COLOUR IMPROVEMENT OF BI-COLOURED PEARS

By

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DECLARATION

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

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SUMMARY

Poor colour development in bi-coloured pears is a big concern for the South African deciduous fruit industry, resulting in low Class 1 packouts with huge financial implications. The objective of this study was, therefore, to evaluate manipulation practices to improve colour development.

The effect of summer pruning on the colour development of 'Rosemarie' pear fruit was studied over two seasons. Pruning consisted of removing the upright shoots of the current season's growth on the lateral branches. The effect of the time of summer pruning on Class 1 packout percentage and average fruit mass were determined. The percentage blushed fruit (colour grading 1-10) of the trees pruned in November or pruned repeatedly from November to just before harvest were significantly higher than for unpruned control trees or trees pruned at other times. Fruit mass was not affected by summer pruning. A second study was conducted on 'Rosemarie' and 'Forelle' pears and the treatments consisted of non-pinched and pinched, where the bourse shoots were cut back at petal drop. There were no significant differences in fruit colour, fruit size, flesh firmness and total soluble solids after pinching compared to the control. In a third study on 'Rosemarie' and 'Forelle' trees, bourse shoots were removed in combination with defoliation. Spur leaves were removed at different times throughout the season from petal drop towards harvest. Both bourse shoots (Rosemarie), or one bourse shoot (Forelle) per cluster was removed as control, one treatment where no bourse shoots were removed served as a secondary control. Spur leaf removal on 'Rosemarie' and 'Forelle' did not have any significant effect on fruit set, fruit size or total soluble solids. In 'Rosemarie', there was also no significant effect on fruit colour. In 'Forelle', colour improved significantly between unmanipulated branches (control 1) and branches were one bourse shoot was removed (control 2). However, all treatments compared to control 1, improved red colour, indicated by a significant decrease in the hue angle values and an increase in Class 1 packout.

A fourth study was conducted on 'Flamingo', 'Forelle' and 'Rosemarie' pears. A number of urea applications were made onto the fruit. Fruit nitrogen content increased with urea sprays. Urea sprays did not affect red colour of 'Flamingo',

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'Forelle' and 'Rosemarie' pears. Urea sprays had no effect on the anthocyanin concentration ($\mu g.g^{-1}$) of 'Rosemarie' and 'Flamingo'. However, there was a quadratic relationship between number of urea sprays and anthocyanin concentration of 'Forelle'. There were not significant differences in the chlorophyll a and b concentrations of 'Flamingo' and 'Forelle'. Urea applications significantly increased the chlorophyll a concentration of 'Rosemarie'. In contrast there was no significant difference in the chlorophyll b concentration of 'Rosemarie'. The carotenoid concentration of 'Flamingo' and 'Forelle' was not affected by urea applications. In contrast the carotenoid concentration of 'Rosemarie' increased significantly after the urea applications.

Overhead cooling was applied with a micro-irrigation system to 'Rosemarie' pears. The water was applied with pulsed irrigation for a three-week period (24 December 1998 to 14 January 1999) before harvest. The system was activated when internal fruit temperature reached 24°C (day) and 19°C (night), and irrigation continued until internal fruit temperature reached 21°C (day) and 16°C (night). No significant differences were found in colour, soluble solids, fruit size, firmness or yields.

A study was conducted on 'Bon Rouge', 'Red d' Anjou' and 'Forelle' pears to assess the effect of storage period for 6 and 8 weeks at -0.5°C and ripening at 21°C for 1 week on anthocyanins, carotenoids, chlorophyll a and chlorophyll b content. There were no significant differences in the anthocyanin concentration in 'Bon Rouge' after cold storage compared to fruit at harvest, whilst for 'Red d' Anjou' anthocyanin concentration increased significantly after cold storage. Results with 'Forelle' were inconsistent. During ripening anthocyanin of 'Red d' Anjou' did not change, whereas the results for 'Bon Rouge' and 'Forelle' were inconsistent. However, cold storage had no effect on the anthocyanin concentrations of 'Bon Rouge' and 'Forelle'. Cold storage significantly decreased the carotenoid concentrations of 'Bon Rouge', but not in 'Red d' Anjou' and 'Forelle'. The carotenoids of 'Bon Rouge', 'Red d' Anjou' and 'Forelle' decreased significantly more during ripening at 21°C. The chlorophyll concentrations of 'Bon Rouge' decreased significantly during storage at -0.5°C, compared to fruit at harvest, but not in 'Red d' Anjou' and 'Forelle'. During ripening at 21°C chlorophyll a and chlorophyll b decreased significantly in 'Bon Rouge', 'Red d' Anjou' and 'Forelle'.

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In conclusion it is clear from this study, that although light is important for initial colour development, high December and January temperatures remain the biggest problem in maintaining good red colour at harvest. Other factors, e.g. fertilisation are secondary.

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OPSOMMING

Kleurverbetering van twee-kleur pere

Onvoldoende kleur ontwikkeling in twee-kleur pere is 'n groot probleem vir die Suid Afrikaanse sagtevrugtebedryf aangesien dit lae Klas 1-uitpakke tot gevolg het. Die doel van hierdie studie was dus om manipulasie tegnieke te evalueer om sodoende kleur ontwikkeling te verbeter.

Die effek van somersnoei op die kleur ontwikkeling van 'Rosemarie' pere is ondersoek oor twee seisoene. Die snoei het bestaan uit die verwydering van regop lote van die huidige seisoen se groei op laterale takke. Die effek van die tyd van somersnoei op Klas 1-uitpakpersentasie en gemiddelde vrugmassa is bepaal. Die persentasie vrugte met 'n blos (kleur gradering 1-10) van die bome wat in November of voortdurend vanaf November tot net voor oes gesomersnoei is, was betekenisvol hoër as die onbehandelde bome of bome wat op ander tye gesnoei was. Vrugmassa was nie beïnvloed deur somersnoei nie. 'n Tweede studie is gedoen op 'Rosemarie' en 'Forelle' bome. Die behandelings was nie-getop of getop, waar die beurslote teruggesny is na blomblaarval. Daar was geen betekenisvolle verskille in vrugkleur, vruggrootte, vrugfermheid en totale oplosbare suikers tussen die getopte en onbehandelde bome se vrugte nie. In 'n derde studie op 'Rosemarie' en 'Forelle' is beurslote verwyder in kombinasie met die verwydering van spoorblare. Spoorblare is verwyder op verskillende tye gedurende die seisoen vanaf blomblaarval tot oes. By 'Rosemarie' (beide beurslote per tros) en by 'Forelle' (een beursloot per tros) is beurslote verwyder om te dien as die kontrole, een behandeling waar geen beurslote verwyder was nie het gedien as die sekondêre kontrole. Spoorblaarverwydering by 'Rosemarie' en by 'Forelle' het geen betekenisvolle effek op vrugset, vruggrootte of totale oplosbare suikers gehad nie. By 'Rosemarie' was daar ook geen betekenisvolle effek op vrugkleur nie. By 'Forelle' is vrugkleur betekenisvol verhoog tussen onbehandelde takke (kontrole1) en takke waar een beursloot verwyder was (kontrole 2). Alle behandelings het egter vrugkleur verhoog in vergelyking met kontrole 1. Dit is waarneembaar in 'n betekenisvolle verlaging in die kleurskakering en 'n verhoging in die Klas 1-uitpakke.

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'n Vierde studie is uitgevoer op 'Flamingo' en 'Forelle' en 'Rosemarie' pere. 'n Aantal ureumtoedienings is gemaak op die vrugte. Die stikstofinhoud van die vrugte is verhoog met ureumbespuitings. Ureum spuite het nie rooi vrugkleur van 'Flamingo', 'Forelle' of 'Rosemarie' pere beïnvloed nie. Ureumbespuiting het geen effek op die antosianienkonsentrasie ($\mu g.g^{-1}$) van 'Rosemarie' en 'Flamingo' gehad nie. Daar was egter 'n kwadratiese verwantskap tussen die aantal ureumbespuitings en die antosianienkonsentrasie van 'Forelle'. Daar was geen betekenisvolle verskille in die chlorofiel a en b konsentrasies van 'Flamingo' en 'Forelle' nie. Ureumtoedienings het die chlorofiel a konsentrasie van 'Rosemarie' betekenisvol verhoog, daar was egter in teenstelling hiermee geen verskil in die chlorofiel b konsentrasie van 'Rosemarie' nie. Die karotenoiedkonsentrasie van 'Flamingo' en 'Forelle' is nie beïnvloed deur die ureum toedienings nie. In teenstelling hiermee het die karotenoiedkonsentrasie van 'Rosemarie' betekenisvol toegeneem na die ureum toedienings.

Oorhoofse verkoeling is toegedien met 'n mikro-besproeiingsstelsel op 'Rosemarie' pere. Die water is toegedien met puls besproeiing vir 'n periode van drie weke (24 Desember 1998 tot 14 Januarie 1999) voor oes. Die stelsel is geaktiveer wanneer die interne vrugtemperatuur 24°C (dag) en 19°C (nag) bereik het, die besproeiing het aangehou totdat die interne vrugtemperatuur 21°C (dag) en 16°C (nag) bereik het. Daar was geen betekenisvolle verskil in kleur, totale oplosbare suikers, vruggrootte, vrugfermheid of opbrengs nie.

'Bon Rouge' 'Red d' Anjou' en 'Forelle' pere is gebruik om die effek van opbergingsperiodes van 6 en 8 weke by –0.5°C en rypwording by 21°C vir 1 week op die antosianiene, karotonoied, chlorofiel a en chlorofiel b konsentrasies te bepaal. Daar was geen betekenisvolle verskille in die antosienien konsentrasie van 'Bon Rouge' na koue opberging nie, terwyl die konsentrasie antosianien by 'Red d' Anjou' betekenisvol toegeneem het na koue opberging. Die resultate by 'Forelle' was nie konstant nie. Gedurende rypwording was daar geen verskille in die antosianienkonsentrasie van 'Red d' Anjou' nie, die resultate by 'Bon Rouge' en 'Forelle' was nie konstant nie. Koue opberging het egter geen effek gehad op die antosianienkonsentrasies van 'Bon Rouge'en 'Forelle' nie. Die karonotoied-

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konsentrasies van 'Bon Rouge', 'Red d' Anjou' en 'Forelle' het betekenisvol meer afgeneem gedurend rypwording by 21°C. Die chlorofielkonsentrasies van 'Bon 'Rouge' het betekenisvol afgeneem gedurende opberging by –0.5°C teenoor die vrugte by oes, maar nie by 'Red d' Anjou' en 'Forelle' nie. Gedurende rypwording by 21°C het die chlorofiel a en chlorofiel b konsentrasies by 'Bon Rouge', Red 'd Anjou' en 'Forelle' betekenisvol afgeneem.

Om op te som dit is duidelik uit hierdie studie dat alhoewel lig belangrik is vir vroeë kleurontwikkeling, hoë temperature gedurende Desember en Januarie steeds die grootste faktor is wat finale rooi vrugkleur by oes bepaal. Ander faktore soos bemesting is van sekondêre belang.

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1. LITERATURE REVIEW: COLOUR IMPROVEMENT OF BI-COLOURED PEARS

1.1. INTRODUCTION

The consumer's preferences in terms of taste and especially fruit colour in this case, determines the guidelines which producers have to follow to get optimum prices on overseas markets. Thus it is very important for the producer to understand the factors involved in red colouration, which will increase their class 1 packout percentages. The pigments responsible for the red colouration in apples and pears are mainly anthocyanins.

The aim of producers should be to optimise their orchard management practices, which would lead to high quality fruit in terms of colour and taste, thus satisfying the consumer's needs. Apple and pear production areas in S.A. are of the world's warmer fruit producing areas. These warm temperatures contribute to the dissatisfactory fruit colour of our red- and bi-coloured apples and pears, therefore mechanical and orchard management manipulations are necessary to obtain optimum fruit colour.

Firstly, in this review the pigment anthocyanin, which is responsible for the red colouration in fruit is discussed in terms of it's location in deciduous fruit, different types, biosynthesis, the influence of the chemical structure on fruit colour, key regulating enzymes and the effect of light and temperature on the regulation of anthocyanin biosynthesis. Secondly, orchard and mechanical management practices in terms of light and temperature are also discussed to obtain optimum fruit colour.

