis

Pigment analysis done by spectrophotometry incorporates the findings of Lichtenthaler (1987) in that chlorophyll *a* (C_a), chlorophyll *b* (C_b), total chlorophylls (C_{a+b}) and total carotenoid (C_{x+c}) concentrations can be calculated. Pigment concentrations are determined by comparing absorbance readings of pigment extractions at various wavelengths with the absorbance of known standards.

Individual rind pigments can be quantified by using Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) (Schwartz and Patroni-Killam, 1985). TLC separates individual carotenoids depending on their characteristics. The various bands obtained can then be identified and quantified by HPLC (Pupin et al., 1999; Schwartz and Patroni-Killam, 1985).

2.7 Conclusion

Rind colour of *Citrus* spp. is affected by various exogenous factors and nutrients, viz. temperature, light intensity, nitrogen, water and carbohydrates, as well as endogenous hormones, viz. gibberellins, cytokinins and ethylene. Some of these factors also stimulate vegetative vigour. The possibility exists that low night air and soil temperatures do not stimulate rind colour formation directly, but rather slows vegetative growth by reducing the formation of vegetative growth promoting hormones (GA_3), which in turn, is antagonistic to the conversion of chloroplasts to chromoplasts.

CHAPTER 3

VEGETATIVE GROWTH RESPONSES OF *CITRUS* NURSERY TREES TO VARIOUS GROWTH RETARDANTS

Abstract

As part of a larger study to improve rind colour of citrus fruit, an initial study was conducted to determine the concentration of various gibberellin biosynthesis inhibitors required to get a biological response in citrus trees, as measured by vegetative growth. Repeated foliar applications of ProGibb® (4% v/v GA_3) increased growth of 'Eureka' lemon [*Citrus limon* (L.) Burm. f.] shoots by 63%, with no significant effect on rootstock and scion diameters. Repeated applications of Regalis® (10% v/v Prohexadione-calcium) at various concentrations (1, 2, 4 and 8 $g \cdot L^{-1}$) as well as Sunny® (5% v/v uniconazole) (at 10 and 20 $mL \cdot L^{-1}$) and Cultar® (25% v/v paclobutrazol) (at 10 $mL \cdot L^{-1}$) had no effect on the rootstock or scion diameters 8 months after the first application. Both the 4 and 8 $g \cdot L^{-1}$ Regalis® treatments, both Sunny® treatments and the Cultar® treatment significantly reduced shoot growth. Sunny® at 20 $mL \cdot L^{-1}$ resulted in the most growth retardation which resulted in 34% shorter shoot length than the control. Although the number of nodes on the longest shoot did not differ from the untreated control, internode length differed significantly among treatments. Regalis® at 4 and 8 $g \cdot L^{-1}$, Sunny® at 20 $mL \cdot L^{-1}$ and Cultar® at 10 $mL \cdot L^{-1}$ reduced internode length relative to the control by 31%, 56%, 50% and 28%, respectively. Vegetative growth of 'Eureka' lemon nursery trees was retarded following the repeated (x4) application of gibberellin biosynthesis inhibitors. Regalis® at 4 to 8 $g \cdot L^{-1}$ and Sunny® at 10 to 20 $mL \cdot L^{-1}$ are potential candidates for further field studies to test their effects on rind colour enhancement of citrus fruit.

Introduction

Rind colour is an important cosmetic preference of consumers when purchasing citrus fruit. In general consumers prefer a deep orange rind colour (Krajewski, 1996). As citrus fruit mature, changes in rind colour are due to increased carotenoid and decreased chlorophyll concentrations in the flavedo. This change in rind pigments is mainly due to the senescence of chlorophyllous tissue in the flavedo, and results in the transformation of chloroplasts into chromoplasts. Chloro-chromoplast transformation is a major physiological response affected by environmental, nutritional and hormonal factors (Goldschmidt, 1988).

As part of a larger study to improve rind colour of citrus fruit, an initial study was conducted to determine the concentration of various gibberellin biosynthesis inhibitors required to get a biological response in citrus trees, as measured by vegetative growth. Goldschmidt (1988) showed that factors contributing to invigorating growing conditions are antagonistic to optimal rind colour development.

Vegetative growth in *Citrus* spp. is stimulated by various exogenous factors and nutrients, viz. high temperature, high light intensity, nitrogen and water, as well as endogenous hormones, viz. gibberellins and cytokinins. Young leaves are a major site of gibberellin biosynthesis (Salisbury and Ross, 1992; Spiegel-Roy and Goldschmidt, 1996). High endogenous gibberellin concentrations enhance stem elongation (Mudzunga, 2000; Salisbury and Ross, 1992), and delay rind colour development of citrus fruit (Garcia-Luis et al., 1985).

Growth retardants, sometimes referred to as gibberellin biosynthesis inhibitors, inhibit vegetative growth in plants by disrupting gibberellin biosynthesis. Aron et al. (1985) demonstrated that when paclobutrazol (Cultar®) was applied at 1 $g \cdot L^{-1}$ on citrus trees just

before the onset of the summer flush it reduced shoot length, internode length and the number of shoots developed by 41%, 76% and 44%, respectively. Gilfillan and Lowe (1985) also reported that paclobutrazol increased 'Satsuma' mandarin (*C. unshiu* Marc.) rind colour by 1-2 colour rating units. Uniconazole (Sunny®) reduced shoot length, number of lateral shoots per terminal, number of nodes per terminal and internode length in 'Wichita' pecan [*Carya illinoinensis* (Wangenh.) K. Koch] and Cleopatra mandarin (*C. reticulata* Blanco) trees by blocking the steps before the formation of GA_{12} (Graham and Storey, 2000; Lee et al, 1998; Wheaton, 1989). Prohexadione-calcium (ProCa traded as Regalis®) is used on pome fruit trees (*Malus* and *Pyrus* spp.) to reduce and control vegetative growth (Miller, 2002). Prohexadione-calcium acts primarily as a gibberellin biosynthesis inhibitor, especially 3β-hydroxylation of GA_{20} to GA_1 (Fig. 2.8) (Nakayama et al., 1992). ProCa applied on 'Navelina Navel' orange [*C. sinensis* (L.) Osbeck], 2 weeks before anticipated harvest, at 100 $mg \cdot L^{-1}$ improved rind colour. This application of ProCa aided in chlorophyll degradation and carotenoid biosynthesis (Barry and Van Wyk, 2004). Stover et al. (2004) found that two 500 $mg \cdot L^{-1}$ ProCa applications reduced the vegetative growth by ~ 40% across six citrus genotypes tested.

The primary objective of this study was to determine the concentration of various gibberellin biosynthesis inhibitors required to get a vegetative growth response in citrus nursery trees. This information could then be used in a field study to test the effects of gibberellin biosynthesis inhibitors on rind colour of citrus.

Materials and Methods

Plant material and site. During the 2005-06 summer growing season, 108 potted nursery trees of 'Eureka' lemon [*C. limon* (L.) Burm. f.] budded on X639 rootstock [Cleopatra mandarin

(*C. reticulata* Blanco) × trifoliate orange (*Poncirus trifoliata* Raf.)] of similar size and with at least three strong primary branches were selected at Nucellar Nursery, Simondium, Western Cape province, South Africa (33°50'S, 18°58'E; 160 m alt.). These trees were 21 months old at the start of the experiment.

Treatments applied. Potted nursery trees were randomly allocated to treatments, viz. untreated control, 1.6 $mL \cdot L^{-1}$ ProGibb® (4% v/v GA_3), 1, 2, 4 and 8 $g \cdot L^{-1}$ Regalis® (10% v/v prohexadione-calcium), 10 and 20 $mL \cdot L^{-1}$ Sunny® (5% v/v uniconazole) and 10 $mL \cdot L^{-1}$ Cultar® (25% v/v paclobutrazol). Kaolin particle film (Surround®) at 20 $g \cdot L^{-1}$ was applied together with all treatments to easily distinguish new growth flushes throughout the assessment period. Application dates of the treatments (15 Nov. 2005, 27 Dec. 2005, 16 Feb. 2006 and 31 Mar. 2006) were planned to coincide with various growth flushes during the summer growing season.

Data collection. Rootstock and scion diameters were measured 2 cm below and 3 cm above the bud union, at the start of the experiment (15 Nov. 2005), 6 weeks thereafter (27 Dec. 2005) and at the end of the experiment (20 July 2006). Three shoots per tree were selected, marked and measured at the start of the experiment. Thereafter, only the length of the new growth was measured and internodes were counted at each assessment date. Since all shoots did not flush and grow out, data analysis was done on the longest shoot to quantify the treatment effects on growth retardation.

Statistical design and analysis. Experimental layout was a completely randomised block design (CRBD) consisting of twelve single-tree replicates. Blocking was used to reduce the possible effect of experimental error due to lighting and microclimate on within-site variation.

Analysis of variance was conducted using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS Inc., Cary, N.C., USA) and least significant difference (LSD) values were used to separate treatment means. Analysis of covariance was conducted with initial stem diameter and shoot growth as covariates.

Results and Discussion

Rootstock diameter did not differ among treatments throughout the experiment (Table 3.1). Significant differences in scion diameter were measured at the onset of the trial and 6 weeks thereafter, but there were no significant differences among treatments at the final measurement (Table 3.1). When the initial rootstock and scion diameter was fixed by covariance analysis, there was no significant difference on the final rootstock and scion diameters.

Repeated applications of the treatments during the summer growing season did not have an effect on the final rootstock and scion diameters. In this short-term study, i.e. 8 months, there was too little time for a treatment response in rootstock and scion diameters.

Shoot length of the longest shoot was longer for the ProGibb® treatment than for the control, whereas shoot length of the two low concentrations of Regalis® (1 and 2 $g \cdot L^{-1}$) did not differ from the control (Fig. 3.1). However, the two high concentrations of Regalis® (4 and 8 $g \cdot L^{-1}$), both Sunny® treatments (10 and 20 $mL \cdot L^{-1}$) and the Cultar® treatment (10 $mL \cdot L^{-1}$) resulted in shorter shoot lengths than the control. Sunny® at 20 $mL \cdot L^{-1}$ significantly retarded growth, resulting in 34% shorter shoot length than the control.

ProGibb® significantly increased shoot length compared to the control by 63%. The present results confirm previous reports that ProGibb® applied at 1.6 $mL \cdot L^{-1}$ stimulates citrus shoot growth (Mudzunga, 2000). This response is not unexpected given the role of gibberellins in enhancing stem elongation (Salisbury and Ross, 1992).

Although the number of nodes on the longest shoot did not differ in any of the treatments from the untreated control (Fig. 3.2), internode length differed significantly among treatments (Fig. 3.3). Regalis® at 4 and 8 $g \cdot L^{-1}$, Sunny® at 20 $mL \cdot L^{-1}$ and Cultar® at 10 $mL \cdot L^{-1}$ reduced internode length relative to the control by 31%, 56%, 50% and 28%, respectively (Figs. 3.3 and 3.4). These findings compare favourably with previous results by Aron et al. (1985) where Cultar® reduced the total growth and internode length of 'Minneola' tangelo (*C. reticulata* Blanco x *C. paradisi* Macf.) trees.

In conclusion, vegetative growth of 'Eureka' lemon nursery trees was retarded following the repeated (x4) application of gibberellin biosynthesis inhibitors. Since it is unlikely that Cultar® would be registered on citrus due to its persistence in the environment and the plant (Goulston and Shearing, 1985), Regalis® at 4 to 8 $g \cdot L^{-1}$ and Sunny at 10 to 20 $mL \cdot L^{-1}$ are potential candidates for further field studies to test their effects on rind colour enhancement of citrus fruit.

Table 3.1. Mean rootstock and scion diameter of 'Eureka' lemon on X639 nursery trees at the start of the experiment (15 Nov. 2005), 6 weeks thereafter (27 Dec. 2005) and at the end of the experiment (20 July 2006).

Treatment	Rootstock diameter (mm)			Scion diameter (mm)		
(per L)	15 Nov. 05	27 Dec. 05	20 Jul. 06	15 Nov. 05	27 Dec. 05	20 Jul. 06
Control	14.3 ns[z]	13.9 ns	14.5 ns	11.1 bc	11.0 bc	11.8 ns
ProGibb 1.6 mL	14.6	14.2	15.3	12.3 a	11.5 abc	12.1
Regalis 1 g	14.1	14.2	14.4	10.9 c	11.2 bc	11.2
Regalis 2 g	14.3	14.2	14.5	11.5 abc	11.0 bc	11.8
Regalis 4 g	15.4	15.3	15.7	11.8 abc	11.3 bc	11.6
Regalis 8 g	13.4	13.5	14.2	11.2 bc	10.7 c	11.3
Sunny 10 mL	15.0	15.3	15.6	12.1 ab	11.9 ab	12.2
Sunny 20 mL	14.8	14.6	15.1	11.4 abc	11.0 bc	11.4
Cultar 10 mL	14.3	14.8	15.3	12.2 a	12.4 a	12.2
P-value	0.2780	0.3159	0.3179	0.0430	0.0482	0.2207
LSD	1.45	1.60	1.41	1.00	1.02	0.91

[z] Means within columns followed by different letters are significantly different (P≤0.05; ns = non significant).

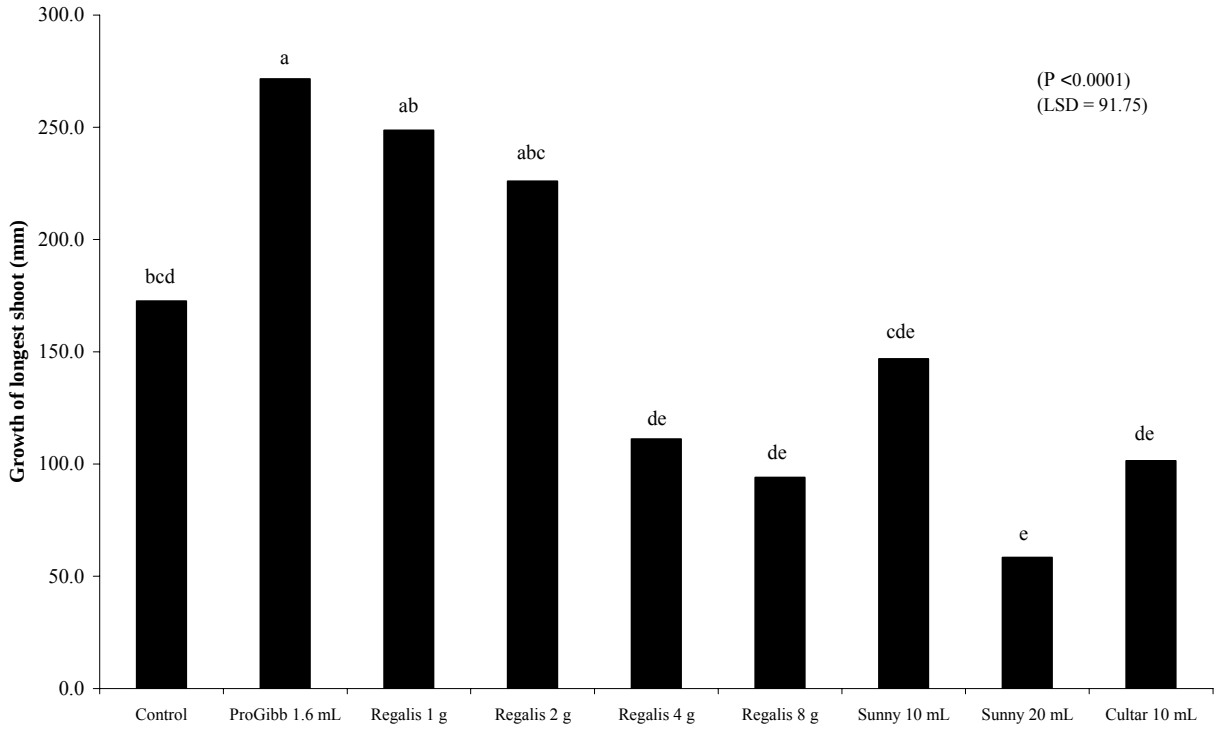


Fig. 3.1. Shoot length of the longest shoot of 'Eureka' lemon on X639 nursery trees at the end of experiment on 20 July 2006. (Means followed by a different letter are significantly different (P≤0.05).

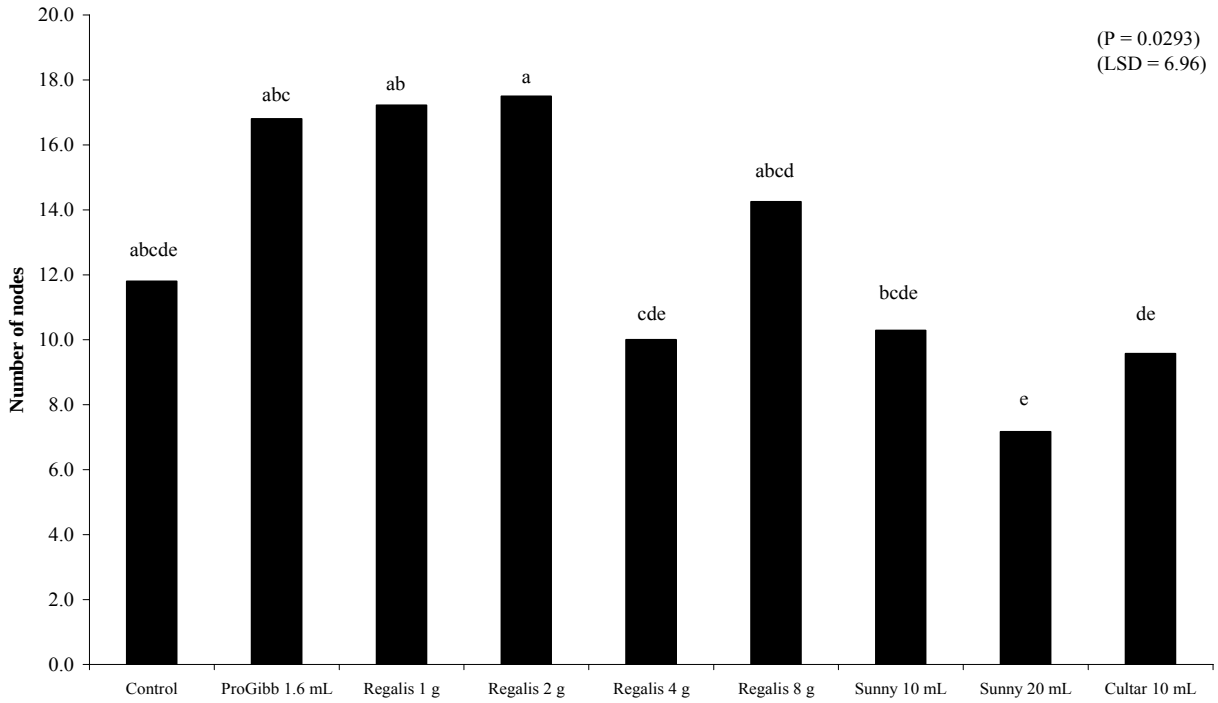


Fig. 3.2. Number of nodes on the longest shoot of 'Eureka' lemon on X639 nursery trees the end of experiment on 20 July 2006. (Means followed by a different letter are significantly different (P≤0.05).

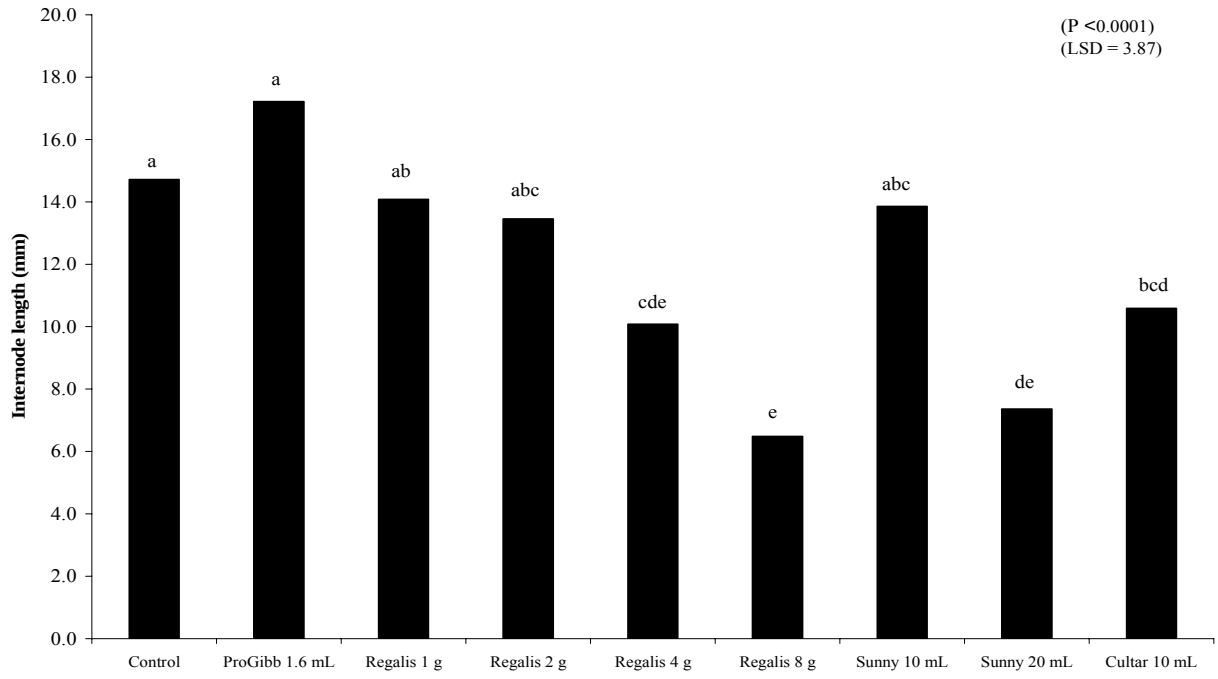


Fig. 3.3. Internode length of the longest shoot of 'Eureka' lemon on X639 nursery trees the end of experiment on 20 July 2006. (Means followed by a different letter are significantly different (P≤0.05).

Fig. 3.4. Photographs of 'Eureka' lemon shoots to illustrate the effect of growth retardants on vegetative growth. A: untreated control; B: 4 $g \cdot L^{-1}$ Regalis®. Note the shortening of internode length by > 30% in the Regalis® treatment compared to the untreated control treatment.

CHAPTER 4

PREHARVEST MANIPULATION OF CHLORO-CHROMOPLAST TRANSFORMATION BY GIBBERELLIN BIOSYNTHESIS INHIBITOR PROHEXADIONE-CALCIUM

Abstract

Rind colour is one of the main cosmetic preferences for the marketing of fresh citrus fruit. Acceptable rind colour is obtained when an adequate amount of carotenoids are synthesised together with chlorophyll degradation. Tree vegetative vigour, as well as high gibberellin and cytokinin levels, are thought to adversely affect rind colour. Thus, methods to increase preharvest rind colour by manipulating vegetative vigour were investigated. Prohexadione-calcium (ProCa; Regalis®) was applied to 'Nules Clementine' mandarin (*Citrus reticulata* Blanco), 'Navelina Navel' and 'Palmer Navel' oranges [*C. sinensis* (L.) Osbeck], and 'Eureka' lemon [*C. limon* (L.) Burm.f.] during the 2005 and 2006 seasons at 200 and 400 $mg \cdot L^{-1}$ active ingredient. Rind colour rating, colorimeter measurements and pigment analysis were done after harvest, after ethylene degreening, and 3 weeks after cold-storage. During the 2005 season, ProCa significantly increased rind colour by increasing carotenoid and decreasing chlorophyll concentrations in flavedo of fruit before and after ethylene degreening for all *Citrus* spp. tested, except 'Eureka' lemon. However, after cold-storage, rind colour was not significantly different among treatments. During the 2006 season, rind colour was significantly increased after harvest and chlorophyll degradation plus carotenoid biosynthesis were stimulated by the late 400 $mg \cdot L^{-1}$ ProCa application on all *Citrus* spp. tested. Foliar spray application of ProCa at a concentration of 400 $mg \cdot L^{-1}$ applied 6 plus 3 weeks before anticipated harvest has the potential to increase preharvest rind colour of early-maturing citrus

cultivars and these results support the hypothesis that there may be a relationship between vegetative vigour and rind colour development of citrus fruit.

Introduction

Rind colour is an important cosmetic preference of consumers when purchasing citrus fruit. In general, consumers prefer a deep-orange rind colour (Krajewski, 1996). As citrus fruit mature, changes in rind colour are due to increased carotenoid and decreased chlorophyll concentrations (Goldschmidt, 1988). “Colour break” of the rind, a colloquial term generally used in the citrus industries of the world, occurs when a decrease in chlorophyll concentration unmasks the presence of carotenoid pigments (Fig. 2.6) (El-Zeftawi, 1978; Goldschmidt, 1988). Various factors affect rind colour development, viz. genetic, tree age, soil type, temperature, light, irrigation, nutritional and hormonal.

Besides the direct effects of some of these factors on rind colour, various indirect effects may also be important to rind colour development, while the interaction of various seemingly minor factors may delay rind colour development. Factors contributing to invigorating growing conditions are antagonistic to optimal rind colour development (Goldschmidt, 1988). For example, young trees tend to be more vigorous than older, mature trees. This vigour difference may be a major reason why fruit borne on young trees have poorer colour compared to fruit borne on old trees. Colour development is also adversely affected by growth flushes during stage III of fruit development, caused by high autumn temperatures. Such flushes are more common in trees bearing a low crop and in young trees of vigorous rootstock/scion combinations (Krajewski, 1997). Peng and Rabe (1996) found that when deficit irrigation caused the soil water tension to reach -70 kPa, better coloured fruit were obtained, compared to normal irrigation with a soil water tension of -30 kPa. Fruit harvested

was not only better coloured, but also had lower chlorophyll levels. Koo (1988) established that excess N (>160 kg/ha/annum) increased the amount of green fruit (from 18% to 32%) when the fruit were physiologically mature and ready for harvest.

Goldschmidt (1988) showed that high gibberellin levels in fruit during maturation delayed chloroplast to chromoplast transformation. Gilfillan et al. (1974) found that when GA_3 was applied at colour break it resulted in unacceptably green fruit at harvest. Gibberellin-treated fruit also resulted in lower carotenoid concentration after full colour development, resulting in paler coloured fruit (Lewis and Coggins, 1964; Rasmussen, 1973). Gilfillan and Lowe (1985), however, reported that paclobutrazol (a gibberellin biosynthesis inhibitor) increased 'Satsuma' mandarin (*C. unshiu* Marc.) rind colour by 1 to 2 colour rating units. These results were achieved when paclobutrazol was applied at 1 $g \cdot L^{-1}$ during November, after fruit drop, January and February, suggesting that paclobutrazol suppressed the November-December growth flush, which may be more important for rind colour development than the January-February growth flush.

Prohexadione-calcium [ProCa; BAS-125W (3-oxido-4propionyl-5-oxo-3-cyclohexene-carboxylate)] traded as Regalis® and Apogee® and developed by BASF (Limburgerhof, Germany) is used on pome fruit trees (*Malus* and *Pyrus* spp.) to reduce and control vegetative growth (Miller, 2002; Stover et al., 2004). Prohexadione-calcium acts primarily as a gibberellin biosynthesis inhibitor, especially 3β-hydroxylation of GA_{20} to GA_1 (Fig. 2.8) (Nakayama et al., 1992). Costa et al. (2001) demonstrated that repeated applications of 100 $mg \cdot L^{-1}$ ProCa significantly reduced shoot growth and increased fruit size in pears (*P. communis* L.). Preliminary research by Barry and Van Wyk (2004) showed that when ProCa

was applied at 100 mg·L^{-1}, 2 weeks before anticipated harvest, rind colour was improved due to chlorophyll degradation and carotenoid synthesis.

In an attempt to reduce vegetative vigour, although this was not measured, and thereby improve rind colour of citrus fruit, various early-maturing citrus cultivars were treated with different concentrations of ProCa at various stages of fruit development. The main objective of this study was to establish the concentration and timing of ProCa applications necessary to improve rind colour by enhancing chlorophyll degradation and carotenoid synthesis.

Materials and Methods

Sites and plant material. Four citrus cultivars at different locations in the Western Cape province, South Africa, were used during the 2005 season, viz. 'Nules Clementine' mandarin (*C. reticulata* Blanco) at Welgevallen Experimental Farm (Stellenbosch) (33°57'S, 18°53'E; 120 m alt.), 'Eureka' lemon [*C. limon* (L.) Burm.f.] at Jericho (Gt. Drakenstein) (33°52'S, 19°01'E; 160 m alt.), 'Palmer Navel' orange [*C. sinensis* (L.) Osbeck] at Landau (Wellington) (33°35'S, 18°59'E; 120 m alt.) and 'Navelina Navel' orange at Hexrivier (Citrusdal) (32°28'S, 18°58'E; 180 m alt.). The same cultivars were used during the 2006 season, however, 'Nules Clementine' mandarin from Diamant (Paarl) (33°46'S, 18°55; 140 m alt.) and 'Palmer Navel' orange from Hexrivier (Citrusdal) were used. The main reason for using different sites and plant materials was to test the treatments on different cultivars and to minimise the possibility of experimental loss.

Treatments and experimental design. 'Nules Clementine' mandarin. Prohexadione-calcium (ProCa; Regalis® containing 10% ProCa) was applied as a medium-cover spray with a hand-held spray gun with application rates of 200 and 400 mg·L^{-1} ProCa on 8 and 28 Dec. 2004, 1

Feb. 2005, and 4 (8 Apr. 2005) and 2 (28 Apr. 2005) weeks before anticipated harvest (13 May 2005) during the 2005 season. During the 2006 season, rates of 200 and 400 $mg \cdot L^{-1}$ ProCa were applied on 19 Dec. 2005 and 17 Jan. 2006 constituting an early application treatment, and for the late application treatment rates of 200 and 400 $mg \cdot L^{-1}$ ProCa were applied 6 (28 Mar. 2006) and 3 (12 Apr. 2006) weeks before anticipated harvest (8 May 2006), and compared with an untreated control treatment.

'Navelina Navel' orange. Prohexadione-calcium was applied as a medium-cover spray with a hand-held spray gun with application rates of 200 and 400 $mg \cdot L^{-1}$ ProCa applied 4 (7 Apr. 2005) and 2 (21 Apr. 2005) weeks before anticipated harvest (5 May 2005) in the 2005 season. During the 2006 season rates of 200 and 400 $mg \cdot L^{-1}$ ProCa were applied on 14 Dec. 2005 and 16 Jan. 2006 constituting an early application treatment, and for the late application treatment rates of 200 and 400 $mg \cdot L^{-1}$ ProCa were applied 6 (8 Mar. 2006) and 3 (23 Mar. 2006) weeks before anticipated harvest (3 May 2006), and compared to an untreated control treatment.

'Palmer Navel' orange. Prohexadione-calcium was applied as a medium-cover spray with a hand-held spray gun with application rates of 200 and 400 $mg \cdot L^{-1}$ on 8 and 28 Dec. 2004, 1 Feb. 2005, and 4 (22 Apr. 2005) and 2 (6 May 2005) weeks before anticipated harvest (12 May 2005). During the 2006 season of 200 and 400 $mg \cdot L^{-1}$ ProCa were applied on 14 Dec. 2005 and 16 Jan. 2006 constituting an early application treatment, and for the late application treatment rates of 200 and 400 $mg \cdot L^{-1}$ ProCa were applied 6 (4 Apr. 2006) and 3 (25 Apr. 2006) weeks before anticipated harvest on 31 May 2006, and compared to an untreated control treatment.

‘Eureka’ lemon. Prohexadione-calcium was applied as a medium-cover spray with a hand-held spray gun with application rates of 200 and 400 mg·L^{-1} on 8 and 28 Dec. 2004, 1 Feb. 2005, 4 (8 Apr. 2005) and 2 (28 Apr. 2005) weeks before anticipated harvest (25 May 2005). In addition, individual fruit and fruit plus leaves were dipped on 4 May 2005 in 200 and 400 mg·L^{-1} ProCa solutions. During the 2006 season rates of 200 and 400 mg·L^{-1} ProCa were applied on 15 Dec. 2005 and 17 Jan. 2006 constituting an early application treatment, and for the late application treatment rates of 200 and 400 mg·L^{-1} ProCa were applied 6 (23 Mar. 2006) and 3 (11 Apr. 2006) weeks before anticipated harvest (12 May 2006), and compared to an untreated control treatment.

Fruit sampling. To limit unwanted, natural variation in rind colour, fruit were sampled from specific canopy positions. Fruit were sampled from the outer, eastern side of trees at a height of 1.5 to 2.0 m. During the 2005 season, 30 fruit were sampled from each tree for ‘Nules Clementine’ mandarin and ‘Palmer Navel’ orange of which 10 fruit were used for immediate analysis, and the remaining 20 fruit were degreened. After degreening, 10 fruit were analysed and the remaining 10 fruit were stored at 7.5 °C for 2 weeks followed by 1 week at 18 °C to simulate early season commercial shipping conditions. For ‘Navelina Navel’ orange, 20 fruit were sampled at a height of 1.5 to 2.0 m. Ten fruit were used for immediate analysis and the remaining 10 fruit were degreened and then analysed. For ‘Eureka’ lemon, only the dipped fruit were sampled as the bulk of the crop had been commercially harvested prior to sampling. Ten fruit per replicate were sampled for immediate analysis.

During the 2006 season, 30 fruit from each replicate from both the eastern and western sides of trees were sampled at a 1.5 to 2.0 m height from ‘Nules Clementine’ mandarin, and ‘Navelina Navel’ and ‘Palmer Navel’ orange trees. Ten fruit were used for immediate analysis

and the remaining 20 fruit were degreened. After degreening, 10 fruit were analysed and the remaining 10 fruit were stored at 4.5 °C for 2 weeks followed by 1 week at 18 °C. For 'Eureka' lemon, 20 fruit were sampled on both the eastern and western sides of trees at a height of 1.0 to 1.5 m. Ten fruit were used for immediate analysis and 10 fruit were degreened and then analysed.

Degreening was done at 23 °C with a relative humidity of 95%, an ethylene concentration of 2 $mg \cdot L^{-1}$ and a carbon dioxide (CO_2) concentration <0.3% (Krajewski and Pittaway, 2002). Fruit were subjected to a degreening time of 48 hours for 'Nules Clementine' mandarin, and for 72 hours for 'Navelina Navel' and 'Palmer Navel' oranges and 'Eureka' lemon.

Stored fruit were treated with 125 $mg \cdot L^{-1}$ 2,4-D (2,4-dichlorophenoxyacetic acid), 500 $mg \cdot L^{-1}$ Tecto® (thiabendazole) and 120 $mg \cdot L^{-1}$ Sporekill™ (didecyldimethylammonium chloride) drench and waxed with a polyethylene wax.

Data collection. Rind colour. Fruit were colour-rated with the "CRI colour charts, set no. 34, 36 or 37, 2004" for oranges, soft citrus and lemons, respectively (CRI, 2004a, 2004b, 2004c; Appendix 1-3). To limit the variation in rind colour on different sides of fruit, rind colour was also measured objectively on both the "vivid" (orange) and "dull" (green) sides of fruit with a Minolta chromameter (Model CR-400, Minolta Co. Ltd., Tokyo, Japan).

Rind pigments. Rind sampling was done by cutting the flavedo from the fruit. This was done either with a potato peeler ('Nules Clementine' mandarin) or with a citrus rind zester ('Navelina Navel' and 'Palmer Navel' oranges and 'Eureka' lemon) during the 2005 season. During the 2006 season, only citrus rind zesters were used for rind sampling on all cultivars.

Sampling was done from all 10 fruit in the eight replicates, the pooled flavedo was then immersed into liquid nitrogen and stored at -80 °C until completely frozen for a period of at least one day, whereafter the samples were freeze-dried at -56 °C until all moisture was removed from the rinds, which lasted 4 days. The samples were then milled (A10 Kika Labortechnic, Kika Werke, GMBH & Co., Staufen, Germany) and sieved through a 500 µm sieve, to a homogenous powder. Samples were then stored in polyethylene vials at -80 °C until analysed. All preparation activities were carried out under low light conditions to inhibit the degradation of carotenoids and chlorophyll.

From the freeze-dried rind sample, a 0.1 g sub-sample was added to 10 mL 96 % (v/v) aqueous ethanol solvent containing 0.1 $g \cdot L^{-1}$ butylated hydroxytoluene (BHT) and 0.2 $g \cdot L^{-1}$ diethyldithiocarbamate (DDC), both antioxidants to prevent carotenoid degradation. The sample was then vortexed for two 1-minute intervals, whereafter it was stored for 1.5 hours at 4 °C to allow the pigment to extract into the solvent. After 1.5 hour storage, the extraction was poured through ashless filter paper (Schleicher & Schuell, Dassel, Germany) to remove rind particles. The filtrated solution was then poured into plastic cuvettes placed into a spectrophotometer, zeroed with a ethanol/antioxidant solvent (Cary 50 conc UV-visible spectrophotometer, Varian Australia (Pty) Ltd, Mulgrave, Victoria, Australia). Absorbance readings were taken at 470, 649 and 664 nm. Absorbance values were used to determine the chlorophyll a (C_a), chlorophyll b (C_b), total chlorophyll (C_{a+b}) and total carotenoids (C_{x+c}) concentrations in $\mu g \cdot g^{-1}$ dry weight, using the Lightenthaler equations (Lichtenthaler, 1987):

$$C_a = 13.36\ A_{664} - 5.19A_{649}$$

$$C_b = 27.43A_{649} - 8.12A_{664}$$

$$C_{a+b} = A_{664} + 22.24A_{649}$$

$$C_{x+c} = \frac{100A_{470} - 2.13\ C_a - 97.64\ C_b}{209}$$

Statistical design and analysis. Experimental layout was a complete randomised block design (CRBD) consisting of eight single-tree replicates. Analysis of variance was conducted using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS Inc., Cary, N.C., USA) and least significant difference (LSD) values were used to indicate any significant differences among treatments.