1.2. ANTHOCYANIN

Anthocyanins are glycosides of some twenty naturally occurring anthocyanidins, which are responsible for the red colouring of fruit (Harborne, 1967). There are two peaks of anthocyanin formation in apple: a first peak during the phase of intense cell division in

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the fruit, which has been widely neglected so far because it is economically unimportant; and a second peak coinciding with ripening of red cultivars (Saure, 1990). This pigment is situated in the cells beneath the epidermis of apples and pears (Asen et al., 1971). Anthocyanin is mainly located in the sub-epidermal cells, although there are also pigments in the epidermal layer. In apples these pigments occur in the hypodermal cells (Mazza & Miniati, 1993). The red colour usually occurs in the top 3-4 layers of the epidermis (Overholser, 1917). This pigment is located in the cell vacuole and belongs to the flavonoid group. It absorbs visible light, which leads to a variety of colours of the media in which it occurs (Brouillard, 1982; 1983). In an aqueous medium most of the anthocyanins behave like pH indicators, being red at a low pH, bluish at a neutral pH and colourless at a high pH (Mazza & Miniati, 1993).

1.2.1. Anthocyanin pigments in apples and pears

Cyanidin 3-galactoside is the major red pigment in apple peel, but the structures of the minor anthocyanins also present are less certain (Mazza & Miniati, 1993; Siegelman & Hendricks, 1958; Timberlake & Bridle, 1971). The identity of the other pigments is very controversial, and it differs from cultivar to cultivar. The pigments are cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 7-arabinoside, cyanidin 3-xyloside and their acylated derivatives (Brouilliard, 1982; Macheix et al., 1990; Mazza & Miniati, 1993).

Less work has been done on pears to identify the specific anthocyanin pigments. Cyanidin 3-galactoside is, as in apples, the major red pigment in pears. Cyanidin 3-arabinoside also occurs in red pear peel (Francis, 1970; Macheix et al., 1990; Mazza & Miniati, 1993). According to Redelinghuys (1969) peonidin 3-galactoside appears in 'Bon Chretien' pears. In pears, these pigments are not situated in the first two cell layers beneath the epidermis, but in the third to seventh cell layers (Dayton, 1966; Mazza & Miniati, 1993). The pear cultivar, 'Starkrimson' differs from the other pear cultivars in terms of anthocyanin location in that the anthocyanin occurs in the epidermal cells of the fruit and not in the cell layers beneath the epidermis (Dayton, 1966).

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1.2.2. Types of anthocyanin

The anthocyanins differ from each other in the number of hydroxyl groups attached to the molecules, the number and nature of the sugars substituted at the molecule, the degree of methylation and the position of substitution and the nature of aliphatic or aromatic acids attached to the sugars in the molecule. The most common anthocyanins contributing to the pigmentation of plant organs are pelargonidin, cyanidin, peonidin, petunidin and malvidin (Macheix et al., 1990; Mazza & Miniati, 1993).

1.3. BIOSYNTHESIS OF ANTHOCYANIN

According to Lancaster (1992) and Macheix et al. (1990) the aromatic amino acid, phenylalanine, which is produced via the shikimic acid pathway, is the common precursor of all the flavonoids in higher plants. Anthocyanins belong to the general class of phenolic compounds known as flavonoids. The first committed step in flavonoid biosynthesis is the condensation of three molecules of malonyl CoA and one of p-coumaroyl CoA by the enzyme chalcone synthase (CHS) to produce a yellow chalcone. The second step, the isomerisation of the chalcone into a colourless flavanone, proceeds spontaneously at a low rate, but it is accelerated by the enzyme chalcone-flavanone isomerase (CHI). The flavanone so formed is hydroxylated at the C_3 position by the action of flavanone 3-hydroxylase (F_3H) to give an unpigmented dihydroflavonol that is reduced by dihydro-flavonol 4-reductase (DFR) to yield a still colourless leucocyanidin. This compound is then converted into a coloured anthocyanidin. The last step is the glycosylation of the anthocyanidin to give anthocyanin and is catalyzed by the enzyme UDP-glucose flavonoid 3-oxyglucosyltransferase (UF_3GT).

1.4. THE CHEMICAL STRUCTURE OF ANTHOCYANIN

Anthocyanin consists of a 3-ring structure with various substitutions, and a positive charge delocalized over the entire structure (Mazza & Miniati, 1993). There is a direct connection between the intensity of the peel colour and the concentration of the anthocyanins and molecular substitution, but the final colour is determined by the interaction between the anthocyanin molecules and other components (Harborne, 1988).

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Mazza & Brouillard (1990) concluded that the changes in the fruit colour of apples might be the result of co-pigmentation or the hydrogen-ion concentration in the vacuoles (Asen et al., 1971) where the anthocyanins are concentrated.

The colour of anthocyanin-containing media depends on the structure and the concentration of the pigment, pH, temperature, presence of co-pigments, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products, sulfur dioxide and other factors. Hydroxyl groups, methoxyl groups, sugars and acylated sugars have a marked effect on colour intensity and stability of anthocyanins (Mazza & Miniati, 1993).

1.4.1. Hydroxylation

A free hydroxyl at positions 5, 7 or 4 is essential for the formation of a quinoidial structure, which is responsible for the coloured pigmentation of fruit (Mazza & Miniati, 1993). The colour of anthocyanins is determined by the degree of hydroxylation in the B-ring of the anthocyanidin, and the greater the substitution the bluer the colour (Asen, 1976; Mazza & Miniati, 1993). Pelargonidin has a hydroxyl at C-4', cyanidin has hydroxyls at C-3', C-4', and delphinidin has hydroxyls at C-3', C-4' and C-5' (Asen, 1976). Delphinidin is, therefore, the bluest of these pigments, because it has the most substitution of hydroxyl groups (Macheix et al., 1990).

1.4.2. Glycosylation

The 3-hydroxyl of the anthocyanin is almost always replaced by a sugar (Francis, 1970; Lancaster, 1992; Timberlake, 1980). According to Macheix et al., (1990) the cyanidin 7-arabinoside in the peel of apple cultivars is the exception. The four monosaccharides involved in the formation of the cyanidin 3-monoglycoside in apples and pears are glucose, galactose, arabinose and xylose (Van Buuren, 1970). These sugars are usually attached to the 3, or/and the 5 carbons of the anthocyanin molecule (Asen, 1976). Mazza & Miniati (1993) concluded that the anthocyanin 3-glycosides are more coloured than 3, 5- and 5-glycosides at a given pH.

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1.4.3. Methoxylation

This structural feature includes the methylation of hydroxyl groups, usually those at C-3' and C-5'. The 3'-0-methyl cyanidin is known as peonidin, the 3'-0-methyl delphinidin as petunidin and the 3', 5'-0-dimethyl delphinidin as malvidin. Cyanidin and delphinidin glycosides are slightly bluer than their corresponding methyl ethers (Asen, 1976). Mazza & Brouillard (1987) concluded that an increase in methyl groups leads to a decrease in blue colour.

1.4.4. Acylation

Anthocyanins in apple peel are not acylated with aromatic acyl residues, and acylation of anthocyanins in pear peel is negligible (Lancaster, 1992; Macheix et al., 1990). Acylation stabilises the anthocyanin pigment in the acidic medium of the vacuole (Macheix et al., 1990). Different parts of the sugars can take part in the formation of the ester, but it is always the C-3' position which is acylated (Harborne, 1967; Timberlake, 1980). Thus, there are a variety of aliphatic dicarboxylic acids, which are involved in acylation of pigments, i.e. malonic, malic, oxalic and succinic acids (Harborne & Grayer, 1988).

1.4.5. Co-pigmentation

Co-pigmentation of anthocyanins with other flavonoids and related compounds produces an increase in colour intensity and a shift in the wave-length of maximum absorbency toward higher wave-lengths, giving purple to blue colours (Asen et al., 1971; Mazza & Miniati, 1993). The effect of co-pigmentation has been shown to be a molecular interaction occurring between the coloured anthocyanins and the co-pigments (Mazza & Miniati, 1993). The molecules change the structure of the anthocyanins, thus their colour also changes, and they tend to be colourless at a pH of 4-6. The concentration of the co-pigment in relation to the anthocyanin determines if co-pigmentation will take place at a specific pH, where the pigments are usually colourless (Asen et al., 1971; Mazza & Brouilliard, 1987). Mazza & Brouillard (1990) have found a positive correlation between fruit colour and anthocyanin concentration.

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Co-pigmentation also increases when the anthocyanin concentration increases; with a higher co-pigment concentration, the co-pigmentation also increases (Asen, 1976; Mazza & Brouillard, 1990). Co-pigmentation occurs from pH values close to 1, the pH value for maximum effect is about 3.5 and may vary slightly depending on the pigment-co-pigment system (Mazza & Miniati, 1993). By controlling the pH and the anthocyanin concentration, and by manipulating the co-pigmentation most of the flower colours can be simulated (Asen, 1976).

1.5. KEY ENZYMES INVOLVED IN ANTHOCYANIN SYNTHESIS

1.5.1. Phenylalanine ammonia-lyase

Phenylalanine ammonia-lyase (PAL), is under certain circumstances, the rate-limiting enzyme of anthocyanin synthesis in apple peel (Faragher & Chalmers, 1977). PAL is also believed to be the main limiting factor in the synthesis of flavonoids (Zucker, 1965). This enzyme catalyses the conversion of L-phenylalanine to trans-cinnamic acid and ammonia (Koukol & Conn, 1961). PAL was proposed as a key enzyme in regulating anthocyanin synthesis in many plants, but its role in apple colouration has been controversial (Saure, 1990). Ju et al., (1995) have shown that the importance of PAL in regulating anthocyanin synthesis depends on the concentrations of simple phenols or flavonoids. PAL would be critical for anthocyanin synthesis, only when anthocyanin was synthesised beginning with the deamination of phenylalanine. When precursors are present in sufficient quantities during fruit maturation, anthocyanin synthesis will not depend on PAL activity. Thus Ju et al., (1995) agree with Lancaster (1994) that anthocyanin production in red apples is likely to involve additional enzymes between leucocyanidin and cyanidin glycosides, and that these may be the key light-inducible enzymes involved in apple peel reddening. Lister et al., (1996) also showed that PAL and CHS were not critical in regulating anthocyanin synthesis in apples, especially during the fruit maturation period.

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PAL is strongly inhibited by cinnamic acid. Control of lignification or flavonoid synthesis could be affected by the regulation of the levels of PAL or of phenylalnine production, or both. It may be expected that PAL has regulatory properties because cinnamic acid is the first compound strictly committed to the biogenesis of flavonoids. Cycloheximide, the protein synthesis inhibitor, prevents PAL formation when applied at the beginning of the inductive period. However when applied later enzyme levels do not decrease. From this work it is inferred that PAL inactivation is dependent upon protein synthesis and perhaps on the synthesis of a specific protein or enzyme, which inactivates or destroys PAL (Zucker, 1965). PAL variations depend on the genotype or plasmotype, but also on the age and development, organ and even tissue of the plant (Camm & Towers, 1973). Creasy (1976) suggested that phenylalanine ammonia-lyase inactivating system (PAL-IS) and inhibitor might regulate PAL. It is also known that wounding has an increased effect on the PAL level, this effect might be ascribed to the production of ethylene because wounding a tissue stimulates the endogenous production of ethylene (Camm & Towers, 1973). PAL activity is affected by wounding (ethylene), but also by light, temperature, growth regulators, inhibitors of RNA and protein synthesis and by mineral nutrition (Saure, 1990).

1.5.2. Chalcone isomerase

Chalcone isomerase (CHI) stimulates ethylene production in plant tissues (Faragher and Chalmers, 1977). Ethylene is responsible for stimulating anthocyanin synthesis in plants. Faragher and Chalmers (1977) also found that one microgram per milliliter CHI doubled the anthocyanin accumulation, while ten micrograms per milliliter increased the anthocyanin accumulation six fold.

1.5.3. Chalcone synthase

Chalcone synthase (CHS) activity was not affected significantly by an ethephon treatment, thus indicating that CHS-activity was not a rate-limiting factor in flavonoid or anthocyanin synthesis in apple fruit (Ju et al., 1995).

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1.5.4. Flavonoid-3-0-glycosyltransferase

Flavonoid-3-0-glycosyltransferase (UFGalT) catalyzes the transformation from unstable anthocyanidin to stable anthocyanin. UFGalT activity was positively correlated with anthocyanidin accumulation during fruit maturation. The importance of this enzyme in regulation of anthocyanin probably depends on the availability of its precursor, cyanidin (Ju et al., 1995).

1.6. INFLUENCE OF LIGHT ON THE REGULATION OF ANTHOCYANIN BIOSYNTHESIS

1.6.1. General

Anthocyanin formation in apple peel is absolutely light dependent (Macheix et al., 1990; Saure, 1990). Pears are even more dependent on light than apples (Wagenmakers, 1987). The poor colour development of fruit in the interior of the tree canopy points to the major importance of light (Saure, 1990). There is a positive correlation between the amount of direct sunlight received and the colour intensity and the size of the blush on the fruit (Jacyna, 1978). Two radiation dependent phases can be distinguished in anthocyanin synthesis in apple peel. There is an induction period of 20 hours before the onset of anthocyanin formation. Secondly, anthocyanin increased linearly with time under continuous irradiation (Siegelman & Hendricks, 1958). Faragher & Chalmers (1977) confirmed a lag phase of 20 hours, and a linear function of anthocyanin accumulation for a further 100 hours.

1.6.2. Light intensity

The intensity of incident photosynthetically active radiation (PAR) within the canopy is primarily decreased by the mutual shading of leaves (Palmer, 1977). Light intensities decrease logarithmically with LAI (leaf area index) from the top to the bottom of the tree (Jackson et al., 1977). Chandler and Watson (1954) concluded that overshadowing decreased the sugar content of plants, therefore decreasing the pigmentation. There is a

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negative correlation between chlorophyll concentration and light intensity, it is more UV sensitive and fruit colour is also better (Marais, 1991). The anthocyanin concentration increases linearly with light intensities higher than 0.5 $mW.cm^{-2}$ (Proctor, 1974) and stop at energy levels of 3.24 $mW.cm^{-2}$ (Arakawa et al., 1985). According to Saure (1990) decreasing light intensities have a more negative effect on colour development with early cultivars than with late cultivars. This might be the result of a longer period of maturation in late cultivars, thus a longer period for anthocyanin accumulation. There is a good positive correlation between the amount of direct sunlight and the colour intensity of the fruit (Jacyna & Soczek, 1980).

1.6.3. Light interception

Light interception is the driving force for leaf photosynthesis and fruit production. There is a linear relationship between light interception and dry mass production (Johnson & Lakso, 1991). The ideal percentage of light interception, for optimal fruit production and fruit colour is 60-70%. The planting of bigger trees or at higher densities will result in higher levels of light interception (Warner, 1993).

1.6.4. Light distribution

The light exposure requirements for fruit colour development require that light be distributed to all fruiting sites in the canopy (Jackson et al., 1977). The best coloured fruit are found in those parts of the canopy receiving 70-80% full sunlight, the average coloured fruit at 40-70% and the worst coloured fruit at the parts receiving less than 40% full sunlight (Jacyna & Soczek, 1980; Macheix et al., 1990). It varies from cultivar to cultivar, but in general 50-75% full sunlight is essential for optimal colour development (Barrit et al., 1987). When sunlight reaches the leaves, approximately 85% of the light is absorbed or reflected by the leaves; only 15% of the light energy penetrates through the leaves. Thus light energy decreases from the outside to the inside of the tree as a result of the absorption and reflection. It leads to the production of fruit with lower TSS, smaller fruit and poor red colour development. Light must be directed onto the fruit for the necessary chemical reactions to take place and thus optimum anthocyanin synthesis.

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1.6.5. Photoreceptors

There are three photoreceptors in apple peel: (1) UV-B light (290-320 nm), (2) UV-A light (320-400 nm) and (3) red (R) (600-690 nm) / far-red (FR) (710-760 nm) - phytochrome. The receptors work together in flavonoid synthesis. In special cases only one photoreceptor will be sufficient for flavonoid synthesis (Heller & Forkmann, 1988). Maximum anthocyanin production is obtained with UV-light, then with R light and the least (even inhibitory) with FR light (Saure, 1990). According to Faragher & Chalmers (1977) anthocyanin synthesis in apples is stimulated by light in the UV-range. There is more UV radiation in the upper half of the tree. The dust and moisture in the air absorb UV. The presence of UV-light explains the better colouration at high light intensities and the accelerated colouration after periods of rain and cloudy weather conditions (Saure, 1990). The bad light penetration of UV-B light might be the reason for the weak colouration within the tree canopy (Arakawa et al., 1986). According to Heller & Forkmann (1988), UV-B light (290-320 nm) is the best for anthocyanin synthesis. Mazza & Miniati (1993) suggested however, that the most effective light for maximum anthocyanin formation is blue-violet, with wavelengths around 650 nm and 430-480 nm.

Phytochrome is responsible for anthocyanin synthesis with R and FR radiation (Mancinelli et al., 1991). Phytochrome is a pigment bound to a protein and acts as a photoreceptor in the light-dependent anthocyanin synthesis (Mancinelli, 1985). UV-B light is able to stimulate anthocyanin synthesis on its own, thus independent of phytochrome (Arakawa et al., 1985). However, Arakawa (1988) showed that the reaction of UV-B light accelerated when it was followed by a radiation period with R light. This effect is reversible with FR treatments. Radiation with both receptors results in more induced anthocyanin. It seems that photosynthesis plays a role in this reaction (Mancinelli & Rabino, 1984). The decreasing anthocyanin levels when FR is used, indicates that phytochrome is involved in the R reaction (Arakawa et al., 1985).

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1.6.6 Phenyl ammonia-lyase

Zucker (1965) found an increased activity of PAL when potato slices were incubated in light. Phytochrome involvement was demonstrated in etiolated pea, mustard and radish seedlings. A short period of illumination with R followed by a return to darkness causes an increase in PAL activity. A brief illumination with FR suppresses the response to R. In addition to this effect of R and its reversibility by FR, continuous FR illumination increases PAL levels in etiolated tissue (Bellini & Van Poucke, 1970). Irradiation for 6 hrs with FR light significantly increases the extractable activity of PAL, although Pfr levels were very low. They concluded that the FR effect in their system couldn't be explained solely by formation and maintenance of Pfr. Schopfer and Mohr (1972) claimed that as little as one 5-min irradiation with R light induces PAL formation and the criteria for the involvement of Pfr in the induction of this response are fulfilled. Gibberellin treatments increase PAL production and lignification in dwarf pea plants, but only when the plants are held in the light (Cheng & Marsh, 1968). In many of the R-FR light experiments there is a lag phase before PAL levels start to increase. In nearly all cases of the light activated enzyme, the initial increase in activity is followed by a significant decline, even in the presence of the continuous light (Camm & Towers, 1973). White + UV 312 light stimulates PAL activity more than white light alone, even at a lower fluency rate (Arakawa et al., 1986).

1.7. ORCHARD MANAGEMENT PRACTISES WHICH INFLUENCE LIGHT INTERCEPTION AND DISTRIBUTION.

1.7.1. Summer pruning

The best coloured fruit are found in the parts of the canopy receiving 70-80% of full sunlight (Jacyna, 1978; Jacyna & Soczek, 1980). Summer pruning to increase light intensity in the canopy generally improves red colour development of fruits via improved anthocyanin production (Jackson, 1980; Tymoszuk et al., 1984), and in some cultivars or training systems it is essential for good fruit colour (Robinson et al., 1991).

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Summer pruning is carried out in addition to winter pruning and it is done to remove unwanted current season's growth with the primary objective of increasing fruit exposure to light. It includes the removal of all unproductive vertical shoots and the pruning back of horizontal shoots to leave 5-10 cm of the new growth (Warrington et al., 1984). Summer pruning especially enhances the development of red colouration or blush and controls vigour (Aselage & Carlson, 1977). Morgan et al. (1984) concluded that summer pruning does not increase the background colour, but decreases the fresh weight of fruit from the early pruned trees, and decreases the soluble solids concentration of the fruit from the late pruned trees. In pruned trees, the fruit peel colour presented a highly significant negative correlation with light intensity in the canopy i.e. the more light available, the redder, darker, less intense and more blushed the fruit were (Dussi & Huysamer, 1995). Saure (1990) concluded that summer pruning decreases GA, which is an inhibitor of anthocyanin synthesis.

The effectiveness of summer pruning is dependent on the cultivar, growing season, and crop load, but particularly the timing and severity of the summer pruning (Marini and Barden, 1982). According to Lord and Greene (1982) the effect of summer pruning depends on the amount of leaf surface removed, and tree vigour. Tymoszuk et al. (1984) suggested one has to be very careful with summer pruning, because it suppresses tree size more than dormant pruning, and also accelerates fruit ripening by exposing the fruit to sunshine.

Other benefits of summer pruning are faster picking rates, less orchard culling, better spray penetration and lower pesticide volumes (Dussi & Huysamer, 1995). However, negative effects i.e. sunburn and a reduction in fruit size may occur if summer pruning is too severe or badly timed (Miller, 1982; Tymozuk, et al., 1984).

1.7.2. Root pruning

Schupp (1991) found that root pruning at full bloom, doubled light penetration in the lower parts of the tree canopy. Root pruning improves fruit colour, but decreases fruit

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size (Saure, 1990; Schupp & Ferree, 1989; Schupp, 1991). According to Saure (1990) root pruning decreases the amount of GA in the plant, which results in better colouration of fruit.

1.7.3. Girdling

The effect of girdling is to weaken or to stop active root growth and thereby reduce vigour indirectly through stopping the manufacture of gibberellins by the roots. In addition, carbohydrates manufactured by the leaves are held in the tree above the girdling cut and diverted from root growth to bud development, secondary thickening of shoot growth and supplying resources for fruit sizing and colour development. The severity of this practice is determined by the time of application, the distance between the cuts and the amount of removal (Wilton, 1997).

1.7.4. Trellis systems and row direction

The purpose of trellis systems is to optimise light interception, light penetration and light distribution inside the tree canopy. The total amount of light intercepted is a function of the leaf area index (LAI) and the orientation of the leaves. Light distribution inside the tree canopy is responsible for fruit set, fruit size and fruit colour (Jackson, 1980; Robinson et al., 1983).

There are two approaches which one can follow to improve light distribution inside the tree canopy. Firstly, you can use small tree forms with good light penetration through small openings in the tree canopy, like the central leader, double leader and slender spindle (Heinicke, 1975; McKenzie, 1972). This approach is successful, but requires high management skills. Secondly, one can look at tree forms with bigger, permanent openings in the tree canopy, like the A, V, Y, or T forms (Lakso et al., 1989; McKenzie, 1972; Palmer, 1988). This approach is however more expensive and needs a support

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system. According to Robinson & Lakso, (1989) light interception decreases from the Y to the slender spindle and from the slender spindle to the central leader systems.

Anthocyanin formation in apple peel is absolutely light dependent (Macheix et al., 1990; Saure, 1990) and pears are even more dependent on light than apples (Wagenmakers, 1987). The poor colour development of fruit in the interior of the tree canopy stress the major importance of light (Saure, 1990). There is a high positive correlation between the amount of direct sunlight received and the colour intensity and the size of the blush on the fruit (Jacyna, 1978). Cain (1972) concluded that angled (70° to the horizontal) hedgerow surfaces give much more even light distribution than vertical ones. Fruit colour formation is directly dependent on light falling on the fruit surface (Jackson & Palmer, 1972). Jackson (1970) found that the estimated percentage interception by east-west hedgerows varied over the season, while that for north-south hedgerows was fairly consistent. The percentage of available direct light, which is intercepted, is controlled by the position of the sun in relation to the hedges and by the distance between these. The effect of row orientation on interception therefore varies with the time of the day and season, latitude and orchard geometry (Jackson, 1970). In the majority of cases, east-west orientation of rows will result in a poorly illuminated north side and a pronounced seasonal pattern of radiation on the south side. In the northern hemisphere, the north-south orientation of hedgerows is preferable because of a more even distribution of light (Jackson & Palmer, 1972).