Results

'Nules Clementine' mandarin. In the 2005 season, the 400 mg·L^{-1} ProCa treatment significantly improved rind colour rating compared to the untreated control treatment by 0.9 colour units after harvest(Table 4.1). The 400 mg·L^{-1} ProCa treatment reduced relative greenness (as evidenced by the lower hue angle), and resulted in fruit appearing brighter (higher lightness), and more intensely coloured (higher chroma) after harvest. These differences in rind colour were smaller following ethylene degreening, but rind colour was still significantly better for the 400 mg·L^{-1} ProCa treatment than the control. After cold-storage however, no significant differences were observed among treatments (Table 4.1). The perceived improvement in rind colour was due to a lower chlorophyll concentration (by ~ 57%), resulting in the improvement of the carotenoid to chlorophyll ratio (P = 0.0860) (Table 4.2).

In the 2006 season, rind colour rating after harvest was significantly improved by the late 400 mg·L^{-1} ProCa treatment by 0.6 colour units for fruit sampled from the eastern side of trees, and for fruit sampled from the western side of trees by the early 400 mg·L^{-1} treatment by 0.4

colour units, compared to the untreated control treatment (Table 4.3; Fig. 4.1). Relative greenness of the rind was reduced by the late 400 mg·L^{-1} ProCa treatment on the eastern side of trees and by the early 400 mg·L^{-1} treatment on the western side of trees (as evidenced by the lower hue angle), brighter fruit (higher lightness) and more intensely coloured fruit (higher chroma) were also the results of the treatments, particularly on the dull side of fruit (Table 4.3). Ethylene degreening improved rind colour in such a way that no significant differences in hue angle among treatments could be observed after degreening and after cold-storage compared to the untreated control treatment (Tables 4.4 to 4.6). The improvement in rind colour after harvest of fruit sampled from the eastern side of trees from the late 400 mg·L^{-1} ProCa treatment (Table 4.3) was due to significantly higher carotenoid concentrations (by ~ 25%), resulting in a higher carotenoid to chlorophyll ratio (P = 0.0645) (Table 4.6).

'Navelina Navel' orange. In the 2005 season, rind colour rating was significantly improved by the 400 mg·L^{-1} ProCa treatment by 1.3 colour units compared with the untreated control treatment (Table 4.7). Relative greenness of fruit was reduced (as evidenced by the significantly lower hue angle), and fruit were brighter (higher lightness) and more intensely coloured (higher chroma) at harvest for the 400 mg·L^{-1} ProCa treatment. These differences in rind colour were smaller following ethylene degreening (Table 4.7). The perceived improvement in rind colour of fruit was due to significantly higher carotenoid concentration (by ~ 15%) and significantly lower chlorophyll concentration (by ~ 41%), resulting in a significantly higher carotenoid to chlorophyll ratio after harvest, and due to a significantly higher carotenoid concentration (by ~ 35%) after ethylene degreening (Table 4.8).

In the 2006 season, rind colour rating after harvest of fruit sampled from the eastern and western sides of trees was significantly improved by the late 400 mg·L^{-1} ProCa treatment by

0.2 and 0.3 colour units, respectively, compared to the untreated control treatment (Table 4.9; Fig. 4.2). As this was the only treatment that improved rind colour, only the late 400 $mg \cdot L^{-1}$ ProCa treatment will be discussed in detail. The late 400 $mg \cdot L^{-1}$ ProCa treatment had a significantly lower hue angle, higher lightness and chroma on both sides of trees, resulting in lower relative greenness of rinds, as well as brighter and more intense coloured fruit (Table 4.9; Fig. 4.2). After ethylene degreening (Table 4.10) and after cold-storage (Table 4.11), rind colour of the late 400 $mg \cdot L^{-1}$ ProCa treatment was not better than that of the control treatment. This rind colour improvement (Table 4.9) was due to a lower chlorophyll concentration (by ~ 21%) of fruit sampled from the eastern side of trees, resulting in a significantly higher carotenoid to chlorophyll ratio (Table 4.12).

'Palmer Navel' orange. In the 2005 season, rind colour rating of fruit after harvest was significantly improved by both the 200 and 400 $mg \cdot L^{-1}$ ProCa treatments compared to the untreated control treatment by 0.8 colour units (Table 4.13). After ethylene degreening, the 200 $mg \cdot L^{-1}$ ProCa treatment had a significantly better rind colour rating than the control (by 0.4 colour units), with no differences in rind colour rating after cold-storage (Table 4.13). Colorimeter measurements indicated that the 200 $mg \cdot L^{-1}$ ProCa treatment improved rind colour of fruit the most, and will therefore be discussed in detail. The 200 $mg \cdot L^{-1}$ ProCa treatment reduced the hue angle, and increased the lightness and chroma of rinds, resulting in a reduction of relative greenness in rinds, as well as brighter and more intensely coloured fruit, after harvest. This perceived rind colour improvement was due to increased carotenoid concentration (by ~ 15%) and a reduction in chlorophyll concentration (by ~ 40%) after harvest, and an increased carotenoid concentration after ethylene degreening (by ~ 25%) and after cold-storage (by ~ 16%), resulting in significantly higher carotenoid to chlorophyll ratios after harvest, after ethylene degreening and after cold-storage (Table 4.14).

In the 2006 season, rind colour rating after harvest of fruit sampled from the eastern side of trees was significantly improved by the late 200 and 400 mg·L^{-1} ProCa treatments compared to the untreated control treatment by 0.4 colour units (Table 4.15; Fig. 4.3). However, these treatments did not affect rind colour rating of fruit sampled from the western side of trees. Colorimeter measurements showed that the late 400 mg·L^{-1} ProCa treatment significantly improved rind colour of fruit, and will therefore be discussed in detail. Relative greenness of fruit sampled from the eastern and western sides of trees was reduced, as evidenced by the lower hue angle. Fruit appeared brighter (higher lightness) and more intensely coloured (higher chroma) on the dull side of fruit sampled from the western side of trees (generally the worst case scenario for rind colour) (personal observation), possibly contributing to a reduction in rind colour variation within trees (Table 4.15). After ethylene degreening, no significant differences in rind colour occurred between the late 400 mg·L^{-1} ProCa and the untreated control treatment (Tables 4.16 and 4.18). After cold-storage, however, all treatments delayed rind colour development, resulting in higher relative greenness (higher hue angle) and less intensely coloured fruit (lower chroma) (Table 4.17). This perceived rind colour improvement (Table 4.15) was due to higher carotenoid concentration (by ~ 18%) of fruit sampled from the eastern and western sides of trees, resulting in a higher carotenoid to chlorophyll ratio on the western side of trees (Table 4.18). The poorer rind colour (Tables 4.16 and 4.17) was due to higher chlorophyll concentration of fruit sampled from the western side of trees (Table 4.18).

'Eureka' lemon. In the 2005 season, rind colour rating of fruit was significantly improved (by 0.5 colour units) by both the 200 and 400 mg·L^{-1} ProCa treatments when the fruit was dipped and (by 0.4 colour units) by the 400 mg·L^{-1} ProCa treatment when the fruit and leaves were

dipped compared to the untreated control treatment (Table 4.19). Colorimeter measurements (Table 4.19) and pigment concentrations (Table 4.20), however, did not differ among treatments.

In the 2006 season, rind colour rating of fruit sampled from the western side of trees was significantly improved by the early 200 mg·L^{-1} and both the late 200 and 400 mg·L^{-1} ProCa treatment by 0.5 colour units compared to the untreated control treatment (Table 4.21; Fig. 4.4). However, after degreening there were no significant differences in rind colour rating among treatments (Table 4.22) nor in pigment concentration when compared to the untreated control treatment (Table 4.23). Colorimeter measurements indicated that the late 400 mg·L^{-1} ProCa treatment significantly improved the rind colour on both the eastern and western sides of trees, and will therefore be discussed in detail (Table 4.21; Fig. 4.4). The late 400 mg·L^{-1} ProCa treatment significantly reduced the relative greenness of fruit (as evidenced by the lower hue angle) after harvest and after ethylene degreening, fruit also appeared brighter (higher lightness) after harvest, but duller (lower lightness) after ethylene degreening, and were more intensely coloured (higher chroma) after harvest and after ethylene degreening of fruit sampled from both the eastern and western sides of trees, on the vivid and dull sides of fruit (Tables 4.21 and 4.22). This perceived improvement in rind colour after harvest was due to a significant reduction in chlorophyll concentration (by ~ 38%), resulting in a significantly lower chlorophyll to carotenoid ratio as well as a significantly higher carotenoid to chlorophyll ratio of fruit sampled from the eastern side of trees (Table 4.23).

Discussion

The late 400 mg·L^{-1} ProCa treatment consistently improved rind colour on all *Citrus* spp. tested. However, these effects were more pronounced after harvest, as ethylene degreening

and cold-storage stimulated additional chlorophyll degradation, unmasking the carotenoids, resulting in overall better coloured fruit (El-Zeftawi, 1978; Goldschmidt, 1988; Van Wyk, 2004). Prohexadione calcium in most instances stimulated chlorophyll degradation and carotenoid biosynthesis confirming the preliminary results of Barry and Van Wyk (2004). These changes in pigment concentration resulted in a higher carotenoid to chlorophyll ratio and, therefore, improved rind colour. Gilfillan and Lowe (1985) demonstrated the same response when paclobutrazol, also a gibberellin biosynthesis inhibitor, improved 'Satsuma' mandarin rind colour.

Prohexadione-calcium has been shown to reduce vegetative growth in *Citrus* spp. (Stover et al., 2004; Chapter 3), similar to paclobutrazol (Aron et al., 1985; Smeirat and Qrunfleh, 1989) and uniconazole (Wheaton, 1989). Therefore, the improvement of rind colour of citrus fruit in the current study following the application of a gibberellin biosynthesis inhibitor (400 $mg \cdot L^{-1}$ ProCa applied 6 plus 3 weeks before harvest) supports the hypothesis that there may be a relationship between vegetative vigour and rind colour development of citrus fruit, although vegetative vigour was not measured in this study.

Table 4.1. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest, after ethylene degreening and after cold-storage on the vivid (yellow) and dull (green) sides of 'Nules Clementine' mandarin fruit sampled from the eastern side of trees during the 2005 season.

Treatment	After harvest		After degreening		After storage	
			Colour rating[z]			
Control	3.5 a[y]		1.3 a		1.1 ns	
ProCa (200 mg·L^{-1})	3.2 a		1.2 b		1.1	
ProCa (400 mg·L^{-1})	2.6 b		1.1 b		1.0	
P-value	0.0006		0.0006		0.2389	
LSD	0.46		0.12		0.07	
	Vivid	Dull	Vivid	Dull	Vivid	Dull
			Hue angle (°)			
Control	74.2 a	83.0 a	64.5 ns	67.7 a	61.0 ns	62.5 ns
ProCa (200 mg·L^{-1})	70.2 b	76.4 b	64.0	66.3 b	61.7	63.2
ProCa (400 mg·L^{-1})	70.3 b	75.8 b	64.7	66.7 ab	61.9	62.7
P-value	<0.0001	<0.0001	0.4553	0.0447	0.3125	0.5606
LSD	1.66	2.23	1.11	1.13	1.23	1.30
			Lightness			
Control	69.9 a	67.1 b	66.2 ns	68.3 a	63.8 ns	64.5 ns
ProCa (200 mg·L^{-1})	68.6 b	67.2 b	65.9	67.0 b	63.7	64.4
ProCa (400 mg·L^{-1})	70.2 a	69.7 a	66.8	67.5 b	64.0	64.4
P-value	<0.0001	0.0006	0.0795	0.0072	0.7285	0.9502
LSD	0.73	1.47	0.77	0.79	0.74	0.73
			Chroma			
Control	69.9 b	64.4 c	71.2 a	71.3 a	68.6 a	69.2 a
ProCa (200 mg·L^{-1})	70.9 ab	67.7 b	69.9 b	69.5 b	67.4 b	67.8 b
ProCa (400 mg·L^{-1})	71.4 a	70.3 a	70.1 b	69.9 b	68.3 a	67.8 b
P-value	0.0456	<0.0001	0.0028	<0.0001	0.0039	<0.0001
LSD	1.23	2.07	0.78	0.80	0.70	0.69

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.2. Total chlorophyll concentration, total carotenoid concentration, chlorophyll carotenoid ratio and carotenoid chlorophyll ratio following different Prohexadione-calcuim treatments on 'Nules Clementine' mandarin fruit after harvest, after ethylene degreening and after cold-storage of fruit during the 2005 season.

Treatment	After harvest	After degreening	After storage
		Carotenoid ($\mu g \cdot g^{-1}$ DW)	
Control	488.8 b[z]	626.0 ns	961.1 ns
ProCa (200 $mg \cdot L^{-1}$)	595.3 a	665.6	1011.1
ProCa (400 $mg \cdot L^{-1}$)	503.7 b	624.0	937.6
P-value	0.0448	0.5849	0.3754
LSD	89.19	92.92	108.85
		Chlorophyll ($\mu g \cdot g^{-1}$ DW)	
Control	130.0 ns	25.4 ns	32.0 ns
ProCa (200 $mg \cdot L^{-1}$)	81.0	34.5	35.9
ProCa (400 $mg \cdot L^{-1}$)	55.3	28.9	28.1
P-value	0.0639	0.1210	0.0890
LSD	62.94	8.87	6.89
		Chlorophyll/Carotenoid Ratio	
Control	0.3 a	0.042 ns	0.033 ns
ProCa (200 $mg \cdot L^{-1}$)	0.1 b	0.054	0.036
ProCa (400 $mg \cdot L^{-1}$)	0.1 b	0.047	0.031
P-value	0.0134	0.4600	0.5810
LSD	0.10	0.02	0.01
		Carotenoid/Chlorophyll Ratio	
Control	4.7 ns	28.9 a	30.7 ns
ProCa (200 $mg \cdot L^{-1}$)	12.2	20.1 b	29.6
ProCa (400 $mg \cdot L^{-1}$)	10.7	21.8 ab	34.9
P-value	0.0860	0.0483	0.3581
LSD	6.97	7.32	7.90

[z] Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = non significant).

Table 4.3. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest on the vivid (yellow) and dull (green) sides of 'Nules Clementine' mandarin fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	4.6 a[y]		4.3 ab	
ProCa early (200 mg·L^{-1})	4.5 a		4.0 bc	
ProCa early (400 mg·L^{-1})	4.7 a		3.9 c	
ProCa late (200 mg·L^{-1})	4.7 a		4.0 c	
ProCa late (400 mg·L^{-1})	4.0 b		4.4 a	
P-value	<0.0001		0.0065	
LSD	0.28		0.29	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	83.2 b	93.0 a	81.4 ab	92.3 a
ProCa early (200 mg·L^{-1})	84.8 ab	94.5 a	79.1 bc	89.7 ab
ProCa early (400 mg·L^{-1})	86.6 a	94.1 a	78.2 c	87.4 b
ProCa late (200 mg·L^{-1})	85.9 ab	95.0 a	80.2 abc	88.1 b
ProCa late (400 mg·L^{-1})	76.3 c	84.5 b	81.8 a	88.3 b
P-value	<0.0001	<0.0001	0.0424	0.0094
LSD	2.97	2.97	2.62	3.01
		Lightness		
Control	67.5 a	59.8 bc	66.6 ab	61.2 c
ProCa early (200 mg·L^{-1})	66.0 ab	59.8 bc	67.3 a	63.6 ab
ProCa early (400 mg·L^{-1})	66.1 ab	61.1 ab	67.5 a	65.5 a
ProCa late (200 mg·L^{-1})	65.0 b	58.4 c	66.7 ab	62.4 bc
ProCa late (400 mg·L^{-1})	66.1 ab	63.0 a	65.4 b	62.2 bc
P-value	0.0329	0.0012	0.0950	0.0002
LSD	1.59	2.12	1.53	1.92
		Chroma		
Control	64.6 a	54.7 bc	64.5 b	56.7 b
ProCa early (200 mg·L^{-1})	62.8 ab	53.9 bc	67.3 a	59.0 ab
ProCa early (400 mg·L^{-1})	63.0 ab	55.9 b	67.0 a	61.5 a
ProCa late (200 mg·L^{-1})	61.2 b	52.3 c	64.4 b	58.2 b
ProCa late (400 mg·L^{-1})	64.9 a	59.2 a	62.1 c	57.6 b
P-value	0.0055	<0.0001	<0.0001	0.0016
LSD	2.14	2.69	2.01	2.45

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.4. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after ethylene degreening on the vivid (yellow) and dull (green) sides of 'Nules Clementine' mandarin orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	1.3 ns[y]		1.4 ns	
ProCa early (200 mg·L^{-1})	1.4		1.2	
ProCa early (400 mg·L^{-1})	1.3		1.3	
ProCa late (200 mg·L^{-1})	1.3		1.3	
ProCa late (400 mg·L^{-1})	1.1		1.4	
P-value	0.1144		0.0524	
LSD	0.20		0.17	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	64.8 ns	67.9 ns	64.2 b	68.2 ns
ProCa early (200 mg·L^{-1})	65.3	68.5	62.9 c	66.9
ProCa early (400 mg·L^{-1})	64.5	68.5	66.0 a	68.7
ProCa late (200 mg·L^{-1})	65.4	68.8	66.0 a	68.4
ProCa late (400 mg·L^{-1})	63.7	67.0	65.1 ab	68.4
P-value	0.2692	0.3025	<0.0001	0.0574
LSD	1.59	1.71	1.30	1.33
		Lightness		
Control	65.2 ns	66.6 a	64.7 b	66.9 ab
ProCa early (200 mg·L^{-1})	65.1	66.8 a	63.4 c	66.2 bc
ProCa early (400 mg·L^{-1})	64.2	66.7 a	65.9 a	67.2 a
ProCa late (200 mg·L^{-1})	65.2	67.1 a	66.1 a	65.6 c
ProCa late (400 mg·L^{-1})	64.1	64.6 b	64.3 b	66.7 ab
P-value	0.1217	0.0013	<0.0001	0.0088
LSD	1.10	1.21	0.95	1.03
		Chroma		
Control	76.6 a	75.4 a	75.8 ab	74.9 a
ProCa early (200 mg·L^{-1})	76.9 a	75.5 a	75.0 b	74.4 ab
ProCa early (400 mg·L^{-1})	74.8 b	74.3 a	76.3 a	74.7 a
ProCa late (200 mg·L^{-1})	74.6 b	74.2 a	75.1 b	73.2 b
ProCa late (400 mg·L^{-1})	74.5 b	71.7 b	73.5 c	73.9 ab
P-value	<0.0001	<0.0001	<0.0001	0.0201
LSD	1.10	1.38	0.84	1.19

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.5. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after cold-storage on the vivid (yellow) and dull (green) sides of 'Nules Clementine' mandarin orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	1.2 ns[y]		1.1 b	
ProCa early (200 mg·L^{-1})	1.0		1.1 b	
ProCa early (400 mg·L^{-1})	1.1		1.1 b	
ProCa late (200 mg·L^{-1})	1.1		1.1 b	
ProCa late (400 mg·L^{-1})	1.0		1.3 a	
P-value	0.1141		0.0391	
LSD	0.13		0.12	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	63.4 ab	65.4 ns	62.5 ns	65.4 ns
ProCa early (200 mg·L^{-1})	62.4 b	65.8	62.2	65.3
ProCa early (400 mg·L^{-1})	63.6 ab	65.8	63.1	66.4
ProCa late (200 mg·L^{-1})	64.6 a	65.7	63.9	66.2
ProCa late (400 mg·L^{-1})	64.1 a	66.2	63.0	65.7
P-value	0.0167	0.8606	0.0730	0.3793
LSD	1.40	1.38	1.35	1.38
		Lightness		
Control	64.2 ab	64.1 bc	62.9 ns	64.9 ab
ProCa early (200 mg·L^{-1})	63.1 c	65.2 a	62.9	65.0 ab
ProCa early (400 mg·L^{-1})	64.0 abc	64.8 ab	63.5	65.7 a
ProCa late (200 mg·L^{-1})	64.6 a	63.2 c	63.4	64.1 b
ProCa late (400 mg·L^{-1})	63.3 bc	64.2 abc	63.5	64.0 b
P-value	0.0101	0.0018	0.5763	0.0040
LSD	0.98	1.09	1.00	0.99
		Chroma		
Control	69.5 a	68.3 ab	68.7 ns	68.1 a
ProCa early (200 mg·L^{-1})	68.6 ab	69.1 a	68.3	68.4 a
ProCa early (400 mg·L^{-1})	68.7 a	68.2 ab	68.8	68.7 a
ProCa late (200 mg·L^{-1})	68.8 a	66.7 c	68.2	67.6 ab
ProCa late (400 mg·L^{-1})	67.6 b	67.3 bc	68.6	66.9 b
P-value	0.0242	0.0038	0.5831	0.0453
LSD	0.99	1.31	0.90	1.14

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.6. Carotenoid, chlorophyll, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest, after ethylene degreening and after cold-storage of 'Nules Clementine' mandarin fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	After harvest	After degreening	After storage	After harvest	After degreening	After storage
	Eastern			Western		
	Carotenoid ($\mu g \cdot g^{-1}$ DW)					
Control	461.5 bc[x]	890.2 ns	928.5 ns	498.3 ns	930.6 ns	865.5 ns
ProCa early (200 $mg \cdot L^{-1}$)	456.0 bc	831.1	884.9	507.4	898.5	793.9
ProCa early (400 $mg \cdot L^{-1}$)	431.5 c	811.6	845.9	503.8	851.8	892.3
ProCa late (200 $mg \cdot L^{-1}$)	538.0 ab	862.1	838.8	502.4	880.4	814.7
ProCa late (400 $mg \cdot L^{-1}$)	617.1 a	968.5	833.0	575.2	985.4	815.6
P-value	0.0093	0.5211	0.7107	0.7511	0.5177	0.5133
LSD	105.11	178.47	173.69	121.81	146.42	137.64
	Chlorophyll ($\mu g \cdot g^{-1}$ DW)					
Control	243.0 b	45.6 ns	nd[z]	237.1 ns	34.4 b	nd
ProCa early (200 $mg \cdot L^{-1}$)	294.0 b	39.8	nd	193.8	28.0 b	nd
ProCa early (400 $mg \cdot L^{-1}$)	291.6 b	53.8	nd	189.6	26.8 b	nd
ProCa late (200 $mg \cdot L^{-1}$)	403.6 a	36.8	nd	290.6	38.7 b	nd
ProCa late (400 $mg \cdot L^{-1}$)	236.0 b	33.9	nd	349.6	59.1 a	nd
P-value	0.0277	0.4062	nd	0.2332	0.0082	nd
LSD	109.01	22.35	nd	154.74	15.74	nd
	Chlorophyll/Carotenoid Ratio					
Control	0.55 ns	0.05 ns	nc[y]	0.51 ns	0.04 ns	nc
ProCa early (200 $mg \cdot L^{-1}$)	0.68	0.05	nc	0.41	0.03	nc
ProCa early (400 $mg \cdot L^{-1}$)	0.69	0.07	nc	0.40	0.03	nc
ProCa late (200 $mg \cdot L^{-1}$)	0.78	0.04	nc	0.60	0.05	nc
ProCa late (400 $mg \cdot L^{-1}$)	0.38	0.04	nc	0.58	0.06	nc
P-value	0.0710	0.3147	nc	0.5302	0.0888	nc
LSD	0.28	0.03	nc	0.31	0.02	nc
	Carotenoid/Chlorophyll Ratio					
Control	2.19 ns	20.04 ns	nc	2.31 ns	28.48 ns	nc
ProCa early (200 $mg \cdot L^{-1}$)	1.95	22.40	nc	3.13	40.61	nc
ProCa early (400 $mg \cdot L^{-1}$)	1.61	17.80	nc	4.65	32.98	nc
ProCa late (200 $mg \cdot L^{-1}$)	1.41	27.58	nc	2.01	23.98	nc
ProCa late (400 $mg \cdot L^{-1}$)	2.95	29.26	nc	2.75	20.45	nc
P-value	0.0645	0.2092	nc	0.2408	0.0543	nc
LSD	1.05	11.00	nc	2.61	14.02	nc

[z] Chlorophylls were not detectable (nd) by spectrophotometry.

[y] Ratios could not be calculated (nc) due to the non detectable chlorophylls.

[x] Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = non significant).

Table 4.7. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest and after ethylene degreening on the vivid (yellow) and dull (green) sides of 'Navelina Navel' orange fruit sampled from the eastern side of trees during the 2005 season.

Treatment	After harvest		After degreening	
			Colour rating[z]	
Control	5.2 a[y]		2.2 a	
ProCa (200 mg·L^{-1})	4.4 b		2.1 a	
ProCa (400 mg·L^{-1})	3.9 c		1.8 b	
P-value	<0.0001		0.0001	
LSD	0.29		0.19	
	Vivid	Dull	Vivid	Dull
			Hue angle (°)	
Control	91.4 a	104.0 a	76.0 ns	78.4 b
ProCa (200 mg·L^{-1})	87.9 b	99.9 b	76.0	80.0 a
ProCa (400 mg·L^{-1})	84.2 c	96.9 c	76.2	79.2 ab
P-value	<0.0001	<0.0001	0.9277	0.0398
LSD	1.89	1.95	1.02	1.19
			Lightness	
Control	68.4 b	56.9 b	70.0 ns	67.4 b
ProCa (200 mg·L^{-1})	69.6 ab	58.6 b	69.7	66.6 b
ProCa (400 mg·L^{-1})	70.0 a	61.2 a	70.7	68.6 a
P-value	0.0013	<0.0001	0.0545	0.0019
LSD	1.37	1.76	0.82	1.12
			Chroma	
Control	64.8 c	52.5 c	71.8 ns	69.7 ab
ProCa (200 mg·L^{-1})	66.9 b	54.9 b	71.7	68.8 b
ProCa (400 mg·L^{-1})	69.0 a	58.2 a	72.4	70.8 a
P-value	<0.0001	<0.0001	0.2839	0.0352
LSD	1.74	2.18	0.95	1.49

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.8. Total chlorophyll concentration, total carotenoid concentration, chlorophyll carotenoid ratio and carotenoid chlorophyll ratio following different Prohexadione-calcium treatments on 'Navelina Navel' orange fruit after harvest and after ethylene degreening of fruit during the 2005 season.

Treatment	After harvest	After degreening
	Carotenoid ($\mu g \cdot g^{-1}$ DW)	
Control	193.9 b[z]	187.5 b
ProCa (200 $mg \cdot L^{-1}$)	210.0 ab	283.5 a
ProCa (400 $mg \cdot L^{-1}$)	229.3 a	289.5 a
P-value	0.0462	0.0069
LSD	27.54	66.73
	Chlorophyll ($\mu g \cdot g^{-1}$ DW)	
Control	211.1 a	22.0 ns
ProCa (200 $mg \cdot L^{-1}$)	158.7 ab	25.1
ProCa (400 $mg \cdot L^{-1}$)	124.7 b	22.9
P-value	0.0343	0.7470
LSD	64.15	8.60
	Chlorophyll/Carotenoid Ratio	
Control	1.1 a	0.5 ns
ProCa (200 $mg \cdot L^{-1}$)	0.8 b	0.1
ProCa (400 $mg \cdot L^{-1}$)	0.6 b	0.1
P-value	0.0105	0.3062
LSD	0.30	0.68
	Carotenoid/Chlorophyll Ratio	
Control	1.1 b	10.0 ns
ProCa (200 $mg \cdot L^{-1}$)	1.5 ab	12.4
ProCa (400 $mg \cdot L^{-1}$)	2.1 a	13.6
P-value	0.0211	0.3256
LSD	0.74	4.98

[z] Means within columns followed by different letters are significantly different ($P \leq 0.05$; ns = non significant).

Table 4.9. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest on the vivid (yellow) and dull (green) sides of 'Navelina Navel' orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	5.0 b[y]		5.4 a	
ProCa early (200 mg·L^{-1})	5.3 a		5.4 a	
ProCa early (400 mg·L^{-1})	5.1 ab		5.3 a	
ProCa late (200 mg·L^{-1})	5.0 bc		5.3 a	
ProCa late (400 mg·L^{-1})	4.8 c		5.1 b	
P-value	<0.0001		0.0030	
LSD	0.17		0.21	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	99.2 a	110.5 a	101.5 a	111.2 a
ProCa early (200 mg·L^{-1})	100.2 a	111.6 a	98.8 b	110.5 ab
ProCa early (400 mg·L^{-1})	99.3 a	110.1 a	97.4 b	109.7 b
ProCa late (200 mg·L^{-1})	96.6 b	108.3 b	99.3 ab	108.1 c
ProCa late (400 mg·L^{-1})	92.9 c	102.4 c	94.9 c	104.3 d
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	2.26	1.69	2.35	1.48
		Lightness		
Control	64.5 b	53.5 c	62.4 b	54.8 c
ProCa early (200 mg·L^{-1})	65.0 b	53.5 c	64.9 a	54.9 c
ProCa early (400 mg·L^{-1})	65.4 b	54.7 bc	65.3 a	55.5 c
ProCa late (200 mg·L^{-1})	68.0 a	56.0 b	65.0 a	57.2 b
ProCa late (400 mg·L^{-1})	69.1 a	59.8 a	66.5 a	59.3 a
P-value	<0.0001	<0.0001	0.0004	<0.0001
LSD	1.82	1.53	1.81	1.44
		Chroma		
Control	59.2 c	47.4 cd	57.6 c	48.7 c
ProCa early (200 mg·L^{-1})	58.3 c	46.0 d	59.7 b	48.3 c
ProCa early (400 mg·L^{-1})	59.6 c	48.1 bc	60.8 b	49.4 c
ProCa late (200 mg·L^{-1})	62.2 b	49.7 b	59.4 bc	51.30 b
ProCa late (400 mg·L^{-1})	64.1 a	54.8 a	63.1 a	53.90 a
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.90	1.86	2.14	1.72

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.10. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after ethylene degreening on the vivid (yellow) and dull (green) sides of 'Navelina Navel ' orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	2.0 c[y]		2.3 c	
ProCa early (200 mg·L^{-1})	2.2 b		2.6 ab	
ProCa early (400 mg·L^{-1})	2.3 b		2.5 b	
ProCa late (200 mg·L^{-1})	2.5 a		2.7 a	
ProCa late (400 mg·L^{-1})	2.2 b		2.3 c	
P-value	<0.0001		<0.0001	
LSD	0.18		0.20	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	76.8 bc	78.2 c	78.9 a	80.3 a
ProCa early (200 mg·L^{-1})	76.6 c	79.2 bc	77.6 dc	79.8 ab
ProCa early (400 mg·L^{-1})	76.9 bc	78.9 bc	77.8 bc	80.4 a
ProCa late (200 mg·L^{-1})	78.5 a	81.4 a	78.8 ab	80.7 a
ProCa late (400 mg·L^{-1})	77.7 ab	79. b	76.8 d	78.6 b
P-value	0.0011	<0.0001	0.0002	0.0332
LSD	1.01	1.26	1.03	1.37
		Lightness		
Control	70.8 a	70.1 ns	70.7 ns	69.8 ns
ProCa early (200 mg·L^{-1})	69.5 b	69.1	69.7	68.8
ProCa early (400 mg·L^{-1})	70.7 a	69.6	70.3	69.3
ProCa late (200 mg·L^{-1})	71.3 a	69.6	70.5	68.9
ProCa late (400 mg·L^{-1})	70.9 a	69.8	70.4	69.2
P-value	0.0001	0.5096	0.1043	0.4403
LSD	0.78	1.10	0.75	1.16
		Chroma		
Control	71.9 a	71.5 a	71.4 ns	70.1 a
ProCa early (200 mg·L^{-1})	70.4 b	69.5 b	70.2	68.1 b
ProCa early (400 mg·L^{-1})	71.1 ab	69.8 b	70.6	68.5 ab
ProCa late (200 mg·L^{-1})	70.3 b	68.9 b	69.9	67.5 b
ProCa late (400 mg·L^{-1})	70.8 b	69.9 b	71.0	69.8 a
P-value	0.0032	0.0069	0.0992	0.0092
LSD	0.93	1.40	1.19	1.68

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.11. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after cold-storage on the vivid (yellow) and dull (green) sides of 'Navelina Navel ' orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	1.0 c[y]		1.2 ns	
ProCa early (200 mg·L^{-1})	1.1 ab		1.3	
ProCa early (400 mg·L^{-1})	1.1 bc		1.2	
ProCa late (200 mg·L^{-1})	1.2 a		1.2	
ProCa late (400 mg·L^{-1})	1.0 bc		1.2	
P-value	0.0155		0.4630	
LSD	0.09		0.15	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	71.8 c	73.0 b	73.8 ns	74.1 b
ProCa early (200 mg·L^{-1})	72.6 bc	73.1 b	73.4	74.2 b
ProCa early (400 mg·L^{-1})	72.3 bc	73.6 b	72.7	73.3 b
ProCa late (200 mg·L^{-1})	73.9 a	74.7 a	74.1	75.6 a
ProCa late (400 mg·L^{-1})	72.9 ab	73.7 b	73.2	73.4 b
P-value	0.0027	0.0134	0.0706	0.0002
LSD	1.11	1.05	1.01	1.07
		Lightness		
Control	66.4 ns	66.8 ns	66.9 ns	66.8 ns
ProCa early (200 mg·L^{-1})	66.5	66.5	66.5	66.5
ProCa early (400 mg·L^{-1})	66.7	66.9	66.8	66.6
ProCa late (200 mg·L^{-1})	67.4	67.2	67.3	67.2
ProCa late (400 mg·L^{-1})	67.0	66.6	66.5	66.4
P-value	0.1399	0.4157	0.1992	0.2163
LSD	0.81	0.81	0.72	0.77
		Chroma		
Control	66.5 ns	67.0 ns	67.9 ns	67.5 ns
ProCa early (200 mg·L^{-1})	67.5	67.7	67.2	67.3
ProCa early (400 mg·L^{-1})	67.2	67.4	67.5	67.6
ProCa late (200 mg·L^{-1})	67.2	66.7	66.8	66.6
ProCa late (400 mg·L^{-1})	66.6	66.0	66.9	66.7
P-value	0.2921	0.0601	0.1603	0.2721
LSD	1.06	1.17	1.03	1.12

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.12. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest, after degreening and after storage of 'Navelina Navel' orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	After harvest	After degreening	After storage	After harvest	After degreening	After storage
	Eastern			Western		
	Carotenoid ($\mu g \cdot g^{-1}$ DW)					
Control	263.3 ns[z]	298.5 ns	454.5 ns	238.3 ns	326.5 ns	390.5 ns
ProCa early (200 $mg \cdot L^{-1}$)	261.6	268.5	427.8	248.7	304.7	390.5
ProCa early (400 $mg \cdot L^{-1}$)	258.8	327.1	447.1	250.2	313.0	395.0
ProCa late (200 $mg \cdot L^{-1}$)	262.2	316.2	437.2	240.2	336.8	370.1
ProCa late (400 $mg \cdot L^{-1}$)	255.0	297.5	407.9	262.4	354.6	393.7
P-value	0.9425	0.1070	0.4180	0.4662	0.2097	0.8130
LSD	22.16	34.85	54.34	28.82	45.23	50.37
	Chlorophyll ($\mu g \cdot g^{-1}$ DW)					
Control	384.7 ab	21.8 ns	18.1 ab	418.1 ns	18.7 ns	21.1 ns
ProCa early (200 $mg \cdot L^{-1}$)	443.8 a	21.1	15.2 b	436.4	21.4	19.5
ProCa early (400 $mg \cdot L^{-1}$)	409.8 a	24.9	13.9 b	416.2	21.5	16.5
ProCa late (200 $mg \cdot L^{-1}$)	402.3 a	20.7	18.3 ab	402.6	14.7	21.8
ProCa late (400 $mg \cdot L^{-1}$)	302.5 b	24.9	23.7 a	359.1	16.1	18.7
P-value	0.0252	0.8703	0.0295	0.5829	0.1832	0.8546
LSD	84.93	10.69	6.12	98.32	7.02	11.11
	Chlorophyll/Carotenoid Ratio					
Control	1.47 a	0.07 ns	0.04 b	1.75 ns	0.06 ns	0.05 ns
ProCa early (200 $mg \cdot L^{-1}$)	1.69 a	0.08	0.04 b	1.74	0.07	0.05
ProCa early (400 $mg \cdot L^{-1}$)	1.57 a	0.08	0.03 b	1.67	0.07	0.04
ProCa late (200 $mg \cdot L^{-1}$)	1.53 a	0.07	0.04 b	1.67	0.04	0.06
ProCa late (400 $mg \cdot L^{-1}$)	1.18 b	0.09	0.06 a	1.37	0.05	0.05
P-value	0.0102	0.8742	0.0208	0.1096	0.1147	0.8269
LSD	0.28	0.04	0.02	0.32	0.03	0.03
	Carotenoid/Chlorophyll Ratio					
Control	0.71 b	15.69 ns	27.48 ab	0.57 ns	20.46 ns	23.67 ns
ProCa early (200 $mg \cdot L^{-1}$)	0.59 b	13.66	38.99 a	0.61	16.02	27.08
ProCa early (400 $mg \cdot L^{-1}$)	0.65 b	13.70	38.13 a	0.61	16.00	26.00
ProCa late (200 $mg \cdot L^{-1}$)	0.67 b	16.56	24.48 ab	0.63	24.34	24.48
ProCa late (400 $mg \cdot L^{-1}$)	0.93 a	16.03	17.70 b	0.77	24.44	23.95
P-value	0.0017	0.7960	0.0323	0.0845	0.0659	0.9913
LSD	0.16	5.99	14.95	0.14	7.80	15.68

[z] Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = non significant).

Table 4.13. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest, after ethylene degreening and after cold-storage on the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the eastern side of trees during the 2005 season.