1.7.5. Fruit thinning

According to Wertheim (1987) winter pruning and/or fruit thinning causes a high leaf/ fruit ratio, which gives rise to good fruit colouration (Saure, 1990; Varga & Wertheim, 1976; Wertheim, 1987). A higher leaf/fruit ratio is also linked to higher sugar levels (Saure, 1990). Early defoliation only increases fruit size, but not fruit colour, whereas later thinning increases both (Saure, 1990). Defoliation 51 days before harvest increased the anthocyanin concentration of 'Fuji' apples but reduced acidity. The thinning of the leaves around the fruit will also expose the fruit to light and thus improve colouration

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(Walter, 1967). Baart & Joosse (1989) found that fruit colour and fruit weight increased linearly with the severity of thinning. This can be explained by increased exposure to light and decreased fruit-fruit competition. Fruit thinning increases fruit colour because it increases the leaf/fruit ratio (Saure, 1990). The disadvantage of fruit thinning is that it reduces yield per hectare (Baart & Joosse, 1989).

1.7.6. Rootstocks

Light interception and light distribution can be partially controlled by the choice of rootstock, which gives trees of different sizes (Schechter et al., 1990). Dwarfing rootstocks inhibit vegetative growth and tree size, the fruit are well exposed and benefit from this in size and soluble solids, and consequently in more red fruit (Schupp & Ferree, 1988). Saure (1990) found that dwarfing rootstocks, and growth reducing interstocks, compared with vigorous rootstocks generally have a positive effect on anthocyanin synthesis in apples. M9 is one of the most dwarfing apple rootstocks, but it is not recommended for the South African climate, because it might cause sunburn in certain cultivars and it might not be vigorous enough for the low and medium potential soils (J. Jacobs, personal communication). Quince A is a dwarfing pear rootstock and is recommended for 'Forelle'. This rootstock is, however, not compatible with other cultivars like 'Rosemarie', etc. Growth reducing interstocks, like the 'Beure Hardy-Quince A' combination will probably solve this problem (J. Jacobs, personal communication).

1.7.7. Fertilisation

1.7.7.1. Nitrogen

Nitrogen (N) and chlorophyll contents of the leaves are important factors dominating fruit colour, and the lower the content the better the fruit colour. N fertilisation was the most important of many factors affecting leaf N and chlorophyll (Marcelle, 1995). Kvale (1968) found a positive correlation between the amount of carotenoids and chlorophylls with increasing nitrogen levels of the trees. He also suggested that a high nitrogen

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concentration in the cell should increase the amino acid formation, thus increasing the concentration of substrate for chlorophyll formation. The nitrogen level of the trees strongly influenced the content of soluble solids as well as pigment concentration and ground colour of the fruit (Kvale, 1968). Fruit nitrogen is negatively correlated with different parameters of eating quality such as the percentage of dry matter, the soluble solids content and the acidity. The susceptibility to many physiological disorders is also dramatically increased when fruit nitrogen content is too high (Marcelle, 1995).

1.7.7.2. Potassium

Marcelle (1995) found a positive correlation between fruit potassium content, acidity and soluble sugars content in 'Jonagold' and 'Cox's Orange Pippin' apples. He also found in 'Jonagold' fruit, which were rich in potassium, that the green background colour of the peel turned yellow faster than in the fruit poor in potassium.

1.7.7.3. Calcium

In 'Cox's Orange Pippin' negative correlations were found between fruit calcium content and the percentage of dry matter, the refractometric index and the content of soluble sugars. The green background colour is correlated with the fruit calcium content, in the sense that fruit rich in calcium remains greener (Marcelle, 1995). Calcium retards senescence by decreasing the lipoxygenase activity, ACC content and ethylene emission (Marcelle, 1991).

1.7.7.4. Phosphorus

Marcelle (1995) found that fruit phosphorus content in 'Cox's O.P.' apples is positively correlated with the refractometric index and the acid content.

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1.7.7.5. Magnesium

Most of the effects of magnesium on fruit quality can be explained by two facts: Mg is an antagonist of K, and it competes with Ca for fixation sites during its transport. It means that correlations between parameters of fruit quality and magnesium content look like those found with potassium and/or with calcium except for the storage quality (Marcelle, 1995).

1.7.7.6. Manganese

After application of Mn to 'Jonagold' apples, Marcelle (1995) concluded that the fruit maintained the green background colour without delaying the red colour formation. It is well known that Mn plays an important role in the oxygen evolving system during photosynthesis and can delay the senescence of chloroplasts. However, it seems that too many applications of Mn can impede the red colour formation of the fruit (Marcelle, 1995).

1.7.7.7. Mineral deficiencies

Nitrogen and potassium deficiencies promote the accumulation of PAL and decrease the accumulation of PAL-IS (Stout, 1969). Magness et al. (1928) indicated that the increase of red fruit colour by low nitrogen content might be due to less shading and less vegetative growth which in turn promoted fruit maturity in low nitrogen trees. The present results suggest that low nitrogen and potassium increase colour formation because they may promote the accumulation of PAL, which is a key enzyme in the synthesis of anthocyanin.

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1.7.8. Application of chemicals

1.7.8.1. Alar (SADH or daminocide)

Alar treated apples were better coloured than the unsprayed ones, and apple thinning on these trees improved fruit colour (Basak et al., 1986).

1.7.8.2. Paclobutrazol (Cultar)

Paclobutrazol is one of several sterol-inhibiting compounds that can retard shoot growth (Greene, 1978), so its stimulating effect on red colour development may be due to less shade (Saure, 1990).

1.7.8.3. Ethephon (Ethrel)

The dependence of red coloring on ethylene has been exploited by applying ethephon, an ethylene-releasing chemical on the fruit. Ethephon improves red colour formation, but it unfortunately also increases softening and it enhances the soluble solids content of the fruit (Blanpied et al., 1975; McBride & Faragher, 1978). Faragher and Chalmers (1977) found that treatments that stimulate ethylene production also stimulate PAL levels in apples.

There is also a positive correlation between ethylene and anthocyanin accumulation and the level of PAL in the peel (Faragher & Brohier, 1984). The application of ethylene stimulates PAL in several plant tissues (Camm & Towers, 1973). Thus it seems that during ripening changes occur, with increased ethylene levels leading to increases in PAL activity and anthocyanin synthesis (Faragher & Brohier, 1984).

1.7.8.4. Cycocel (CCC)

CCC reduces shoot growth dramatically. The retarding effect of CCC has been ascribed to the partial blocking of GA production, as GA enhances vegetative growth (Volz & Knight, 1986).

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1.8. MECHANICAL MANAGEMENT PRACTISES WHICH INFLUENCE LIGHT AND THUS FRUIT COLOUR.

1.8.1. Artificial reflectors

Moreshet et al. (1975) found that fruit size, colour and sugar content of fruit in the lower parts of the canopy increased when the soil surface between the rows of a high density orchard were covered with reflective aluminum-coated plastic. Viljoen (1996) concluded after the use of two kinds of reflectors, namely Supersisalation and Tyvek, that more light was reflected to the interior of the canopy. There was also a significant difference in colour between the fruit from the upper and lower parts of the canopy. Mika (1980) also found that the fruit nearest to the reflectors had a darker red colour, thus indicating an increase in the anthocyanin concentration.

Reflected light has been found to constitute as much as 30% of light available to a shaded lower leaf (Lakso, 1975). Reflectors do not provide a uniform source of light, being very dependent on environmental conditions such as time of day, weather conditions and the characteristics of the tree such as stage of development (Doud & Ferree, 1980). The use of an aluminium mulch increased light levels in the lower parts of the tree canopies by about 20%. After two years, the aluminium mulch lost most of its reflectivity due to the development of algae and lichens on the surface. Photometric measurements showed that newly laid aluminised tar paper reflected 94% of the incident light, whilst the reflectivity of bare ground measured under the tree canopy was around 25% (Mika, 1980). The total weight of fruit harvested per tree was not affected until the following season when a large increase was recorded as a result of improved flowering and fruit set. The gain from any specific under-tree or within-alley arrangement obviously will depend on overall canopy geometry and density and may be very low in dense orchards (Jackson, 1980).

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1.8.2. Bagging

Bagging is the practice of enclosing young fruitlets in several layers of paper to promote colour development after the bag is removed just prior to harvest. Proctor and Lougheed (1976) suggested an optimum time for bag removal to obtain maximum red colour formation, possibly the time of the respiratory preclimateric minimum respiration rate and the onset of the ripening phase. The optimum temperature for colouration of bagged fruit is 20-25°C (Arakawa, 1991). Covering apples with foil bags for about 1 month after full bloom to harvest had no effect on fruit size or starch content, had inconsistent effects on fruit firmness, and reduced soluble solids, anthocyanin and chlorophyll levels (Proctor and Lougheed, 1976). Fruit bagging significantly inhibited both PAL activity and anthocyanin synthesis, while bag removal enhanced both (Ju et al., 1995). Arakawa et al. (1985) showed that under white + UV312 light, bagged fruit which had been covered with paper bags since about one month after flowering produced much higher anthocyanin levels at immature and mature stages than non-bagged ones. Proctor and Lougheed (1976) suggested that the better colour formation, which was obtained by the use of bagging, was due to the high anthocyanin to chlorophyll ratio. Saure (1990) concluded that the better fruit colour was rather the effect of decreased chlorophyll concentrations, than a higher anthocyanin concentration.

1.8.3. Post harvest irradiation of fruit

Very little work, if any, has been done on postharvest irradiation treatments on pears, therefore I will discuss only apples. There are two phases in anthocyanin synthesis that depend on irradiation. Siegelman and Hendricks (1958) found a lag phase of 20 hours in apples. This phase is followed by a linear period of about 100 hours, in which anthocyanin formation is a function of time and continuous irradiation (Mancinelli, 1983; Siegelman & Hendricks, 1958). Dong et al. (1995) irradiated 'Royal Gala' apples post harvest with UV- and white light. They found a red-striped pattern with white light only. However, when UV-light (150 $\mu W.cm^{-2}$) and white light were applied together they observed a significant increase in the red colouring. The relative changes in the fruit colour (a *-values) and the total amount of extracted anthocyanin during the irradiation

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period, were the same. Saks et al. (1990) irradiated 'Anna' apples with white-fluorescent light (14.5 $W.m^{-2}$) at 2, 13, and 20°C. They observed the most intense fruit colour at 13°C after three days of irradiation, and also found that the colouring reaction was the best at 20°C after one day of irradiation.

1.9. INFLUENCE OF TEMPERATURE ON ANTHOCYANIN BIOSYNTHESIS

1.9.1. General

Temperature determines the rate of anthocyanin synthesis (Creasy, 1968). Low temperatures enhance, and high temperatures inhibit anthocyanin synthesis (Mazza & Miniati, 1993). Saks et al. (1990) concluded that there is an inverse relationship between anthocyanin synthesis and temperature. Faragher and Brohier (1983) concurred, but they also concluded that the increasing anthocyanin levels might be the result of the higher ethylene levels during maturation. According to Siegelman and Hendricks (1958), temperature influences both the rate of anthocyanin synthesis and the induction period before anthocyanin synthesis starts. Biran and Halevy (1974) reported that high temperature (36°C) during the development of 'Bacara' roses decreased red colour. The colour did not increase at the optimum temperature (18°C) for anthocyanin synthesis.

The effect of temperature varies for the fruit on the tree and for the fruit after harvest, depending on maturity, light intensity, cultivar and stage of fruit development (Mazza & Miniati, 1993). Biran and Halevy (1974) suggested that stress conditions such as high temperatures decreased the availability of sugars. The high tissue temperatures blocked the transport of sugars to the fruit, and thus anthocyanin synthesis (Kliewer, 1977). The better colour at low temperatures is ascribed to the reduced respiration rate at these temperatures, leading to the lower consumption of carbohydrates, and therefore an increase in the available carbohydrates for anthocyanin synthesis (Lancaster, 1992; Mazza & Miniati, 1993; Varga & Wertheim, 1976). According to Macheix (1990) continuous temperatures of 15°C are very effective in the colouring of apples. Alternating temperatures of 6°C and 18°C doubled the anthocyanin concentration in fruit compared to a constant 18°C (Mazza & Miniati, 1993).

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1.9.2. Phenylalanine ammonia -lyase

Temperature plays an important part in the regulation of PAL (Macheix et al., 1990). Low temperatures (6°C) stimulate PAL and anthocyanin synthesis in apple peel (Tan, 1979). Tan (1979) suggests that PAL-IS is activated at high temperatures and inhibited at low temperatures. It is well established that low temperatures are necessary to insure good colouration in apples (Magness, 1928). Low temperatures (6°C) generally reduced the level of PAL-IS. Cold treatments might destroy or suppress the development of PAL-IS; therefore low temperatures result in anthocyanin accumulation. The initial rate of PAL-IS accumulation is higher under very high temperatures (25°C and above), and under these high temperatures it also declines at a faster rate after its peak value has been reached (Saure, 1990; Tan, 1980). According to Faragher (1983) the regulation of PAL is rather the effect of higher inhibition levels for PAL at high temperatures than the effect of direct stimulation of PAL at low temperatures.

1.10. MECHANICAL MANAGEMENT PRACTISES WHICH INFLUENCE TEMPERATURE AND THUS FRUIT COLOUR

1.10.1. Evaporative cooling

Evaporative cooling involves application of water through an overhead sprinkler system to the leaves and fruit of the tree. The water evaporates from the fruit surface using heat (Evans, 1993). Evaporative cooling increases red peel colour, soluble solids and titratable acids of 'Fuji' apples (Andrews, 1993; Warner, 1993). Evaporative cooling can also delay maturation and harvest, which adds to increased colour and size (Hinman, 1993).

The production of anthocyanins (idaein) depends on the carbon products, mainly carbohydrates, formed during photosynthesis, and glucose metabolism. During very hot weather, the leaves shut down photosynthesis in the day, and the carbohydrate concentration decreases. By the use of evaporative cooling the temperature can be regulated to obtain maximum colour development (Williams & Mayles, 1993). Andrews

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(1993) found that the net photosynthesis was about 30% higher for evaporatively cooled trees, compared to uncooled trees. He also concluded that the stomatal conductance of the evaporatively cooled trees had increased about 35% since the morning measurement, whereas the stomatal conductance of uncooled leaves had decreased about 10% in the same period.

The activity of phytochrome, the photoreceptor in the light dependent reaction in anthocyanin biosynthesis, decreases at high temperatures. This effect could be stabilised by the use of evaporative cooling (Saure, 1990). According to Williams and Mayles (1990) evaporative cooling commence when the air temperature is 31°C and internal core temperature is 34°C to 36°C. The irrigation system should be turned off when the internal core temperature has decreased by 3°C (Williams & Mayles, 1990).

1.11. CONCLUSION

The producer must have a basic knowledge of the factors influencing anthocyanin metabolism as well as the different management practices influencing fruit colour, to produce fruit with optimum red colour. There is still a lot of work to be done regarding the regulation of anthocyanin biosynthesis in apples, but especially in pears and more specifically on the bi-coloured cultivars like 'Forelle', 'Rosemarie' and 'Flamingo'. Management - and manipulation practices at the right time should result in well coloured fruit and thus higher income.

Producers cannot change the climate of the fruit producing areas, but they can farm more effectively with light. They can achieve this by managing the light interception and light distribution within the tree canopies. Red- and bi-coloured fruit are realising good prices on the overseas markets, thus producers have to use management- and manipulation practices to gain from this. If producers do not act accordingly, South Africa will not be able to compete on the overseas markets. This will have huge economic implications for the fruit industry.

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By the use of summer pruning unnecessary branches and shoots are removed. This improves light distribution, and thus fruit colour, because of the more direct light on the fruit at earlier stages of fruit development. Root pruning is not very practical because of the small root systems in high-density plantings. It also has a negative effect on fruit size.

Trees have to be planted in a north-south row direction. By doing this, more direct and uniform light distribution on the fruit for a longer period will result in optimum red colouration. The correct trellis system for optimum colour is very important. Planar systems like the V, Y and Tatura intercept the most light because of a higher leaf area index (LAI). These systems are, however, more expensive than the traditional central leader system. Fruit thinning is feasible, but the advantages must compensate for the potential exportable fruit that will be lost. Thinning (fruit and leaf) results in better coloured fruit, because of the more exposed fruit. Anthocyanin formation must be stimulated with light in the small fruit because pears seem to lose their red colour as they mature.

Dwarfing rootstocks must be used to minimize vegetative growth, expose more fruit to direct light and benefit from this in fruit size and soluble solids, and consequently in more red coloured fruit. The M.9 rootstock is not recommended for South African soils and M793 is the generally used rootstock, however a suitable dwarfing rootstock must be found especially for red apple cultivars. The 'Quince A-Beurre Hardy' interstock combination, which is compatible with the red- and bi-coloured pear cultivars, is recommended for pears (J. Jacobs, personal communication).

High potassium and calcium, low nitrogen, phosphorus and manganese levels enhance anthocyanin formation and thus red colouration in fruit.

Chemicals such as Alar®, Paclobutrazol®, and Cycocel® can retard shoot growth, which stimulates red colour development due to better light penetration. Ethephon is an ethylene-releasing chemical, which also results in better-coloured fruit, but unfortunately also in softening.

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Bagging is not a common practice because it is very expensive and labour intensive. The use of reflective material is more economic than bagging. Too expensive, is however, a relative concept as one looks at the successful results with the use of these two techniques on high income cultivars like 'Forelle','Rosemarie', 'Flamingo' and 'Cripps Pink'.

Post harvest irradiation of fruit is in theory, and thus for research purposes, very interesting. Very little research has been done on post harvest irradiation treatments on pears, while post harvest treatments on apples showed very promising results. This method is, however, not yet commercially implemented because of its financial implications. Post harvest irradiation has great potential, especially on the high income cultivars. Great care should however be taken on the effect of irradiation on fruit quality and senescence.

When used correctly, overhead cooling could increase red colour, but it can cause more problems than it solves. Most of the work on evaporative cooling has been done on apples, thus work on pears would be of great interest. The use of water for cooling is a luxury consumption of water, and growers in S.A. must be aware of the pressures on the industry to conserve water.

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2. PAPER I - THE EFFECT OF SUMMER PRUNING, PINCHING AND SPUR LEAF REMOVAL ON COLOUR DEVELOPMENT OF 'ROSEMARIE' AND 'FORELLE' PEARS.

Abstract

Development of red peel colour in 'Rosemarie' pears is dependent on adequate light distribution within the tree canopy. The effect of summer pruning on the colour development of 'Rosemarie' pear fruit was studied over two seasons in a commercial orchard in the Grabouw area of the Western Cape, South Africa (34°55'S; 19°02'E). The treatments consist of an (1) unpruned control, (2) a late October pruning, 37 days after full bloom (DAFB), (3) an early November pruning (60 DAFB), (4) an early December pruning (84 DAFB), (5) an early January pruning (109 DAFB), and (6) a combination of above dates. Pruning consisted of removing the upright shoots of the current season's growth on the lateral branches. The effect of the time of summer pruning on Class 1 packout percentage and average fruit mass were determined. The percentage blushed fruit (colour grading 1-10) of the trees pruned in November or pruned repeatedly were significantly higher than for unpruned control trees or trees pruned at other times. Fruit mass was not affected by summer pruning. A second study was conducted on 'Rosemarie' and 'Forelle' pears in their fifth and fourth leaf in commercial orchards in the Koue Bokkeveld area of the Western Cape, South Africa (33°10'S; 19°20'E). The treatments consisted of non-pinched and pinched, where the bourse shoots were cut back at petal drop. There were no significant differences in fruit colour, fruit size, flesh firmness and total soluble solids after pinching compared to the control. In a third study on 'Rosemarie' and 'Forelle' trees, bourse shoots were removed in combination with leaf defoliation. Spur leaves were removed at different times throughout the season from petal drop towards harvest. Both bourse shoots ('Rosemarie') or one bourse shoot ('Forelle') per cluster was removed as control, one treatment were no bourse shoots were removed served as a secondary control. Spur leaf defoliation on 'Rosemarie' and 'Forelle' did not have any significant effect on fruit set, fruit size or total soluble solids. In 'Rosemarie', there was no significant effect on fruit colour, in terms of L, C and H-values or colour chart ratings. In 'Forelle', the ratings on the Capespan colour chart, P 16, the L, C and H-values decreased significantly between unmanipulated branches (control 1) and branches where one bourse shoot was removed (control 2), indicating better coloured fruit as is also confirmed by the higher class 1 packout. However,

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all treatments compared to control 1, improved red colour, indicated by a significant decrease in the H – values and increase in class 1 packout.

INTRODUCTION

Light is essential for anthocyanin formation, as indicated by the poor colour formation in the interior of the tree (Ferree, 1998; Macheix et al., 1990; Saure, 1990). Pears are even more dependent on light than apples for colour formation (Wagenmakers, 1987). A high positive correlation exists between the amount of direct sunlight received with both colour intensity and the area of the blush on apple fruit (Jacyna, 1978). Two radiation dependent phases can be distinguished in anthocyanin synthesis in apple peel. There is an induction period of 20 hours before the onset of anthocyanin formation. After the induction period, anthocyanin increases linearly with time under continuous irradiation (Faragher & Chalmers, 1977; Siegelman & Hendricks, 1958). There are two peaks of anthocyanin formation in apples: a first peak during the phase of intense cell division in the fruit, and a second peak coinciding with maturation of the fruit and contributing most to the final colour (Lancaster, 1992; Saure, 1990). In bi-colour pears, which do not display the second peak of anthocyanin synthesis under South African climatic conditions, the fruitlets should be exposed to direct sunlight during the period from petal drop to approximately 40 days thereafter to ensure optimum colour development (Viljoen, 1996).

According to Dayton (1966) the red colour in fruit is determined by the anthocyanin concentration in the vacuoles of epidermal cells in apples or in the vacuoles of the cells in the third to fifth cell layers in pears. The best coloured fruit are found in parts of the canopy receiving 70-80% full sunlight (approximately 1120 $\mu mol.m^{-2}.s^{-1}$ at mid day under S.A conditions), medium coloured fruit at 40-70% and worst coloured fruit in parts receiving less than 40% full sunlight (Barrit et al., 1987; Jacyna & Soczek, 1980; Macheix et al., 1990). According to Saure (1990), lower light intensities have a more negative effect on colour development in early apple cultivars than in late ones. This might be due to a longer period of maturation in late apple cultivars, thus a longer period for anthocyanin accumulation. The intensity of incident photosynthetically active radiation (PAR) within the canopy is decreased primarily by the mutual shading of leaves (Palmer, 1977), which according to

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Chandler and Watson (1954) decreases the sugar content of plants and thus leads to a decrease in pigmentation. Light interception is the driving force for photosynthesis and there is a linear relationship between light interception and dry mass production (Johnson & Lakso, 1991) and the distribution of light influences fruit quality (Warner, 1993).

The quality of the light, thus the wavelengths of light, is also important in colour development (Arakawa et al., 1985; Fletcher, 1929; Magness, 1928; Siegelman and Hendricks, 1958). Researchers have determined that blue-violet (BV) and ultraviolet (UV-A) (320-400 nm), but especially UV-B (280-320 nm), were the most effective at inducing colour, red (R) (600-690 nm) and blue light were less effective, and far-red (FR) (710-760 nm) was the least effective and even inhibitory to colour development (Faragher & Chalmers, 1977; Fletcher 1929; Heller & Forkmann, 1988; Magness, 1928; Mazza & Miniati, 1993 and Saure, 1990). In contrast, however, Siegelman and Hendricks (1958) found that within the visible light, the red light spectrum between 640 and 670 nm was more effective than other wavelengths for anthocyanin production. Arakawa et al. (1985) found that combining UV-B light with white or red light is synergistic in anthocyanin synthesis while combining UV-B with blue light was only additive. There is more UV radiation in the upper half of the tree. The poor penetration of UV-B light into the canopy is probably the reason for the poor colour formation on fruit within the tree canopy (Arakawa et al., 1986). The dust and moisture in the air absorb UV-light and anthocyanin synthesis is stimulated after periods of rain and cloudy weather conditions (Magness, 1928; Saure, 1990). The effectiveness of different wavelengths of light in stimulating colour development is however influenced by the stage of fruit development (Saure, 1990).

Phytochrome is responsible for anthocyanin synthesis with R and FR radiation (Mancinelli et al., 1991). Phytochrome is a pigment bound to a protein and acts as a photoreceptor in the light-dependent anthocyanin synthesis (Mancinelli, 1985). Downs et al. (1965) suggested that besides phytochrome the photosynthesis system is also a photoreceptor promoting colour development. UV-B light is able to stimulate anthocyanin synthesis on its own, thus independent of phytochrome (Arakawa et al., 1985). However, Arakawa (1988) showed that the reaction to UV - B light was accelerated when it was followed by a radiation period with R light. This effect is reversible with FR treatments. It seems that photosynthesis plays a role in this reaction (Mancinelli & Rabino, 1984).