Treatment	After harvest		After degreening		After storage	
			Colour rating[z]			
Control	5.3 a[y]		2.9 a		1.9 ns	
ProCa (200 mg·L^{-1})	4.5 b		2.5 b		1.8	
ProCa (400 mg·L^{-1})	4.5 b		2.8 ab		1.9	
P-value	<0.0001		0.0129		0.3968	
LSD	0.24		0.30		0.19	
	Vivid	Dull	Vivid	Dull	Vivid	Dull
			Hue angle (°)			
Control	90.7 a	105.4 a	77.1 a	82.2 a	73.8 a	78.0 a
ProCa (200 mg·L^{-1})	83.8 b	95.7 c	75.0 b	78.4 c	72.8 ab	75.0 c
ProCa (400 mg·L^{-1})	84.2 b	98.2 b	74.5 b	79.9 b	72.3 b	76.6 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0258	<0.0001
LSD	2.28	2.09	1.23	1.37	1.11	1.10
			Lightness			
Control	67.6 b	61.6 c	70.9 ab	74.2 a	69.2 ab	72.7 a
ProCa (200 mg·L^{-1})	70.6 a	66.7 a	71.2 a	73.4 a	69.5 a	71.5 b
ProCa (400 mg·L^{-1})	70.1 a	63.6 b	70.2 b	71.3 b	68.6 b	71.1 b
P-value	<0.0001	<0.0001	0.0263	<0.0001	0.0331	<0.0001
LSD	1.49	1.75	0.75	1.09	0.66	0.70
			Chroma			
Control	67.7 b	56.7 c	75.8 b	73.0 b	73.5 a	75.1 a
ProCa (200 mg·L^{-1})	73.3 a	63.3 a	78.3 a	75.6 a	73.8 a	74.9 a
ProCa (400 mg·L^{-1})	72.6 a	60.5 b	76.8 b	73.2 b	72.7 b	73.6 b
P-value	<0.0001	<0.0001	0.0016	0.0003	0.0175	0.0002
LSD	2.36	2.15	1.36	1.42	0.80	0.76

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.14. Total chlorophyll concentration, total carotenoid concentration, chlorophyll carotenoid ratio and carotenoid chlorophyll ratio following different Prohexadione-calcium treatments on 'Palmer Navel' orange fruit after harvest, after ethylene degreening and after cold-storage of fruit during the 2005 season.

Treatment	After harvest	After degreening	After storage
		Carotenoid ($\mu g \cdot g^{-1}$ DW)	
Control	298.1 ns[z]	256.8 b	510.5 b
ProCa (200 $mg \cdot L^{-1}$)	349.3	340.7 a	605.5 a
ProCa (400 $mg \cdot L^{-1}$)	298.3	331.9 a	566.1 ab
P-value	0.0543	<0.0001	0.0161
LSD	47.33	32.77	62.48
		Chlorophyll ($\mu g \cdot g^{-1}$ DW)	
Control	334.7 a	67.9 ns	39.6 ns
ProCa (200 $mg \cdot L^{-1}$)	201.3 b	52.2	37.5
ProCa (400 $mg \cdot L^{-1}$)	230.1 b	68.2	52.7
P-value	0.0247	0.3242	0.1397
LSD	97.99	24.66	16.42
		Chlorophyll/Caroteniod	
Control	1.1 a	0.3	0.08 ns
ProCa (200 $mg \cdot L^{-1}$)	0.6 b	0.2	0.07
ProCa (400 $mg \cdot L^{-1}$)	0.8 b	0.2	0.09
P-value	0.0036	0.0647	0.3211
LSD	0.29	0.09	0.04
		Carotenoid/Chlorophyll	
Control	0.9 b	4.0 b	13.2 b
ProCa (200 $mg \cdot L^{-1}$)	2.3 a	8.7 a	20.5 a
ProCa (400 $mg \cdot L^{-1}$)	1.4 b	5.5 b	11.7 b
P-value	0.0049	0.0177	0.0303
LSD	0.76	3.16	6.81

[z]Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = non significant).

Table 4.15. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest on the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	4.0 b[y]		4.6 bc	
ProCa early (200 mg·L^{-1})	4.4 a		4.9 a	
ProCa early (400 mg·L^{-1})	4.5 a		4.9 ab	
ProCa late (200 mg·L^{-1})	3.6 c		4.6 c	
ProCa late (400 mg·L^{-1})	3.6 c		4.5 c	
P-value	<0.0001		0.0027	
LSD	0.33		0.25	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	77.8 bc	87.9 b	84.7 b	94.5 b
ProCa early (200 mg·L^{-1})	81.3 a	91.3 a	87.1 a	98.5 a
ProCa early (400 mg·L^{-1})	79.6 ab	90.1 ab	85.2 ab	95.4 b
ProCa late (200 mg·L^{-1})	77.8 bc	85.2 c	83.3 bc	91.8 c
ProCa late (400 mg·L^{-1})	76.2 c	82.0 d	82.3 c	88.9 d
P-value	<0.0001	<0.0001	0.0005	<0.0001
LSD	1.85	2.49	2.26	2.27
		Lightness		
Control	69.9 ns	66.3 ns	69.2 ns	62.4 b
ProCa early (200 mg·L^{-1})	68.8	65.0	67.9	61.0 b
ProCa early (400 mg·L^{-1})	69.5	65.3	67.8	62.3 b
ProCa late (200 mg·L^{-1})	69.2	66.6	68.9	64.6 a
ProCa late (400 mg·L^{-1})	69.0	66.1	68.7	64.6 a
P-value	0.0897	0.1616	0.0656	<0.0001
LSD	0.88	1.46	1.12	1.54
		Chroma		
Control	73.3 a	65.2 ab	68.8 a	59.1 b
ProCa early (200 mg·L^{-1})	69.6 c	62.0 c	66.1 c	56.3 c
ProCa early (400 mg·L^{-1})	70.4 bc	63.2 bc	66.5 bc	58.6 b
ProCa late (200 mg·L^{-1})	71.3 b	65.9 a	68.5 a	61.4 a
ProCa late (400 mg·L^{-1})	71.6 b	66.6 a	67.9 ab	61.7 a
P-value	<0.0001	0.0001	0.0044	<0.0001
LSD	1.43	2.17	1.68	2.12

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.16. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after ethylene degreening on the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	3.0 cd[y]		3.5 c	
ProCa early (200 mg·L^{-1})	3.4 b		4.4 a	
ProCa early (400 mg·L^{-1})	3.8 a		4.0 b	
ProCa late (200 mg·L^{-1})	3.3 bc		3.9 b	
ProCa late (400 mg·L^{-1})	2.8 d		3.8 bc	
P-value	<0.0001		<0.0001	
LSD	0.39		0.34	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	72.1 cd	80.3 b	76.6 ns	85.6 bc
ProCa early (200 mg·L^{-1})	73.4 bc	80.4 b	78.6	88.7 a
ProCa early (400 mg·L^{-1})	77.3 a	84.6 a	77.2	86.0 b
ProCa late (200 mg·L^{-1})	74.7 b	80.6 b	76.8	84.0 bc
ProCa late (400 mg·L^{-1})	71.5 d	76.7 c	77.3	83.4 c
P-value	<0.0001	<0.0001	0.1214	<0.0001
LSD	1.50	2.09	1.60	2.28
		Lightness		
Control	69.2 bc	68.2 bc	71.2 ns	66.7 a
ProCa early (200 mg·L^{-1})	69.9 ab	69.6 a	70.5	64.7 b
ProCa early (400 mg·L^{-1})	68.9 a	67.0 c	70.8	66.1 ab
ProCa late (200 mg·L^{-1})	70.3 a	68.1 bc	70.5	66.8 a
ProCa late (400 mg·L^{-1})	70.6 c	68.7 ab	70.6	67.0 a
P-value	<0.0001	0.0072	0.2867	0.0448
LSD	0.76	1.36	0.74	1.64
		Chroma		
Control	75.1 a	69.5 a	74.0 a	65.8 a
ProCa early (200 mg·L^{-1})	74.1 b	69.8 a	72.5 bc	62.2 b
ProCa early (400 mg·L^{-1})	73.1 c	66.2 b	73.3 ab	65.0 a
ProCa late (200 mg·L^{-1})	72.7 c	68.8 a	71.9 c	65.7 a
ProCa late (400 mg·L^{-1})	73.5 bc	70.5 a	71.5 d	65.9 a
P-value	<0.0001	0.0017	<0.0001	0.0151
LSD	0.78	2.08	0.92	2.45

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.17. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after cold-storage on the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	1.5 c[y]		1.7 c	
ProCa early (200 mg·L^{-1})	1.9 b		2.4 b	
ProCa early (400 mg·L^{-1})	2.2 a		2.6 ab	
ProCa late (200 mg·L^{-1})	2.2 a		2.8 a	
ProCa late (400 mg·L^{-1})	2.3 a		2.6 ab	
P-value	<0.0001		<0.0001	
LSD	0.29		0.30	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	70.6 b	74.2 b	72.7 b	77.1 b
ProCa early (200 mg·L^{-1})	72.4 a	76.3 a	74.2 a	79.8 a
ProCa early (400 mg·L^{-1})	72.7 a	76.7 a	73.7 ab	78.2 b
ProCa late (200 mg·L^{-1})	71.9 a	76.0 a	74.8 a	79.9 a
ProCa late (400 mg·L^{-1})	70.6 b	74.5 b	73.7 ab	77.6 b
P-value	<0.0001	0.0002	0.0032	<0.0001
LSD	1.05	1.30	1.09	1.52
		Lightness		
Control	67.4 bc	69.4 a	68.3 c	69.2 a
ProCa early (200 mg·L^{-1})	68.5 a	68.8 ab	69.0 ab	66.8 c
ProCa early (400 mg·L^{-1})	68.6 a	69.0 a	68.6 bc	68.0 b
ProCa late (200 mg·L^{-1})	68.0 ab	68.1 bc	69.3 a	67.2 bc
ProCa late (400 mg·L^{-1})	67.1 c	67.6 c	68.8 abc	68.2 ab
P-value	<0.0001	0.0001	0.0364	<0.0001
LSD	0.67	0.85	0.68	1.09
		Chroma		
Control	73.6 a	74.6 a	74.2 a	73.5 a
ProCa early (200 mg·L^{-1})	73.7 a	72.1 bc	73.3 b	69.0 bc
ProCa early (400 mg·L^{-1})	73.4 ab	72.7 b	73.1 bc	70.8 b
ProCa late (200 mg·L^{-1})	72.6 bc	71.0 c	72.9 bc	68.4 c
ProCa late (400 mg·L^{-1})	72.0 c	70.9 c	72.4 c	70.0 bc
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	0.79	1.45	0.75	1.86

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.18. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest, after degreening and after storage of 'Palmer Navel' orange fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	After harvest	After degreening	After storage	After harvest	After degreening	After storage
	Eastern			Western		
	Carotenoid ($\mu g \cdot g^{-1}$ DW)					
Control	368.9 b[z]	431.6 ns	703.8 ns	379.5 b	414.3 ns	688.1 ns
ProCa early (200 $mg \cdot L^{-1}$)	385.7 b	449.6	702.1	366.4 b	394.2	639.2
ProCa early (400 $mg \cdot L^{-1}$)	409.9 ab	424.7	693.8	354.1 b	410.4	625.3
ProCa late (200 $mg \cdot L^{-1}$)	440.2 a	483.0	726.6	410.5 ab	433.7	687.0
ProCa late (400 $mg \cdot L^{-1}$)	448.7 a	499.8	766.9	460.8 a	440.3	660.0
P-value	0.0041	0.4073	0.5258	0.0089	0.3495	0.6192
LSD	46.82	94.93	96.40	62.09	51.11	95.53
	Chlorophyll ($\mu g \cdot g^{-1}$ DW)					
Control	115.4 ns	25.4 ns	25.6 ns	192.6 ns	55.4 ns	19.1 b
ProCa early (200 $mg \cdot L^{-1}$)	123.1	45.3	23.2	193.7	89.4	38.6 a
ProCa early (400 $mg \cdot L^{-1}$)	128.4	72.1	30.5	223.1	74.7	44.7 a
ProCa late (200 $mg \cdot L^{-1}$)	122.7	50.2	37.9	160.5	65.3	29.7 ab
ProCa late (400 $mg \cdot L^{-1}$)	118.2	41.4	32.6	151.3	68.7	36.7 a
P-value	0.9893	0.1213	0.4151	0.4782	0.5828	0.0315
LSD	52.79	34.01	16.70	83.43	43.86	15.88
	Chlorophyll/Carotenoid Ratio					
Control	0.32 ns	0.06 ns	0.04 ns	0.52 ns	0.15 ns	0.03 c
ProCa early (200 $mg \cdot L^{-1}$)	0.32	0.10	0.03	0.55	0.23	0.06 ab
ProCa early (400 $mg \cdot L^{-1}$)	0.32	0.18	0.05	0.62	0.18	0.07 a
ProCa late (200 $mg \cdot L^{-1}$)	0.28	0.11	0.05	0.39	0.15	0.04 bc
ProCa late (400 $mg \cdot L^{-1}$)	0.27	0.09	0.04	0.34	0.17	0.06 ab
P-value	0.9234	0.0743	0.5566	0.0926	0.5584	0.0221
LSD	0.14	0.08	0.03	0.22	0.12	0.03
	Carotenoid/Chlorophyll Ratio					
Control	3.33 ns	18.32 ab	28.32 ns	2.30 b	13.02 ns	37.13 a
ProCa early (200 $mg \cdot L^{-1}$)	5.07	10.26 b	34.50	2.20 b	5.97	18.43 b
ProCa early (400 $mg \cdot L^{-1}$)	3.25	10.72 b	25.25	1.70 b	6.06	15.97 c
ProCa late (200 $mg \cdot L^{-1}$)	3.88	10.81 b	25.32	3.02 ab	8.76	30.20 a
ProCa late (400 $mg \cdot L^{-1}$)	4.41	19.95 a	24.36	4.41 a	13.21	18.79 b
P-value	0.3960	0.0374	0.2387	0.0196	0.3106	<0.0001
LSD	2.02	8.15	9.84	1.63	9.22	8.87

[z] Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = non significant).

Table 4.19. Hue angle, lightness and chroma responses following different Prohexadione-calcium treatments on 'Eureka' lemons after harvest for yellow and green sides of fruit during the 2005 season.

Treatment	Fruit dipped		Fruit and leaves dipped	
	Colour rating[z]			
Control	5.2 a[y]		4.8 a	
ProCa (200 mg·L^{-1})	4.7 b		4.9 a	
ProCa (400 mg·L^{-1})	4.7 b		4.4 b	
P-value	0.0047		0.0086	
LSD	0.38		0.36	
	Fruit dipped Vivid	Fruit and leaves dipped Vivid	Fruit dipped Dull	Fruit and leaves dipped Dull
	Hue angle (°)			
Control	105.8 ns[z]	103.7 ns	110.6 ns	106.8 ns
ProCa (200 mg·L^{-1})	103.1	102.3	108.0	108.5
ProCa (400 mg·L^{-1})	104.1	102.7	108.6	106.4
P-value	0.1611	0.7164	0.1478	0.3358
LSD	2.81	3.63	2.70	2.89
	Lightness			
Control	67.8 a	70.4 ns	60.5 ns	62.3 ns
ProCa (200 mg·L^{-1})	69.9 a	69.2	61.8	63.2
ProCa (400 mg·L^{-1})	70.0 b	69.8	61.8	62.1
P-value	0.0413	0.5929	0.6897	0.8150
LSD	1.93	2.36	3.62	3.69
	Chroma			
Control	51.8 ns	52.1 ns	50.0 ns	52.1 ns
ProCa (200 mg·L^{-1})	51.8	53.7	50.2	50.3
ProCa (400 mg·L^{-1})	51.0	53.2	50.7	52.0
P-value	0.5781	0.2579	0.7832	0.1616
LSD	1.76	2.04	1.81	2.09

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 37, 2004c).

[y] Means within columns followed by different letters are significantly different ($P<0.05$; ns = non significant).

Table 4.20. Total chlorophyll concentration, total carotenoid concentration, chlorophyll carotenoid ratio and carotenoid chlorophyll ratio following different Prohexadione-calcium treatments on 'Eureka' lemons after harvest of fruit during the 2005 season.

Treatment	Fruit dipped	Fruit and leaves dipped
	Carotenoid ($\mu g \cdot g^{-1}$ DW)	
Control	82.8 ns[z]	81.0 b
ProCa (200 $mg \cdot L^{-1}$)	86.7	100.5 a
ProCa (400 $mg \cdot L^{-1}$)	82.5	84.5 b
P-value	0.8997	0.0136
LSD	24.62	11.70
	Chlorophyll ($\mu g \cdot g^{-1}$ DW)	
Control	301.8 ns	224.8 ns
ProCa (200 $mg \cdot L^{-1}$)	276.2	286.9
ProCa (400 $mg \cdot L^{-1}$)	252.3	236.2
P-value	0.6549	0.3781
LSD	126.99	106.68
	Chlorophyll/Carotenoid Ratio	
Control	3.6 ns	2.8 ns
ProCa (200 $mg \cdot L^{-1}$)	3.1	2.8
ProCa (400 $mg \cdot L^{-1}$)	3.0	2.8
P-value	0.1755	0.9745
LSD	0.72	1.24
	Carotenoid/Chlorophyll Ratio	
Control	0.3 ns	0.3 ns
ProCa (200 $mg \cdot L^{-1}$)	0.3	0.4
ProCa (400 $mg \cdot L^{-1}$)	0.3	0.4
P-value	0.2626	0.9573
LSD	0.08	0.16

[z]Means within columns followed by different letters are significantly different ($P \leq 0.05$; ns = non significant).

Table 4.21. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours of harvest on the vivid (yellow) and dull (green) sides of 'Eureka' lemon fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	4.9 ns[y]		4.8 a	
ProCa early (200 mg·L^{-1})	4.4		4.3 b	
ProCa early (400 mg·L^{-1})	4.7		4.5 ab	
ProCa late (200 mg·L^{-1})	4.7		4.3 b	
ProCa late (400 mg·L^{-1})	4.6		4.3 b	
P-value	0.1039		0.0318	
LSD	0.36		0.40	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	104.3 a	108.1 a	104.4 a	108.3 a
ProCa early (200 mg·L^{-1})	103.8 ab	106.0 abc	102.2 b	105.5 b
ProCa early (400 mg·L^{-1})	102.5 ab	106.5 ab	101.6 b	106.5 ab
ProCa late (200 mg·L^{-1})	101.6 bc	104.7 bc	101.8 b	104.7 b
ProCa late (400 mg·L^{-1})	100.1 c	104.3 c	99.6 c	104.6 b
P-value	0.0003	0.0034	<0.0001	0.0011
LSD	1.95	2.17	1.96	2.04
		Lightness		
Control	71.3 ns	62.7 b	69.7 c	63.5 c
ProCa early (200 mg·L^{-1})	71.6	65.9 a	72.2 ab	67.7 a
ProCa early (400 mg·L^{-1})	70.0	65.6 a	71.3 b	65.1 bc
ProCa late (200 mg·L^{-1})	71.4	67.2 a	71.6 b	68.1 a
ProCa late (400 mg·L^{-1})	71.9	67.8 a	73.3 a	67.1 ab
P-value	0.1792	0.0004	<0.0001	0.0004
LSD	1.55	2.45	1.50	2.35
		Chroma		
Control	52.9 b	51.2 c	53.6 ns	51.6 bc
ProCa early (200 mg·L^{-1})	51.8 b	51.1 c	53.1	52.3 abc
ProCa early (400 mg·L^{-1})	52.0 b	51.7 bc	52.4	51.4 c
ProCa late (200 mg·L^{-1})	53.0 b	52.7 ab	52.6	52.7 ab
ProCa late (400 mg·L^{-1})	54.8 a	53.4 a	53.9	53.2 a
P-value	0.0004	0.0037	0.2173	0.0287
LSD	1.49	1.43	1.51	1.24

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 37, 2004c).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.22. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after ethylene degreening on the vivid (yellow) and dull (green) sides of 'Eureka' lemon fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	3.4 ns[y]		3.2 ns	
ProCa early (200 mg·L^{-1})	3.4		3.0	
ProCa early (400 mg·L^{-1})	3.0		3.0	
ProCa late (200 mg·L^{-1})	3.5		3.3	
ProCa late (400 mg·L^{-1})	3.4		3.0	
P-value	0.2239		0.3489	
LSD	0.40		0.40	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	94.8 a	96.6 a	95.0 a	96.2 ab
ProCa early (200 mg·L^{-1})	94.6 a	96.6 a	94.3 a	95.3 bc
ProCa early (400 mg·L^{-1})	94.2 ab	94.8 b	94.6 a	95.7 ab
ProCa late (200 mg·L^{-1})	94.9 a	96.3 a	94.5 a	96.7 a
ProCa late (400 mg·L^{-1})	93.2 b	95.3 ab	92.3 b	94.6 c
P-value	0.0075	0.0292	<0.0001	0.0019
LSD	1.10	1.33	1.10	1.15
		Lightness		
Control	76.3 a	72.2 ns	75.3 a	74.1 ns
ProCa early (200 mg·L^{-1})	75.6 a	72.5	75.3 a	73.4
ProCa early (400 mg·L^{-1})	75.6 a	73.6	75.1 ab	74.3
ProCa late (200 mg·L^{-1})	75.7 a	73.3	75.6 a	73.5
ProCa late (400 mg·L^{-1})	74.5 b	73.4	74.3 b	74.0
P-value	0.0005	0.3452	0.0276	0.6968
LSD	0.89	1.63	0.89	1.40
		Chroma		
Control	54.8 b	54.8 ns	54.2 b	54.2 b
ProCa early (200 mg·L^{-1})	52.8 b	53.9	53.4 b	54.6 b
ProCa early (400 mg·L^{-1})	53.0 b	54.2	53.1 b	53.8 b
ProCa late (200 mg·L^{-1})	54.4 b	54.9	54.7 b	54.9 b
ProCa late (400 mg·L^{-1})	57.4 a	55.8	58.6 a	57.3 a
P-value	<0.0001	0.1114	<0.0001	<0.0001
LSD	2.03	1.60	2.07	1.50

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 37, 2004c).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 4.23. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest and after degreening of 'Eureka' lemon fruit sampled from the eastern and western sides of trees during the 2006 season.

Treatment	After harvest	After degreening	After harvest	After degreening
	Eastern		Western	
	Carotenoid ($\mu g \cdot g^{-1}$ DW)			
Control	140.0 a[z]	75.6 ab	125.5 ns	73.0 b
ProCa early (200 $mg \cdot L^{-1}$)	114.9 b	69.4 b	112.5	74.1 b
ProCa early (400 $mg \cdot L^{-1}$)	121.6 b	69.5 b	127.6	79.2 ab
ProCa late (200 $mg \cdot L^{-1}$)	126.5 ab	76.6 ab	116.3	76.0 b
ProCa late (400 $mg \cdot L^{-1}$)	116.4 b	84.0 a	124.8	91.0 a
P-value	0.0286	0.0276	0.2696	0.0352
LSD	16.89	10.20	16.83	12.87
	Chlorophyll ($\mu g \cdot g^{-1}$ DW)			
Control	333.4 a	49.3 ns	274.6 ns	44.2 ns
ProCa early (200 $mg \cdot L^{-1}$)	275.1 ab	53.8	230.8	39.7
ProCa early (400 $mg \cdot L^{-1}$)	272.4 ab	43.0	282.9	46.1
ProCa late (200 $mg \cdot L^{-1}$)	242.0 bc	52.1	251.9	53.0
ProCa late (400 $mg \cdot L^{-1}$)	206.3 c	48.5	208.9	42.2
P-value	0.0028	0.6267	0.2320	0.5942
LSD	63.33	13.89	75.32	17.50
	Chlorophyll/Carotenoid Ratio			
Control	2.36 a	0.65 ns	2.18 ns	0.61 ns
ProCa early (200 $mg \cdot L^{-1}$)	2.36 a	0.79	2.06	0.55
ProCa early (400 $mg \cdot L^{-1}$)	2.28 ab	0.62	2.24	0.60
ProCa late (200 $mg \cdot L^{-1}$)	1.94 bc	0.68	2.16	0.71
ProCa late (400 $mg \cdot L^{-1}$)	1.77 c	0.58	1.71	0.49
P-value	0.0077	0.2294	0.2673	0.5239
LSD	0.40	0.20	0.58	0.26
	Carotenoid/Chlorophyll Ratio			
Control	0.43 b	1.55 ns	0.47 ns	1.90 ns
ProCa early (200 $mg \cdot L^{-1}$)	0.44 b	1.35	0.51	1.92
ProCa early (400 $mg \cdot L^{-1}$)	0.45 b	1.79	0.48	1.97
ProCa late (200 $mg \cdot L^{-1}$)	0.53 ab	1.62	0.47	1.48
ProCa late (400 $mg \cdot L^{-1}$)	0.59 a	1.75	0.67	2.62
P-value	0.0145	0.3158	0.1511	0.1316
LSD	0.11	0.46	0.20	0.89

[z] Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = non significant).

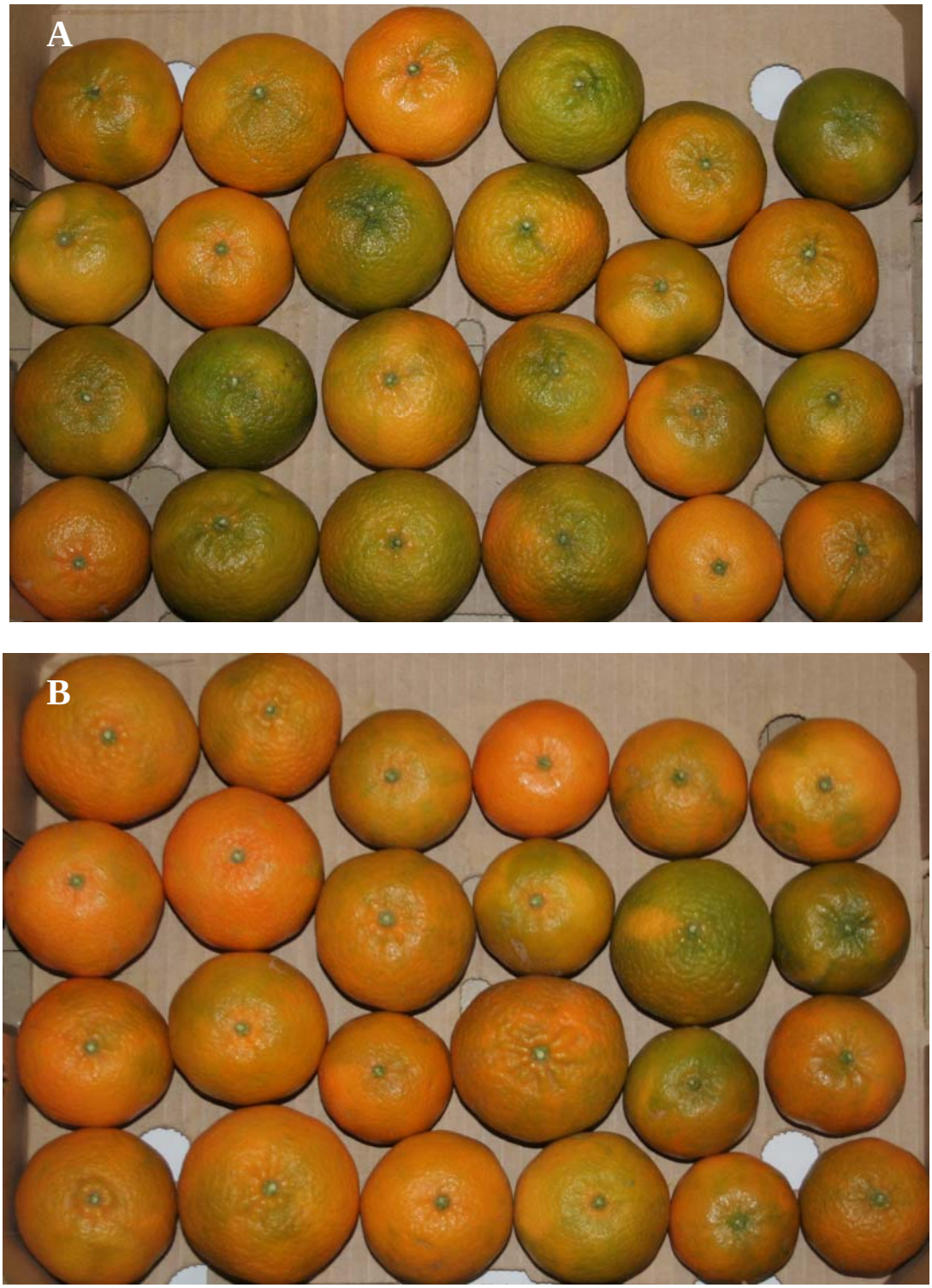


Fig. 4.1. Photographs of 'Nules Clementine' mandarin fruit taken after harvest of fruit sampled during the 2006 season from the eastern side of trees to illustrate the effect of ProCa on rind colour enhancement. A: untreated control; B: late 400 mg·L^{-1} ProCa treatment. Note the more intensely coloured fruit of the ProCa treatment compared to the untreated control treatment.

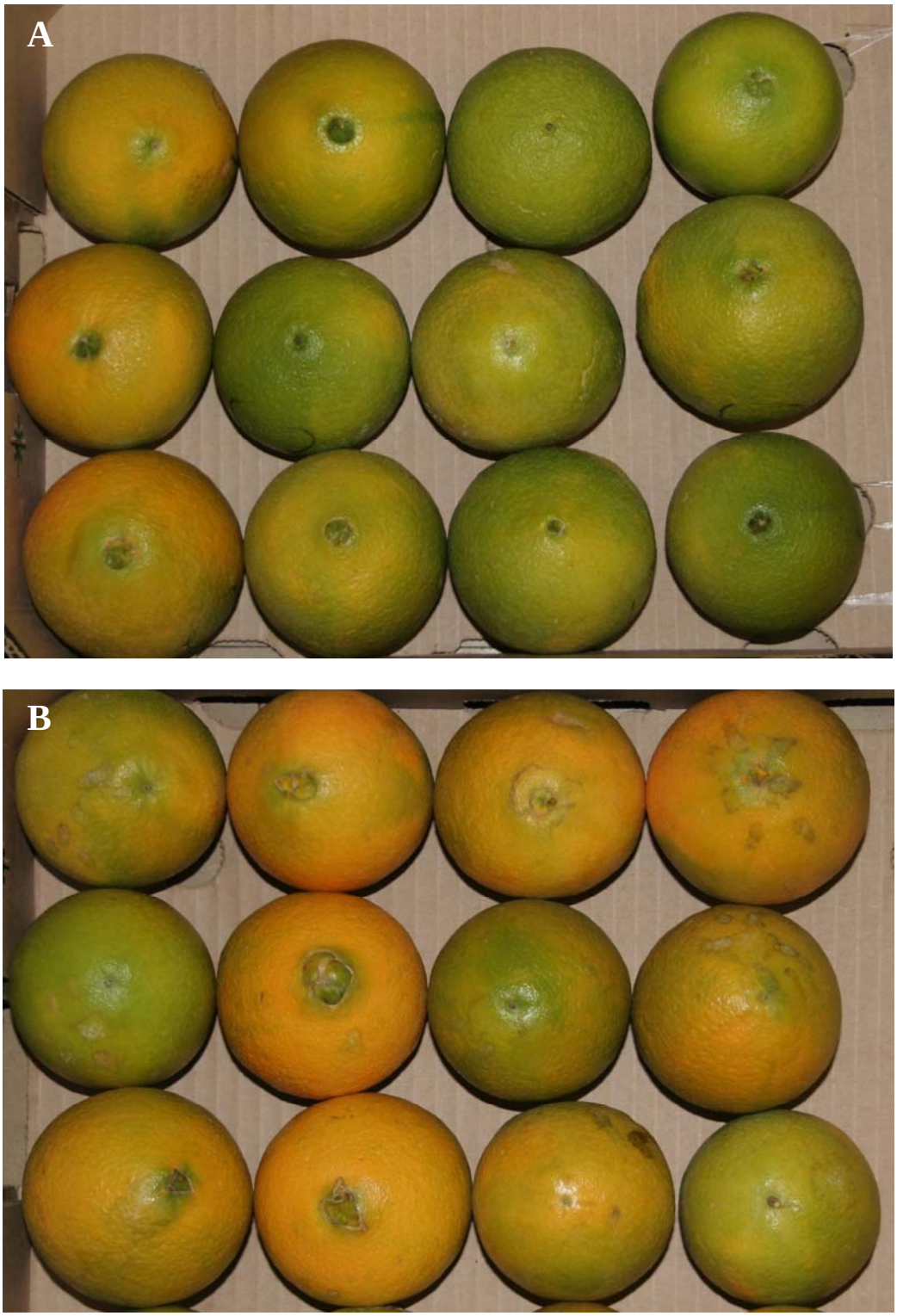


Fig. 4.2. Photographs of 'Navelina Navel' orange fruit taken after harvest of fruit sampled during the 2006 season from the eastern side of trees to illustrate the effect of ProCa on rind colour enhancement. A: untreated control; B: late 400 mg·L^{-1} ProCa treatment. Note the more intensely coloured fruit of the ProCa treatment compared to the untreated control treatment.

Fig. 4.3. Photographs of 'Palmer Navel' orange fruit taken after harvest of fruit sampled during the 2006 season from the eastern side of trees to illustrate the effect of ProCa on rind colour enhancement. A: untreated control; B: late 400 mg·L^{-1} ProCa treatment. Note the more intensely coloured fruit of the ProCa treatment compared to the untreated control treatment.

Fig. 4.4. Photographs of 'Eureka' lemon fruit taken after harvest of fruit sampled during the 2006 season from the eastern side of trees to illustrate the effect of ProCa on rind colour enhancement. A: untreated control; B: late 400 $mg \cdot L^{-1}$ ProCa treatment. Note the more intensely coloured fruit of the ProCa treatment compared to the untreated control treatment.

CHAPTER 5

PRODUCT SCREENING FOR THE ENHANCEMENT OF RIND COLOUR IN *CITRUS* SPP.

Abstract

Rind colour development (chlorophyll degradation and carotenoid biosynthesis) of fruit of early-maturing citrus (*Citrus* spp.) cultivars is often less than ideal, justifying the search for chemical products that stimulate rind colour development. 'Miho Wase Satsuma' mandarin (*C. unshiu* Marc.), 'Nules Clementine' mandarin (*C. reticulata* Blanco), 'Navelina Navel' and 'Palmer Navel' oranges [*C. sinensis* (L.) Osbeck] and 'Eureka' lemon [*C. limon* (L.) Burm.f.] fruit were treated over three consecutive seasons (2003-04 to 2005-06) with various nutritional [boric acid, Thiovit® (elemental sulphur), ColourUp® (neutralised calcium carbonate) and Carotenol® (hydrocarbon substances)], hormonal [Figaron® (ethyclozate), Regalis® (prohexadione-calcium) and Ethrel® (48% ethephon)] and possible physiological stress-inducer products [ammonium thiosulphate (ATS)] and some combination treatments thereof. Boric acid stimulated the degradation of chlorophyll in yellow-rinded fruit, e.g. 'Eureka' lemon, by ~ 40%, and stimulated carotenoid biosynthesis in orange rinded-fruit, e.g. 'Miho Wase Satsuma' mandarin, by ~ 24%. Thiovit® aided in the degradation of chlorophyll and stimulated carotenoid biosynthesis when applied in combination with ATS and Ethrel® on both orange- and yellow-rinded fruit. ColourUp® applied 3 weeks before anticipated harvest stimulated the degradation of chlorophyll on 'Nules Clementine' mandarin fruit. Carotenol® did not improve rind colour whether it was applied alone or in combination with various other chemical products. Figaron® stimulated chloro-chromoplast transformation by aiding in the degradation of chlorophyll (by ~ 32%) in 'Navelina Navel' orange fruit, but did not improve carotenoid biosynthesis. Regalis® applied in combination with ColourUp® or

Ethrel® did not add to the positive effect the latter two products had on improving rind colour of citrus fruit. ATS aided in the degradation of chlorophyll and the biosynthesis of carotenoids especially when applied in combination with Thiovit® plus Ethrel®. Ethrel®, applied at half the recommended rate, in combination with Thiovit® plus ATS stimulated chlorophyll degradation (by ~ 40%) in both orange- and yellow-rinded fruit and stimulated carotenoid biosynthesis (by ~ 20%) in orange-rinded fruit. Screening of chemical products which stimulated carotenoid biosynthesis (e.g. Thiovit® plus ATS plus Ethrel®) in orange-rinded fruit in combination with products which stimulated chlorophyll degradation (e.g. boric acid, ColourUp® and Figaron®) warrant further testing.

Introduction

Rind colour development (chlorophyll degradation and carotenoid biosynthesis) of fruit of early-maturing citrus (*Citrus* spp.) cultivars is often less than ideal. Various factors adversely affect rind colour development, including environmental factors, viz. day, night and soil temperatures above 20°C, 13°C and 12°C, respectively (Young and Erickson, 1961), low light intensities (Sites and Reitz, 1949), low soil water tension (Peng and Rabe, 1996), nutritional factors, viz. excess N, P and K (Koo, 1988), and hormonal factors, viz. low ethylene and auxin concentrations (Goldschmidt et al., 1993; Kamuro and Hirai, 1981) and high gibberellin and cytokinin concentrations (Goldschmidt, 1988).

Shipping fruit at sub-zero (-0.6 °C) temperatures, necessary for cold-sterilisation to some export markets, results in no further rind colour development or even a loss in rind colour during the voyage (Le Roux, 1997; Van Wyk, 2004). Some export companies have stricter rind colour requirements for fruit shipped at these sub-zero temperatures (Maritz, 2000). For this reason, rind colour prior to shipping needs to be enhanced (Van Wyk, 2004). To address

the commercial problem of poor rind colour development, a search for chemical products to trigger chlorophyll degradation and/or carotenoid biosynthesis was initiated. This search for chemical products to enhance rind colour development was subdivided into nutritional, hormonal and possible physiological stress-inducer products.

Of the nutritional products, boric acid could act on improving rind colour by possibly increasing the indole acetic acid (IAA)/cytokinin ratio (Puzina, 2004). In doing so, rind colour development may be triggered since more promotors and less inhibitors are present for chloroplast-chromoplast transformation (Goldschmidt, 1988). ColourUp® (neutralised calcium carbonate) at 1 $mL \cdot L^{-1}$ improved rind colour of 'Palmer Navel' orange [*C. sinensis* (L.) Osbeck] by almost 1 colour plate when applied 2 weeks before anticipated harvest under South African conditions (Barry, 2005). ColourUp® is reported by the manufacturers (Miller, USA) to enhance rind colour of citrus fruit and anthocyanin concentration of grape berries (*Vitis vinifera* L.). Carotenol® (hydrocarbon substances) allegedly improved citrus rind colour in Spain, by stimulating chlorophyll degradation (Lida Quimica, 2006).

Of the hormonal products, ethyclozate (ethyl 5-chloro-1H-3-indazolylacetate), a synthetic auxin, enhanced rind colour of 'Satsuma' mandarin (*C. unshiu* Marc.) (Kamuro and Hirai, 1981). Ethyclozate decreased chlorophyll concentration and increased carotenoid concentration (Kamuro and Hirai, 1981; Tominaga and Diato, 1981). Two foliar sprays, applied 90 and 105 days after full bloom, had the best effect on colour enhancement with no difference in rind colour between the two concentrations (67 and 200 $mg \cdot L^{-1}$) used (Iwahori et al., 1986). Ethyclozate seems to enhance rind colour due to its stimulation of ethylene biosynthesis (Cooper and Henry, 1968). When ethephon (2-chloroethylphosphonic acid) was applied at 480 $mg \cdot L^{-1}$ to trees after colour break, an improvement in rind colour was observed

(El-Otmani et al., 1996; El-Zeftawi and Garret, 1978). Unfortunately, ethephon causes leaf abscission, possibly due to an increase in respiration (El-Otmani et al., 1996; Protopapadakis and Manseka, 1992). Preliminary research by Barry and Van Wyk (2004) showed that prohexadione calcium (ProCa) (Regalis®), proven to be a gibberellin biosynthesis inhibitor (Nakayama et al., 1992; Rademacher, 2001), applied at 100 $mg \cdot L^{-1}$, 2 weeks before anticipated harvest, improved rind colour of 'Navelina Navel' orange due to chlorophyll degradation and carotenoid synthesis.

Ammonium thiosulphate (ATS), a desiccant used for fruit thinning in apples (*Malus domestica*), could trigger endogenous ethylene evolution through the induction of physiological stress, thereby stimulating improved colour during the maturation phase of fruit development.

The objective of this study was to screen various chemical products for their possible improvement and hastening of citrus rind colour development by enhancing chlorophyll degradation and carotenoid biosynthesis.

Materials and Methods

Sites, plant material and treatments. Pre-harvest applications of various chemical products and combinations thereof were applied to 'Miho Wase Satsuma' and 'Nules Clementine' mandarins, 'Navelina Navel' and 'Palmer Navel' oranges, and 'Eureka' lemon at various sites, as summarised in Tables 5.1 to 5.5, in the Western Cape province, South Africa, as medium-cover foliar sprays with a hand-held spray gun.

Fruit sampling. Prior to fruit sampling for laboratory analysis, rind colour of fruit was rated on the tree to identify which treatments were worth sampling for detailed laboratory analysis. To limit unwanted, natural variation in rind colour, fruit were sampled from specific canopy positions. In the 2004 and 2005 seasons, 10 fruit were sampled at a height of 1.5 to 2.0 m from both the outer eastern and western sides of trees for all cultivars tested except for 'Palmer Navel' orange in the 2004 season when 20 fruit were sampled from only the eastern side of trees. In the 2006 season, 20 fruit were sampled from the two canopy positions, of which 10 fruit were used for immediate analysis and the remaining 10 fruit were degreened and analysed after degreening.

Degreening was done at 23 °C with a relative humidity of 95%, an ethylene concentration of 2 $mg \cdot L^{-1}$ and a carbon dioxide (CO_2) concentration <0.3% (Krajewski and Pittaway, 2002). Fruit were subjected to a degreening time of 48 hours for 'Miho Wase Satsuma' and 'Nules Clementine' mandarins, and for 72 hours for 'Navelina Navel' and 'Palmer Navel' oranges and 'Eureka' lemon.

Data collection. Rind colour. Fruit were colour-rated with the "CRI colour charts, set no. 34, 36 or 37, 2004" for oranges, soft citrus and lemons, respectively (CRI, 2004a, 2004b, 2004c; Appendix 1 to 3). To limit the variation in rind colour on different sides of fruit, rind colour was also measured objectively on both the "vivid" (orange) and "dull" (green) sides of fruit with a Minolta chromameter (Model CR-400, Minolta Co. Ltd., Tokyo, Japan).

Rind pigments. Rind sampling was done by cutting the flavedo from the fruit. This was done either with a potato peeler ('Nules Clementine' mandarin) or with a citrus rind zester ('Navelina Navel' and 'Palmer Navel' oranges and 'Eureka' lemon) during the 2005 season.

During the 2006 season, only citrus rind zesters were used for rind sampling on all cultivars. Sampling was done from all 10 fruit in the eight replicates, the pooled flavedo was then immersed into liquid nitrogen and stored at -80 °C until completely frozen for a period of at least 1 day, whereafter the samples were freeze-dried at -56 °C until all moisture was removed from the rinds, which lasted 4 days. The samples were then milled (A10 Kika Labortechnic, Kika Werke, GMBH & Co., Staufen, Germany) and sieved through a 500 µm sieve, to a homogenous powder. Samples were then stored in polyethylene vials at -80 °C until analysed. All preparation activities were carried out under low light conditions to inhibit the degradation of carotenoids and chlorophyll.

From the freeze-dried rind sample, a 0.1 g sub-sample was added to 10 mL 96 % (v/v) aqueous ethanol solvent containing 0.1 $g \cdot L^{-1}$ butylated hydroxytoluene (BHT) and 0.2 $g \cdot L^{-1}$ diethyldithiocarbamate (DDC), both antioxidants to prevent carotenoid degradation. The sample was then vortexed for two 1-minute intervals, whereafter it was stored for 1.5 hours at 4 °C to allow the pigment to extract into the solvent. After 1.5 hour s`torage, the extraction was poured through ashless filter paper (Schleicher & Schuell, Dassel, Germany) to remove rind particles. The filtrated solution was then poured into plastic cuvettes placed into a spectrophotometer, zeroed with a ethanol/antioxidant solvent (Cary 50 conc UV-visible spectrophotometer, Varian Australia (Pty) Ltd, Mulgrave, Victoria, Australia). Absorbance readings were taken at 470, 649 and 664 nm. Absorbance values were used to determine the chlorophyll a (C_a), chlorophyll b (C_b), total chlorophyll (C_{a+b}) and total carotenoids (C_{x+c}) concentrations in $\mu g \cdot g^{-1}$ dry weight, using the Lightenthaler equations (Lichtenthaler, 1987):

$$C_a = 13.36\ A_{664} - 5.19A_{649}$$

$$C_b = 27.43A_{649} - 8.12A_{664}$$

$$C_{a+b} = A_{664} + 22.24A_{649}$$

$$C_{x+c} = \frac{100A_{470} - 2.13\ C_a - 97.64\ C_b}{209}$$

Statistical design and analysis. Experimental layout was a complete randomised block design (CRBD) consisting of eight single-tree replicates. Analysis of variance was conducted using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS Inc., Cary, N.C., USA) and least significant difference (LSD) values were used to indicate any significant differences among treatments.

Results and Discussion

Boric acid 'Miho Wase Satsuma' mandarin. In the 2004 season, boric acid at 2 $g \cdot L^{-1}$ reduced relative greenness of fruit as evidenced by significantly lower hue angle on both the vivid and dull sides of fruit sampled from both the eastern and western sides of trees (Table 5.6). Boric acid at 2 $g \cdot L^{-1}$ also resulted in fruit appearing brighter (significantly higher lightness) and more intensely coloured (significantly higher chroma) on the dull side of fruit sampled from the western side of trees (generally the worst case scenario for rind colour) (G.H. Barry, personal communication), possibly reducing within-tree variation in rind colour. The lower application rate (1 $g \cdot L^{-1}$) of boric acid, was less effective than the higher rate in reducing relative greenness of fruit sampled from the eastern side of trees, but was as effective as the higher rate for fruit sampled from the western side of trees.

In the 2006 season, boric acid at 1 $g \cdot L^{-1}$ significantly improved the rind colour rating by 0.9 colour units as well as improving the objectively measured rind colour (significantly lower hue angle, and higher lightness and chroma) of fruit sampled from the western side of trees, but not from the eastern side of trees (Table 5.10). These differences in rind colour of fruit

sampled from the western side of trees were smaller following ethylene degreening, but still significantly better (Table 5.11). The perceived improvement in rind colour of fruit from the western side of trees was due to significantly higher carotenoid concentration (by ~ 23%) and significantly lower chlorophyll concentration (by ~ 43%), and therefore a lower chlorophyll to carotenoid ratio and a higher carotenoid to chlorophyll ratio (P = 0.0590) (Table 5.12). However, when boric acid was applied in combination with Thiovit® or Regalis® the treatments did not improve rind colour (data not shown).

When boric acid was applied in combination with Carotenol®, boric acid at 1 $g \cdot L^{-1}$ overcame the adverse effects of Carotenol® on rind colour (see Carotenol® section) (Tables 5.13 to 5.15), but did not improve rind colour compared to the untreated control treatment.

'Nules Clementine' mandarin. In the 2006 season, boric acid at 1 $g \cdot L^{-1}$ significantly improved rind colour rating by 0.3 and 0.4 colour units of fruit sampled from the eastern and western sides of trees, respectively (Table 5.19). When applied in combination with (Regalis®) an improvement of 0.4 and 0.7 colour units was obtained on fruit sampled from the eastern and western sides of trees, respectively, and by 0.7 colour units when applied in combination with (Thiovit®). Objectively measured rind colour was also improved (lower hue angle, higher lightness and higher chroma) by the boric acid alone and combination treatments, especially on the dull sides of fruit from both the eastern and western sides of trees after harvest (Table 5.20), possibly reducing variation in rind colour between the vivid and dull sides of fruit. After cold-storage rind colour was significantly improved by the boric acid treatments on fruit sampled from the western side of trees, evident by the lower hue angle (Table 5.21). None of the boric acid treatments stimulated carotenoid biosynthesis, however, they did stimulate

chlorophyll degradation (P = 0.0772) of fruit sampled from the eastern side of trees (Table 5.22).

When boric acid was applied in combination with Carotenol®, boric acid partially overcame the negative effect of Carotenol® on rind colour, although rind colour was not significantly better than that of the untreated control treatment (Tables 5.23 to 5.24).

'Navelina Navel' orange. In the 2006 season, boric acid applied alone or in combination with ColourUp® or Regalis® did not improve rind colour (data not shown).

'Palmer Navel' orange. In the 2006 season, boric acid alone or in combination with ColourUp® or Regalis® did not improve rind colour (data not shown). When boric acid was applied in combination with Carotenol®, the boric acid did not aid in overcoming the adverse effects of Carotenol® on rind colour compared to the untreated control treatment, both after harvest and after ethylene degreening (Tables 5.39 to 5.41).

'Eureka' lemon. In the 2006 season, boric acid applied at 1 $g \cdot L^{-1}$ significantly improved the rind colour rating after harvest by 0.5 colour units of fruit sampled from the eastern side of trees and by 0.7 colour units of fruit sampled from the western side of trees, reducing within-tree differences between the eastern and western sides of trees (Table 5.43). The boric acid application also reduced relative greenness of fruit (as evidenced by the lower hue angle), and resulted in brighter (higher lightness) and more intensely coloured fruit (higher chroma) (Table 5.43). These differences in rind colour were smaller after ethylene degreening, but still significantly better than that of the control (Table 5.44). The perceived rind colour improvement of fruit was due to a significant reduction in chlorophyll concentration of fruit

from the eastern (by ~ 28%) and western (by ~ 36%) sides of trees, resulting in a significantly lower chlorophyll to carotenoid ratio after harvest (Table 5.45).

Since boric acid plays a role in increasing the IAA/cytokinin ratio (Puzina, 2004), boric acid may trigger chloro-chromoplast transformation, and hence promote rind colour development, via a change in balance between growth promotors and inhibitors. In yellow-rinded fruit, e.g. 'Eureka' lemon, applications of boric acid resulted in reduced chlorophyll concentration with no effect on the yellow carotenoid pigments. Whereas in orange-rinded fruit, e.g. 'Miho Wase Satsuma' mandarin, applications of boric acid resulted in increased carotenoid concentration and a reduction in chlorophyll concentration, but not consistently. Combination treatments of boric acid with other products that improve rind colour development should be screened.

Thiovit® 'Miho Wase Satsuma' mandarin. In the 2004 season, Thiovit® reduced relative greenness of fruit as evidenced by the significantly lower hue angle on both the vivid and dull sides of fruit sampled from both the eastern and western sides of trees (Table 5.7). Thiovit® also resulted in fruit appearing brighter (significantly higher lightness) and more intensely coloured (significant higher chroma) when compared to the untreated control treatment. When Thiovit® was applied in combination with Ethrel®, this combination treatment significantly reduced relative greenness (lower hue angle), but resulted in fruit appearing duller (lower lightness) and less intensely coloured (significantly lower chroma) (Table 5.7).

In the 2006 season, Thiovit® alone and in combination with boric acid did not improve rind colour (data not shown). However, when applied in combination with Ethrel®, ATS, and Ethrel® plus ATS the treatments significantly improved rind colour rating by 0.8, 0.9 and 1.3 colour units, respectively, of fruit sampled from the western side of trees (generally the worst

coloured side of trees) (G.H. Barry, personal communication) (Table 5.10). These Thiovit® combination treatments reduced relative greenness (significantly lower hue angle) of fruit sampled from both the eastern and western sides of trees. Fruit brightness and colour intensity were also improved (significantly higher lightness and chroma) of fruit sampled from the western side of trees, but not of fruit sampled from the eastern side of trees (Table 5.10). These differences in rind colour of fruit from the western side of trees were smaller following ethylene degreening, but still significantly better than that of the control fruit (Table 5.11). The perceived improvement in rind colour of fruit from the western side of trees treated with Thiovit® in combination with Ethrel®, ATS, and Ethrel® plus ATS were due to significantly higher carotenoid concentration (by ~ 26%, ~ 25% and ~ 32%, respectively) and significantly lower chlorophyll concentration (by ~ 46%, ~ 49% and ~ 63%, respectively), resulting in a higher carotenoid to chlorophyll ratio (P = 0.0590) (Table 5.12).

'Nules Clementine' mandarin. In the 2004 season, Thiovit® alone and in combination with Ethrel® did not significantly improve rind colour compared to the untreated control treatment, on both the vivid and dull sides of fruit sampled from both the eastern and western sides of trees (Table 5.16).

In the 2006 season, the rind colour rating of fruit was significantly improved by Thiovit® in combination with ATS, Ethrel® plus ATS and boric acid treatments by 0.6, 1.3 and 0.7 colour units, respectively, of fruit sampled from the eastern and western sides of trees (Table 5.19). Relative greenness was also reduced by the Thiovit® combination treatments (as evidenced by the significantly lower hue angle) on both vivid and dull sides of fruit sampled from the eastern and western sides of trees (Table 5.20). Fruit brightness and colour intensity was also significantly improved (higher lightness and chroma) on the dull side of fruit sampled from

the eastern and western sides of trees (Table 5.20). These differences in rind colour were smaller following cold-storage, but still significantly better than that of the control (Table 5.21). The Thiovit® combination treatments did not stimulate carotenoid biosynthesis, but tended to reduce the chlorophyll concentration (P = 0.0772) (Table 5.22).

'Navelina Navel' orange. In the 2006 season, Thiovit® in combination with ATS and Ethrel® plus ATS significantly improved the rind colour rating by 0.3 and 0.6 colour units, respectively, of fruit sampled from the eastern side of trees compared to the untreated control treatment (Table 5.25). The two Thiovit® combination treatments reduced relative greenness of fruit (as evidenced by the lower hue angle), improved brightness (higher lightness) and improved rind colour intensity (higher chroma) on both the vivid and dull sides of fruit sampled from the eastern side of trees after harvest, but not of fruit sampled from the western side of trees (Table 5.25). After ethylene degreening rind colour of fruit from the Thiovit® combination treatments was poorer than that of the control (higher colour rating, higher hue angle, lower lightness and lower chroma) (Table 5.26).The perceived rind colour improvement (Table 5.25) of the Thiovit® in combination with Ethrel® plus ATS was due to significantly higher carotenoid concentration of fruit sampled from the eastern (by ~ 25%) and western (by ~ 19%) sides of trees, respectively (Table 5.27).

'Palmer Navel' orange. In the 2004 season, Thiovit® alone or in combination with Ethrel® did not improve rind colour on both the vivid and dull sides of fruit sampled from the eastern sides of trees after harvest (Table 5.31).

In the 2006 season, Thiovit® in combination with ATS significantly reduced rind colour after harvest or after ethylene degreening (Tables 5.36 to 5.38). Thiovit® in combination with

Ethrel® plus ATS significantly improved rind colour rating by 0.4 and 0.8 colour units for fruit sampled from the eastern and western sides of trees, respectively (Table 5.36). This treatment reduced relative greenness (as evidenced by the significantly lower hue angle) on both the vivid and dull sides of fruit, and improved fruit brightness (significantly higher lightness) and rind colour intensity (significantly higher chroma) on the dull side of fruit sampled from both the eastern and western sides of trees (Table 5.36). This difference in rind colour was smaller after ethylene degreening, but still significantly better than that of the control (Table 5.37). The perceived rind colour improvement of fruit from the eastern side of trees after harvest was due to the significantly higher carotenoid concentration (by ~ 11%) and lower chlorophyll concentration ($P = 0.0545$), resulting in a higher carotenoid to chlorophyll ratio (Table 5.38).

'Eureka' lemon. In the 2004 season, the Thiovit® application significantly improved rind colour on the vivid side of fruit sampled from the eastern side of trees as evidenced by the significantly lower hue angle (Table 5.42). Fruit brightness and colour intensity were not improved on both the vivid and dull sides of fruit sampled from the eastern and western sides of trees (Table 5.42).

In the 2006 season, Thiovit® in combination with ATS and Ethrel® plus ATS significantly improved rind colour rating by 0.8 and 1.3 colour units, respectively, of fruit sampled from the eastern side of trees, and by 0.4 and 0.6 colour units, respectively, of fruit sampled from the western side of trees, thereby reducing the difference found in rind colour between the two sides of trees (Table 5.43). Both treatments also reduced relative greenness (as evidenced by the lower hue angle), increased fruit brightness (higher lightness) and improved colour intensity of fruit (higher chroma) after harvest (Table 5.43). These differences in rind colour

were smaller following ethylene degreening, but were still significantly better than those of the control (Table 5.44). The perceived rind colour improvement of fruit following the Thiovit® in combination with ATS and Ethrel® plus ATS treatments was due to a reduction in chlorophyll concentration by ~ 46% and by ~ 63%, respectively, for the two treatments compared to the untreated control treatment, for fruit sampled from the eastern side of trees, resulting in a significantly higher carotenoid to chlorophyll ratio (Table 5.45). For fruit sampled from the western side of trees, a significant reduction in chlorophyll concentration by ~ 45% for the Thiovit® in combination with Ethrel® plus ATS treatment compared to the untreated control treatment, resulting in a significantly higher carotenoid to chlorophyll ratio (Table 5.45).

Thiovit® alone applied at 3 $g \cdot L^{-1}$ did not consistently improve rind colour development, but when applied in combination with Ethrel® plus ATS the combination treatment did improve rind colour. This improvement in rind colour was mainly due to a stimulation in carotenoid biosynthesis in orange-rinded fruit, e.g. 'Miho Wase Satsuma' mandarin, 'Navelina Navel' and 'Palmer Navel' oranges, but not in yellow-rinded fruit, e.g. 'Eureka' lemon. Chlorophyll degradation was, however, enhanced in both orange- and yellow-rinded fruit.

ColourUp®. 'Miho Wase Satsuma' mandarin. In the 2005 season, both the 0.5 and 1 $mL \cdot L^{-1}$ ColourUp® applications significantly reduced the relative greenness (as evidenced by the significantly lower hue angle) when compared to the untreated control treatment of fruit sampled from the eastern side of trees (Table 5.9). These differences in rind colour were more pronounced when ColourUp® was applied 3 weeks before anticipated harvest than when applied 4 weeks before anticipated harvest. There was relatively little difference in rind colour among treatments of fruit sampled from the western side of trees (Table 5.9).

'Nules Clementine' mandarin. In the 2005 season, both ColourUp® treatments (applied at 0.5 and 1 $mL \cdot L^{-1}$) significantly reduced relative greenness (as evidenced by the lower hue angle) and improved rind colour intensity (higher chroma) on both the vivid and dull sides of fruit sampled from the eastern side of trees (Table 5.17). These differences in rind colour of fruit were most evident when 1 $mL \cdot L^{-1}$ was applied 3 weeks before anticipated harvest (Table 5.17). Fruit sampled from the western side of trees did not have a distinct difference in rind colour compared to the untreated control treatment (Table 5.18).

In the 2006 season, ColourUp® applied at 0.5 $mL \cdot L^{-1}$ did not improve rind colour (data not shown). However, ColourUp® applied at 0.75 $mL \cdot L^{-1}$ significantly improved rind colour rating by 0.5 and 0.7 colour units of fruit sampled from the eastern and western sides of trees, respectively (Table 5.19). The ColourUp® treatment reduced the relative greenness of fruit as evidenced by the significantly lower hue angle on the dull side of fruit (greenest side) sampled from both the eastern and western sides of trees (Table 5.20). The ColourUp® treatment also resulted in fruit appearing brighter (significantly higher lightness) and more intensely coloured (significantly higher chroma) on the dull side of fruit sampled from the both the eastern and western sides of trees, possibly reducing variation in rind colour between vivid and dull sides of fruit (Table 5.20). The perceived improvement in rind colour after harvest was due to the reduction in chlorophyll concentration ($P = 0.0772$) (Table 5.22). After cold-storage, however, there were no consistent differences in rind colour between the ColourUp® treatment and the control (Tables 5.19, 5.21 and 5.22).

'Navelina Navel' orange. In the 2006 season, ColourUp® at 0.75 and 1.0 $mL \cdot L^{-1}$ or in combination with boric acid did not improve rind colour (data not shown). However, when

ColourUp® was applied in combination with Regalis® rind colour rating of fruit sampled from the eastern side of trees was improved by 0.5 colour units (Table 5.25). This treatment reduced the relative greenness of fruit (as evidenced by the lower hue angle), and improved the brightness (higher lightness) and rind colour intensity (higher chroma) on both the vivid and dull sides of fruit sampled form the eastern side of trees, compared to the untreated control treatment. The perceived rind colour improvement could not be attributed to significant changes in rind pigment concentration (Table 5.27). No significant differences in rind colour were observed on fruit sampled from the western side of trees (Table 5.25). No significant improvement in rind colour was observed following ethylene degreening when comparing ColourUp® in combination with Regalis® treatment with the untreated control treatment (Table 5.26).

'Palmer Navel' orange. In the 2005 season, relative greenness of fruit was reduced (significantly lower hue angle) by both the 0.5 and 1.0 $mL \cdot L^{-1}$ ColourUp® treatments when applied 2 weeks before anticipated harvest on the dull sides of fruit sampled from the eastern and western sides of trees when applied 2 weeks before anticipated harvest (Table 5.32 and 5.33). The 0.5 $mL \cdot L^{-1}$ application had the best result. The dull side of fruit from both the eastern and western sides of trees also appeared brighter (higher lightness) and more intensely coloured (higher chroma) compared to the untreated control treatment (Tables 5.32 and 5.33). When ColourUp® was applied 6 weeks before anticipated harvest, relative greenness of fruit was significantly reduced (lower hue angle) by both the 0.5 and 1.0 $mL \cdot L^{-1}$ treatments on the eastern side of trees (Table 5.34). Fruit brightness and rind colour intensity were not affected by the treatments. No significant improvements in rind colour were observed for fruit sampled from the western side of trees (Table 5.35).

In the 2006 season, no improvement in rind colour rating was observed for the ColourUp® alone [0.5 mL·L^{-1} (data not shown) and 1.0 mL·L^{-1}] or ColourUp® in combination with boric acid (data not shown) or Regalis® treatments, both after harvest (Table 5.36) and after ethylene degreening (Table 5.37). ColourUp® alone at 1.0 mL·L^{-1} and in combination with Regalis® did however reduce the relative greenness (as evidenced by the significantly lower hue angle) of fruit from the eastern side of trees, both after harvest (Table 5.36) and after ethylene degreening (Table 5.37). Fruit brightness and colour intensity was not affected by the treatments (Tables 5.36 and 5.37). This slight improvement in rind colour was not detected by spectrophotometry, although ColourUp® alone tended to lower the chlorophyll concentration in the rind (P = 0.0545) (Table 5.38).

Rind colour of orange-rinded citrus cultivars, except 'Navelina Navel' orange, was improved following the application of ColourUp® at 0.75 to 1.0 mL·L^{-1} confirming the results of Barry (2005). ColourUp® stimulated chlorophyll degradation on 'Nules Clementine' mandarin, suggesting that ColourUp® may promote chloro-chromoplast transformation. The best application concentration and timing appears to be 0.75 to 1.0 mL·L^{-1} applied 3 weeks before anticipated harvest. ColourUp® in combination with other products that improve rind colour should be screened.

Carotenol®. 'Miho Wase Satsuma' mandarin. In the 2006 season, Carotenol® alone and in combination with Regalis® did not improve rind colour (Table 5.13). On the contrary, it delayed rind colour development, resulting in greener fruit (significantly higher hue angle) after harvest and after ethylene degreening (Tables 5.13 and 5.14). When Carotenol® was applied in combination with boric acid, boric acid partially overcame the negative effects of Carotenol® on rind colour development, resulting in fruit with similar colour development to

that of the untreated control treatment (Tables 5.13 to 5.14). The Carotenol® treatments had no effect on rind pigment expression (Table 5.15).

'Nules Clementine' mandarin. In the 2006 season, Carotenol® significantly delayed rind colour development compared to the untreated control treatment, but when applied in combination with boric acid, boric acid partially overcame the negative effects of Carotenol® on rind colour (Table 5.23). The fruit from the Carotenol® treatment which were perceived to be greener than fruit from the control treatment had a higher chlorophyll concentration ($P = 0.0690$) resulting in a significantly higher chlorophyll to carotenoid ratio of fruit sampled from the eastern side of trees (Table 5.24).

'Navelina Navel' orange. In the 2006 season, Carotenol® significantly delayed rind colour development of fruit from the eastern side of trees compared to the control treatment (Table 5.28). After harvest, fruit from the eastern side of trees were significantly greener (higher hue angle), duller (lower lightness) and less intensely coloured (lower chroma) (Table 5.28). The delayed rind colour development after harvest was due to the significantly higher chlorophyll concentration (by ~ 31%) resulting in a significantly higher chlorophyll to carotenoid ratio (Table 5.30). Fruit from the western side of trees had significantly better rind colour rating by 0.6 colour units; with lower hue angle and higher lightness and chroma on the vivid side of fruit (Table 5.28). However, these perceived differences in rind colour of fruit from the western side of trees could not be accounted for by spectrophotometry (Table 5.30).

'Palmer Navel' orange. In the 2006 season, Carotenol® alone and in combination with boric acid significantly delayed rind colour development (Table 5.39). Fruit appeared greener, duller and less intensely coloured (higher hue angle, lower lightness and lower chroma) after

harvest and after ethylene degreening than untreated control fruit. However, when Carotenol® was applied in combination with Regalis®, the Regalis® partially overcame the adverse effects of Carotenol® on rind colour development of fruit both after harvest and after ethylene degreening (Tables 5.39 and 5.40). The perceived greener fruit from the Carotenol® treatment was due to a significantly higher chlorophyll concentration by ~ 44%, resulting in a significantly higher chlorophyll to carotenoid ratio of fruit sampled from the eastern side of trees (Table 5.41).

Carotenol® application at 3 $g \cdot L^{-1}$ delayed rind colour development in all cases tested. When Carotenol® was applied in combination with boric acid or Regalis®, both the boric acid and Regalis® partially overcame the adverse effects of Carotenol® on rind colour development, suggesting that the possible manipulation in some growth promotors like IAA, cytokinin and gibberellins by boric acid and Regalis® (Nakayama et al., 1992; Puzina, 2004) could improve chloro-chromoplast transformation.

Figaron®. ‘Miho Wase Satsuma’ mandarin. In the 2004 season, Figaron® significantly reduced the relative greenness (as evidenced by the lower hue angle) and improved the brightness (significantly higher lightness) on the vivid side of fruit sampled from the eastern side of trees (Table 5.8). No significant differences in rind colour were observed on fruit sampled from the western side of trees when compared with the untreated control treatment (Table 5.8).

‘Nules Clementine’ mandarin. In the 2006 season, Figaron® did not improve the rind colour of fruit sampled from the eastern and western sides of trees (Tables 5.23 to 5.24).

‘Navelina Navel’ orange. In the 2006 season, Figaron® significantly improved rind colour rating after harvest by 0.4 and 0.9 colour units of fruit sampled from the eastern and western sides of trees, respectively (Table 5.28). Figaron® reduced the relative greenness of fruit (as evidenced by the significantly lower hue angle), and improved the brightness (significantly higher lightness) and colour intensity (significantly higher chroma) of fruit sampled from both the eastern and western side of trees (Table 5.28). These improvements in rind colour were smaller following ethylene degreening, but still significantly better than the control (Table 5.29). The perceived improvement in rind colour after harvest was due to a significant reduction in chlorophyll concentration of fruit sampled from the eastern (by ~ 28%) and western (by ~ 36%) sides of trees, resulting in a significantly higher carotenoid to chlorophyll ratio both sides of trees (Table 5.30).

‘Palmer Navel’ orange. In the 2006 season, Figaron® did not affect rind colour rating, but significantly reduced the relative greenness of fruit as evidenced by the lower hue angle of fruit sampled from both the eastern and western sides of trees (Table 5.39). Fruit brightness and rind colour intensity were significantly improved (higher lightness and higher chroma) on the dull side (greenest side) of fruit, both after harvest and after ethylene degreening (Tables 5.39 and 5.40). The apparent improvement in rind colour (lower hue angle) was due to a reduction in chlorophyll concentration (by ~ 15%) of fruit sampled from the eastern side of trees (Table 5.41).

Figaron® stimulates the biosynthesis of ethylene (Cooper and Henry, 1968), thereby possibly triggering chloro-chromoplast transformation, and hence promoted rind colour development of ‘Miho Wase Satsuma’ mandarin and ‘Navel’ orange fruit. Figaron® stimulated the degradation of chlorophyll in ‘Navelina Navel’ and ‘Palmer Navel’ oranges, confirming

findings of Kamuro and Hirai (1981) and Tominaga and Diato (1981). Combination treatments of Figaron® with other products that improved rind colour development should be screened.

Regalis®. 'Miho Wase Satsuma' mandarin. In the 2004 season, Regalis® in combination with Ethrel® significantly reduced relative greenness of fruit as evidenced by the lower hue angle (Table 5.7). Fruit brightness and rind colour intensity were also significantly improved (higher lightness and higher chroma) on the vivid and dull sides of fruit sampled from the eastern and western sides of trees (Table 5.7).

In the 2006 season, Regalis® alone or in combination with boric acid (data not shown) or in combination with Carotenol® did not improve rind colour (Table 5.13). In fact, the Regalis®-Carotenol® combination treatment delayed rind colour formation compared with the untreated control treatment both after harvest and after ethylene degreening (Table 5.13).

'Nules Clementine' mandarin. In the 2004 season, Regalis® in combination with Ethrel® reduced relative greenness (significantly lower hue angle), improved brightness and rind colour intensity (significantly higher lightness and chroma) on the vivid side of fruit sampled from the eastern side of trees (Table 5.16). No differences were observed on fruit sampled from the western side of trees.

In the 2006 season, Regalis® in combination with boric acid significantly improved rind colour rating of fruit sampled from the eastern and western sides of trees by 0.4 and 0.7 colour units, respectively (Table 5.19), but Regalis® did not improve the effect of boric acid when applied alone. Fruit relative greenness was also reduced by the treatment as evidenced by the

lower hue angle, and brightness and rind colour intensity were also improved by the treatment, although not significantly in most cases (Table 5.20). This improvement in rind colour was smaller following cold-storage (Table 5.21).

'Navelina Navel' orange. In the 2006 season, Regalis® in combination with boric acid did not improve rind colour (data not shown). However, Regalis® in combination with ColourUp® improved rind colour rating of fruit sampled from the eastern side of trees by 0.5 colour units (Table 5.25). This treatment reduced the relative greenness of fruit (as evidenced by the lower hue angle), and improved the brightness (higher lightness) and rind colour intensity (higher chroma) on both the vivid and dull sides of fruit sampled form the eastern side of trees compared to the untreated control treatment. No significant differences in rind colour were observed on fruit sampled from the western side of trees (Table 5.25). No significant improvement in rind colour was observed following ethylene degreening (Table 5.26). However, the perceived rind colour improvement of fruit sampled from the eastern side of trees after harvest was not detected by spectrophotometry (Table 4.27).

'Palmer Navel' orange. In the 2004 season, Regalis® in combination with Ethrel® did not improve rind colour on either the vivid or dull sides of fruit (Table 5.31).

In the 2006 season, Regalis® in combination with boric acid did not improve rind colour (data not shown). However, Regalis® in combination with ColourUp® reduced the relative greenness (as evidenced by the significantly lower hue angle) of fruit sampled from the eastern side of trees, both after harvest and after ethylene degreening (Table 5.36), but Regalis® did not enhance the effect of ColourUp® when the latter was applied alone (Table 5.36). Fruit brightness and colour intensity were not affected by the treatment (Tables 5.36

and 5.37) and the slight improvement in rind colour was not detected by spectrophotometry (Table 5.38). When Regalis® was applied in combination with Carotenol®, the Regalis® partially overcame the adverse effects of Carotenol® on rind colour development of fruit both after harvest and after ethylene degreening (Tables 5.39 and 5.41).

'Eureka' lemon. In the 2004 season, Regalis® in combination with Ethrel® or ATS did not improve rind colour of fruit sampled from both the eastern and western sides of trees (Table 5.42).

Regalis®, a gibberellin biosynthesis inhibitor (Nakayama et al., 1992; Rademacher, 2001), possibly promotes rind colour development via a change in balance between growth promotors and inhibitors. The effect of Regalis® on improving rind colour when applied as a single application or at a lower concentration than that used by Barry and Van Wyk (2004) and in Chapter 4 was poor. When Regalis® was applied in combination with ColourUp® or Ethrel®, both products were shown to trigger chloro-chromoplast transformation and hence rind colour development. The addition of Regalis® to the treatment did not enhance the effects of these products when applied alone.

Ammonium thiosulphate. 'Miho Wase Satsuma' mandarin. In the 2004 season, ATS alone and in combination with Ethrel® significantly reduced the relative greenness of fruit as evidenced by the lower hue angle on both the vivid and dull sides of fruit sampled from the eastern and western sides of trees compared to the untreated control treatment (Table 5.7). Fruit brightness and rind colour intensity were also improved by both treatments (significantly higher lightness and chroma) (Table 5.7).

In the 2006 season, ATS in combination with Thiovit® and Thiovit® plus Ethrel® significantly improved rind colour rating by 0.9 and 1.3 colour units, respectively, of fruit sampled from the western side of trees (generally the worst coloured side) (G.H. Barry, personal communication) (Table 5.10). These combination treatments with ATS reduced relative greenness (significantly lower hue angle) of fruit sampled from the eastern and western sides of trees. Fruit brightness and colour intensity were also improved (significantly higher lightness and chroma) of fruit sampled from the western side of trees, but not of fruit sampled from the eastern side of trees (Table 5.10). These differences in rind colour of fruit sampled from the western side of trees were smaller following ethylene degreening, but still significantly better than the control (Table 5.11). The perceived improvement in rind colour of fruit sampled from the western side of trees treated with ATS in combination with Thiovit® and Thiovit® plus Ethrel® were due to significantly higher carotenoid concentrations by ~ 25% and ~ 32%, respectively, and significantly lower chlorophyll concentration by ~ 49% and ~ 63%, respectively, resulting in a higher carotenoid to chlorophyll ratio (P = 0.0590) (Table 5.12).

‘Nules Clementine’ mandarin. In the 2004 season, ATS alone did not significantly improve rind colour of fruit sampled from both the eastern and western sides of trees (Table 5.16), but when ATS was applied in combination with Ethrel® the treatment significantly reduced the relative greenness of fruit as evidenced by the lower hue angle on both the vivid and dull sides of fruit from both the eastern and western sides of trees compared to the untreated control treatment (Table 5.16). Fruit brightness (significantly higher lightness) and rind colour intensity (significantly higher chroma) were also improved by the ATS plus Ethrel® combination treatment (Table 5.7).

In the 2006 season, rind colour rating of fruit sampled from the eastern and western sides of trees was significantly improved by the ATS in combination with Thiovit® and Thiovit® plus Ethrel® treatments by 0.6 and 1.3 colour units, respectively (Table 5.19). Relative greenness was also reduced by the two treatments (as evidenced by a significantly lower hue angle) on both vivid and dull sides of fruit sampled from both the eastern and western sides of trees (Table 5.20), and brightness and colour intensity of fruit were significantly improved (higher lightness and chroma) on the dull side of fruit sampled from the eastern and western sides of trees (Table 5.20). These differences in rind colour were smaller following cold-storage, but still significantly better than that of the control (Table 5.21). The ATS-combination treatments did not stimulate carotenoid biosynthesis, but tended to reduce the chlorophyll concentration in the rind ($P = 0.0772$) (Table 5.22).