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Summer pruning involves the removal of all non-bearing vertical shoots and the pruning back of horizontal shoots to leave 5-10 cm stumps of the new growth (Marini & Barden, 1982b; Warrington et al., 1984). Summer pruning increases light intensity in the cropping zone of the canopy and generally improves red colour development of fruit (Ferree, 1981; Jackson, 1980; Lord & Greene, 1982; Marini & Barden, 1982b; Morgan et al., 1984). The effectiveness of summer pruning is dependent on the cultivar, growing season, and crop load, but particularly the timing and severity of the summer pruning (Marini and Barden, 1982b). In apples, summer pruning decreased gibberellic acid levels thereby removing its inhibitory effect on anthocyanin synthesis (Lord & Greene, 1982; Saure, 1990). Summer pruning on mature trees where radiation penetration is inadequate and blush development is poor has been shown to improve red colour development of apples (Aselage & Carlson, 1977; Jackson, 1980; Morgan, et al., 1984; Tymoszuk, et al., 1984) and in 'Forelle' pears (Dussi & Huysamer, 1995).

Other benefits of summer pruning are faster picking rates, less orchard culling, better spray penetration and lower pesticide volumes (Dussi & Huysamer, 1995). However, negative effects i.e. sunburn and a reduction in fruit size may occur if summer pruning is too severe or badly timed (Miller, 1982; Tymozuk, et al., 1984).

This paper reports on a study undertaken to determine the effect of different times of summer pruning, bourse shoot pinching and spur leaf removal on colour development in 'Rosemarie' pears.

MATERIALS AND METHODS

Plant material

Summer pruning trial: The trial was performed in a commercial orchard situated in the Grabouw area of the Western Cape, South Africa (34°55'S; 19°02'E). The area is characterised by a Mediterranean climate with cold, wet winters and warm, dry summers. 'Rosemarie' trees in their sixth leaf on BP 1 rootstock were used. The trees were planted in 1992 at a spacing of 4 m x 2 m (1250 trees.ha^{-1}) in a North–South row orientation. The trees were trained to a trellised central leader.

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Pinching and spur leaf removal trials: These trials were conducted on 'Rosemarie' and 'Forelle' pears in their fifth and fourth leaf, respectively in commercial orchards in the Koue Bokkeveld, Western Cape (33°10'S; 19°20'E). This area has a Mediterranean climate with wet winters and dry hot summers. The 'Rosemarie' trees on BP 1 rootstock were planted at a spacing of 4.5 m x 1.5 m (1481 trees.ha^{-1}) in a North-South row orientation. The 'Forelle' trees on BP 1 rootstock were planted at a spacing of 4.5 m x 1.5 m (1481 trees.ha^{-1}) in an East-West row orientation. The trees of both orchards were trained to a freestanding central leader.

Experimental design and treatments

Summer pruning trial: A randomised complete block design was used with six treatments and six single tree replicates. The treatments were: (1) control, where no summer pruning was done; (2) a late October pruning, 37 days after full bloom (DAFB); (3) an early November pruning (60 DAFB); (4) an early December pruning (84 DAFB); (5) an early January pruning (109 DAFB); and (6) a combination of above dates. The pruning consisted of removing the current season's upright growth from the lateral branches. The trial was first performed in 1997 and repeated on the same trees in 1998. The October pruning treatment, 37 days after full bloom (DAFB) was omitted in 1997.

'Rosemarie and Forelle' pinching trial: A randomised complete block design was used with two treatments and nine replicates. The treatments consist of non-pinched and a pinched treatment, where all bourse shoots on all the fruit clusters per tree were cut back to three leaves at petal drop (PD) (approximately 3.5 cm remaining).

'Rosemarie and Forelle' bourse shoot removal and defoliation trial: A randomised complete block design was used with ten blocks and sixteen treatments. Two bearing units were chosen on each tree, one on the eastern and one on the western side. Treatments for 'Rosemarie' and 'Forelle' are summarised in Tables 1 and 2, respectively.

Data collected

Summer pruning trial: The trees were strip-picked at harvest and fruit were individually graded for colour using the Capespan (P.O. Box. 505, Bellville, 7530) colour chart P 26. The colour chart rates fruit blush from 1 to 12 with 1 the best and 12 the worst coloured fruit. Originally fruit categorised as 1 to 9 was marketed as Cape export quality and 10 to 12 as

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Crown for the local market. Marketing standards were changed in 1998 to include category 10 as export. The class 1 packout (Cape) of each treatment was determined by converting the number of fruit categorised from 1 to 9 or 1 to 10 as a percentage of the total number of fruit per tree. Fruit size and other defects were not considered in determining the class 1 packout. Average fruit mass was determined.

'Rosemarie' and 'Forelle' pinching trial: At harvest ten fruit per tree were analysed. The following data were recorded on each sample: (1) fruit diameter, measured with a caliper, (2) total soluble solids, expressed as percentage Brix as measured with a refractometer, (3) colour development using the Capespan (P.O. Box. 505, Bellville, 7530) colour chart, P 26 for 'Rosemarie' and P 14 for 'Forelle'. The colour chart, rates fruit from 1 to 12 with 1 the best and 12 the worst coloured fruit. Fruit categorised as 1 to 10 are marketed as Cape export quality and 11 to 12 as Crown.

'Rosemarie' and 'Forelle' bourse shoot removal and defoliation trial: At harvest the bearing units of each tree were picked separately, fruit set determined and further data recorded as above. In addition a Minolta colorimeter (model NR-3000, Nippon Denshoku Kogyo Co., Tokyo, Japan) was used to measure the colour of the fruit. Chromaticity (fruit colour variables independent of brightness) was recorded in L*, a* and b* colour space coordinates (Dussi et al., 1995). The meter was calibrated at illuminant condition C (6774K) with a white standard (Minolta calibration plate). The a* and b* values were converted to hue angle ($\tan^{-1} a^*/b^*$) and chroma ($(a^{*2} + b^*2)^{1/2}$) (McGuire, 1992). Hue angle is a measure of colour, with 0 = red, 90 = yellow, 180 = green and 270 = blue, chroma is a measure of intensity or vividness of the colour, and L* is a measure of the lightness of the colour.

Data analysis

The data were analysed using the GLM (General Linear Models) Procedure of the Statistical Analysis Systems (SAS) Program (SAS Inc., 1990). Packout percentages were transformed using the arcsin √(percentage) transformation before data analysis.

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RESULTS AND DISCUSSION

Summer pruning trial

Compared to the unpruned control trees summer pruning did not significantly increase the percentage fruit in the categories 1 to 9 in 1997 and 1998 (Table 3). In both years however there was a slight increase in the percentage of well-coloured fruit following summer pruning, except where summer pruning was delayed until January. When fruit in category 10 were included early summer pruning and repeated pruning in 1997 improved the pack-out, but not in 1998.

The efficacy of the early and repeated pruning on improving the red colour is possibly related to the seasonal pattern of anthocyanin synthesis. Viljoen (1996) found that the synthesis of anthocyanin in pears occurred predominantly during the first 40 days after petal drop and that light is essential for colour development. Improving light penetration of trees by mid to late summer pruning would therefore be less effective due to the reduced ability of the fruit to synthesise anthocyanin. It is also possible that gibberellins produced by the young leaves, which inhibit anthocyanin synthesis were reduced. The packout percentages in 1998 were higher than in 1997. This may be an indication of an improved penetration of light into trees and the reason why pruning failed to improve colour of the fruit compared to the unpruned control.

Fruit size was not affected by the pruning treatments, which removed relatively few leaves located near the fruit. Fruit size and soluble solids of apple (Lord & Greene, 1982) and peach (Marini & Barden, 1982a) were reduced by severe heading cuts near the fruit, but not by thinning cuts at a greater distance from the fruit. It seems that adequate leaf area near the fruit is important for fruit development (Marini & Barden, 1982a). In contrast it was shown in several studies that early summer pruning decreased fresh weight of fruit and total soluble solids (Morgan et al., 1984). In other studies summer pruning did reduce fruit size and soluble solids due to the loss of leaf area (Lord & Greene, 1982; Marini & Barden, 1982a; Morgan et al., 1984). Early or repeated pruning to improve the exposure of fruit to sunlight may be beneficial for colour development in vigorous 'Rosemarie' pear trees.

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'Rosemarie' and 'Forelle' pinching trial

Pinching to enhance the exposure of fruit to light did not affect yield, fruit size, flesh firmness and class 1 packout percentages of 'Rosemarie' and 'Forelle' (Tables 4 and 5). A small but significant reduction in total soluble solids was found in response to pinching in 'Rosemarie', but not in 'Forelle'. The lack of a significant improvement in red colour formation might be due to high temperatures (above 30°C), resulting in the degradation of anthocyanin concentration (W. Steyn, personal communication). It seems that apart from light, temperature is the most important factor, influencing red colour formation. Therefore, it is important to expose the fruitlets to direct sunlight during the first period of anthocyanin formation (petal drop to approximately 40 days after petal drop) to optimise red colour development, and that temperatures should be below 30°C until harvest to minimise anthocyanin degradation.

'Rosemarie' bourse shoot removal and defoliation trial

Spur leaf defoliation on 'Rosemarie' did not have any significant effect on fruit set, fruit size or total soluble solids (Table 6). There was also no significant effect on fruit colour, in terms of L, C and H-values or colour chart ratings (Table 7).

'Forelle' bourse shoot removal and defoliation

Spur leaf defoliation on 'Forelle' did not have any significant effect on fruit set, fruit size or total soluble solids (Table 8). The ratings on the Capespan colour chart, P 16, the L, C and H-values decreased significantly between unmanipulated branches (control 1) and branches were one bourse shoot was removed (control 2), indicating better coloured fruit. This was confirmed by the higher class1 packout (Table 9). However, all treatments compared to control 1, improved red colour, indicated by a significant decrease in the H – values and increase in class 1 packout.

The number of leaves necessary for colour development differs between apple cultivars; 20-25 leaves per fruit in 'Jonathan' and 40 leaves per fruit in 'Delicious' were necessary for colour development and adequate fruit size. In several cultivars with 10–20 leaves per fruit, colour development was insufficient despite optimum light conditions (Magness, 1928). Greater leaf area per fruit resulted in a higher proportion of well-coloured fruit in 'Jonagold' apples (Wertheim, 1987). The defoliation of 'Fuji' apples 51 days before harvest increases

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the anthocyanin concentration, due to better light exposure, however no literature could be found on the effect of defoliation on red colour development of pear fruit. It appears that pears are less sensitive to defoliation in terms of fruit size than apples as the average size of 'Rosemarie' and 'Forelle' was not negatively influenced, even with the most severe treatments. This indicates that long distance transport of photosynthates from non-defoliated branches on the tree is adequate to support fruit growth on defoliated branches.

The disappointing improvement in red colour formation in 'Rosemarie' may be due to an over exposure of fruitlets to high light intensities and high temperatures (above 30°C), resulting in the degradation of anthocyanin. In direct sunlight fruit temperatures can be 15°C higher than ambient temperature. At higher temperature a greater amount of energy is required to synthesize anthocyanin, i.e. that the lower efficiency of light at higher temperature must be substituted by more light for comparable results (Saure, 1990).

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Table 1. Summary of treatments in defoliation trails for 'Rosemarie'.

Treatment code	Treatment	Date
Petal Drop (PD)		
Control 1	No bourse shoots or spur leaves removed	22 Sept. 1998
Control 2	Two bourse shoots removed	22 Sept. 1998
7 Days after PD		
1 leaf	Two bourse shoots and one spur leaf removed	28 Sept. 1998
2 leaves	Two bourse shoots and two spur leaves removed	28 Sept. 1998
14 Days after PD		
1 leaf	Two bourse shoots and one spur leaf removed	5 Oct. 1998
2 leaves	Two bourse shoots and two spur leaves removed	5 Oct. 1998
21 Days after PD		
1 leaf	Two bourse shoots and one spur leaf removed	12 Oct. 1998
2 leaves	Two bourse shoots and two spur leaves removed	12 Oct. 1998
28 Days after PD		
1 leaf	Two bourse shoots and one spur leaf removed	19 Oct. 1998
2 leaves	Two bourse shoots and two spur leaves removed	19 Oct. 1998
35 Days after PD		
1 leaf	Two bourse shoots and one spur leaf removed	26 Oct. 1998
2 leaves	Two bourse shoots and two spur leaves removed	26 Oct. 1998
42 Days after PD		
1 leaf	Two bourse shoots and one spur leaf removed	2 Nov.1998
2 leaves	Two bourse shoots and two spur leaves removed	2 Nov.1998
49 Days after PD		
1 leaf	Two bourse shoots and one spur leaf removed	9 Nov. 1998
2 leaves	Two bourse shoots and two spur leaves removed	9 Nov. 1998

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Table 2. Summary of treatments in defoliation trails for 'Forelle'.