'Navelina Navel' orange. In the 2006 season, ATS in combination with Thiovit® and Thiovit® plus Ethrel® significantly improved rind colour rating of fruit sampled from the eastern side of trees by 0.3 and 0.6 colour units, respectively, compared to the untreated control treatment (Table 5.25). The two ATS-combination treatments significantly reduced relative greenness of fruit (as evidenced by the lower hue angle), improved brightness (higher lightness) and rind colour intensity (higher chroma) of both the vivid and dull sides of fruit sampled from the eastern side of trees after harvest, but not of the fruit sampled from the western side of trees (Table 5.25). The perceived rind colour improvement after harvest of ATS in combination with Thiovit® plus Ethrel® was due to significantly higher carotenoid concentration of fruit sampled from the eastern (by ~ 25%) and western (by ~ 19%) sides of trees (Table 5.27). However, after ethylene degreening, the two ATS-combination treatments delayed further rind colour development (poorer colour rating, higher hue angle, lower lightness and lower chroma) compared to the untreated control treatment (Table 5.26).

'Palmer Navel' orange. In the 2006 season, ATS in combination with Thiovit® did not improve rind colour after harvest or after ethylene degreening (Tables 5.36 to 5.38). ATS in combination with Thiovit® plus Ethrel® significantly improved rind colour rating of fruit sampled from the eastern and western sides of trees by 0.4 and 0.8 colour units, respectively (Table 5.36). This treatment reduced relative greenness (as evidenced by the significantly lower hue angle) on both the vivid and dull sides of fruit, and improved fruit brightness (significantly higher lightness) and rind colour intensity (significantly higher chroma) on the dull side of fruit sampled from both the eastern and western sides of trees (Table 5.36). This difference in rind colour was smaller following ethylene degreening, but still significantly better than that of the control (Table 5.37). The perceived rind colour improvement of fruit sampled from the eastern side of trees after harvest was due to significantly higher carotenoid concentration (by ~ 11%), resulting in a higher carotenoid to chlorophyll ratio (Table 5.38).

'Eureka' lemon. In the 2004 season, ATS alone reduced the relative greenness (as evidenced by the lower hue angle) on the vivid side of fruit sampled from the eastern side of trees (Table 5.42). Fruit brightness was also improved (higher lightness) of fruit sampled from the eastern side of trees, but not of fruit sampled from the western side of trees. When ATS was applied in combination with Ethrel® or Regalis® the treatments did not improve rind colour on both the vivid and dull sides of fruit sampled from the eastern and western sides of trees (Table 5.42).

In the 2006 season, ATS in combination with Thiovit® and Thiovit® plus Ethrel® significantly improved rind colour rating of fruit sampled from the eastern side of trees by 0.8 and 1.3 colour units, respectively, and by 0.4 and 0.6 colour units, respectively, of fruit

sampled from the western side of trees (Table 5.43). Both treatments also reduced relative greenness (as evidenced by the lower hue angle), increased fruit brightness (higher lightness) and improved colour intensity of fruit (higher chroma) after harvest (Table 5.43). These differences in rind colour were smaller following ethylene degreening, but still significantly better than that of the control (Table 5.44). The perceived rind colour improvement of fruit following the ATS in combination with Thiovit® and Thiovit® plus Ethrel® treatments was due to a reduction in chlorophyll concentration by ~ 46% and ~ 63%, respectively, for the two treatments for fruit sampled from the eastern side of trees and a reduction in chlorophyll concentration by ~ 20% and ~ 45%, respectively, for the two treatments for fruit sampled from the western side of trees (Table 5.45). These differences in chlorophyll concentration resulted in a significantly higher carotenoid to chlorophyll ratio for fruit sampled from the eastern and western sides of trees (Table 5.45).

ATS improved rind colour by possibly triggering the endogenous ethylene evolution through the induction of physiological stress, stimulating chloro-chromoplast transformation. ATS stimulated carotenoid biosynthesis in orange-rinded fruit, e.g. 'Miho Wase Satsuma' mandarin, 'Navelina Navel' and 'Palmer Navel' oranges, but not in yellow-rinded fruit, e.g. 'Eureka' lemon. However, ATS stimulated chlorophyll degradation in both orange- and yellow-rinded fruit. ATS in combination with other products that improved rind colour, e.g. Thiovit® plus Ethrel®, was more effective in improving rind colour than when applied alone.

Ethrel® in combination with other potential colour-enhancing treatments. 'Miho Wase Satsuma' mandarin. In the 2004 season, Ethrel® in combination with Thiovit®, Regalis® or ATS significantly reduced the relative greenness on both the vivid and dull sides of fruit sampled from both the eastern and western sides of trees of fruit as evidenced by the lower

hue angle, compared to the untreated control treatment (Table 5.7). Fruit brightness (significantly higher lightness) and rind colour intensity (significantly higher chroma) were also improved by the Ethrel® in combination with Regalis® and ATS treatments (Table 5.7). Ethrel® had an additive effect when applied in combination with Thiovit® and ATS, compared with the Thiovit® and ATS alone.

In the 2006 season, Ethrel® in combination with Thiovit® and Thiovit® plus ATS significantly improved rind colour rating by 0.8 and 1.3 colour units, respectively, of fruit sampled from the western side of trees (generally the worst coloured side) (G.H. Barry, personal communication) (Table 5.10). These combination treatments with Ethrel® reduced relative greenness (significantly lower hue angle), improved the brightness (higher lightness) and colour intensity (higher chroma) of fruit sampled from the western side of trees, but on the eastern side of trees only the Ethrel® in combination with Thiovit® plus ATS significantly improved rind colour (Table 5.10). These differences in rind colour of fruit sampled from the western side of trees were smaller following cold-storage, but still significantly better than that of the control (Table 5.11). The perceived improvement in rind colour of fruit sampled from the western side of trees treated with Ethrel® in combination with Thiovit® and Thiovit® plus ATS was due to significantly higher carotenoid concentration by ~ 26% and ~ 32%, respectively, and significantly lower chlorophyll concentration by ~ 46% and ~ 63%, respectively, resulting in a higher carotenoid to chlorophyll ratio ($P = 0.0590$) (Table 5.12).

‘Nules Clementine’ mandarin. In the 2004 season, Ethrel® in combination with Thiovit®, Regalis® or ATS significantly reduced relative greenness as evidenced by the lower hue angle on the vivid side of fruit sampled from the eastern side of trees (Table 5.16). Relative

greenness of fruit sampled from the western side of trees (generally the worst coloured side) (G.H. Barry, personal communication) was only reduced by the Ethrel® in combination with ATS treatment. Fruit brightness was not improved compared to the untreated control treatment. Fruit rind colour intensity was improved (significantly higher chroma) by the Ethrel® in combination with ATS treatment (Table 5.16). Ethrel® tended to have an additive effect on rind colour improvement when applied in combination with ATS.

In the 2006 season, Ethrel® in combination with Thiovit® plus ATS significantly improved rind colour rating of fruit sampled from both the eastern and western sides of trees by 1.3 colour units (Table 5.19). The additive of Ethrel® to the Thiovit® plus ATS treatment improved rind colour more than the Thiovit® plus ATS treatment without Ethrel®. Relative greenness was significantly reduced as evidenced by the lower hue angle on both the vivid and dull sides of fruit sampled from the eastern and western sides of trees. Fruit brightness (significantly higher lightness) and rind colour intensity (significantly higher chroma) were improved on the dull side of fruit sampled from both the eastern and western sides of trees (Table 5.20). These differences in rind colour were smaller following ethylene degreening, but still significantly better than that of the control (Table 5.21). The Ethrel® combination application did not stimulate carotenoid biosynthesis, but tended to reduce chlorophyll concentration ($P = 0.0772$) (Table 5.22).

‘Navelina Navel’ orange. In the 2006 season, Ethrel® in combination with Thiovit® plus ATS significantly improved rind colour rating of fruit sampled from the eastern side of trees by 0.6 colour units (Table 5.25), and this response in colour improvement was larger than the Thiovit® plus ATS treatment. Fruit relative greenness was reduced (significantly lower hue angle), brightness was improved (significantly higher lightness) and rind colour intensity was

improved (significantly higher chroma) of fruit sampled from the eastern side of trees. No significant improvement in rind colour of fruit sampled from the western side of trees was observed, when the Ethrel® in combination with Thiovit® plus ATS treatment was compared to the untreated control treatment (Table 5.25). However, following ethylene degreening, the Ethrel® combination resulted in poorer rind colour rating than the untreated control (Table 5.26). The apparent improvement in rind colour after harvest of fruit sampled from the eastern side of trees was due to a significant higher carotenoid concentration by ~ 25% and ~ 19%, respectively (Table 5.27).

'Palmer Navel' orange. In the 2004 season, Ethrel® in combination with Thiovit® and Regalis® caused a reduction in rind colour on both the vivid and dull sides of fruit (Table 5.31).

In the 2006 season, the Ethrel® in combination with Thiovit® plus ATS significantly improved the rind colour rating of fruit sampled from the eastern and western sides of trees by 0.4 and by 0.8 colour units, respectively (Table 5.36). This treatment reduced relative greenness (as evidenced by the significantly lower hue angle) on both the vivid and dull sides of fruit, and improved fruit brightness (significantly higher lightness) and rind colour intensity (significantly higher chroma) on the dull side of fruit sampled from both the eastern and western sides of trees (Table 5.36). This difference in rind colour was smaller after ethylene degreening, but still significantly better than that of the control (Table 5.37). The perceived rind colour improvement of fruit sampled from the eastern side of trees after harvest was due to a significantly higher carotenoid concentration by ~ 11%, lower chlorophyll concentration ($P = 0.0545$), resulting in a higher carotenoid to chlorophyll ratio (Table 5.38).

'Eureka' lemon. In the 2004 season, Ethrel® in combination with Regalis® or ATS did not improve rind colour on both the vivid and dull sides of fruit sampled from the eastern and western sides of trees (Table 5.42).

In the 2006 season, Ethrel® in combination with Thiovit® plus ATS significantly improved rind colour rating of fruit sampled from the eastern side of trees by 1.3 colour units, and by 0.6 colour units of fruit sampled from the western side of trees (Table 5.43). The treatment also reduced relative greenness (as evidenced by the lower hue angle), increased fruit brightness (higher lightness) and improved colour intensity of fruit (higher chroma) after harvest on both the vivid and dull sides of fruit sampled from the eastern and western sides of trees (Table 5.43). The addition of Ethrel® to the Thiovit® plus ATS treatment enhanced the effect of Thiovit® plus ATS on colour development. These differences in rind colour were smaller following ethylene degreening, but still significantly better than that of the control (Table 5.44). The perceived rind colour improvement of fruit following the Ethrel® in combination with Thiovit® plus ATS treatment was due to a reduction in chlorophyll concentration for fruit sampled from the eastern (by ~ 63%) and western (by ~ 45%) sides of trees, resulting in a significantly higher carotenoid to chlorophyll ratio (Table 5.45).

Ethrel®, applied at half the recommended rate, in combination with other colour enhancing treatments, especially Thiovit® plus ATS, stimulated the biosynthesis of carotenoids of orange-rinded fruit, e.g. 'Miho Wase Satsuma' mandarin, 'Nules Clementine' mandarin, and 'Navelina Navel' and 'Palmer Navel' oranges, but not of yellow-rinded fruit, e.g. 'Eureka' lemon. Chlorophyll concentration was reduced in both orange- and yellow-rinded fruit types. Although the effects of Ethrel® on rind colour improvement are known (El-Otmani et al., 1996; El-Zeftawi and Garret, 1978), by reducing the concentration of Ethrel® required to get

a rind colour improvement and combining Ethrel® with other colour-enhancing treatments, the amount of leaf drop due to Ethrel® application could be reduced and an additive effect of Ethrel® on other colour enhancing products was achieved.

Conclusions

Rind colour of citrus fruit can be enhanced through pre-harvest applications of various chemical products. Boric acid at 1 $g \cdot L^{-1}$ applied 6 plus 3 weeks before anticipated harvest improved rind colour by stimulating the degradation of chlorophyll in orange- and yellow-rinded fruit, e.g. 'Miho Wase Satsuma' mandarin and 'Eureka' lemon, by ~ 30% and ~ 40%, respectively, and stimulating carotenoid biosynthesis in orange-rinded fruit (by ~ 24%). Rind colour rating was improved by 0.7 colour units for 'Eureka' lemon and by 0.5 colour units for 'Miho Wase Satsuma' mandarin. Thiovit® at 3 $g \cdot L^{-1}$ applied 6 + 3 weeks before anticipated harvest did not consistently improve rind colour. However, when applied in combination treatments, especially with ATS plus Ethrel®, Thiovit® added to the improvement in rind colour by aiding in the degradation of chlorophyll and through enhanced carotenoid biosynthesis. ColourUp® at 0.75 to 1.0 $mL \cdot L^{-1}$ stimulated the degradation of chlorophyll on 'Nules Clementine' mandarin, suggesting that ColourUp® may promote chloro-chromoplast transformation. The best timing seems to be 3 weeks before anticipated harvest. Carotenol® alone at 3 $g \cdot L^{-1}$ or in various combination treatments did not improve rind colour of citrus fruit. Figaron® at 1 $mL \cdot L^{-1}$ triggered chloro-chromoplast transformation possibly by stimulating the biosynthesis of ethylene (Cooper and Henry, 1968). Figaron® stimulated the degradation of chlorophyll (by ~ 30%) on 'Navelina Navel' orange, but did not stimulate carotenoid biosynthesis. The addition of Regalis® to ColourUp® or Ethrel® treatments did not enhance the positive effects the latter two products had on rind colour improvement when they were applied alone. Ammonium thiosulphate stimulated the degradation of chlorophyll

and carotenoid biosynthesis when applied alone, but not to the extent when applied in combination with Thiovit® plus Ethrel®. Ethrel®, applied at half the recommended rate, in combination with other colour-enhancing treatments, especially Thiovit® plus ATS, stimulated the biosynthesis of carotenoids (by ~ 20%) of orange-rinded fruit and chlorophyll degradation (by ~ 40%) in both orange- and yellow-rinded fruit.

Therefore, this study provides a range of novel chemical products with the potential to improve rind colour of citrus fruit. The screening of chemical products which stimulated carotenoid biosynthesis (e.g. Thiovit® plus ATS plus Ethrel®) in combination with products which stimulated chlorophyll degradation (e.g. boric acid, ColourUp® and Figaron®) warrant further evaluation.

Table 5.1. Summary of sites used and treatments applied to improve rind colour of 'Miho Wase Satsuma' mandarin during the 2004 to 2006 seasons. Fruit were sampled at physiological maturity on 31 Mar. 2004, 11 Apr. 2005, and 29 Mar. and 6 Apr. 2006.

Site details	Season	Product[z]	Rate (per L)	Application date	Development stage
Welgevallen Experimental Farm, Stellenbosch (33º57'S, 18º53'E; 120 m alt.)	2004	• [y] Boric acid	1 g	27 Feb. + 13 Mar.	4 + 2 weeks BH[x]
		• Boric acid	2 g	27 Feb. + 13 Mar.	4 + 2 weeks BH
		o Thiovit	3 g	28 Feb.	4 weeks BH
		o ATS	10 mL	28 Feb.	4 weeks BH
		o ATS + Ethrel	5 mL + 0.26 mL	28 Feb.	4 weeks BH
		o Regalis + Ethrel	2 g + 0.26 mL	28 Feb.	4 weeks BH
		o Thiovit + Ethrel	3 g + 0.26 mL	28 Feb.	4 weeks BH
		▪ Figaron	1 mL	10 Dec. 03	20 mm diameter
Welgevallen Experimental Farm, Stellenbosch	2005	ColourUp	0.5 mL	15 Mar. + 22 Mar.	4 + 2 weeks BH
		ColourUp	1.0 mL	15 Mar. + 22 Mar.	4 + 2 weeks BH
Diamant, Paarl (33º46'S; 18°55'E, 140 m alt.)	2006	Boric acid	1 g	14 Feb. + 9 Mar.	6 + 3 weeks BH
		Thiovit + Ethrel[w]	3 g + 0.26 mL	14 Feb. + 9 Mar.	6 + 3 weeks BH
		Thiovit + ATS[w]	3 g + 7.5 mL	14 Feb. + 9 Mar.	6 + 3 weeks BH
		Thiovit + Ethrel + ATS[w]	3 g + 0.26 mL + 7.5 mL	14 Feb. + 9 Mar.	6 + 3 weeks BH
		Thiovit	3 g	14 Feb. + 9 Mar.	6 + 3 weeks BH
		Boric acid + Regalis	1 g + 3 g	14 Feb. + 9 Mar.	6 + 3 weeks BH
		Boric acid + Thiovit	1 g + 3 g	14 Feb. + 9 Mar.	6 + 3 weeks BH
Welgevallen Experimental Farm, Stellenbosch	2006	Carotenol	3 g	14 Nov. + 12 Dec. 05 + 2 Mar.[v]	15 mm + 20 mm + 4 weeks BH
		Regalis	4 g	8 Mar.	4 weeks BH
		Carotenol + Regalis	3 g + 4 g	8 Mar.	4 weeks BH
		Carotenol + Boric acid	3 g + 1 g	30 Feb.	6 weeks BH

[z] Boric acid = 99% boric acid; Thiovit® = 80% elemental sulphur; ColourUp® = neutralised calcium carbonate; Carotenol® = hydrocarbon substances; Ethrel® = 48% ethephon; Figaron® = ethyclozate; Regalis® = 10% prohexadione calcium; ATS = ammoniumthiosulphate.

[y] Within a season, treatments with the same symbols form part of a single experiment with its own control treatment.

[x] Before anticipated harvest

[w] Ethrel® and ATS: single applications only, applied 3 weeks BH.

[v] Application dates for Carotenol® alone also apply to Carotenol® when applied in combination with other products.

Table 5.2. Summary of sites used and treatments applied to improve rind colour of 'Nules Clementine' mandarin during the 2004 to 2006 seasons. Fruit were sampled at physiological maturity on 21 May 2004, 10 May 2005, and 8 May and 15 May 2006.

Site details	Season	Product[z]	Rate (per L)	Application date	Development stage
Welgevallen	2004	Thiovit	3 g	1 Apr.	7 weeks BH[y]
Experimental Farm,		ATS	10 mL	1 Apr.	7 weeks BH
Stellenbosch		ATS + Ethrel	0.26 mL + 5 mL	1 Apr.	7 weeks BH
(33º57'S, 18º53'E;		Regalis + Ethrel	0.26 mL + 2 g	1 Apr.	7 weeks BH
120 m alt.)		Thiovit + Ethrel	3 g + 0.26 mL	1 Apr.	7 weeks BH
Saratoga, Robertson	2005	ColourUp	0.5 mL	19 Apr. + 26 Apr. + 3 May	3 + 2 + 1 weeks BH
(33º50'S, 19º59'E;		ColourUp	1.0 mL	19 Apr. + 26 Apr. + 3 May	3 + 2 + 1 weeks BH
200 m alt.)					
Môrelig, Franschhoek	2006	Boric acid	1 g	20 Mar. + 19 Apr.	6 + 2 weeks BH
(33º51'S, 18º19'E;		ColourUp	0.75 mL	19 Apr.	2 weeks BH
170alt)		Thiovit + ATS[x]	3 g + 7.5 mL	20 Mar. + 19 Apr.	6 + 2 weeks BH
		Thiovit + Ethrel + ATS[x]	3 g + 0.26 mL + 7.5 mL	20 Mar. + 19 Apr.	171+ 2 weeks BH
		Boric acid + Regalis	1 g + 3 g	20 Mar. + 19 Apr.	6 + 2 weeks BH
		Boric acid + Thiovit	1 g + 3 g	20 Mar. + 19 Apr.	6 + 2 weeks BH
		ColourUp	0.5 mL	19 Apr.	2 weeks BH
Saratoga, Robertson	2006	Carotenol	3 g	14 Nov. + 12 Dec. 05 + 20 Apr.[w]	5 mm + 10 mm + 4 weeks BH
		Figaron	1 mL	14 Nov. + 12 Dec. 05	172mm + 10 mm
		Carotenol + Boric acid	3 g + 1 g	30 Mar.	6 weeks BH

[z] Boric acid = 99% boric acid; Thiovit® = 80% elemental sulphur; ColourUp® = neutralised calcium carbonate; Carotenol® = hydrocarbon substances; Ethrel® = 48% ethephon; Figaron® = ethyclozate; Regalis® = 10% prohexadione calcium; ATS = ammoniumthiosulphate.

[y] Before anticipated harvest

[x] Ethrel® and ATS: single applications only, applied 2 weeks BH.

[w] Application dates for Carotenol® alone also apply to Carotenol® when applied in combination with other products.

Table 5.3. Summary of sites used and treatments applied to improve rind colour of 'Navelina Navel' orange during the 2006 season. Fruit were sampled at physiological maturity on 3 May 2006.

Site details	Season	Product[z]	Rate (per L)	Application date	Development stage
Hexrivier, Citrusdal (32º28'S, 18º59'E; 180 m alt.)	2006	• [y] Thiovit + ATS[x]	3 g + 7.5 mL	7 Mar. + 4 Apr.	6 + 2 weeks BH[w]
		• Thiovit + Ethrel + ATS[y]	3 g + 0.26 mL + 7.5 mL	7 Mar. + 4 Apr.	6 + 2 weeks BH
		• ColourUp[y] + Regalis	0.75 mL + 3 g	7 Mar. + 4 Apr.	6 + 2 weeks BH
		• Boric acid	1g	7 Mar. + 4 Apr.	6 + 2 weeks BH
		• Boric acid + Regalis	1 g + 3 g	7 Mar. + 4 Apr.	6 + 2 weeks BH
		• Boric acid + ColourUp	1 g + 0.75 mL	7 Mar. + 4 Apr.	6 + 2 weeks BH
		• ColourUp	0.75 mL	4 Apr.	6 + 2 weeks BH
		• ColourUp	1.0 mL	4 Apr.	6 + 2 weeks BH
		o Carotenol	3 g	16 Nov. + 14 Dec. 05 + 23 Mar.	30 mm + 35 mm + 4 weeks BH
		o Figaron	1 mL	16 Nov. + 14 Dec. 05	30 mm + 35 mm

[z] Boric acid = 99% boric acid; Thiovit® = 80% elemental sulphur; ColourUp® = neutralised calcium carbonate; Carotenol® = hydrocarbon substances; Ethrel® = 48% ethephon; Figaron® = ethyclozate; Regalis® = 10% prohexadione calcium; ATS = ammoniumthiosulphate.

[y] Treatments with the same symbols form part of a single experiment with its own control treatment.

[x] Ethrel®, ATS and ColourUp®: single applications only, applied 2 weeks BH.

[w] Before anticipated harvest

Table 5.4. Summary of sites used and treatments applied to improve rind colour of 'Palmer Navel' orange during the 2004 to 2006 seasons. Fruit were sampled at physiological maturity on 11 May 2004, 12 May and 31 May 2005, and 31 May 2006.

Site details	Season	Product[z]	Rate (per L)	Application date	Development stage
Landau, Wellington (33º35'S, 18º59'E; 120 m alt.)	2004	Thiovit	3 g	15 Apr.	6 weeks BH[x]
		Regalis + Ethrel	0.26 mL + 2 g	15 Apr.	6 weeks BH
		Thiovit + Ethrel	3 g + 0.26 mL	15 Apr.	6 weeks BH
Landau, Wellington	2005	ColourUp	0.5 mL	22 Apr. + 29 Apr. + 6 May	3 + 2 + 1 week BH
		ColourUp	1.0 mL	22 Apr. + 29 Apr. + 6 May	3 + 2 + 1 week BH
Hexrivier, Citrusdal (32º28'S, 18º59'E; 180 m alt.)	2005	ColourUp	0.5 mL	21 Apr. + 28 Apr. + 5 May	6 + 5 + 3 weeks BH
		ColourUp	1.0 mL	21 Apr. + 28 Apr. + 5 May	6 + 5 + 3 weeks BH
Hexrivier, Citrusdal	2006				
		• [y] Thiovit + ATS[w]	3 g + 7.5 mL	4 Apr. + 11 May	8 + 2 weeks BH
		• Thiovit + Ethrel + ATS[w]	3 g + 0.26 mL + 7.5 mL	4 Apr. + 11 May	8 + 2 weeks BH
		• ColourUp + Regalis[w]	0.75 mL + 3 g	4 Apr. + 11 May	8 + 2 weeks BH
		• Boric acid	1 g	4 Apr. + 11 May	6 + 2 weeks BH
		• Boric acid + Regalis	1 g + 3 g	4 Apr. + 11 May	8 + 2 weeks BH
		• Boric acid + ColourUp	1 g + 0.75 mL	4 Apr. + 11 May	8 + 2 weeks BH
		• ColourUp	0.5 mL	11 May	2 weeks BH
		• ColourUp	1.0 mL	11 May	2 weeks BH
		o Carotenol	3 g	16 Nov. + 14 Dec. 05 + 18 Apr.[v]	20 mm + 25 mm + 4 weeks BH
		o Figaron	1 mL	16 Nov. + 14 Dec. 05	20 mm + 25 mm
		o Carotenol + Regalis	3 g + 3 g	11 May	2 weeks BH
		o Carotenol + Boric acid	3 g + 1 g	18 Apr.	6 weeks BH

[z] Boric acid = 99% boric acid; Thiovit® = 80% elemental sulphur; ColourUp® = neutralised calcium carbonate; Carotenol® = hydrocarbon substances; Ethrel® = 48% ethephon; Figaron® = ethyclozate; Regalis® = 10% prohexadione calcium; ATS = ammoniumthiosulphate.

[y] Within a season, treatments with the same symbols form part of a single experiment with its own control treatment.

[x] Before anticipated harvest

[w] Ethrel®, ATS and ColourUp: single applications only, applied 2 weeks BH.

[v] Application dates for Carotenol® alone also apply to Carotenol® when applied in combination with other products.

Table 5.5. Summary of sites used and treatments applied to improve rind colour of 'Eureka' lemon during the 2004 and 2006 seasons: Fruit were sampled at physiological maturity on 27 May 2004 and 18 May 2006.

Site details	Season	Product[z]	Rate (per L)	Application date	Development stage
Jericho, Gt. Drakenstein	2004	Thiovit	3 g	1 May	4 weeks BH[y]
(33º52'S,19º01'E;		ATS	10 mL	1 May	4 weeks BH
160 m alt.)		ATS + Ethrel	0.26 mL + 5 mL	1 May	4 weeks BH
		Regalis + Ethrel	0.26 mL + 2 g	1 May	4 weeks BH
		Regalis + ATS	2 g + 5 mL	1 May	4 weeks BH
Jericho, Gt. Drakenstein	2006	Boric acid	1 g	17 Mar + 19 Apr	8 + 3 weeks BH
		Thiovit + ATS[x]	3 g + 7.5 mL	17 Mar + 19 Apr	8 + 3 weeks BH
		Thiovit + Ethrel + ATS[x]	3 g + 0.26 mL + 7.5 mL	17 Mar + 19 Apr	8 + 3 weeks BH

[z] Boric acid = 99% boric acid; Thiovit® = 80% elemental sulphur; Ethrel® = 48% ethephon; Regalis® = 10% prohexadione calcium; ATS = ammoniumthiosulphate.
[y] Before anticipated harvest
[x] Ethrel® and ATS: single applications only, applied 3 weeks BH.

Table 5.6. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Miho Wase Satsuma' mandarin fruit sampled from the eastern and western sides of trees during the 2003-04 season to determine the effects of boric acid on rind colour.

Treatment	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	90.0 a[z]	104.4 a	91.9 a	103.9 a
Boric acid (1 g·L^{-1})	91.5 a	104.2 a	85.0 b	101.1 b
Boric acid (2 g·L^{-1})	85.7 b	101.4 b	86.0 b	101.8 b
P-value	<0.0001	0.0056	<0.0001	0.0181
LSD	2.53	2.04	2.22	1.97
		Lightness		
Control	69.4 ns	57.2 ns	67.9 b	54.3 b
Boric acid (1 g·L^{-1})	67.8	55.2	71.2 a	59.9 a
Boric acid (2 g·L^{-1})	69.8	57.9	71.3 a	58.3 a
P-value	0.2393	0.1513	0.0054	0.0004
LSD	2.46	2.89	2.28	2.77
		Chroma		
Control	73.6 ns	66.0 ns	71.8 ns	62.8 b
Boric acid (1 g·L^{-1})	72.7	64.2	73.2	67.4 a
Boric acid (2 g·L^{-1})	72.8	66.8	73.2	67.4 a
P-value	0.4499	0.0644	0.1428	<0.0001
LSD	1.42	2.30	1.58	1.96

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.7. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Miho Wase Satsuma' mandarin fruit sampled from the eastern and western sides of trees during the 2003-04 season to determine the effects of Thiovit®, ATS, Regalis® and combinations thereof with Ethrel® on rind colour.

Treatment	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	83.5 a[z]	102.3 a	87.2 a	102.7 a
Thiovit	80.3 b	96.9 b	80.9 b	97.2 bc
ATS	80.3 b	98.6 b	85.7 a	103.3 a
ATS + Ethrel	78.1 c	94.1 c	80.6 b	96.3 c
Regalis + Ethrel	79.5 bc	98.2 b	80.9 b	99.4 b
Thiovit + Ethrel	74.7 d	91.4 d	78.0 c	92.3 d
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	2.09	2.50	2.16	2.33
		Lightness		
Control	65.9 b	51.5 c	65.5 c	52.7 c
Thiovit	67.8 b	56.0 b	68.6 b	55.8 b
ATS	72.3 a	59.0 a	70.7 ab	57.4 b
ATS + Ethrel	70.7 a	60.4 a	71.9 a	60.6 a
Regalis + Ethrel	71.5 a	56.0 b	69.8 b	55.9 b
Thiovit + Ethrel	62.1 c	50.8 c	60.9 d	51.2 c
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.92	2.67	2.07	2.37
		Chroma		
Control	67.8 d	60.3 d	67.6 d	60.5 d
Thiovit	69.1 c	63.9 c	69.2 c	63.5 c
ATS	73.4 a	67.1 ab	72.9 ab	66.8 ab
ATS + Ethrel	71.7 b	68.3 a	73.2 a	68.4 a
Regalis + Ethrel	72.4 ab	65.2 bc	71.9 b	65.2 bc
Thiovit + Ethrel	59.2 e	55.2 e	58.6 e	55.1 e
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.16	2.11	1.21	1.93

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.8. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Miho Wase Satsuma' mandarin fruit sampled from the eastern and western sides of trees during the 2003-04 season to determine the effects of Figaron® on rind colour.

Treatment	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	83.2 a	99.3 ns	85.6 ns	101.3 ns
Figaron	80.5 b	98.3	83.4	99.9
P-value	0.0065	0.4728	0.0734	0.2014
LSD	1.96	2.62	2.34	2.14
		Lightness		
Control	69.9 b	57.6 ns	70.7 ns	57.5 ns
Figaron	72.7 a	58.3	70.4	57.2
P-value	0.0285	0.6916	0.7871	0.8733
LSD	2.54	3.18	1.99	3.15
		Chroma		
Control	72.6 ns	66.4 ns	72.9 ns	65.8 ns
Figaron	73.0	66.0	73.2	65.9
P-value	0.6330	0.7401	0.6538	0.9582
LSD	1.58	2.32	1.22	2.27

[z] Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = non significant).

Table 5.9. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Miho Wase Satsuma' mandarin fruit sampled from the eastern and western sides of trees during the 2004-05 season to determine the effects of ColourUp® at two different concentrations on rind colour.

Treatment	4 Weeks before harvest		3 Weeks before harvest		4 Weeks before harvest		3 Weeks before harvest	
	Eastern				Western			
	Vivid	Dull	Vivid	Dull	Vivid	Dull	Vivid	Dull
				Hue angle (°)				
Control	82.2 a	94.7 ns	82.2 a	94.7 a	77.2 ns	89.0 ab	77.2 ns	89.0 a
ColourUp (0.5 mL·L^{-1})	79.1 b	93.1	77.4 b	91.2 b	78.7	86.8 b	75.3	86.4 b
ColourUp (1.0 mL·L^{-1})	78.6 b	91.0	77.8 b	90.1 b	79.1	91.2 a	76.4	89.1 a
P-value	0.0215	0.1528	0.0001	0.0255	0.2712	0.0178	0.2053	0.0191
LSD	2.76	3.87	2.15	3.12	2.44	3.03	1.98	2.36
				Lightness				
Control	72.1 ns	65.8 ns	72.1 ns	65.8 ab	72.6 ns	70.6 ns	72.6 a	70.6 ns
ColourUp (0.5 mL·L^{-1})	71.8	65.9	70.7	65.3 b	72.2	70.4	71.5 b	69.1
ColourUp (1.0 mL·L^{-1})	71.6	68.4	71.4	68.0 a	72.5	69.0	71.9 ab	69.6
P-value	0.7923	0.1220	0.0700	0.0128	0.7205	0.2518	0.0487	0.2219
LSD	1.42	2.89	1.13	2.19	1.11	2.19	0.87	1.67
				Chroma				
Control	68.3 b	57.8 ns	68.3 b	57.8 b	71.8 ns	63.9 ab	71.8 ns	63.9 ns
ColourUp (0.5 mL·L^{-1})	70.7 a	60.2	69.8 a	59.0 ab	71.0	66.2 a	70.9	63.9
ColourUp (1.0 mL·L^{-1})	70.0 ab	61.3	70.5 a	61.4 a	71.2	62.5 b	71.5	64.0
P-value	0.0474	0.1623	0.0272	0.0328	0.6894	0.0392	0.3274	0.9877
LSD	1.85	3.74	1.48	2.85	1.71	2.84	1.19	2.25

[z] Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = non significant).

Table 5.10. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hour after harvest on the vivid (yellow) and dull (green) sides of 'Miho Wase Satsuma' mandarin fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of boric acid, Thiovit® and combinations with Thiovit® with ATS and Ethrel® plus ATS on rind colour.

Treatment	Eastern		Western	
			Colour rating[z]	
Control	4.8 ns[y]		5.0 a	
Boric acid	4.8		4.1 b	
Thiovit + Ethrel	4.8		4.2 b	
Thiovit + ATS	4.7		4.1 b	
Thiovit + Ethrel + ATS	4.6		3.7 c	
P-value	0.2907		<0.0001	
LSD	0.25		0.25	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
			Hue angle (°)	
Control	92.5 a	106.8 a	94.4 a	106.3 a
Boric acid	91.8 ab	103.8 bc	85.4 b	99.6 b
Thiovit + Ethrel	89.3 bc	104.5 abc	84.7 b	99.2 b
Thiovit + ATS	87.3 c	105.7 ab	83.4 bc	99.7 b
Thiovit + Ethrel + ATS	89.9 b	103.3 c	82.1 c	94.1 c
P-value	0.0006	0.0237	<0.0001	<0.0001
LSD	2.54	2.39	2.04	2.40
			Lightness	
Control	66.3 ns	60.3 ns	66.0 c	60.0 c
Boric acid	67.0	61.0	69.3 ab	64.6 b
Thiovit + Ethrel	67.5	59.8	69.1 ab	64.2 b
Thiovit + ATS	68.7	61.1	68.9 b	64.2 b
Thiovit + Ethrel + ATS	67.4	62.0	70.2 a	67.4 a
P-value	0.0920	0.1822	<0.0001	<0.0001
LSD	1.74	1.86	1.18	1.76
			Chroma	
Control	66.1 b	56.4 ns	65.5 c	56.2 c
Boric acid	67.2 b	58.3	71.7 b	62.0 b
Thiovit + Ethrel	68.6 ab	56.3	71.9 b	61.7 b
Thiovit + ATS	70.4 a	56.6	71.4 b	60.6 b
Thiovit + Ethrel + ATS	68.5 ab	58.5	73.9 a	65.6 a
P-value	0.0252	0.1770	<0.0001	<0.0001
LSD	2.64	2.41	1.89	2.34

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.11. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after degreening on the vivid (yellow) and dull (green) sides of 'Miho Wase Satsuma' mandarin fruit sampled from the eastern and western sides of trees during the 2005-06 season determine the effects of boric acid, Thiovit® and combinations with Thiovit® with ATS and Ethrel® plus ATS on rind colour.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	2.5 a[y]		2.5 a	
Boric acid	2.5 a		2.0 b	
Thiovit + Ethrel	2.3 ab		1.8 bc	
Thiovit + ATS	2.2 b		1.6 c	
Thiovit + Ethrel + ATS	2.2 b		1.3 d	
P-value	0.0132		<0.0001	
LSD	0.24		0.23	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	79.8 ns	86.1 ns	80.3 a	86.2 a
Boric acid	79.3	85.6	75.9 b	83.4 b
Thiovit + Ethrel	79.2	85.3	75.9 b	83.8 b
Thiovit + ATS	78.3	85.1	75.8 b	84.2 b
Thiovit + Ethrel + ATS	79.0	84.6	75.1 b	80.4 c
P-value	0.2372	0.3373	<0.0001	<0.0001
LSD	1.22	1.47	1.12	1.43
		Lightness		
Control	71.4 a	74.2 a	71.9 a	73.7 ab
Boric acid	71.6 a	74.4 a	70.7 b	74.3 a
Thiovit + Ethrel	70.3 bc	73.8 ab	70.3 b	73.4 b
Thiovit + ATS	71.1 ab	74.3 a	70.3 b	74.3 a
Thiovit + Ethrel + ATS	70.1 c	73.1 b	70.1 b	72.1 c
P-value	0.0004	0.0128	<0.0001	<0.0001
LSD	0.80	0.85	0.75	0.86
		Chroma		
Control	75.5 ab	75.8 ns	76.5 ns	75.1 b
Boric acid	76.6 a	76.6	77.3	76.8 a
Thiovit + Ethrel	75.4 ab	76.3	77.4	76.1 a
Thiovit + ATS	76.5 a	76.9	77.2	76.0 a
Thiovit + Ethrel + ATS	75.0 b	75.8	77.0	75.9 ab
P-value	0.0301	0.1508	0.2217	0.0109
LSD	1.17	0.98	0.87	0.95

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.12. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest and after degreening of 'Miho Wase Satsuma' mandarin fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of of boric acid, Thiovit® and combinations with Thiovit® with ATS and Ethrel® plus ATS on rind pigments.

Treatment	After harvest	After degreening	After harvest	After degreening
	Eastern		Western	
	Carotenoid (µg·g^{-1} DW)			
Control	520.8 ns[z]	627.2 ns	536.7 b	702.7 b
Boric acid	544.2	662.2	695.1 a	911.7 a
Thiovit + Ethrel	639.9	725.2	726.7 a	960.6 a
Thiovit + ATS	507.0	703.8	717.8 a	947.6 a
Thiovit + Ethrel + ATS	596.2	682.8	785.5 a	1004.4 a
P-value	0.2130	0.6258	0.0105	0.0024
LSD	128.24	134.01	131.41	153.05
	Chlorophyll (µg·g^{-1} DW)			
Control	653.8 ns	56.3 ns	603.7 a	69.2 a
Boric acid	514.6	45.3	344.4 b	41.9 b
Thiovit + Ethrel	585.1	60.8	325.2 b	41.6 b
Thiovit + ATS	455.8	48.3	307.5 b	32.6 b
Thiovit + Ethrel + ATS	497.0	60.5	225.5 b	40.6 b
P-value	0.3342	0.4854	<0.0001	0.0010
LSD	205.14	22.16	125.51	16.60
	Chlorophyll/Carotenoid Ratio			
Control	1.30 ns	0.10 ns	1.18 a	0.11 a
Boric acid	0.97	0.07	0.53 b	0.05 b
Thiovit + Ethrel	1.01	0.10	0.49 b	0.05 b
Thiovit + ATS	0.91	0.07	0.46 b	0.04 b
Thiovit + Ethrel + ATS	0.95	0.09	0.32 b	0.04 b
P-value	0.4512	0.7183	<0.0001	<0.0001
LSD	0.47	0.05	0.29	0.03
	Carotenoid/Chlorophyll Ratio			
Control	0.84 ns	12.27 ns	0.94 ns	12.37 b
Boric acid	1.10	16.42	2.23	23.89 a
Thiovit + Ethrel	1.46	16.05	3.23	25.18 a
Thiovit + ATS	1.14	15.47	3.19	32.95 a
Thiovit + Ethrel + ATS	1.95	11.54	4.48	25.88 a
P-value	0.3887	0.3695	0.0590	0.0089
LSD	1.22	6.28	2.34	10.66

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.13. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Miho Wase Satsuma' mandarin fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Carotenol®, Regalis® and combinations with Carotenol® with Regalis® and boric acid on rind colour.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	4.3 c[y]		4.7 ns	
Carotenol	4.6 ab		4.8	
Regalis	4.5 bc		4.9	
Carotenol + Regalis	4.8 a		4.8	
Carotenol + Boric acid	4.4 bc		4.8	
P-value	0.0006		0.6322	
LSD	0.26		0.26	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	89.3 cd	104.4 c	93.7 a	106.1 c
Carotenol	93.7 a	109.7 a	92.8 a	110.1 a
Regalis	90.6 bc	106.5 bc	93.3 a	108.3 ab
Carotenol + Regalis	93.2 ab	108.4 ab	93.5 a	107.6 bc
Carotenol + Boric acid	86.7 d	106.6 bc	89.1 b	108.2 ab
P-value	<0.0001	0.0002	0.0142	0.0039
LSD	2.63	2.42	2.85	2.07
		Lightness		
Control	69.5 a	62.8 a	69.7 a	64.0 a
Carotenol	65.7 b	57.5 c	67.1 b	57.7 c
Regalis	69.3 a	60.6 b	67.9 ab	60.7 b
Carotenol + Regalis	66.0 b	58.4 c	67.0 b	61.0 b
Carotenol + Boric acid	68.9 a	58.7 bc	67.1 b	58.6 c
P-value	<0.0001	<0.0001	0.0333	<0.0001
LSD	1.94	2.04	1.96	1.97
		Chroma		
Control	71.1 a	59.2 a	69.5 ns	60.5 a
Carotenol	64.8 b	52.9 c	66.8	53.0 c
Regalis	69.8 a	56.6 b	67.3	56.2 b
Carotenol + Regalis	64.9 b	53.6 c	66.5	56.8 b
Carotenol + Boric acid	70.9 a	54.6 bc	67.8	53.8 c
P-value	<0.0001	<0.0001	0.2609	<0.0001
LSD	2.96	2.60	2.87	2.37

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).

[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = nonsignificant).

Table 5.14. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after degreening on the vivid (yellow) and dull (green) sides of 'Miho Wase Satsuma' mandarin fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Carotenol®, Regalis® and combinations with Carotenol® with Regalis® and boric acid on rind colour.

Treatment	Eastern		Western	
			Colour rating[z]	
Control	3.0 c[y]		2.9 c	
Carotenol	3.9 a		3.9 a	
Regalis	3.5 b		3.7 ab	
Carotenol + Regalis	3.9 a		3.7 b	
Carotenol + Boric acid	3.7 ab		3.9 a	
P-value	<0.0001		<0.0001	
LSD	0.26		0.23	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
			Hue angle (°)	
Control	79.2 b	89.5 c	82.0 b	89.6 b
Carotenol	83.1 a	94.1 ab	84.3 a	93.8 a
Regalis	82.0 a	92.3 b	84.1 a	91.8 a
Carotenol + Regalis	82.9 a	95.1 a	83.8 a	92.4 a
Carotenol + Boric acid	79.0 b	92.2 b	81.1 b	92.4 a
P-value	<0.0001	<0.0001	0.0001	0.0007
LSD	1.55	2.25	1.57	1.95
			Lightness	
Control	71.5 a	72.5 a	72.5 a	75.2 a
Carotenol	69.6 b	69.6 bc	70.7 bc	70.6 c
Regalis	72.1 a	72.6 a	72.0 a	73.7 ab
Carotenol + Regalis	71.2 a	70.6 b	71.8 ab	73.2 b
Carotenol + Boric acid	69.0 b	68.7 c	69.9 c	70.6 c
P-value	<0.0001	<0.0001	0.0002	<0.0001
LSD	1.20	1.76	1.20	1.54
			Chroma	
Control	77.5 a	73.9 a	77.4 a	75.9 a
Carotenol	72.4 c	70.5 bc	74.0 b	71.6 b
Regalis	76.2 ab	72.5 ab	75.0 b	72.6 b
Carotenol + Regalis	74.4 b	69.9 c	75.4 b	72.7 b
Carotenol + Boric acid	74.5 b	70.5 bc	74.4 b	71.85 b
P-value	<0.0001	0.0023	0.0018	<0.0001
LSD	1.80	2.32	1.76	1.91

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = nonsignificant).

Table 5.15. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest and after degreening of 'Miho Wase Satsuma' mandarin fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Carotenol®, Regalis® and combinations with Carotenol® with Regalis® and boric acid on rind pigments.

Treatment	After harvest	After degreening	After harvest	After degreening
	Eastern		Western	
	Carotenoid (µg·g^{-1} DW)			
Control	722.1 ab[z]	909.9 a	543.6 ns	698.1 b
Carotenol	621.6 bc	702.1 b	565.2	676.2 b
Regalis	618.1 bc	695.7 b	586.2	595.8 b
Carotenol + Regalis	566.9 c	626.3 b	583.6	576.0 b
Carotenol + Boric acid	767.4 a	966.2 a	692.3	871.4 a
P-value	0.0191	0.0042	0.1824	0.0107
LSD	124.36	189.89	126.97	152.55
	Chlorophyll (µg·g^{-1} DW)			
Control	494.2 ns	81.0 ns	629.3 ns	83.0 ns
Carotenol	588.4	119.8	778.3	164.3
Regalis	828.3	167.3	919.6	160.2
Carotenol + Regalis	829.5	142.0	748.7	128.1
Carotenol + Boric acid	574.1	166.2	727.1	175.4
P-value	0.0701	0.1472	0.1844	0.0791
LSD	291.66	77.15	225.53	74.05
	Chlorophyll/Carotenoid Ratio			
Control	0.73 c	0.09 b	1.17 ns	0.12 b
Carotenol	0.98 bc	0.18 ab	1.44	0.24 a
Regalis	1.33 ab	0.24 a	1.61	0.27 a
Carotenol + Regalis	1.51 a	0.23 ab	1.34	0.23 a
Carotenol + Boric acid	0.78 c	0.18 ab	1.06	0.20 ab
P-value	0.0187	0.0367	0.2408	0.0134
LSD	0.53	0.10	0.49	0.09
	Carotenoid/Chlorophyll Ratio			
Control	1.78 ns	13.02 a	0.89 ns	10.01 a
Carotenol	1.21	6.83 b	0.76	5.06 b
Regalis	0.84	4.68 b	0.74	3.82 b
Carotenol + Regalis	0.75	4.63 b	0.81	4.62 b
Carotenol + Boric acid	1.74	9.00 ab	1.00	7.58 ab
P-value	0.1423	0.0350	0.5931	0.0352
LSD	1.02	5.93	0.35	4.53

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.16. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Nules Clementine' mandarin fruit sampled from the eastern and western sides of trees during the 2003-04 season to determine the effects of Thiovit®, ATS, Regalis® and combinations thereof with Ethrel® on rind colour.

Treatment	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	71.1 a[z]	79.7 ns	71.2 a	80.5 a
Thiovit	69.8 ab	78.8	69.8 a	79.8 a
ATS	70.0 ab	78.6	71.7 a	79.6 a
ATS + Ethrel	65.5 c	86.0	66.9 b	74.8 b
Regalis + Ethrel	68.1 b	78.1	71.2 a	80.1 a
Thiovit + Ethrel	68.9 b	78.1	70.7 a	79.3 a
P-value	<0.0001	0.8746	<0.0001	0.0013
LSD	1.94	13.94	1.93	2.86
		Lightness		
Control	67.7 a	64.0 ab	67.8 ab	64.8 ns
Thiovit	66.7 ab	63.2 bc	68.1 a	64.4
ATS	66.4 b	65.5 a	67.4 abc	65.4
ATS + Ethrel	64.8 c	63.1 bc	66.3 c	64.8
Regalis + Ethrel	66.0 bc	61.3 c	66.8 bc	63.6
Thiovit + Ethrel	66.4 b	63.5 ab	68.5 a	65.9
P-value	0.0012	0.0113	0.0036	0.3485
LSD	1.28	2.14	1.21	2.10
		Chroma		
Control	71.3 b	62.6 b	70.9 b	62.7 b
Thiovit	71.3 b	63.3 ab	70.7 b	61.4 b
ATS	71.4 b	66.2 a	70.3 b	62.4 b
ATS + Ethrel	73.4 a	66.4 a	73.4 a	67.0 a
Regalis + Ethrel	72.5 ab	61.4 b	70.1 b	62.4 b
Thiovit + Ethrel	72.6 ab	64.1 ab	71.2 b	64.5 ab
P-value	0.0494	0.0252	0.0053	0.0184
LSD	1.61	3.37	1.75	3.35

[z] Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = non significant).

Table 5.17. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Nules Clementine' mandarin fruit sampled from the eastern sides of trees during the 2004-05 season to determine the effects of ColourUp® at two concentrations on rind colour.

Treatment	3 Weeks before harvest		2 Weeks before harvest		1 Week before harvest	
	Vivid	Dull	Vivid	Dull	Vivid	Dull
			Hue angle (°)			
Control	79.0 a[z]	88.8 a	77.7 a	89.4 a	75.2 ns	87.1 a
ColourUp (0.5 mL·L^{-1})	73.2 b	83.2 b	73.5 b	85.5 b	72.0	82.6 b
ColourUp (1.0 mL·L^{-1})	70.0 c	79.7 c	71.6 b	81.3 c	72.6	82.4 b
P-value	<0.0001	<0.0001	0.0008	<0.0001	0.0806	0.0038
LSD	2.51	2.63	3.29	3.48	2.93	3.08
			Lightness			
Control	61.0 ns	54.6 ns	58.4 b	53.1 b	60.6 ns	54.7 ns
ColourUp (0.5 mL·L^{-1})	60.2	53.3	62.1 a	58.8 a	59.4	53.7
ColourUp (1.0 mL·L^{-1})	60.1	54.2	62.6 a	60.0 a	59.1	53.0
P-value	0.4562	0.5884	<0.0001	<0.0001	0.1293	0.3878
LSD	1.44	2.57	1.65	2.01	1.48	2.36
			Chroma			
Control	56.0 b	46.2 b	55.3 b	44.8 b	57.6 ns	46.7 b
ColourUp (0.5 mL·L^{-1})	58.6 a	46.6 b	60.8 a	52.0 a	58.0	49.9 a
ColourUp (1.0 mL·L^{-1})	59.7 a	49.7 a	60.9 a	53.3 a	57.1	47.8 ab
P-value	0.0017	0.0276	<0.0001	<0.0001	0.7079	0.0359
LSD	2.05	2.80	2.33	2.68	2.06	2.47

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.18. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Nules Clementine' mandarin fruit sampled from the western sides of trees during the 2004-05 season to determine the effects of ColourUp® at two concentrations on rind colour.

Treatment	3 Weeks before harvest		2 Weeks before harvest		1 Week before harvest	
	Vivid	Dull	Vivid	Dull	Vivid	Dull
			Hue angle (°)			
Control	70.3 ns[z]	84.9 a	67.9 b	80.9 b	70.5 a	83.2 a
ColourUp (0.5 mL·L^{-1})	69.2	82.9 ab	72.6 a	86.7 a	69.8 a	78.7 b
ColourUp (1.0 mL·L^{-1})	69.8	80.8 b	68.8 b	82.8 b	66.6 b	81.3 ab
P-value	0.6562	0.0126	0.0023	0.0003	0.0112	0.0135
LSD	2.40	2.74	2.77	2.89	2.71	2.99
			Lightness			
Control	60.5 a	54.6 ns	60.4 ns	57.5 ns	59.1 ns	55.5 a
ColourUp (0.5 mL·L^{-1})	60.0 a	54.8	61.4	57.9	57.5	55.2 a
ColourUp (1.0 mL·L^{-1})	57.5 b	56.1	60.9	59.5	58.6	52.5 b
P-value	<0.0001	0.3347	0.2912	0.1000	0.0728	0.0201
LSD	1.14	2.16	1.25	1.94	1.36	2.28
			Chroma			
Control	59.4 a	47.8 ns	60.8 ns	52.1 ns	57.9 ns	48.6 ns
ColourUp (0.5 mL·L^{-1})	59.4 a	48.3	60.0	51.5	58.7	50.8
ColourUp (1.0 mL·L^{-1})	56.3 b	49.9	60.4	52.4	58.1	49.2
P-value	0.0001	0.2360	0.6622	0.8069	0.7106	0.1699
LSD	1.62	2.55	1.76	2.60	1.87	2.39

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.19. Rind colour rating were made within 24 hours after harvest and after storage on fruit sampled from the eastern and western sides of 'Nules Clementine' mandarin trees during the 2005-06 season to determine the effects of boric acid, ColourUp®, Thiovit® and combinations thereof with ATS, Ethrel® plus ATS and Regalis® on rind colour .

Treatment	After harvest		After storage	
	Colour rating[z]			
	Eastern	Western	Eastern	Western
Control	3.5 a[y]	3.5 a	1.0 b	1.0 ns
Boric acid	3.2 b	3.1 b	1.0 b	1.0
ColourUp (0.75 mL·L^{-1})	3.0 bcd	2.8 bc	1.1 a	1.0
Thiovit + ATS	2.9 cd	2.8 bc	1.0 b	1.0
Thiovit + Ethrel + ATS	2.2 e	2.2 e	1.0 b	1.0
Boric acid + Regalis	3.1 bc	2.8 bc	1.0 a	1.0
Boric acid + Thiovit	2.8 d	2.8 c	1.0 b	1.0
P-value	<0.0001	<0.0001	0.0121	0.4345
LSD	0.25	0.26	0.06	0.05

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.20. Hue angle, lightness and chroma measurements were made within 24 hour after harvest on the vivid (yellow) and dull (green) sides of 'Nules Clementine' mandarin fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of boric acid, ColourUp®, Thiovit® and combinations thereof with ATS, Ethrel® plus ATS and Regalis® on rind colour.

Treatment	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	71.7 a[z]	81.5 a	70.6 a	81.6 a
Boric acid	70.7 ab	78.0 b	70.3 a	78.2 b
ColourUp (0.75 mL·L^{-1})	70.5 ab	77.8 b	69.6 ab	75.4 c
Thiovit + ATS	69.9 b	77.6 b	70.2 a	77.1 bc
Thiovit + Ethrel + ATS	66.2 c	71.7 c	66.2 c	70.8 d
Boric acid + Regalis	71.2 a	77.9 b	68.6 b	75.2 c
Boric acid + Thiovit	69.8 b	77.0 b	70.1 a	77.2 bc
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.27	2.07	1.26	2.19
		Lightness		
Control	68.5 b	66.8 b	67.8 bc	65.4 c
Boric acid	68.7 ab	69.4 a	68.6 a	67.7 ab
ColourUp (0.75 mL·L^{-1})	69.0 ab	69.3 a	68.5 ab	69.1 a
Thiovit + ATS	67.4 c	68.6 a	67.9 ab	67.8 ab
Thiovit + Ethrel + ATS	67.0 c	68.6 a	67.1 c	69.1 a
Boric acid + Regalis	69.4 a	66.9 b	67.9 ab	66.9 bc
Boric acid + Thiovit	68.5 b	69.4 a	68.0 ab	68.7 a
P-value	<0.0001	<0.0001	0.0012	<0.0001
LSD	0.72	1.41	0.74	1.45
		Chroma		
Control	72.8 cd	66.3 d	71.8 bc	65.0 c
Boric acid	74.3 a	70.5 a	72.8 a	67.6 b
ColourUp (0.75 mL·L^{-1})	74.0 ab	68.8 abc	72.1 abc	69.3 b
Thiovit + ATS	71.9 e	68.2 bc	71.7 bc	67.3 b
Thiovit + Ethrel + ATS	72.5 cde	70.5 a	72.6 ab	71.3 a
Boric acid + Regalis	71.6 e	67.3 cd	71.7 c	67.5 b
Boric acid + Thiovit	73.2 bc	69.4 ab	71.7 c	67.7 b
P-value	<0.0001	<0.0001	0.0492	<0.0001
LSD	0.91	1.85	0.91	1.92

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.21. Hue angle, lightness and chroma measurements were made within 24 hour after storage on the vivid (yellow) and dull (green) sides of 'Nules Clementine' mandarin fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of boric acid, ColourUp®, Thiovit® and combinations thereof with ATS, Ethrel® plus ATS and Regalis® on rind colour.

Treatment	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	60.2 ab[z]	62.7 ab	60.6 a	64.0 a
Boric acid	60.3 a	62.8 ab	59.1 b	62.1 b
ColourUp (0.75 mL·L^{-1})	60.8 a	63.0 a	59.5 b	61.3 bc
Thiovit + ATS	59.1 b	62.3 ab	58.5 bc	61.3 bc
Thiovit + Ethrel + ATS	58.0 c	60.6 c	57.6 c	60.4 c
Boric acid + Regalis	60.0 ab	63.4 a	59.4 b	61.7 b
Boric acid + Thiovit	59.2 b	61.8 bc	59.1 b	61.9 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.10	1.14	1.07	1.17
		Lightness		
Control	63.1 a	64.1 a	63.5 a	64.9 a
Boric acid	63.1 a	64.0 ab	62.4 b	64.3 ab
ColourUp (0.75 mL·L^{-1})	62.9 ab	64.2 a	62.0 bc	63.6 bc
Thiovit + ATS	62.3 bc	63.8 ab	62.0 bc	63.6 bc
Thiovit + Ethrel + ATS	61.5 d	62.7 c	61.3 c	63.6 bc
Boric acid + Regalis	63.0 ab	62.9 c	62.4 b	62.6 d
Boric acid + Thiovit	61.9 cd	63.2 bc	61.6 c	63.3 cd
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	0.76	0.76	0.77	0.83
		Chroma		
Control	67.0 ns	67.0 ab	67.1 ns	67.7 a
Boric acid	66.3	67.6 a	66.9	66.8 ab
ColourUp (0.75 mL·L^{-1})	66.9	66.8 abc	65.8	65.7 bc
Thiovit + ATS	66.5	66.4 bc	65.8	66.7 ab
Thiovit + Ethrel + ATS	66.1	65.9 bc	66.1	65.5 c
Boric acid + Regalis	66.1	65.7 c	66.4	66.0 bc
Boric acid + Thiovit	65.3	66.8 abc	66.3	67.3 a
P-value	0.0507	0.0233	0.0881	0.0005
LSD	1.07	1.14	1.09	1.16

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.22. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest and after storage of 'Nules Clementine' mandarin fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of boric acid, ColourUp®, Thiovit® and combinations thereof with ATS, Ethrel® plus ATSand Regalis® on rind pigments.

Treatment	After harvest	After storage	After harvest	After storage
	Eastern		Western	
		Carotenoid (µg·g^{-1} DW)		
Control	639.7 ns[z]	1016.9 b	692.7 ns	1080.7 ns
Boric acid	614.5	876.0 b	603.7	1013.9
ColourUp (0.75 mL·L^{-1})	552.4	943.0 b	668.8	1070.2
Thiovit + ATS	606.4	994.7 b	650.5	1055.9
Thiovit + Ethrel + ATS	667.2	1188.8 a	686.6	1175.3
Boric acid + Regalis	568.5	996.5 b	623.5	1026.5
Boric acid + Thiovit	544.0	971.6 b	579.1	1063.7
P-value	0.2970	0.0075	0.2731	0.4761
LSD	116.12	144.03	108.94	160.49
		Chlorophyll (µg·g^{-1} DW)		
Control	95.5 ns	nd[y]	97.0 ns	nd
Boric acid	54.8	nd	71.8	nd
ColourUp (0.75 mL·L^{-1})	48.0	nd	51.9	nd
Thiovit + ATS	68.3	nd	79.3	nd
Thiovit + Ethrel + ATS	45.8	nd	42.5	nd
Boric acid + Regalis	85.8	nd	75.6	nd
Boric acid + Thiovit	75.9	nd	63.1	nd
P-value	0.0772	nd	0.1527	nd
LSD	37.90	nd	40.60	nd
		Chlorophyll/Carotenoid Ratio		
Control	0.16 ns	nc[x]	0.14 ns	nc
Boric acid	0.09	nc	0.12	nc
ColourUp (0.75 mL·L^{-1})	0.10	nc	0.08	nc
Thiovit + ATS	0.11	nc	0.13	nc
Thiovit + Ethrel + ATS	0.07	nc	0.06	nc
Boric acid + Regalis	0.15	nc	0.12	nc
Boric acid + Thiovit	0.14	nc	0.12	nc
P-value	0.0782	nc	0.2637	nc
LSD	0.07	nc	0.07	nc
		Carotenoid/Chlorophyll Ratio		
Control	10.88 ns	nc	10.27 ns	nc
Boric acid	14.65	nc	9.66	nc
ColourUp (0.75 mL·L^{-1})	13.06	nc	17.32	nc
Thiovit + ATS	13.00	nc	14.09	nc
Thiovit + Ethrel + ATS	16.14	nc	18.16	nc
Boric acid + Regalis	8.66	nc	9.40	nc
Boric acid + Thiovit	9.46	nc	13.97	nc
P-value	0.6492	nc	0.2370	nc
LSD	9.07	nc	8.78	nc

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).
[y] Chlorophylls were not detectable (nd) by spectrophotometry.
[x] Ratios could not be calculated (nc) due to the non detectable chlorophylls.

Table 5.23. Rind colour rating, hue angle, lightness and chroma measurements were made within 48 hours after harvest on the vivid (yellow) and dull (green) sides of 'Nules Clementine' mandarin fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Carotenol®, Figaron® and combinations with boric acid on rind colour.

Treatment	Eastern		Western	
			Colour rating[z]	
Control	4.6 b[y]		4.4 b	
Carotenol	5.2 a		5.0 a	
Figaron	5.0 ab		4.4 b	
Carotenol + Boric acid	4.8 bc		5.0 a	
P-value	0.0011		<0.0001	
LSD	0.32		0.33	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
			Hue angle (°)	
Control	86.4 c	98.0 ns	86.3 b	97.9 ns
Carotenol	101.0 a	99.8	99.2 a	96.5
Figaron	89.1 bc	100.9	86.7 b	98.7
Carotenol + Boric acid	90.8 b	101.1	89.7 b	100.1
P-value	<0.0001	0.2451	<0.0001	0.2621
LSD	3.93	3.48	4.09	3.79
			Lightness	
Control	67.5 a	61.5 ns	67.3 a	63.5 a
Carotenol	61.7 c	59.8	61.5 b	62.4 ab
Figaron	65.0 b	59.6	66.4 a	61.6 ab
Carotenol + Boric acid	66.4 ab	61.0	66.3 a	60.2 b
P-value	<0.0001	0.3462	<0.0001	0.0398
LSD	2.26	2.49	2.03	2.43
			Chroma	
Control	65.9 a	57.2 ns	66.1 a	59.7 ns
Carotenol	55.8 c	54.7	57.1 b	58.5
Figaron	62.8 b	55.3	64.9 a	58.0
Carotenol + Boric acid	63.7 ab	56.7	63.6 a	55.9
P-value	<0.0001	0.2701	<0.0001	0.0559
LSD	2.79	2.92	2.72	2.94

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 36, 2004b).

[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.24. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest of 'Nules Clementine' mandarin fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Carotenol®, Figaron® and combinations with boric acid on rind pigments.

Treatment	Eastern	Western
	Carotenoid (µg·g^{-1} DW)	
Control	433.0 ns[z]	374.3 ns
Carotenol	313.8	370.0
Figaron	366.5	381.7
Carotenol + Boric acid	361.3	340.3
P-value	0.0738	0.9594
LSD	92.27	162.16
	Chlorophyll (µg·g^{-1} DW)	
Control	256.8 ns	273.2 ns
Carotenol	497.7	339.2
Figaron	445.3	282.7
Carotenol + Boric acid	402.8	440.8
P-value	0.0690	0.3273
LSD	191.33	202.26
	Chlorophyll/Carotenoid Ratio	
Control	0.64 b	0.76 ns
Carotenol	1.59 a	1.13
Figaron	1.26 ab	0.80
Carotenol + Boric acid	1.13 ab	1.34
P-value	0.0291	0.2900
LSD	0.63	0.72
	Carotenoid/Chlorophyll Ratio	
Control	1.82 ns	1.69 ns
Carotenol	0.80	2.58
Figaron	0.95	1.40
Carotenol + Boric acid	1.08	0.86
P-value	0.0934	0.6761
LSD	0.89	3.09

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.25. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Navelina Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Thiovit® in combination with ATS and Ethrel plus ATS as well as ColourUp® in combination with Regalis® on rind colour.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	4.4 a[y]		4.8 ns	
Thiovit + ATS	4.1 b		4.6	
Thiovit + Ethrel + ATS	3.8 c		4.6	
ColourUp + Regalis	3.9 bc		4.5	
P-value	<0.0001		0.1361	
LSD	0.22		0.22	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	92.8 a	104.7 a	93.2 ns	105.7 a
Thiovit + ATS	87.1 b	99.3 b	91.5	103.2 b
Thiovit + Ethrel + ATS	83.2 c	92.8 c	91.5	101.0 c
ColourUp + Regalis	85.2 bc	97.1 b	91.3	102.0 bc
P-value	<0.0001	<0.0001	0.4203	0.0001
LSD	2.30	2.29	2.57	2.13
		Lightness		
Control	68.3 b	59.3 c	67.6 ns	59.8 ns
Thiovit + ATS	68.9 ab	61.8 b	67.1	59.6
Thiovit + Ethrel + ATS	67.9 b	64.0 a	65.9	59.1
ColourUp + Regalis	70.2 a	63.5 a	67.4	60.5
P-value	0.0091	<0.0001	0.0889	0.2957
LSD	1.40	1.57	1.49	1.48
		Chroma		
Control	64.1 c	53.9 c	64.6 a	53.9 ns
Thiovit + ATS	66.6 b	56.9 b	63.7 ab	54.1
Thiovit + Ethrel + ATS	67.0 ab	60.6 a	61.9 b	53.3
ColourUp + Regalis	68.7 a	58.5 b	64.7 a	54.8
P-value	<0.0001	<0.0001	0.0425	0.4878
LSD	1.90	2.09	2.15	1.92

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).

[y] Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = non significant).

Table 5.26. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after degreening on the vivid (yellow) and dull (green) sides of 'Navelina Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Thiovit® in combination with ATS and Ethrel plus ATS as well as ColourUp® in combination with Regalis® on rind colour.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	1.9 c[y]		2.2 c	
Thiovit + ATS	2.2 b		2.5 b	
Thiovit + Ethrel + ATS	3.2 a		2.8 a	
ColourUp + Regalis	2.4 b		2.4 b	
P-value	<0.0001		<0.0001	
LSD	0.38		0.16	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	73.7 ns	75.0 bc	75.4 ns	76.1 ns
Thiovit + ATS	72.8	73.9 c	74.8	75.7
Thiovit + Ethrel + ATS	73.1	76.4 a	74.3	76.6
ColourUp + Regalis	74.0	75.8 ab	74.8	76.0
P-value	0.1465	0.0001	0.3189	0.4721
LSD	1.13	1.14	1.09	1.12
		Lightness		
Control	69.4 a	70.1 a	70.2 a	70.5 a
Thiovit + ATS	68.7 a	69.2 b	69.1 b	69.2 bc
Thiovit + Ethrel + ATS	67.5 b	68.1 c	68.2 c	68.8 c
ColourUp + Regalis	69.2 a	70.4 a	67.0 a	69.9 ab
P-value	<0.0001	<0.0001	<0.0001	0.0001
LSD	0.74	0.79	0.69	0.80
		Chroma		
Control	72.7 a	72.5 a	72.7 a	72.2 a
Thiovit + ATS	72.8 a	71.7 a	70.9 b	70.4 c
Thiovit + Ethrel + ATS	70.6 b	69.8 b	70.6 b	70.7 bc
ColourUp + Regalis	72.3 a	71.9 a	72.0 a	71.6 ab
P-value	<0.0001	<0.0001	<0.0001	0.0008
LSD	0.88	0.96	0.92	0.98

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.27. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest and after degreening of 'Navelina Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Thiovit® in combination with ATS and Ethrel plus ATS as well as ColourUp® in combination with Regalis® on rind pigments.

Treatment	After harvest	After degreening	After harvest	After degreening
	Eastern		Western	
	Carotenoid (µg·g^{-1} DW)			
Control	248.0 b[z]	358.6 b	232.3 c	370.5 ns
Thiovit + ATS	263.6 b	407.6 ab	249.8 bc	319.8
Thiovit + Ethrel + ATS	329.3 a	454.3 a	285.3 a	399.7
ColourUp + Regalis	270.3 b	371.5 b	273.1 ab	366.7
P-value	0.0007	0.0193	0.0007	0.0572
LSD	38.45	63.01	24.46	56.57
	Chlorophyll (µg·g^{-1} DW)			
Control	237.4 ns	24.9 ab	237.0 ns	20.5 ns
Thiovit + ATS	191.6	18.4 b	230.8	18.5
Thiovit + Ethrel + ATS	192.1	30.4 a	265.3	24.1
ColourUp + Regalis	213.5	17.2 b	200.5	14.9
P-value	0.5850	0.0498	0.4080	0.2088
LSD	77.32	10.28	76.03	8.65
	Chlorophyll/Carotenoid Ratio			
Control	0.98 ns	0.07 ns	1.03 ns	0.05 ns
Thiovit + ATS	0.73	0.05	0.92	0.06
Thiovit + Ethrel + ATS	0.62	0.07	0.94	0.06
ColourUp + Regalis	0.82	0.05	0.75	0.04
P-value	0.1455	0.1673	0.2833	0.2692
LSD	0.33	0.03	0.31	0.02
	Carotenoid/Chlorophyll Ratio			
Control	1.13 ns	15.27 ns	1.10 ns	20.91 ns
Thiovit + ATS	1.49	22.44	1.14	19.31
Thiovit + Ethrel + ATS	2.20	21.91	1.21	20.30
ColourUp + Regalis	1.52	26.59	1.52	27.27
P-value	0.1059	0.2192	0.3189	0.3012
LSD	0.88	10.80	0.51	9.37

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.28. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Navelina Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Carotenol® and Figaron® on rind colour.

Treatment	Eastern		Western	
	Colour rating[z]			
Control	4.6 b[y]		5.4 a	
Carotenol	5.3 a		4.8 b	
Figaron	4.2 c		4.5 c	
P-value	<0.0001		<0.0001	
LSD	0.22		0.22	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
	Hue angle (°)			
Control	91.0 b	105.5 b	99.1 a	109.1 a
Carotenol	98.7 a	110.4 a	94.0 b	108.0 a
Figaron	87.8 c	100.1 c	90.2 c	102.8 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	2.36	1.95	2.41	1.65
	Lightness			
Control	67.81 a	57.0 b	64.3 b	55.2 b
Carotenol	63.29 b	54.8 c	66.4 a	55.8 b
Figaron	68.60 a	62.5 a	67.9 a	61.0 a
P-value	<0.0001	<0.0001	0.0001	<0.0001
LSD	1.48	1.56	1.69	1.52
	Chroma			
Control	64.5 b	51.3 b	58.6 c	48.8 b
Carotenol	58.8 c	48.6 c	62.5 b	50.1 b
Figaron	68.1 a	58.0 a	66.5 a	55.6 a
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	2.03	1.94	2.04	1.80

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.29. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after degreening on the vivid (yellow) and dull (green) sides of 'Navelina Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Carotenol® and Figaron® on rind colour.

Treatment	Eastern		Western	
	Colour rating[z]			
Control	2.3 b[y]		2.8 a	
Carotenol	2.9 a		2.5 b	
Figaron	1.9 c		2.3 c	
P-value	<0.0001		<0.0001	
LSD	0.16		0.18	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
	Hue angle (°)			
Control	73.4 b	75.5 b	74.8 a	76.4 a
Carotenol	75.6 a	77.4 a	73.4 b	74.8 b
Figaron	70.8 c	72.6 c	73.3 b	74.3 b
P-value	<0.0001	<0.0001	0.0084	0.0010
LSD	1.20	1.18	1.08	1.18
	Lightness			
Control	68.8 a	68.6 ns	68.8 ns	68.3 ns
Carotenol	68.7 a	68.6	68.5	68.7
Figaron	67.3 b	68.4	68.5	68.7
P-value	0.0001	0.8105	0.4832	0.4832
LSD	0.77	0.87	0.69	0.82
	Chroma			
Control	72.2 a	71.5 a	70.9 b	70.2 b
Carotenol	70.5 b	70.0 b	71.8 a	71.7 a
Figaron	72.5 a	72.2 a	72.4 a	71.9 a
P-value	<0.0001	0.0005	0.0033	0.0042
LSD	0.87	1.11	0.90	1.09

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.30. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest and after degreening of 'Navelina Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Carotenol® and Figaron® on rind pigments.

Treatment	After harvest	After degreening	After harvest	After degreening
	Eastern		Western	
	Carotenoid (µg·g^{-1} DW)			
Control	267.0 ns[z]	393.4 b	257.0 ns	321.3 b
Carotenol	250.9	310.8 c	271.3	388.8 a
Figaron	270.3	441.0 a	262.2	371.8 a
P-value	0.1781	<0.0001	0.4615	0.0211
LSD	22.38	43.68	23.72	48.00
	Chlorophyll (µg·g^{-1} DW)			
Control	294.0 b	17.5 ns	365.4 a	21.0 ns
Carotenol	427.4 a	21.0	347.4 a	22.2
Figaron	211.2 c	16.4	233.0 b	23.1
P-value	<0.0001	0.4706	0.0322	0.8243
LSD	73.54	8.02	104.72	7.05
	Chlorophyll/Carotenoid Ratio			
Control	1.11 b	0.05 ab	1.43 ns	0.07 ns
Carotenol	1.71 a	0.07 a	1.28	0.06
Figaron	0.77 c	0.04 b	0.91	0.06
P-value	<0.0001	0.0334	0.0449	0.7150
LSD	0.30	0.03	0.42	0.02
	Carotenoid/Chlorophyll Ratio			
Control	0.99 b	28.11 ns	0.73 b	16.18 ns
Carotenol	0.60 c	18.22	0.83 b	20.03
Figaron	1.40 a	28.76	1.41 a	17.06
P-value	0.0003	0.1077	0.0112	0.5312
LSD	0.34	11.02	0.46	7.07

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.31. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Palmer Navel'orange fruit sampled from the eastern sides of trees during the 2003-04 season to determine the effects of Thiovit® and Regalis® and combinations thereof with Ethrel® on rind colour.

Treatment	Eastern, vivid	Eastern, dull
	Hue angle (°)	
Control	86.1 c[z]	102.3 d
Thiovit	87.0 bc	105.9 b
Regalis + Ethrel	89.3 a	107.6 a
Thiovit + Ethrel	88.1 ab	104.2 c
P-value	0.0085	<0.0001
LSD	1.93	1.70
	Lightness	
Control	72.8 ns	61.1 a
Thiovit	72.7	57.8 bc
Regalis + Ethrel	71.8	56.5 c
Thiovit + Ethrel	71.5	58.3 b
P-value	0.0574	<0.0001
LSD	1.14	1.65
	Chroma	
Control	72.1 a	56.0 a
Thiovit	71.1 ab	51.6 bc
Regalis + Ethrel	68.7 c	49.8 c
Thiovit + Ethrel	70.0 bc	52.7 b
P-value	0.0005	<0.0001
LSD	1.69	2.11

[z] Means within columns followed different letter are significantly different ($P \leq 0.05$; ns = non significant).

Table 5.32. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the eastern sides of trees during the 2004-05 season to determine the effects of ColourUp® at two concentrations on rind colour.