Treatment code	Treatment	Date
Petal Drop (PD)		
Control 1	No bourse shoots or spur leaves removed	1 Oct. 1998
Control 2	One bourse shoot removed	1 Oct. 1998
7 Days after PD		
1 leaf	One bourse shoot and one spur leaf removed	8 Oct. 1998
2 leaves	One bourse shoot and two spur leaves removed	8 Oct. 1998
14 Days after PD		
1 leaf	One bourse shoot and one spur leaf removed	15 Oct. 1998
2 leaves	One bourse shoot and two spur leaves removed	15 Oct. 1998
21 Days after PD		
1 leaf	One bourse shoot and one spur leaf removed	22 Oct. 1998
2 leaves	One bourse shoot and two spur leaves removed	22 Oct. 1998
28 Days after PD		
1 leaf	One bourse shoot and one spur leaf removed	29 Oct. 1998
2 leaves	One bourse shoot and two spur leaves removed	29 Oct. 1998
35 Days after PD		
1 leaf	One bourse shoot and one spur leaf removed	5 Nov. 1998
2 leaves	One bourse shoot and two spur leaves removed	5 Nov. 1998
42 Days after PD		
1 leaf	One bourse shoot and one spur leaf removed	12 Nov. 1998
2 leaves	One bourse shoot and two spur leaves removed	12 Nov. 1998
49 Days after PD		
1 leaf	One bourse shoot and one spur leaf removed	19 Nov. 1998
2 leaves	One bourse shoot and two spur leaves removed	19 Nov. 1998

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Table 3. The effect of different times of summer pruning on colour and fruit mass of 'Rosemarie' pears.

	df	Capespan chart, P26 (1- 9)				Capespan chart, P26 (1- 10)				Fruit mass (g)			
		1997		1998		1997		1998		1997		1998	
TREATMENT													
Control		49.60	a	67.82	ab	57.15	c	75.23	b	125.55	ab	168.29	a
22 October		-		72.43	ab	-		81.33	ab	-		171.07	a
14 November		59.48	a	74.13	ab	69.87	ab	80.80	ab	124.38	ab	166.21	a
8 December		58.56	a	75.50	ab	68.08	abc	81.35	ab	130.42	a	167.68	a
2 January		47.95	a	63.30	b	58.48	bc	71.60	b	122.02	b	170.93	a
Combination		59.65	a	79.65	a	71.38	a	87.13	a	126.02	ab	163.37	a
SIG. LEVEL													
Treatment (1997)	4	0.1885		-		0.0701		-		0.1201		-	
Treatment (1998)	5	-		0.2056		-		0.1174		-		0.8631	
Contrasts													
Control vs Pruned	1	0.2721		0.3077		0.2129		0.2211		0.5036		0.9342	
Pruned linear	1	0.8200		0.2231		0.5320		0.1208		0.1745		0.9412	
Pruned quadratic	1	0.0534		0.1543		0.0399		0.2730		0.0252		0.4156	
Single vs Multiple	1	0.2725		0.1099		0.0819		0.0509		0.8091		0.3100	

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Table 4. The effect of pinching of 'Rosemarie' trees on fruit diameter, fruit mass, firmness, total soluble solids, class1 packout percentage and production.

	df	Fruit diam. (mm)	Fruit mass (g)	Flesh firmness (kg)	TSS (% brix)	% Class1 packout (P26)	Production (fruit per cm circum.)
TREATMENT							
Control		61.13	143.33	15.89	13.88	48.97	5.88
Pinched		63.00	142.37	15.76	13.08	53.46	6.13
SIG. LEVEL							
Treatment	1	0.1993	0.8513	0.6887	0.0076	0.4669	0.7716
LSD (5 %)		2.957	10.732	0.664	0.559	12.214	1.827

Table 5. The effect of pinching of 'Forelle' trees on fruit diameter, fruit mass, firmness, total soluble solids, class1 packout percentage and production.

	df	Fruit diam. (mm)	Fruit mass (g)	Flesh firmness (kg)	TSS (% brix)	% Class1 packout (P16)	Production (fruit per cm circum.)
TREATMENT							
Control		56.94	108.86	14.17	15.54	63.08	5.46
Pinched		55.74	105.77	14.23	15.65	66.58	6.33
SIG. LEVEL							
Treatment	1	0.2901	0.5721	0.4108	0.2587	0.4744	0.3507
LSD (5 %)		2.325	11.36	0.1701	0.1971	10.132	1.916

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Table 6. The effect of defoliation of 'Rosemarie' pears on fruit set, fruit size and total soluble solids.

	df	Fruit set (%)	Fruit diam. (mm)	TSS (% brix)
TREATMENT				
Petal Drop (PD)				
Control 1 *		15.70	53.68	12.08
Control 2 *		16.70	49.67	10.54
7 Days after PD				
1 Leaf removed		16.02	53.69	12.06
2 Leaves removed		21.18	55.85	11.13
14 Days after PD				
1 Leaf removed		13.80	47.66	10.60
2 Leaves removed		13.51	57.28	12.39
21 Days after PD				
1 Leaf removed		25.48	52.08	10.93
2 Leaves removed		19.13	43.59	9.62
28 Days after PD				
1 Leaf removed		23.09	57.33	12.33
2 Leaves removed		25.54	53.94	12.06
35 Days after PD				
1 Leaf removed		17.77	54.40	11.91
2 Leaves removed		12.54	53.91	12.43
42 Days after PD				
1 Leaf removed		18.56	59.61	13.25
2 Leaves removed		14.88	37.93	8.30
49 Days after PD				
1 Leaf removed		15.39	59.14	13.78
2 Leaves removed		15.73	57.23	12.01
SIG. LEVEL				
Treatment	15	0.0431	0.0876	0.1601
Contrasts				
Contr1 vs Contr2	1	0.8187	0.5410	0.3477
Controls vs Rest	1	0.4270	0.6800	0.7084
One vs Two	1	0.5055	0.1641	0.1170
Time Linear	1	0.3752	0.5974	0.2996
Time Quadr.	1	0.0517	0.2282	0.3813
*Treat * Linear*	1	0.3787	0.0554	0.1103
*Treat * Quadr.*	1	0.9522	0.5132	0.2725

P = 0.05

* No bourse shoots or spur leaves removed

** Two bourse shoots removed, but no spur leaves

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Table 7. The effect of defoliation of 'Rosemarie' pears on colour and class 1 packout percentage.

	df	L –value	C –value	H –value	Class 1 %
TREATMENT					
Petal Drop (PD)					
Control 1 *		51.21	36.16	69.78	45.49
Control 2 **		42.17	30.13	52.77	48.14
7 Days after PD					
1 Leaf removed		43.59	32.08	51.12	65.92
2 Leaves removed		49.61	36.13	64.02	46.24
14 Days after PD					
1 Leaf removed		40.69	31.47	46.71	57.54
2 Leaves removed		50.24	37.02	61.64	70.49
21 Days after PD					
1 Leaf removed		46.73	33.95	57.37	48.21
2 Leaves removed		41.73	31.23	51.33	58.08
28 Days after PD					
1 Leaf removed		52.09	37.29	71.36	40.33
2 Leaves removed		49.21	36.06	61.59	61.92
35 Days after PD					
1 Leaf removed		48.29	36.41	58.58	58.07
2 Leaves removed		49.69	35.54	62.36	49.55
42 Days after PD					
1 Leaf removed		52.59	39.31	64.40	69.22
2 Leaves removed		36.91	27.04	48.92	30.79
49 Days after PD					
1 Leaf removed		49.34	36.24	64.29	64.24
2 Leaves removed		49.24	36.48	62.08	59.34
SIG. LEVEL					
Treatment	15	0.4072	0.4352	0.2179	0.0275
Contrasts					
Contr1 vs Contr2	1	0.1590	0.1859	0.0638	0.8154
Contols vs Rest	1	0.8955	0.5138	0.6379	0.1435
One vs Two	1	0.6919	0.5461	0.9363	0.3663
Time Linear	1	0.4998	0.5053	0.2481	0.7333
Time Quadr.	1	0.9237	0.9967	0.8753	0.2189
*Treat * Linear*	1	0.0665	0.0615	0.0472	0.2013
*Treat * Quadr.*	1	0.9013	0.8899	0.9288	0.7546

P = 0.05

* No bourse shoots or spur leaves removed

** Two bourse shoots removed, but no spur leaves

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Table 8. The effect of defoliation of 'Forelle' pears on fruit set, fruit size and total soluble solids.

	df	Fruit set (%)	Fruit diam. (mm)	TSS (% brix)
TREATMENT				
Petal Drop (PD)				
Control 1 *		20.27	50.89	13.91
Control 2 **		15.97	49.62	14.03
7 Days after PD				
1 Leaf removed		17.46	50.91	14.06
2 Leaves removed		12.15	50.03	14.15
14 Days after PD				
1 Leaf removed		15.97	56.28	15.75
2 Leaves removed		15.76	56.79	15.74
21 Days after PD				
1 Leaf removed		15.64	55.41	15.63
2 Leaves removed		18.65	55.75	15.59
28 Days after PD				
1 Leaf removed		19.73	53.39	14.94
2 Leaves removed		16.23	54.29	14.78
35 Days after PD				
1 Leaf removed		12.74	51.07	12.36
2 Leaves removed		12.23	54.16	14.14
42 Days after PD				
1 Leaf removed		14.52	53.11	14.88
2 Leaves removed		17.26	50.68	14.11
49 Days after PD				
1 Leaf removed		15.96	47.31	13.36
2 Leaves removed		20.37	53.97	14.81
SIG. LEVEL				
Treatment	15	0.8049	0.7290	0.4288
Contrasts				
Contr1 vs Cont 2	1	0.3151	0.7497	0.9600
Contr1. vs Rest	1	0.1888	0.5642	0.5480
One vs Two	1	0.9596	0.4819	0.4997
Time Linear	1	0.6486	0.3167	0.1528
Time Quadr.	1	0.8456	0.0894	0.3941
*Treat * Linear*	1	0.1904	0.4052	0.5238
*Treat * Quadr.*	1	0.7898	0.2882	0.3032

P = 0.05

* No bourse shoots or spur leaves removed

** One bourse shoot removed, but no spur leaves

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Table 9. The effect of defoliation of 'Forelle' pears on colour and class 1 packout percentage.

	df	L –value	C –value	H –value	Class 1 %
TREATMENT					
Petal Drop (PD)					
Control 1		53.69	43.98	91.28	56.81
Control 2		47.21	32.52	73.26	87.14
7 Days after PD					
1 Leaf removed		47.66	33.35	76.69	77.23
1 Leaf removed		48.61	34.83	77.90	62.04
14 Days after PD					
1 Leaf removed		52.95	36.86	82.70	85.22
2 Leaves removed		54.34	37.58	89.05	72.26
21 Days after PD					
1 Leaf removed		53.53	37.67	86.68	84.89
2 Leaves removed		55.21	39.32	90.24	73.75
28 Days after PD					
1 Leaf removed		50.80	35.07	78.73	77.95
2 Leaves removed		50.70	35.87	79.81	83.07
35 Days after PD					
1 Leaf removed		48.95	33.50	77.92	75.03
2 Leaves removed		51.61	35.07	82.82	76.74
42 Days after PD					
1 Leaf removed		52.16	37.15	83.20	59.72
2 Leaves removed		47.89	33.60	74.65	73.08
49 Days after PD					
1 Leaf removed		45.60	31.10	72.78	73.08
2 Leaves removed		50.71	34.77	78.16	84.15
SIG. LEVEL					
Treatment	15	0.5421	0.0867	0.1596	0.0714
Contrasts					
Contr1 vs Contr2	1	0.1325	0.0021	0.0150	0.0051
Contr1. vs Rest	1	0.3217	0.0021	0.0439	0.0131
One vs Two	1	0.5004	0.4774	0.4521	0.7665
Time Linear	1	0.3108	0.2004	0.1394	0.9736
Time Quadr.	1	0.0643	0.1032	0.0632	0.3032
*Treat * Linear*	1	0.9242	0.9118	0.6664	0.0068
*Treat * Quadr.*	1	0.2618	0.2086	0.3273	0.7517

P = 0.05

* No bourse shoots or spur leaves removed

** One bourse shoot removed, but no spur leaves

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3. PAPER II – EFFECT OF UREA SPRAYS ON RED COLOUR AND ANTHOCYANIN, CHLOROPHYLL AND CAROTENOID CONCENTRATION IN THE PEEL OF 'ROSEMARIE', 'FLAMINGO' AND 'FORELLE' PEARS.