Treatment	3 Weeks before harvest		2 Weeks before harvest		1 Week before harvest	
	Vivid	Dull	Vivid	Dull	Vivid	Dull
			Hue angle (°)			
Control	85.1 ns[z]	102.6 ns	87.6 a	104.7 a	83.0 b	99.9 ns
ColourUp (0.5 mL·L^{-1})	85.9	102.7	85.6 ab	99.7 b	87.7 a	100.9
ColourUp (1.0 mL·L^{-1})	86.7	102.4	83.6 b	101.2 b	83.1 b	99.0
P-value	0.4495	0.9655	0.0068	<0.0001	0.0087	0.3560
LSD	2.51	2.26	2.49	1.86	3.14	2.61
			Lightness			
Control	72.8 ns	59.2 ns	73.1 a	58.5 b	69.2 b	59.6 b
ColourUp (0.5 mL·L^{-1})	72.4	60.8	72.4 a	63.6 a	72.7 a	64.5 a
ColourUp (1.0 mL·L^{-1})	73.8	61.5	68.3 b	58.2 b	70.8 b	61.4 b
P-value	0.1835	0.0550	<0.0001	<0.0001	0.0003	0.0044
LSD	1.56	1.87	1.38	2.18	1.64	2.77
			Chroma			
Control	68.5 ab	49.8 b	67.6 a	48.9 b	65.8 ns	50.9 c
ColourUp (0.5 mL·L^{-1})	67.5 b	50.9 ab	69.2 a	56.4 a	67.5	56.8 a
ColourUp (1.0 mL·L^{-1})	69.3 a	52.8 a	65.6 b	50.4 b	67.0	54.0 b
P-value	0.0315	0.0430	0.0007	<0.0001	0.1049	0.0003
LSD	1.40	2.33	1.79	2.34	1.69	2.81

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.33. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the western sides of trees during the 2004-05 season to determine the effects of ColourUp® at two concentrations on rind colour.

Treatment	3 Weeks before harvest		2 Weeks before harvest		1 Week before harvest	
	Vivid	Dull	Vivid	Dull	Vivid	Dull
			Hue angle (°)			
Control	85.9 ns[z]	103.8 ns	87.6 a	104.9 a	82.0 b	99.6 ns
ColourUp (0.5 mL·L^{-1})	87.1	104.1	87.7 a	99.8 b	85.1 a	101.9
ColourUp (1.0 mL·L^{-1})	88.0	103.4	83.0 b	100.2 b	86.5 a	101.4
P-value	0.2233	0.7392	0.0002	<0.0001	0.0015	0.0900
LSD	2.43	1.71	2.49	2.18	2.61	2.20
			Lightness			
Control	72.8 ns	61.7 ns	73.2 a	59.7 c	68.2 b	60.7 b
ColourUp (0.5 mL·L^{-1})	72.7	60.1	72.8 a	65.1 a	69.9 a	60.9 b
ColourUp (1.0 mL·L^{-1})	73.4	61.8	70.3 b	62.7 b	70.4 a	64.2 a
P-value	0.4403	0.1300	<0.0001	<0.0001	0.0096	0.0049
LSD	1.08	1.92	1.32	2.19	1.56	2.42
			Chroma			
Control	68.2 ns	52.6 a	67.6 ns	50.6 b	64.9 b	51.6 b
ColourUp (0.5 mL·L^{-1})	67.0	49.9 b	68.6	57.3 a	66.0 ab	52.9 b
ColourUp (1.0 mL·L^{-1})	68.4	51.5 ab	67.0	54.9 a	67.5 a	56.8 a
P-value	0.1614	0.0288	0.1138	<0.0001	0.0174	0.0001
LSD	1.56	2.11	1.53	2.70	1.86	2.52

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.34. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the eastern sides of trees during the 2004-05 season to determine the effects of ColourUp® at two concentrations on rind colour.

Treatment	6 Weeks before harvest		5 Weeks before harvest		3 Week before harvest	
	Vivid	Dull	Vivid	Dull	Vivid	Dull
			Hue angle (°)			
Control	78.3 a[z]	86.7 a	74.1 ns	79.8 ns	74.5 ns	82.9 a
ColourUp (0.5 mL·L^{-1})	74.2 b	80.8 b	73.2	79.4	75.2	83.6 a
ColourUp (1.0 mL·L^{-1})	72.5 b	79.0 b	72.9	79.3	73.0	78.2 b
P-value	<0.0001	<0.0001	0.3199	0.9084	0.0522	<0.0001
LSD	1.81	2.56	1.55	2.01	1.83	2.48
			Lightness			
Control	68.4 a	67.1 a	67.2 a	67.7 a	66.3 c	65.7 c
ColourUp (0.5 mL·L^{-1})	67.1 b	66.5 a	62.0 b	62.2 b	72.2 a	71.5 a
ColourUp (1.0 mL·L^{-1})	63.7 c	63.5 b	67.3 a	68.7 a	68.1 b	68.2 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD	0.97	1.54	1.17	1.30	1.44	1.50
			Chroma			
Control	68.3 a	63.6 ns	68.5 a	66.7 a	67.3 c	63.7 b
ColourUp (0.5 mL·L^{-1})	68.2 a	65.3	64.2 b	61.3 b	72.1 a	68.3 a
ColourUp (1.0 mL·L^{-1})	66.1 b	63.1	69.4 a	67.8 a	70.0 b	67.1 a
P-value	<0.0001	0.0793	<0.0001	<0.0001	<0.0001	<0.0001
LSD	0.96	2.00	1.16	1.53	1.29	1.70

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.35. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the western sides of trees during the 2004-05 season to determine the effects of ColourUp® at two concentrations on rind colour.

Treatment	6 Weeks before harvest		5 Weeks before harvest		3 Weeks before harvest	
	Vivid	Dull	Vivid	Dull	Vivid	Dull
			Hue angle (°)			
Control	85.9 ns[z]	103.8 ns	78.7 a	86.7 ns	77.8 ns	85.3 ab
ColourUp (0.5 mL·L^{-1})	87.1	104.1	73.9 b	84.0	79.4	86.8 a
ColourUp (1.0 mL·L^{-1})	88.0	103.4	77.7 a	84.9	76.7	83.0 b
P-value	0.2233	0.7392	<0.0001	0.2087	0.0625	0.0335
LSD	2.43	1.70	2.13	2.97	2.25	2.86
			Lightness			
Control	72.8 ns	61.7 ns	68.7 a	66.8 a	68.3 b	66.9 b
ColourUp (0.5 mL·L^{-1})	72.7	60.1	62.6 b	62.2 b	73.9 a	70.9 a
ColourUp (1.0 mL·L^{-1})	73.4	61.8	68.6 a	66.2 a	68.5 b	64.6 c
P-value	0.4403	0.1300	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.08	1.92	1.14	1.69	1.29	1.96
			Chroma			
Control	68.2 ns	52.6 a	68.6 a	63.9 a	68.0 b	63.6 b
ColourUp (0.5 mL·L^{-1})	67.0	49.9 b	64.1 b	60.3 b	72.3 a	67.3 a
ColourUp (1.0 mL·L^{-1})	68.4	51.5 ab	68.8 a	64.6 a	68.4 b	62.2 b
P-value	0.1614	0.0288	<0.0001	0.0010	<0.0001	0.0001
LSD	1.56	2.11	1.17	2.38	1.22	2.34

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.36. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hour after harvest of the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of ColourUp®, Thiovit® and combinations thereof with ATS, Ethrel® plus ATS and Regalis® on rind colour.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	3.9 b[y]		4.7 bc	
ColourUp (1 mL·L^{-1})	3.9 b		4.6 c	
Thiovit + ATS	4.6 a		5.2 a	
Thiovit + Ethrel + ATS	3.5 c		3.9 d	
ColourUp + Regalis	4.4 a		5.0 ab	
P-value	<0.0001		<0.0001	
LSD	0.34		0.28	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	80.6 a	90.8 a	84.9 bc	95.4 ab
ColourUp (1 mL·L^{-1})	77.2 b	87.1 b	83.4 c	93.8 b
Thiovit + ATS	81.1 a	90.1 a	88.3 a	97.5 a
Thiovit + Ethrel + ATS	73.3 c	80.6 c	78.7 d	87.1 c
ColourUp + Regalis	78.8 b	85.1 b	86.7 ab	94.9 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.76	2.36	2.17	2.35
		Lightness		
Control	69.7 a	64.5 b	68.9 a	62.2 b
ColourUp (1 mL·L^{-1})	69.3 a	66.3 a	69.2 a	63.8 a
Thiovit + ATS	69.2 a	64.4 b	66.6 c	61.0 b
Thiovit + Ethrel + ATS	67.5 b	67.2 a	67.7 bc	64.8 a
ColourUp + Regalis	69.4 a	66.3 a	68.1 ab	61.5 b
P-value	<0.0001	0.0014	<0.0001	<0.0001
LSD	0.90	1.57	1.14	1.52
		Chroma		
Control	71.0 bc	61.9 c	67.6 a	58.0 c
ColourUp (1 mL·L^{-1})	73.2 a	65.1 b	68.7 a	60.1 b
Thiovit + ATS	69.9 c	62.2 c	64.7 b	56.2 c
Thiovit + Ethrel + ATS	71.4 b	67.5 a	69.2 a	62.8 a
ColourUp + Regalis	70.5 bc	65.3 ab	65.5 b	56.9 c
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.31	2.19	1.62	2.03

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.37. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after degreening on the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of ColourUp®, Thiovit® and combinations thereof with ATS, Ethrel® plus ATS and Regalis® on rind colour.

Treatment	Eastern		Western	
			Colour rating[z]	
Control	2.2 b[y]		2.5 bc	
ColourUp (1 mL·L^{-1})	2.1 b		2.7 ab	
Thiovit + ATS	2.6 a		3.0 a	
Thiovit + Ethrel + ATS	1.6 c		2.3 c	
ColourUp + Regalis	2.6 a		2.9 a	
P-value	<0.0001		<0.0001	
LSD	0.28		0.30	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
			Hue angle (°)	
Control	74.1 a	82.5 a	75.9 a	84.6 a
ColourUp (1 mL·L^{-1})	71.9 b	78.5 b	76.7 a	85.1 a
Thiovit + ATS	73.2 a	81.3 a	75.7 a	84.9 a
Thiovit + Ethrel + ATS	68.8 c	74.2 c	73.4 b	79.4 b
ColourUp + Regalis	73.1 ab	79.3 b	77.0 a	84.5 a
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.23	1.87	1.55	2.08
			Lightness	
Control	69.8 a	68.1 ns	70.3 a	66.6 ab
ColourUp (1 mL·L^{-1})	68.7 b	68.9	70.3 a	66.0 bc
Thiovit + ATS	68.7 b	67.5	69.3 b	65.0 c
Thiovit + Ethrel + ATS	66.6 c	68.5	68.2 c	67.6 a
ColourUp + Regalis	69.2 ab	67.13	70.0 a	66.6 ab
P-value	<0.0001	0.0721	<0.0001	0.0135
LSD	0.65	1.34	0.66	1.54
			Chroma	
Control	74.3 ab	68.5 b	73.6 a	65.1 b
ColourUp (1 mL·L^{-1})	74.8 a	70.9 a	72.6 b	64.9 b
Thiovit + ATS	73.8 bc	67.9 b	72.9 ab	64.1 b
Thiovit + Ethrel + ATS	73.3 c	72.6 a	72.3 bc	68.8 a
ColourUp + Regalis	73.4 c	68.6 b	71.7 c	64.3 b
P-value	0.0002	<0.0001	0.0010	<0.0001
LSD	0.74	2.01	0.91	2.19

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.38. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest and after degreening of 'Palmer Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of ColourUp®, Thiovit® and combinations thereof with ATS, Ethrel® plus ATS and Regalis®on rind pigments.

Treatment	After harvest	After degreening	After harvest	After degreening
	Eastern		Western	
	Carotenoid (µg·g^{-1} DW)			
Control	440.7 b[z]	534.7 b	436.8 ab	447.8 ns
ColourUp (1 mL·L^{-1})	411.7 b	535.9 b	408.4 b	414.0
Thiovit + ATS	425.5 b	527.2 b	405.3 b	433.2
Thiovit + Ethrel + ATS	494.5 a	618.8 a	493.2 a	485.3
ColourUp + Regalis	436.8 b	468.1 b	433.8 b	455.3
P-value	0.0062	0.0185	0.0351	0.1498
LSD	43.64	81.57	59.35	58.57
	Chlorophyll (µg·g^{-1} DW)			
Control	163.8 ns	75.9 ns	219.2 ns	52.2 ns
ColourUp (1 mL·L^{-1})	91.6	30.8	193.2	58.6
Thiovit + ATS	139.6	27.7	247.9	51.1
Thiovit + Ethrel + ATS	66.7	31.2	159.7	40.9
ColourUp + Regalis	117.8	38.3	240.0	54.9
P-value	0.0545	0.2219	0.1370	0.8266
LSD	68.55	49.29	75.78	31.65
	Chlorophyll/Carotenoid Ratio			
Control	0.38 ns	0.14 ns	0.51 ns	0.12 ns
ColourUp (1 mL·L^{-1})	0.22	0.06	0.47	0.13
Thiovit + ATS	0.34	0.05	0.62	0.12
Thiovit + Ethrel + ATS	0.14	0.05	0.35	0.09
ColourUp + Regalis	0.27	0.08	0.56	0.14
P-value	0.0507	0.1616	0.0801	0.8371
LSD	0.17	0.09	0.20	0.08
	Carotenoid/Chlorophyll Ratio			
Control	5.91 ab	16.59 ns	2.81 ns	13.04 ns
ColourUp (1 mL·L^{-1})	5.59 ab	24.72	2.43	8.58
Thiovit + ATS	4.25 b	22.74	1.82	16.45
Thiovit + Ethrel + ATS	8.97 a	21.47	5.11	19.38
ColourUp + Regalis	4.25 b	16.03	1.87	10.36
P-value	0.0298	0.4993	0.1877	0.4801
LSD	3.56	12.18	3.03	13.50

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.39. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hour after harvest on the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of Carotenol®, Figaron® and combinations of Carotenol® with Regalis® and boric acid on rind colour.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	4.0 b[y]		4.6 b	
Carotenol	4.8 a		5.1 a	
Figaron	4.1 b		4.7 b	
Carotenol + Regalis	4.6 a		4.8 b	
Carotenol + Boric acid	4.5 a		5.2 a	
P-value	<0.0001		<0.0001	
LSD	0.32		0.27	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	78.2 bc	87.7 b	82.4 c	92.3 b
Carotenol	83.6 a	93.0 a	86.5 a	95.9 a
Figaron	76.7 c	83.3 c	79.8 d	89.2 c
Carotenol + Regalis	80.0 b	87.1 b	83.3 bc	90.7 bc
Carotenol + Boric acid	82.1 a	91.3 a	85.1 ab	96.1 a
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	2.06	2.53	2.34	2.44
		Lightness		
Control	68.9 ns	65.9 b	69.0 a	63.6 ab
Carotenol	69.5	62.9 c	68.4 ab	61.2 c
Figaron	69.6	67.6 a	69.3 a	65.1 a
Carotenol + Regalis	68.4	65.9 b	67.5 bc	62.7 bc
Carotenol + Boric acid	68.8	65.1 b	66.6 c	62.1 bc
P-value	0.1035	<0.0001	<0.0001	<0.0001
LSD	0.96	1.58	1.09	1.68
		Chroma		
Control	72.1 a	65.0 b	70.1 a	61.4 b
Carotenol	69.7 b	61.0 c	67.6 b	57.8 c
Figaron	73.0 a	68.4 a	71.6 a	63.9 a
Carotenol + Regalis	70.5 b	65.5 b	67.3 b	60.6 b
Carotenol + Boric acid	69.3 b	62.7 c	65.4 c	58.3 c
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.45	2.22	1.59	2.23

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.40. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after degreening on the vivid (yellow) and dull (green) sides of 'Palmer Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season season to determine the effects of Carotenol®, Figaron® and combinations of Carotenol® with Regalis® and boric acid on rind colour.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	2.3 b[y]		2.8 b	
Carotenol	3.8 a		4.0 a	
Figaron	2.6 b		2.6 b	
Carotenol + Regalis	3.5 a		4.2 a	
Carotenol + Boric acid	4.0 a		4.1 a	
P-value	<0.0001		<0.0001	
LSD	0.51		0.34	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	72.7 bc	79.1 b	74.3 b	82.9 b
Carotenol	75.8 a	83.6 a	77.1 a	86.6 a
Figaron	71.6 c	76.6 c	72.7 c	78.7 c
Carotenol + Regalis	73.3 b	79.9 b	76.9 a	85.6 a
Carotenol + Boric acid	76.9 a	84.3 a	77.6 a	86.1 a
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.40	1.99	1.48	2.11
		Lightness		
Control	69.0 b	68.8 b	69.6 ns	66.8 b
Carotenol	69.9 a	67.0 cd	70.0	65.9 b
Figaron	68.9 b	70.3 a	69.3	69.3 a
Carotenol + Regalis	68.6 b	68.2 bc	69.7	64.1 c
Carotenol + Boric acid	70.1 a	66.3 d	69.7	66.6 b
P-value	0.0002	<0.0001	0.4350	<0.0001
LSD	0.75	1.35	0.77	1.67
		Chroma		
Control	74.8 ab	71.3 b	74.1 b	67.2 b
Carotenol	74.3 b	67.9 c	73.5 b	64.7 c
Figaron	75.5 a	74.0 a	75.2 a	72.3 a
Carotenol + Regalis	74.2 b	69.7 bc	72.4 c	62.8 c
Carotenol + Boric acid	72.8 c	65.9 d	71.7 c	65.0 bc
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	0.84	1.98	0.90	2.40

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 34, 2004a).
[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.41. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest and after degreening of 'Palmer Navel' orange fruit sampled from the eastern and western sides of trees during the 2005-06 season season to determine the effects of Carotenol®, Figaron® and combinations of Carotenol® with Regalis® and boric acid on rind pigments.

Treatment	After harvest	After degreening	After harvest	After degreening
	Eastern		Western	
	Carotenoid (µg·g^{-1} DW)			
Control	416.6 ns[z]	519.0 ns	418.2 ns	477.9 ab
Carotenol	433.6	476.7	420.7	423.6 bc
Figaron	478.3	536.6	430.6	493.6 a
Carotenol + Boric acid	378.7	453.4	387.6	415.7 c
Carotenol + Regalis	436.0	498.4	405.0	452.6 abc
P-value	0.0831	0.0517	0.4719	0.0417
LSD	68.45	59.20	49.40	58.72
	Chlorophyll (µg·g^{-1} DW)			
Control	102.8 bc	37.0 ns	123.0 ns	44.2 b
Carotenol	183.0 a	43.8	238.6	49.6 b
Figaron	87.6 c	43.3	149.0	30.3 b
Carotenol + Boric acid	163.6 ab	51.3	222.7	97.8 a
Carotenol + Regalis	121.0 abc	52.2	175.2	47.5 b
P-value	0.0383	0.7431	0.0702	0.0250
LSD	67.26	26.48	90.83	34.23
	Chlorophyll/Carotenoid Ratio			
Control	0.25 b	0.07 ns	0.29 b	0.10 b
Carotenol	0.45 a	0.10	0.57 a	0.12 b
Figaron	0.19 b	0.08	0.35 b	0.06 b
Carotenol + Boric acid	0.44 a	0.12	0.57 a	0.23 a
Carotenol + Regalis	0.29 ab	0.10	0.44 ab	0.11 b
P-value	0.0217	0.4249	0.0409	0.0126
LSD	0.18	0.05	0.22	0.08
	Carotenoid/Chlorophyll Ratio			
Control	4.67 ns	15.00 ns	5.39 a	14.14 ns
Carotenol	4.21	12.88	1.99 b	10.46
Figaron	8.07	16.01	3.26 ab	22.38
Carotenol + Boric acid	2.72	14.36	1.75 b	5.47
Carotenol + Regalis	4.11	17.15	3.10 ab	14.90
P-value	0.1831	0.9686	0.0331	0.3289
LSD	4.54	12.30	2.41	16.34

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.42. Hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Eureka'lemon fruit sampled from the eastern and western sides of trees during the 2003-04 season to determine the effects of Thiovit®, ATS, Regalis® and combinations with Ethrel® as well as Regalis® and combinations with ATS on rind colour.

Treatment	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	105.1 a[z]	110.8 ns	104.1 ns	110.5 ns
Thiovit	103.1 b	110.7	103.9	109.6
ATS	102.1 b	109.2	103.1	110.4
ATS + Ethrel	104.7 a	109.4	104.8	109.9
Regalis + Ethrel	105.1 a	109.9	104.9	110.6
Regalis + ATS	104.9 a	109.7	106.9	111.0
P-value	<0.0001	0.1291	0.0512	0.1819
LSD	1.35	1.40	2.45	1.13
		Lightness		
Control	70.0 bc	57.9 b	69.7 b	60.8 ns
Thiovit	71.0 ab	59.8 ab	71.2 a	62.2
ATS	71.9 a	61.3 a	71.6 a	60.6
ATS + Ethrel	69.0 c	61.0 a	69.3 b	61.1
Regalis + Ethrel	69.0 c	59.8 ab	69.3 b	60.9
Regalis + ATS	68.9 c	60.0 a	68.6 b	59.5
P-value	<0.0001	0.0083	0.0002	0.0644
LSD	1.45	1.85	1.48	1.70
		Chroma		
Control	48.7 b	49.0 ns	49.3 bc	49.8 ns
Thiovit	47.4 b	48.9	48.7 bc	50.0
ATS	47.4 b	50.6	48.0 c	50.5
ATS + Ethrel	50.2 a	50.7	50.0 ab	50.3
Regalis + Ethrel	51.4 a	49.9	49.6 ab	50.6
Regalis + ATS	50.9 a	50.5	50.9 a	50.3
P-value	<0.0001	0.0773	0.0015	0.9296
LSD	1.46	1.62	1.41	1.56

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.43. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after harvest on the vivid (yellow) and dull (green) sides of 'Eureka' lemon fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of boric acid and Thiovit® and combinations of Thiovit® with ATS and Ethrel® plus ATS on rind colour.

Treatment	Eastern		Western	
		Colour rating[z]		
Control	4.8 a[y]		4.7 a	
Boric acid	4.3 b		4.0 b	
Thiovit + ATS	4.0 b		4.3 b	
Thiovit + Ethrel + ATS	3.5 c		4.1 b	
P-value	<0.0001		0.0014	
LSD	0.38		0.39	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
		Hue angle (°)		
Control	104.5 a	110.8 a	105.7 a	110.5 a
Boric acid	101.2 b	106.7 b	100.8 b	105.2 b
Thiovit + ATS	101.5 b	106.1 b	101.4 b	105.4 b
Thiovit + Ethrel + ATS	97.5 c	102.4 c	100.3 b	104.2 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.70	1.77	1.96	2.11
		Lightness		
Control	69.7 b	58.8 c	69.6 b	61.1 b
Boric acid	72.4 a	63.9 b	72.1 a	66.3 a
Thiovit + ATS	71.7 a	65.9 b	71.2 a	65.9 a
Thiovit + Ethrel + ATS	72.8 a	69.0 a	72.0 a	67.7 a
P-value	<0.0001	<0.0001	0.0005	<0.0001
LSD	1.12	2.05	1.34	2.26
		Chroma		
Control	51.1 c	49.7 c	51.5 b	50.4 b
Boric acid	53.3 b	51.9 b	53.5 a	52.7 a
Thiovit + ATS	53.7 ab	52.2 b	53.5 a	52.2 a
Thiovit + Ethrel + ATS	54.8 a	53.4 a	53.5 a	52.3 a
P-value	<0.0001	<0.0001	0.0028	0.0014
LSD	1.37	1.19	1.26	1.24

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 37, 2004c).

[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.44. Rind colour rating, hue angle, lightness and chroma measurements were made within 24 hours after degreening on the vivid (yellow) and dull (green) sides of 'Eureka' lemon fruit sampled from the eastern and western sides of trees during the 2005-06 season to determine the effects of boric acid and Thiovit® and combinations of Thiovit® with ATS and Ethrel® plus ATS on rind colour.

Treatment	Eastern		Western	
			Colour rating[z]	
Control	4.0 a[y]		3.4 a	
Boric acid	3.1 b		3.0 b	
Thiovit + ATS	2.9 b		3.1 b	
Thiovit + Ethrel + ATS	2.4 c		2.5 c	
P-value	<0.0001		<0.0001	
LSD	0.39		0.32	
	Eastern, vivid	Eastern, dull	Western, vivid	Western, dull
			Hue angle (°)	
Control	95.9 a	97.4 a	96.1 a	97.2 a
Boric acid	95.2 a	95.6 b	94.9 b	94.9 b
Thiovit + ATS	93.4 b	94.4 c	94.3 bc	94.6 b
Thiovit + Ethrel + ATS	93.8 b	94.3 c	94.0 c	94.9 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001
LSD	0.83	1.12	0.90	1.03
			Lightness	
Control	75.2 ns	70.2 b	75.6 ab	73.7 ns
Boric acid	75.6	73.9 a	76.0 a	74.4
Thiovit + ATS	75.2	73.0 a	75.2 bc	73.9
Thiovit + Ethrel + ATS	74.7	73.0 a	74.8 c	73.9
P-value	0.0723	<0.0001	0.0055	0.5685
LSD	0.70	1.26	0.70	1.05
			Chroma	
Control	50.9 c	54.0 c	52.2 ns	52.70 b
Boric acid	53.3 b	54.5 bc	52.9	54.7 a
Thiovit + ATS	56.3 a	56.7 a	53.3	54.9 a
Thiovit + Ethrel + ATS	54.8 ab	55.5 ab	54.4	54.8 a
P-value	<0.0001	0.0001	0.0819	0.0060
LSD	1.55	1.21	1.75	1.43

[z] Rind colour rating where 1= fully coloured fruit and 8= totally dark green fruit (CRI colour chart, set no. 37, 2004c).

[y] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

Table 5.45. Carotenoid concentration, chlorophyll concentration, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio analysis were made with a spectrophotometer after harvest and after degreening of 'Eureka' lemon fruit sampled from the eastern and western sides of trees during the 2005-06 season season to determine the effects of boric acid and Thiovit® and combinations of Thiovit® with ATS and Ethrel® plus ATS on rind pigments.

Treatment	After harvest	After degreening	After harvest	After degreening
	Eastern		Western	
	Carotenoid (µg·g^{-1} DW)			
Control	136.9 ns[z]	69.0 ns	120.8 ns	64.0 b
Boric acid	131.7	72.9	113.2	71.8 ab
Thiovit + ATS	123.5	83.5	122.5	74.5 ab
Thiovit + Ethrel + ATS	119.0	77.8	109.9	82.3 a
P-value	0.0558	0.1246	0.2718	0.0488
LSD	13.57	12.27	14.95	12.85
	Chlorophyll (µg·g^{-1} DW)			
Control	384.8 a	58.4 ns	344.4 a	51.6 ns
Boric acid	276.4 b	34.4	221.3 bc	44.3
Thiovit + ATS	205.9 c	28.1	275.1 ab	36.0
Thiovit + Ethrel + ATS	141.2 c	57.9	187.9 c	41.6
P-value	<0.0001	0.0741	0.0004	0.2444
LSD	66.64	28.41	69.78	15.76
	Chlorophyll/Carotenoid Ratio			
Control	2.78 a	0.86 ns	2.81 a	0.81 ns
Boric acid	2.10 b	0.52	1.99 b	0.64
Thiovit + ATS	1.66 c	0.34	2.25 b	0.49
Thiovit + Ethrel + ATS	1.19 d	0.80	1.73 b	0.54
P-value	<0.0001	0.0662	0.0022	0.0676
LSD	0.38	0.43	0.54	0.26
	Carotenoid/Chlorophyll Ratio			
Control	0.37 c	1.43 ns	0.38 c	1.35 b
Boric acid	0.49 c	2.89	0.56 ab	1.82 ab
Thiovit + ATS	0.63 b	4.50	0.45 bc	2.16 a
Thiovit + Ethrel + ATS	0.87 a	2.30	0.62 a	2.17 a
P-value	<0.0001	0.0588	0.0171	0.0365
LSD	0.12	2.20	0.16	0.63

[z] Means within columns followed by a different letter are significantly different (P≤0.05; ns = non significant).

CHAPTER 6

OVERALL DISCUSSION AND CONCLUSIONS

To enhance the cosmetic quality of citrus (*Citrus* spp.) fruit, attempts were made to stimulate preharvest chlorophyll degradation and carotenoid biosynthesis to obtain a deeper, more uniform, orange rind colour in early-maturing citrus cultivars. As part of a larger study to stimulate rind colour enhancement, an initial study was conducted on 'Eureka' lemon [*C. limon* (L.) Burm. f.] nursery trees to determine the concentration of various gibberellin biosynthesis inhibitors required to get a biological response in citrus trees, as measured by vegetative growth. Thereafter, different concentrations of prohexadione-calcium (ProCa; Regalis®) were applied at various stages of fruit development on early-maturing citrus cultivars to establish the concentration and timing of ProCa required to improve rind colour by enhancing chlorophyll degradation and carotenoid biosynthesis. In addition, a search to enhance rind colour development of early-maturing citrus cultivars was conducted by screening various nutritional, hormonal and possible physiological stress-inducer products and some combination treatments thereof.

Multiple applications of gibberellic acid biosynthesis inhibitors on 'Eureka' lemon nursery trees significantly reduced vegetative growth supporting the results of earlier research on vegetative growth retardation of *Citrus* spp. by Aron et al. (1985), Stover et al. (2004) and Wheaton (1989). Regalis® (prohexadione-calcium) applied at 4 to 8 $g \cdot L^{-1}$ and Sunny® (uniconazole) applied at 10 to 20 $mL \cdot L^{-1}$ reduced internode length and hence shoot growth, and therefore were identified as possible candidates for further field studies to test their effect on rind colour enhancement of citrus fruit.

The late, double applications (6 plus 3 weeks before anticipated harvest) of ProCa applied at 400 mg·L^{-1} consistently improved rind colour of all *Citrus* spp. tested. However, these effects were more pronounced after harvest, as ethylene degreening and cold-storage stimulated additional chlorophyll degradation, unmasking the carotenoids, resulting in overall better coloured fruit (El-Zeftawi, 1978; Goldschmidt, 1988; Van Wyk, 2004). In most instances in this study, ProCa stimulated chlorophyll degradation allowing the underlying carotenoids to be expressed. Prohexadione calcium has been shown to reduce vegetative growth in *Citrus* spp. (Stover et al., 2004; Chapter 3). Therefore, the improvement of rind colour of citrus fruit following the application of a gibberellin biosynthesis inhibitor (400 mg·L^{-1} ProCa applied 6 plus 3 weeks before harvest) supports the hypothesis that there may be a relationship between vegetative vigour and rind colour development of citrus fruit, although vegetative vigour was not measured.

Boric acid stimulated the degradation of chlorophyll in orange- and yellow-rinded fruit, e.g. ‘Miho Wase Satsuma’ mandarin and ‘Eureka’ lemon, by ~ 30% and ~ 40%, respectively, and stimulated carotenoid biosynthesis in orange rinded-fruit (by ~ 24%). Thiovit® applied twice (6 plus 3 weeks before anticipated harvest) aided in the degradation of chlorophyll and stimulated carotenoid biosynthesis when applied in combination with ATS and Ethrel® on both orange- and yellow-rinded fruit. ColourUp® applied 3 weeks before anticipated harvest stimulated the degradation of chlorophyll in ‘Nules Clementine’ mandarin fruit, thereby aiding in chloro-chromoplast transformation. Carotenol® did not improve rind colour of fruit of all citrus cultivars tested whether it was applied alone or in combination with other chemical products. Figaron® stimulated chloro-chromoplast transformation possibly by stimulating ethylene biosynthesis (Cooper and Henry, 1968), and thereby stimulating the degradation of chlorophyll (by ~ 32%) in ‘Navelina Navel’ orange fruit, but did not affect

carotenoid biosynthesis. Regalis® applied in combination with ColourUp® or Ethrel® did not add to the positive effect the latter two products had on improving rind colour of citrus fruit when these products were applied alone. ATS aided in the degradation of chlorophyll and biosynthesis of carotenoids especially when applied in combination with Thiovit® plus Ethrel®. Ethrel®, applied at half the recommended rate, in combination with Thiovit® plus ATS stimulated chlorophyll degradation (by ~ 40%) in both orange- and yellow-rinded fruit and stimulated carotenoid biosynthesis (by ~ 20%) in orange-rinded fruit. The screening of chemical products which stimulated carotenoid biosynthesis (e.g. Thiovit® plus ATS plus Ethrel®) in orange-rinded fruit in combination with products which stimulated chlorophyll degradation (e.g. boric acid, ColourUp® and Figaron®) warrants further evaluation.

Worldwide, research on rind colour improvement has received attention for several decades, particularly during the 1980s. Yet, rind colour still remains a problem at the beginning of certain seasons. In the present study, the approach to improving rind colour was to manipulate rind pigments through the reduction of vegetative vigour, which was hypothesised to be an antagonist of chloro-chromoplast transformation. To this end, the preharvest application of prohexadione-calcium stimulated chlorophyll degradation and carotenoid biosynthesis in citrus fruit rinds. Furthermore, preharvest applications of various chemical products provides a novel approach to stimulate chlorophyll degradation and carotenoid biosynthesis. Together, the results of this study provide potential commercial treatments that will result in deeper, more uniform orange rind colour, thereby meeting consumer needs.

LIST OF REFERENCES

Afitlhile, M.M., R.M. Dent and A.K. Cowan, 1993. Changes in carotenoid composition in senescing leaves of *Hordeum vulgare* L. cv. Dyan. J. Plant Physiol. 142:43-49.

Apelbaum, A., E.E. Goldschmidt and S. Ben-Yehoshua, 1976. Involvement of endogenous ethylene in the induction of colour changes in 'Shamouti' orange. Plant Physiol. 57:836-838.

Aron, Y., S.P. Monselise, R. Goren and J. Costo, 1985. Chemical control of vegetative growth in citrus trees by paclobutrazol. HortScience 20(1):96-98.

Aung, L.H., L.G. Houck and S.M. Norman, 1991. The abscisic acid content of citrus with special reference to lemon. J. Exp. Bot. 42:1083-1088.

Bain, J.M., 1958. Morphological, anatomical and physiological changes in the developing fruit of the 'Valencia' orange, *Citrus sinensis* (L.) Osbeck. Austr. J. Bot. 6:1-24.

Baldwin, E.A., 1993. Citrus fruit. *In* G.B. Seymore, J.E. Taylor and G.A. Tucker (eds.), *Biochemistry of Fruit Ripening*. Chapman and Hall, New York.

Barry, G.H., 2005. Effects of ColourUp® on rind colour of citrus fruit. Research report, CRI, Stellenbosch, South Africa.

Barry, G.H. and A.A. Van Wyk, 2004. Novel approaches to rind colour enhancement of citrus. Proc. Int. Soc. Citricult. (In press.).

Boswell, S.B., E.M. Nauer and D.R. Atkin, 1982. Effect of tree density on fruit quality, temperature, light penetration, growth, and production of old-line 'Atwood Navel' orange trees. J. Amer. Soc. Hort. Sci. 107(1):60-65.

Boyer, C.D., 1989. Genetic control of chromoplast formation during fruit development of *Cucurbita pepo* L. p. 241-252. In: C.D. Boyer, J.C. Shannon and R.C. Hardison

(Eds.). Physiology, Biochemistry and genetics of nongreen plastids, Vol. 2, Current Topicks in Plant Physiology, Amer. Soc. Plant Physiol.

Bramley, P.M., C.R. Bird and W. Schuch, 1993. Carotenoid biosynthesis and manipulation, 139-175. In: D. Grierson (ed). Plant biotechnology Vol. 3, Biosynthesis and manipulation of plant products. Blackie Academic and Proffesional, Glasgow.

Brisker, H.E., E.E. Goldschmidt and R. Goren, 1976. Ethylene-induced formation of ABA in citrus peel as related to chloroplast transformations. Plant Physiol. 58:377-379.

Britton, G., 1985. General carotenoid methods. Methods Enzymol. 111:113.

Camara, B., 1984. Terpenoid metabolism in plastids: sites of phytoene synthase activity and synthesis in plant cells. Plant Physiol. 74:112.

Caprio, J.M., 1956. An analysis of the relation between regreening of 'Valencia' oranges and mean monthly temperatures in Southern California. Proc. Amer. Soc. Hort. Sci. 67:222-235.

Chapman, H.D., 1968. The mineral nutrition of citrus, 127-189. In: W. Reuther, L.D. Batchelor, H.J. Webber (eds.). The Citrus Industry, Vol. II. Berkely: Division of agricultural science, University of California.

Chayet, L., R. Pont-Lezica, C. George-Nascimento and O. Cori, 1973. Biosynthesis of sesquiterpene alcohols and aldehydes by cell free extracts from orange flavedo. Phytochemistry 12:95-101.

Clarke, I.E., G. Sandmann, P.M. Bramley, and P. Boger, 1982. Carotene biosynthesis with isolated photosynthetic membranes. FEBS Lett. 140:203.

Coggins, C.W., Jr., 1981. The influence of exogenous growth regulators on rind quality and internal quality of citrus fruit. Proc. Int. Soc. Citricult. 1:214-216.

Coggins, C.W., Jr. and G.L. Henning, 1988. A comprehensive California field study of the influence of preharvest applications of gibberellic acid on the rind quality of Valencia orange. Israel J. Bot. 37:145-154.

Coggins, C.W., Jr. and H.Z. Hield, 1962. Navel orange response to potassium gibberellate. Proc. Amer. Soc. Hort. Sci. 81:227-230.

Coggins, C.W., Jr. and L.N. Lewis, 1962. Regreening of valencia orange as influenced by potassium gibberellate. Plant Physiol. 37(5):625-627.

Coggins, C.W., Jr., A.E. Hall and W.W. Jones, 1981. The influence of temperature on regreening and carotenoid content of the 'Valencia' orange rind. J. Amer. Soc. Hort. Sci. 106(2):251-254.

Coggins, C.W., Jr., G.L. Henning and H. Yokoyama, 1970. Lycopene accumulation induced by 2-(4-chlorophenylthio)-triethylamine hydrochloride. Science 168:1589-1590.

Collado, M., J.M. Sala and P. Inarrea, 1996. Influence of nitrogenous fertilization on the protein content of flavedo of 'Navelina' orange and its relationship with rind colour. J. Hort. Sci. 71(6):971-976.

Cooper, W.C. and W.H. Henry, 1968. Effects of growth regulators on the colouring and abscission of citrus fruit. Israel. J. Agric. Res. 18(4):161-174.

Costa, G., C. Andreotti, F. Bucchi, E. Sabatini, C. Bazzi and S. Malaguti, 2001. Prohexadione-Ca (Apogee®): Growth regulation and reduced fire blight incidence in pear. HortScience 36(5):931-933.

Cowan, A.K. and G.R. Richardson, 1993. 1',4'*trans*-[^{14}C]-abscisic acid diol: major product of R-[2-^{14}C]-mevalonic acid metabolism in extracts of *Citrus sinensis* exocarp. J. Plant Physiol. 142:730-734.

CRI, 1995. Rootstock choice. Production guidelines for export citrus. Vol. 1., Ch 6. Citrus Research International, Nelspruit, South Africa.

CRI, 2004a. Colour-oranges, Set No. 34. Colour prints for blemish standards. Citrus Research International, Nelspruit, South Africa.

CRI, 2004b. Colour-soft citrus, Set No. 36. Colour prints for blemish standards. Citrus Research International, Nelspruit, South Africa.

CRI, 2004c. Colour-lemons, Set No. 37. Colour prints for blemish standards. Citrus Research International, Nelspruit, South Africa.

De Vries, P.J. and R. Bester, 1996. Verwantskap tussen rypheid van Midnight Valencias en vrugposisie op die boom. (Afrikaans) Citrus J. 6(3):26-27.

Dogbo, O., A. Laferriere, A. D'Harlingue and B. Camara, 1988. Carotenoid biosynthesis: isolation and characterisation of a bifunctional enzyme catalysing the synthesis of phytoene. Proc. Natl. Acad. Sci. USA 85:7054.

Dogbo, O., J. Bardat, A. Laferriere, J. Quennemet, J. Brangeon and B. Camara, 1987. Metabolism of terpanoids I. Biosynthesis of phytoene in plastid stroma isolated from higher plants. Plant Sci. 49:89.

Eilati, S.K., E.E. Goldschmidt and S.P. Monselise, 1969a. Hormonal control of colour changes in orange peel. Experientia 25:209-210.

Eilati, S.K., S.P. Monselise and P. Budowski, 1969b. Seasonal development of external colour and carotenoid content in the peel of ripening 'Shamouti' oranges. J. Amer. Soc. Hort. Sci. 52:127-134.

Embleton, T.W., W.W. Jones and C.W. Coggins, Jr., 1973. Aggregate effects of nutrients and gibberellic acid on Valencia orange crop value. J. Amer. Soc. Hort. Sci. 98(3):281-285.

El-Otmani, M., A. Tadili and A. Ait-Oubahou, 1996. Possibilities and limitations of using ethephon to promote colour development of 'Clementine' fruits. Proc. Int. Soc. Citricult. 2:1072-1075.

El-Zeftawi, B.M., 1978. Chemical and temperature control of rind pigment of citrus fruits. Proc. Int. Soc. Citricult. 1:33-36.

El-Zeftawi, B.M. and R.G. Garrett, 1978. Effects of ethephon, GA and light exclusion on rind pigments, plastid ultrastructure and juice quality of Valencia oranges. J. Hort. Sci. 53(3):215-223.

Esau, K.,1965. Plant Anatomy. John Wiley and Sons, Inc., New York.

Evans, J.R., R.R. Evans, C.L. Regusci and W. Rademacher, 1999. Mode of action, metabolism, and uptake of BAS 125W, prohexadione-calcium. HortScience 34(7):1200-1201.

Farin, D., R. Ikan, and J. Gross, 1983. The carotenoid pigments in juice and flavedo of mandarin hybrid (*Citrus reticulata)* cv 'Michal' during ripening. Phytochemistry 22:403-408.

Ferguson, L., T.A. Wheaton, F.S. Davies and M.A. Ismail, 1986. ^{14}C-gibberellic acid uptake, translocation, persistence, and metabolism in grapefruit. J. Amer. Soc. Hort. Sci. 111(6):926-932.

Fishwick, M.J. and A.J. Wright, 1980. Isolation and characterization of amyloplast envelope membranes from *Solanum tuberosum.* Phytochemistery 19:55-59.

Francis, F.J., 1980. Colour quality evaluation of horticultural crops. HortScience 15(1):58-59.

Frey-Wyssling, A. and F. Schwegler, 1965. Ultrastructure of the chromoplasts in the carrot root. J. Ultrastruct. Res. 13:543-559.

Fucik, J.E. and D. Swietlik, 1990. Anti-gibberellins' effect on grapefruit size quality. Proc. Plant Growth Reg. Soc. Amer. 17:107-113.

Garcia-Luis, A., F. Fornes and J.L. Guardiola, 1986. Effects of gibberellin A_3 and cytokinins on natural and post-harvest, ethylene-induced pigmentation of 'Satsuma Mandarin' peel. Physiol. Plantarum 68:271-274.

Garcia-Luis, A., A. Herrero-Villen and J.L. Guardiola, 1992. Effects of applications of gibberellic acid on late growth, maturation and pigmentation of the Clementine mandarin. Scientia Hort. 49:71-82.

Garcia-Luis, A., M. Agusti, V. Almela, E. Romero and J.L. Guardiola, 1985. Effects of gibberellic acid on ripening and peel puffing in Satsuma mandarin. Scientia Hort. 27:75-86.

Gilfillan, I.M., J.K.A. Stevenson and W. Koekemoer, 1974. Gibberellic acid reduces creasing in late-season navels. Citrus Subtrop. Fruit J. 482:4-5.

Gilfillan, I.M. and S.J. Lowe, 1985. Fruit colour improvement in Satsumas with paclobutrazol and ethephon – preliminary studies. Citrus J. 4-8.

Giuliano, G., G.E. Bartley and P.A. Scolnik, 1993. Regulation of carotenoid biosynthesis during tomato development. Plant Cell 5:379-387.

Goldschmidt, E.E., 1988. Regulatory aspects of chloro-chromoplast interconvensions in senescing *Citrus* fruit peel. Isr. J. Bot. 47:123-130.

Goldschmidt, E.E., M. Huberman and R. Goren, 1993. Probing the role of endogenous ethylene in the degreening of citrus fruit with ethylene antagonists. Plant Growth Regulat. 12(3):325-329.

Goldschmitdt, E.E., R. Goren, Z. Even-Chen and S. Bittner, 1973. Increase in free and bound abscisic acid during natural and ethylene-induced senescence of citrus fruit peel. Plant Physiol. 51:879-882.

Gonzalez, C.M. and C.J. Lovatt, 2004. Foliar-applied Aminoethoxyvinylglycine (AVG) reduces albedo breakdown of late-harvested Navel orange fruit – *preliminary results*. Proc. Int. Soc. Citricult. (In press).

Goodwin, T.W., 1958. Studies of carotenogenesis. Biochem. J. 68:503-511.

Goodwin, T.W., 1973. Carotenoids, p. 112-142. In: L.P. Miller (ed.). Phytochemistry Vol. I. Nostrand Reinhold, New York.

Goodwin, T.W., 1980. The Biochemistry of the Carotenoids. 2nd ed. Chapman and Hall, London.

Goodwin, T.W. and G. Britton, 1988. Distribution and analysis of carotenoids, p. 61-131. In: T.W. Goodwin (ed.). Plant Pigments. Academic Press, London. (Cited by Bramley, P.M., C.R. Bird and W. Schuch, 1993).

Goulston, G.H. and S.J. Shearing, 1985. Review of the effects of paclobutrazol on ornamental pot plants. Acta Hort. 167:339-348.

Graham, C.J. and J.B. Storey, 2000. Method of application of Uniconazol affects vegetative growth of pecan. HortScience 35:1199-1201.

Gross, J., R. Trimberg and M. Greaf, 1983. Pigment and ultrastructural changes in the developing pummelo. *Citrus grandis* Goliath. Bot. Gaz. 144:401-406.

Harberlandt, G., 1965. *Physiological Plant Anatomy.* Macmillan and Co. Limited, London.

Harris, M.J. and W.M. Dugger, 1986. The occurrence of abscisic acid and abscisyl-β-D-glucopyranoside in developing and mature citrus fruit as determined by enzyme immunoassay. Plant Physiol. 82:339-345.

Huff, A., 1983. Nutritional control of regreening and degreening in citrus peel segments. Plant Physiol. 73:243-249.

Huff, A., 1984. Sugar regulation of plastid interconversions in epicarp of citrus fruit. Plant Physiol. 76:307-312.

Huff, A., M.Z. Abdel-Bar, D.R. Rodney, R.L. Roth and B.R. Gardner, 1981. Enhancement of citrus regreening and peel lycopene by trickle irrigation. HortScience 16(3):301-302.

Hunt, R.W.G., 1977. The specification of colour appearance. I. Concept and terms. Colour Res. Appl. 2(2):55-68.

Iglesias, D.J., F.R. Tadeo, F. Legaz, E. Primo-Millo and M. Talon, 2001. In vivo sucrose stimulation of colour change in citrus fruit epicarps: Interactions between nutritional and hormonal signals. Physiol. Plant. 112:244-250.

Ihl, M., C. Shene, E. Scheuermann and V. Bifani, 1994. Correlation for pigment content through colour determination using tristimulus values in a green leafy vegetable, swiss chard. J. Sci. Food Agric. 66:527-531.

Ilias, I.F. and N. Rajapakse, 2005. Prohexadione-calcium affects growth and flowering of petunia and impatiens growth under photoselective films. Scientia Hort. 106:190-202.

In-Jung, K., K. Kyong-Cheol, K. Chan-Shick and C. Won-Il, 2001. Isolation and characterization of cDNAs encoding β-carotene hydroxylase in citrus. Plant Sci. 161:1005-1010.

Iwagaki, I. and K. Kudo, 1977. Fruit quality relation to the location of fruit in a canopy of 'Satsuma' mandarin tree. Bull. Shikoku Agric. Exp. Stn. 30:17-23 (abstr).

Iwahori, S., S. Tominaga and J.T. Oohata, 1986. Ethychlozate accelerates colouration and enhances fruit quality of 'Ponkan', *Citrus reticulata* Blanco. Scientia Hort. 28:243-250.

Jacob-Wilk, D., D. Holland, E.E. Goldschmidt, J. Riov and Y. Eyal, 1999. Chlorophyll breakdown by chlorophyllase: isolation and functional expression of the *Chlase1* gene from ethylene treated citrus fruit and its regulation during development. Plant J. 20(6):653-661.

Jahn, O.L. and R. Young, 1972. Influence of the tree on the response of citrus fruit to preharvest applications of (2-chloroethyl)phosphonic acid. J. Amer. Soc. Hort. Sci. 97: 544-549.

Jimenez-Cuesta, M, J. Cuquerella and J.M. Martinez-Javaga, 1981. Determination of a colour index for citrus fruit degreening. Proc. Int. Soc. Citricult. 2:750-753.

Jones, O.T.G., 1973. Chlorophyll, p. 75-111. In: L.P. Miller (ed.) Phytochemistry. NoStrand Reinhold, New York.

Kamuro, Y. and K. Hirai, 1981. Physiological activity of Ethyclozate – fruit thinning and maturity accelerating effects for citrus. Proc. Int. Soc. Citricult. 1:260-263.

Kleinig, H., 1989. The role of plastids in isoprenoid biosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:39.

Koo, R.C.J., 1988. Fertilization and irrigation effects on fruit quality, p. 35-42. In: J.J. Ferguson and W.F. Wardowski (eds.). Factors affecting fruit quality, Citrus Short Course Proc.

Koo, R.C.J. and R.L. Reese, 1977. Influence of nitrogen, potassium, and irrigation on citrus fruit quality. Proc. Int. Soc. Citricult. 1:34-38.

Krajewski, A., 1997. Guidelines for the improvement of fruit colour in citrus. L.A. von Broembsen (ed.) Outspan International, p 1-25.

Krajewski, A.J. and T.M. Pittaway, 2002. Common defects associated with degreening of citrus. In: G.H. Barry (ed.) Citrus Research International, p 4-18.

Kubo, T. and S. Hiratsuka, 1999. Historical study on rind roughness of Satsuma Mandarin fruit. J. Jpn. Soc. Hort. Sci. 68:101-107.

Lancaster, J.E., C.E. Lister, P.F. Reay and C.M. Triggs, 1997. Influence of pigment composition on skin colour in a wide range of fruit and vegetables. J. Amer. Soc. Hort. Sci. 122(4):594-598.

Lee, H.S. and W.S. Castle, 2001. Seasonal changes of carotenoid pigments and colour in ‘Hamlin’, ‘Earlygold’ and ‘Budd blood’ orange juices. J. Agric. Food Chem. 49:877-882.

Lee, I.J., K.R. Foster and P.W. Morgan, 1998. Effect of gibberellin biosynthesis inhibitors on native gibberellin content, growth and floral initiation in sorghum bicolour. J. Plant Growth Regul. 17: 185-195.

Le Roux, C., 1997. Fruit physiology changes during ripening and postharvest handeling of Mandarins. Univ. Stellenbosch, Stellenbosch, South Africa, MScAgric Thesis.

Lewis, L.N. and C.W. Coggins Jr., 1964. The inhibition of carotenoid accumulation in navel oranges by gibberellin A_3, as measured by thin layer chromatography. Plant and Cell Physiol. 5:457-463.

Lewis, L.N., C.W. Coggins, Jr. and M.J. Garber, 1964. Chlorophyll concentration in Navel orange rind as related to potassium gibberellate, light intensity, and time. Proc. Amer. Soc. Hort. Sci. 84:177-180.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology. 148:350-382.

Lichtenthaler, H.K., U. Prenzel and G. Kuhn, 1982. Carotenoid composition of chlorophyll-carotenoids proteins from radish cotyledons. Z. Naturforsch. 37C:10. (Cited by Bramley, P.M., C.R. Bird and W. Schuch, 1993).

Lida Quimica, 2006. Augmentation de l' indice de maturité en clementines extra précoces moyennant l' application foliaire du prodiut. Département de Développement, Agrotechnologique.

Lima, J.E.O. and F.S. Davies, 1984. Secondary-fruit ontogeny in navel orange. Amer. J. Bot. 71:532-541.

Little, A.C., 1976. Physical measurements as predictors of visual appearance. Food Technol. 74-82.

Lois, L.M., M. Rodriguez-Concepcion, F. Gallego, N. Campos and A. Boronat, 2000. Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. Plant J. 22:503-513.

Maritz, C., 2000. USA export manual for citrus supplier. Capespan (Pty) Ltd., South Africa.

Matile, P., S. Hortensteiner, H. Thomas and B. Krautler, 1996. Chlorophyll breakdown in senescent leaves. Plant Physiol. 112:1403-1409.

Mauseth, J.D., 1988. The cell, p. 13-39. In: A. Crowley and R. Williams (eds.). Plant Anatomy. Benjamin/Cummings Publishing Company, Inc., Menlo Park, California, USA.

Mauzerall, D., 1977. Porphyrins, chlorophyll and photosynthesis, p. 117-124. In: A. Trebst and M. Avron (eds.). Encyclopedia of Plant Physiology Vol. 5, Photosynthesis I. Springer-Verlag, Berlin.

Mayfield, S.P. and A. Huff, 1989. Accumulation of chlorophyll, chloroplastic proteins in thylakoid membranes during reversion of chromoplasts to chloroplasts in *Citrus sinensis* epicarp. Plant Physiol. 81:30-35.

Meredith, F.I. and R.H. Young, 1969. Effect of temperature on pigment development in 'Red blush' grapefruit and 'Ruby blood' oranges. Proc. Intl. Citrus Symp. 1:271-276.

Milborrow, B.V., 1974. Biosynthesis of abscisic acid by a cell-free system. Phytochemistry 13:131-136.

Miller, E.V., J.R. Winston and H.A. Schomer, 1940. Physiological studies of plastid pigments in rinds of maturing oranges. J. Agr. Res. 60:259-267.

Miller, S.S., 2002. Prohexadione-calcium control vegetative shoot growth in apple. J. Tree Fruit Production 3(1):11-28.

Mitcham, E.J. and R.E. McDonald, 1993. Changes in grapefruit flavedo cell wall noncellulosic neutral sugar composition. Phytochemistry 34(5):1235-1239.

Molnar, P. and J. Szabolcs, 1980. *β*-citraurin epoxide, a new carotenoid from 'Valencia' orange peel. Phytochemistry 19:633-637.

Morales, P. and F.S. Davies, 2000. Pruning and skirting affect canopy microclimate, yield, and fruit quality of 'Orlando' tangelo. HortScience 35(1):30-35.

Mudzunga, M.J., 2000. Enhancement of vegetative growth in young citrus plantings. Univ. Stellenbosch, Stellenbosch, South Africa, MScAgric Thesis.

Nakayama, I., M. Kobayashi, Y. Kamiya, H. Abe and A. Sakurai, 1992. Effects of plant-growth regulator, prohexadione-calcium (BX-112), on the endogenous levels of gibberellins in rice. Plant Cell Physiol. 33(1): 59-62.

Norman, S.M., 1991. β-Carotene from the abscisic acid producing strain of *Cercospora rosicola.* Plant Growth Regul. 10:103-108.

Norman, S.M., V.P. Maier and D.L. Pon, 1990. Abscisic acid accumulation and carotenoid and chlorophyll content in relation to water stress and leaf age of different types of citrus. J. Agric. Food Chem. 38:1326-1334.

Oberholster, R., A.K. Cowan, P. Molnar and G. Toth, 2001. Biochemical basis of colour as an aesthetic quality in *Citrus sinensis*. J. Agric. Food Chem. 49:303-307.

Parry, A.D., 1993. Abscisic acid metabolism. Methods Plant Biochem. 9:381-402.

Parry, A.D. and R. Horgan, 1991. Carotenoid metabolism and the biosynthesis of abscisic acid. Phytochemistry 30:815-821.

Parry, A.D., M.J. Babiano and R. Horgan, 1990. The role of *cis*- carotenoids in abscisic acid biosynthesis. Planta 182:118-128.

Peng, Y.H. and E. Rabe, 1996a. Effect of summer trunk girdling on fruit quality, maturation, yield, fruit size and tree performance in 'Mihowase' satsumas. J. Hort. Sci. 71(4):581-589.

Peng, Y.H. and E. Rabe, 1996b. Improvement of internal fruit quality in 'Mihowase' satsuma by summer girdling and regulated deficit irrigation. Proc. Int. Soc. Citricult. 2:725-729.

Poling, S.M., W. Hsu and H. Yokoyama, 1973. New chemical inducers of carotenoid biosynthesis. Phytochemistry 12:2665-2667.

Poling, S.M., W. Hsu and H. Yokoyama, 1975. Structure-activity relationships of chemical inducers of carotenoid biosynthesis. Phytochemistry 14:1933-1938.

Poling, S.M., W. Hsu and H. Yokoyama, 1976. Synthetic regulators of carotenoid biosynthesis in *Citrus paradisi*. Phytochemistry 15:1685-1687.

Poling, S.M., W. Hsu, F.J. Koehrn and H. Yokoyama, 1977. Chemical induction of β-carotene biosynthesis. Phytochemistry 16:551-555.

Pons, J., V. Almela, M. Jaun and M. Agusti, 1992. Use of ethephon to promote colour development in early ripening 'Clementine' cultivars. Proc Int. Soc. Citricult. 1:459-462.

Porat, R., B. Weiss, L. Cohen, A. Daus, R. Coren and S. Droby, 1999. Effects of ethylene and 1-methylcyclopropene on the postharvest qualities of 'Shamouti' oranges. Postharvest Biol. Technol. 15:155-163.

Porat, R., X. Feng, M. Huberman, D. Galili, R. Goren and E.E. Goldschmidt, 2001. Gibberellic acid slows postharvest degreening of 'Oroblanco' citrus fruits. HortScience 36(5):937-940.

Pozo, L. and J.K. Burns, 2000. Ethylene action inhibitors reduce Ethrel®-indiced leaf drop and gummosis in *Citrus*. Proc. Int. Soc. Citricult. 3:578-579.

Protopapadakis, E. and V.S. Manseka, 1992. Effect of ethylene-releasing compounds on colour break and abscission in five clones of Clementine. Proc. Int. Soc. Citricult. 1:463-464.

Pupin, A.M., M.J. Dennis and M.C.F. Toledo, 1999. HPLC analysis of carotenoids in orange juice. Food Chem. 64:269-275.

Purvis, A.C., 1980. Sequence of chloroplast degreening in Calamondin fruit as influenced by ethylene and $AgNO_3$. Plant Physiol. 66:624-627.

Puzina, T.I., 2004. Effect of zinc sulphate and boric acid on the hormonal status of potato plants in relation to tuberization. Russian J. Plant Physiol. 51(2):209-215.

Rademacher, W., 2001. BAS 125 10 W*("Regalis")* – General information and biological profile. BASF Global PGR Research and Development, Limburgerhof, Germany.

Rasmussen, G.K., 1973. The effects of growth regulators on degreening and regreening of citrus fruit. Acta Hort. 34:473-479.

Rasmussen, G.K., 1974. Cellulase activity in separation zones of citrus fruit treated with abscisic acid under normal and hypobaric atmospheres. J. Amer. Soc. Hort. Sci. 99:229-231.

Rasmussen, G.K., 1975. Cellulase activity, endogenous abscisic acid, and ethylene in four citrus cultivars during maturation. Plant Physiol. 56:765-767.

Reinbothe, S. and C. Reinbothe, 1996. The regulation of enzymes involved in chlorophyll biosynthesis. Eur. J. Biochem. 237:323-343.

Reitz, H.J. and R.C.J. Koo, 1960. Effect of nitrogen and potassium fertilization on yield, fruit quality, and leaf analysis of 'Valencia' orange. Proc. Amer. Soc. Hort. Sci. 75:244-252.

Reuther, W., 1988. Climate and fruit quality, p. 9-23. In: J.J. Ferguson and W.F. Wardowski (eds.). Factors affecting fruit quality. Citrus Short Course Proc.

Reuther, W. and P.F. Smith, 1952. Relation of nitrogen, potassium, and magnesium fertilization to some fruit qualities of 'Valencia' orange. Proc. Amer. Soc. Hort. Sci. 59:1-11.

Richardson, G.R. and A.K. Cowan, 1995. Abscisic acid content of citrus flavedo in relation to colour development. J. Hort. Sci. 70:769-773.

Rodrigo, M., J.F. Marcos, F. Alferez, M.D. Mallent and L. Zacarias, 2003. Caracterización of Pinalate, a novel *Citrus sinensis* mutant with a fruit-specific alteration that results in yellow pigmentation and decreased ABA content. J. Exp. Bot. 54:727-738.

Rosso, S.W., 1968. The ultrastructure of chromoplast development in red tomatoes. J. Ultrastruct. Res. 25:307-322.

Saidha, T., E.E. Goldschmidt and S.P. Monselise, 1983. Endogenous growth regulators in tracheal sap of citrus. HortScience, 18:231-232.

Sakoda, M., K. Hasegawa and K. Ishizuka, 1991. The occurrence in plants of the growth inhibitors, the raphanusanins. Phytochemistry 30:57-61.

Sala, J.M., P. Cunat, M. Collado and V. Moncholi, 1992. Effect on nitrogenous fertilization (quality and nitrogen form) in precocity of colour change of 'Navelina' orange. Proc. Int. Soc. Citricult. 2:598-602.

Salisbury, F.B. and C.W. Ross, 1992. Plant Physiology. J.C. Carey (ed.), Wadsworth Publishing Company, Belmont, California.

Saunt, J., 2000. Citrus varieties of the world. 2nd ed. Sinclair Intl. Ltd., Norwich, England.

Schneider, H., 1968. The anatomy of citrus, p. 1-22. In: W. Reuther, L.D. Batchelor and H.J. Webber (eds.). The Citrus Industry (v. 2). University of California, Berkeley, California.

Schwartz, S.J. and M. Patroni-Killam, 1985. Detection of cis-trans carotene isomers by two-dimentional thin-layer and high-performance liquid chromatography. J. Agric. Food Chem. 33:1160-1163.

Scora, R.W., 1975. On the history and origin of citrus, Bul. Torrey Bot. Club 102:369-375.

Shimokawa, K, A. Sakanoshita and K. Horiba, 1978. Ethylene-induced changes in chloroplast structure in 'Satsuma' mandarin (*Citrus unshiu* Mark). Plant Cell Physiol. 19:229-236.

Sites, J.W. and H.J. Reitz, 1949. Tha variation in individual Valencia orange from different locations on the tree as a guide to sampling methods and spot-picking for quality I. Soluble solids in the juice. Proc. Amer. Soc. Hort. Sci. 54:1-9.

Sites, J.W. and H.J. Reitz, 1950. The variation in individual Valencia oranges from different locations of the tree as a guide to sampling methods and spot-picking for quality. II. Titratable acid and the soluble solids/titratable acid ratio for the juice. Proc. Amer. Soc. Hort. Sci. 55:73-80.

Smeirat, N. and M. Qrunfleh, 1989. Effect of paclobutrazol on vegetative and reproductive growth of 'Lisbon' lemon. Acta Hort. 239:261-264.

Smith, P.F., G.K. Scudder, Jr. and G. Hrnciar, 1963. Twenty years of differential phosphate application on 'Pineapple' oranges. Proc. Fla. State Hort. Soc. 7-12.

Spiegel-Roy, P. and E.E. Goldschmidt, 1996. Fruit development and maturation p. 92-107. In: P. Spiegel-Roy and E.E. Goldschmidt (eds.). Biology of Horticultural Crops. Cambridge Univ. Press, Great Brittan.

Sponsel, V.M., 1987. Gibberellin biosynthesis and metabolism, p. 43-75. In: P.J. Davies (ed). Plant Hormone and their role in plant growth and development. Martinus Nijhoff Publishers, Boston.

Spurr, A.R. and W.M. Harris, 1968. Ultrastructure of chloroplasts and chromoplasts in *Capsicum annuum.* I. Thylalakoid membrane changes during fruit ripening. Amer. J. Bot. 55:1210-1224.

Stanier, R.Y. and G. Cohen-Bazire, 1957. The role of light in the microbial world: Some facts and speculations, p. 56-89. In Microbial Ecology. Williams, R.E.O., Spicer, C.C. (eds.). London: Cambridge U.P. (Cited by Goodwin, 1980).

Stassen, P.J.C., P.G. Mostert and B.L. Smith, 1999. Mango tree nutrition. A crop perspective. *Neltropika. Bul*. 303:41-51.

Stearns, C.R. and G.T. Young, 1942. The relation of climatic conditions to colour development in citrus fruit. Proc. Fla State Hort. Soc. 55:59-61.

Stover, E.W., S.M. Ciliento and M.E. Myers, 2004. Response of six citrus genotypes to Prohexadione-Ca. Plant Growth Regulat. Soc. Amer. 32: 86.

Syvertsen, J.P., 1981. Hydraulic conductivity of four commercial citrus rootstocks. J. Amer. Soc. Hort. Sci. 106:378-381.

Taiz, L. and E. Zeiger, 2002. Plant Physiology 3rd edition. Sinauer Associates Inc., Sunderland, Massachusetts, USA.

Talon, M., L. Zacarias and E. Primo-Millo, 1990. Hormonal changes associated with fruit set and development in mandarins differing in their parthenocarpic ability. Physiol. Plant. 79:400-406.

Thomas, R.L. and J.J. Jen, 1975. Phytochrome-mediated caroteniod biosynthesis in ripening tomatoes. Plant Physiol. 56:452-453.

Thomson, W.W., L.N. Lewis and C.W. Coggins, 1967. The reversion of chromoplast to chloroplasts in Valencia oranges. Cytologia 32:117-124.

Tominaga, S. and H. Diato, 1981. Effects of Ethychlozate (ethyl 5-chloro-1H-3-indazolylacetate) on fruit quality of Satsuma mandarin (*Citrus unshiu* Marc.). Proc. Int. Soc. Citricult. 1:233-236.

Tucker, G.A. and D. Grierson, 1987. Fruit ripening, p. 265-318 In: D.D. Davies (ed.), The biochemistery of plants, Vol.12. Physiology of Metabolism. Academic Press, New York.

Tukey, L.D., 1989. Uniconazole – a new triazole growth regulant for apple. Acta Hort. 239:249-252.

Van Wyk, A.A., 2004. Time- temperature interaction on postharvest rind colour development of citrus. Univ. Stellenbosch, Stellenbosch, South Africa, MScAgric Thesis.

Verreynne, J.S., 1999. Manipulation of internal fruit quality in citrus. Univ. Stellenbosch, Stellenbosch, South Africa, MScAgric Thesis p. 45-49.

Voss, D.H. and W.N. Hale, 1998. A comparison of the three editions of the royal horticultural society colour chart. HortScience 33(1):13-17.

Walles, B., 1972. An electron microscopy study on photodestruction of plastid ribosomes in β-carotene deficient mutants of *Helianthus annuus*, L. Protoplasma 215-227. (Cited by Goodwin T.W., 1977).

Wang, H., X. Huang, G. Hu, Z.Yang and H. Haung, 2005. A comparative study of chlorophyll loss and its related mechanism during fruit maturation in the pericarp of fast- and slow-degreening litchi pericarp. Scientia Hort. 106:247-257.

Wheaton, T.A., 1989. Triazole bioregulators reduce internode length and increase branch angle of citrus, Acta Hort. 239:277-280.

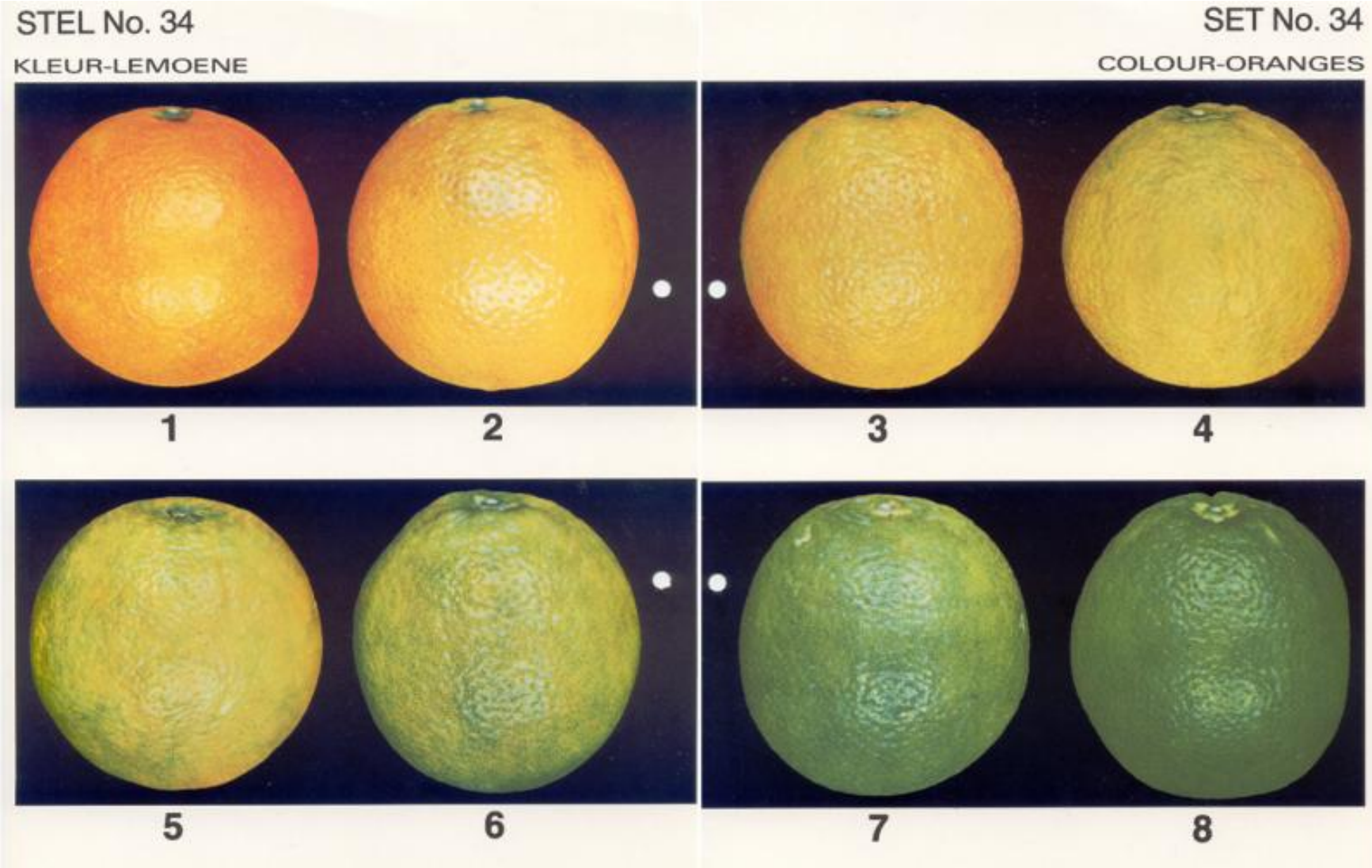
Yokoyama, H. and J.H. Keithley, 1991. Regulation of biosynthesis of carotenoids, p. 19-25. In: H.W. Gausmann (ed). Plant Biochemical Regulators, Marcel Dekker, New York.

Yokoyama, H., C.W. Coggins, Jr. and G.L. Henning, 1971. The effect of 2-(4-chlorophenylthio)-triethylamine hydrochloride on the formation of carotenoids in citrus. Phytochemistry 10:1831-1834.

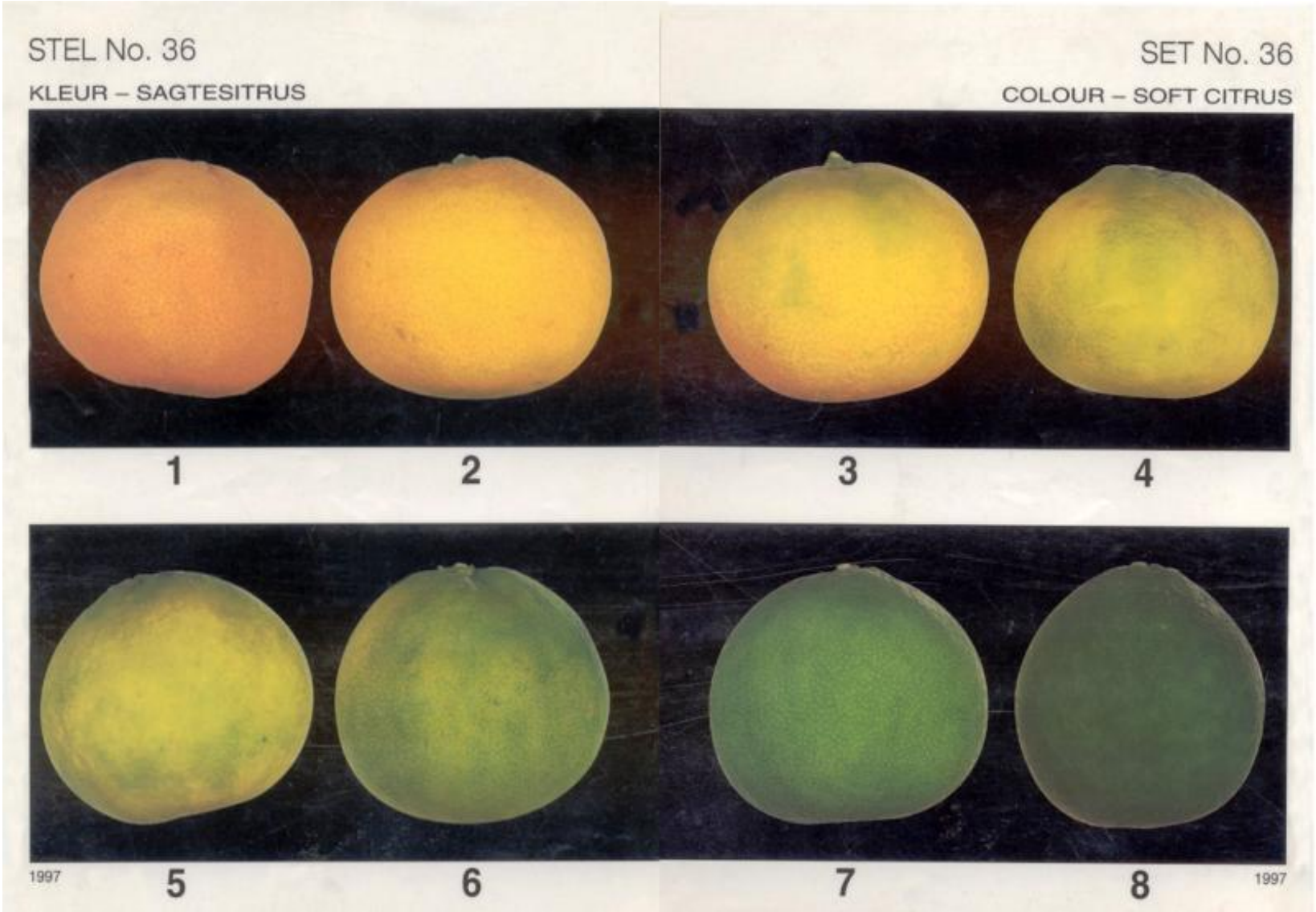
Yokoyama, H., C. DeBenedict, C.W. Coggins, Jr. and G.L. Henning, 1972. Induced colour changes in grapefruit and orange. Phytochemistry 11:1721-1724.

Young, L.B. and L.C. Erickson, 1961. Influence of temperature on colour change in Valencia orange. Proc. Amer. Soc. Hort. Sci. 78:197-200.

Young, R. and O. Jahn, 1972. Ethylene induced carotenoid accumulation in citrus fruit rinds. J. Amer. Soc. Hort. Sci. 97(2):258-261.



Appendix 1. Rind colour rating chart for oranges (CRI, 2004a).



Appendix 2. Rind colour rating chart for soft citrus (CRI, 2004b).

Appendix 3. Rind colour rating chart for lemons (CRI, 2004c).