Abstract

A high nitrogen concentration in the cell may be expected to increase the amino acid pool, thus increasing the concentration of substrate for chlorophyll formation. Therefore, this trial was conducted in the Ceres area of the Western Cape, South Africa (l33°10'S; 19°20'E) to evaluate the effect of urea applications on the peel pigments. The treatments consisted of a control, two urea applications, four urea applications, six urea applications, eight urea applications and ten urea applications. Fruit nitrogen content increased with urea sprays. Fruit colour was measured with a Minolta colorimeter. Urea sprays did not affect red colour of 'Flamingo', 'Forelle' and 'Rosemarie' pears. Anthocyanin, carotenoid, chlorophyll a and chlorophyll b concentrations were determined spectrophotometrically. Urea sprays had no effect on the anthocyanin concentration ($\mu g.g^{-1}$) of 'Rosemarie' and 'Flamingo'. However, there was a quadratic relationship between number of urea sprays and anthocyanin concentration of 'Forelle'. There were no significant differences in the chlorophyll a and b concentrations of 'Flamingo' and 'Forelle'. Urea applications significantly increased the chlorophyll a concentration of 'Rosemarie'. In contrast there was no significant difference in the chlorophyll b concentration of 'Rosemarie'. The carotenoid concentration of 'Flamingo' and 'Forelle' was not affected by urea applications. In contrast the carotenoid concentration of 'Rosemarie' increased significantly after the urea applications.

INTRODUCTION

High nitrogen (N) levels in the fruit result in greener peel colour of apples (Williams and Billingsley, 1974). At harvest peel colour of urea treated 'Newton' apples was greener than the control (Meheriuk, 1990). A high nitrogen concentration in the cell may be expected to increase the amino acid pool, thus increasing the concentration of

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substrate for chlorophyll formation (Saure, 1990). N fertilisation is the most important of many factors affecting leaf N and chlorophyll concentration in the fruit and there is a positive correlation between the carotenoid and chlorophyll concentrations and increasing nitrogen levels of the fruit (Marcelle, 1995; Kvale, 1968). Kvale (1968) found that with increasing N levels soluble solid levels increased in apple fruit, but Meheriuk (1990) found no increase. Urea applications did not affect fruit weight or firmness.

Nitrogen (N) and chlorophyll content of the leaves are important factors determining red colour of apple fruit, and the lower the N concentration the redder the fruit colour (Saure, 1990). Nitrogen deficiencies promote the accumulation of PAL, which is a key enzyme in the synthesis of anthocyanin, and decrease the accumulation of PAL-inactivating system (PAL-IS) (Stout, 1969). The increase in red colour of fruit caused by low nitrogen content may also be due to less vegetative growth and therefore less shading (Magness, 1926). Excessive nitrogen fertilisation generally causes a reduction in the percentage well-coloured fruit at harvest, though the availability of nitrogen is very important for anthocyanin formation (Saure, 1990).

This paper reports on the effect of urea applied to the fruit on the peel colour of bi-coloured pears, without the complication of stimulating vegetative growth.

MATERIAL AND METHODS

Plant material

This trial was conducted in three commercial orchards in the Ceres area of the Western Cape, South Africa (33°10'S; 19°20'E). This area is characterised by a Mediterranean climate of cold, wet winters and warm, dry summers. The 'Forelle' trees in their 14th leaf were planted at a spacing of 5.5 m x 3 m (606 trees.ha^{-1}). The 'Flamingo' trees in their 4th leaf were planted at a spacing of 4.5 m x 1 m (2222 trees.ha^{-1}) and the 'Rosemarie' trees in their fifth leaf were planted at a spacing of 4.5 m x 1.5 m (1481 trees.ha^{-1}). The orchards used in this trial were free-standing, central leader trees, planted in a North - South row direction

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Experimental design and treatments

A randomised complete block design was used with ten blocks and six treatments. Three-fruit replicates were used. Urea was applied twice a week directly onto the fruit with a handheld sprayer, at a concentration of $100g.l^{-1}$. The treatments consisted of a control, two urea applications, four urea applications, six urea applications in the case of 'Rosemarie' and in the case of 'Forelle' and 'Flamingo' eight and ten applications were included. The first applications were done on 15 December 1997.

Data collected

Before the first urea application was made, colorimeter readings were taken on the red side of each fruit. At the commercial harvest dates of 'Rosemarie' (15/01/98), 'Flamingo' (22/01/98) and 'Forelle' (28/02/98), fruit were picked and brought to the laboratory for colorimeter readings and pigment analyses. Thirty fruit of each cultivar were randomly picked to determine the N content of the fruit before urea application started. After colorimeter readings were taken and fruit were peeled the remainder of the fruit were analysed for N concentration.

Colorimeter readings:

A Minolta colorimeter (model NR-3000, Nippon Denshoku Kogyo Co., Tokyo, Japan) was used to measure the colour of the fruit. Measurements were taken on the red side of the fruit before the first application and at harvest date. Chromaticity (fruit colour variables independent of brightness) was recorded in L*, a* and b* colour space co-ordinates (Dussi et al., 1995). The meter was calibrated at illuminant condition C (6774 K) with a white standard (Minolta calibration plate). The a* and b* values were converted to hue angle ($\tan^{-1} a^*/b^*$) and chroma ($(a^{*2} + b^*2)^{1/2}$) (McGuire, 1992). Hue angle is a measure of 'colour', with 0 = red, 90 = yellow, 180 = green and 270 = blue, chroma is a measure of intensity or vividness of the colour, and L* is a measure of the lightness of the colour.

Laboratory procedures for spectrophotometric analysis of pigments

Fruit was peeled with a potato peeler. Fruit peel of the three replicates was pooled and the total fresh mass was determined. The peel was frozen at $-80^{O}C$, and then lyophilised. Dry mass was then determined, and the peel was ground to a fine powder with a 'Maulinex' type 684 grinder.

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Anthocyanin: Anthocyanin extractions were based on the procedure of Siegelman & Hendricks (1958). Fifteen ml of a 1% HCL:MeOH solution was added to 500 mg of sample and left for 1 hour at 5^{O}C in the dark. Following the extraction period, the samples were centrifuged for 5 min. at 550 g. Absorbance of the supernatant was measured at 530 nm on a 'Beckman DU - 64' spectrophotometer.

Anthocyanin concentration was expressed as µg cyanidin 3-galactoside equivalents/g dry peel, as cy 3-gal is the major anthocyanin pigment in pear peel (Francis, 1970; Dussi et al., 1995). A standard curve was obtained with idaein chloride (cy 3-gal) (Carl Roth GmbH and Company, Germany).

Carotenoids and chlorophylls a and b: Carotenoid and chlorophyll extraction was based on the procedures of Goodwin (1958) and Harborne (1973), respectively. Fifteen ml of an 80% acetone: H_2O solution was added to 500 mg of sample and left for 18 hours at 5^{O}C in the dark. Following the extraction period, samples were centrifuged for 5 minutes at 550g. The supernatants were transferred to separation funnels and 15 ml diethyl ether was added. The lower layer was discarded. The ether layer was washed free of acetone with water (3 x 15 ml) and dried by standing for 30 minutes over $MgCO_3$ pentahydrate.

Absorbance of the carotenoids was measured at 436 nm, and chlorophyll at 660 nm and 642 nm on a Beckman DU-64 spectrophotometer with diethyl ether as the blank.

Concentration of carotenoids was expressed as µg B-carotene equivalents/g dry mass of peel. A standard curve was prepared with trans-B-carotene (Sigma). The following equations were used to determine chlorophyll a and b concentrations (Strain et al., 1971).

Chl a (µg/g dry mass) = 9.93 (A660) - 0.777 (A642)

Chl b (µg/g dry mass) = 17.6 (A642) - 2.81 (A660)

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Data analysis

Data were analysed using the GLM (General Linear Means) Procedure in the SAS (Statistical Analysis Systems) Program (SAS Inc., 1990).

RESULTS AND DISCUSSION

The aim of this work was to determine the relationship between fruit nitrogen (N) concentration and fruit colour. The N concentration of 'Rosemarie', 'Flamingo' and 'Forelle' fruit increased with an increasing number of urea applications (Table 1). However, this was not reflected in the hue angle values obtained (Figure 1-3). In 'Rosemarie' a big increase in N concentration of the fruit was found between 0 applications and 2 urea applications, with little increase with further applications (Table 1). The hue angle of these fruit did not change significantly (P=0.6686) (Figure 1). In the case of 'Flamingo', a very gradual increase in N concentration of the fruit was observed (Table 1), whereas the hue angle remained constant (Figure 2). 'Forelle' fruit showed a big increase in fruit N concentration between 0 and 2 urea applications and 8 and 10 urea applications (Table 1), which was again not reflected in the hue angles of the same fruit (Figure 3).

The spectrophotometer analyses of the individual peel pigments at harvest showed no significant differences between different numbers of urea applications in anthocyanin concentration in 'Rosemarie' and 'Flamingo' (Table 2 & 3). In the case of 'Forelle', the anthocyanin concentration increased with up to 6 urea applications whereafter it decreased with more applications (Table 4).

The chlorophyll a concentration of 'Rosemarie' increased significantly after urea applications. In contrast the urea applications did not affect the chlorophyll b concentration of 'Rosemarie' (Table 2). There were no significant differences in the chlorophyll a and b concentrations of 'Flamingo' (Table 3) and 'Forelle' (Table 4) between the number of urea applications and the control.

The carotenoid concentration of 'Rosemarie' increased significantly after urea applications, however there was no difference between the number of applications

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(Table 2). Urea applications did not affect the carotenoid concentration of 'Flamingo' (Table 3) and 'Forelle' (Table 4).

The hue angles before the urea application indicated that 'Forelle' (Figure 3) was more red than Flamingo' (Figure 2), whilst 'Rosemarie' (Figure 1) had the worst fruit colour. This might be due to different stages of fruit maturity and genetic difference between cultivars.

It is clear that although fruit nitrogen content increased with urea applications, no differences were found in red colour of fruit. It appears that poor colour development in fruit in response to high N fertilisation is indirect e.g. stimulation of vegetative growth, resulting in shading of fruit by leaves or higher gibberellin levels in the plant, which inhibit anthocyanin synthesis.

ACKNOWLEDGEMENTS

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Table 1. Nitrogen concentration ($mg.100g^{-1}$ dry mass) at harvest in fruit of three pear cultivars following urea applications ($100g.l^{-1}$).

Treatments	'Rosemarie'	'Flamingo'	'Forelle'
0 applications	84	72	29
2 applications	121	62	74
4 applications	122	101	74
6 applications	128	81	98
8 applications	-	99	190
10 applications	-	112	274
R^2	0.7317	0.6550	0.8768
Prob > F	0.1446	0.0511	0.0057

Table 2. The effect of the number of applications of spray urea ($100g.l^{-1}$) on the colour pigments of 'Rosemarie' pears ($\mu g.g^{-1}$).

TREATMENTS		Anthocyanin	Chlorophyll a	Chlorophyll b	Carotenoid
0 applications		154.60	1.09	0.77	2.86
2 applications		152.20	2.45	0.93	4.84
4 applications		159.05	2.51	0.92	5.22
6 applications		151.37	2.27	0.83	4.19
SIG. LEVEL	df				
Treatment	3	0.8862	0.0034	0.2743	0.0096
Contrast					
Control vs. Urea	1	0.9641	0.0006	0.1166	0.0021
Urea linear	1	0.9378	0.2680	0.2709	0.3492
Urea quadratic	1	0.4335	0.3018	0.6529	0.2373
LSD (5 %)		21.641	0.332	0.1953	1.3957

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Table 3. The effect of the number of applications of spray urea ($100g.l^{-1}$) on the colour pigments of 'Flamingo' pears ($\mu g.g^{-1}$).

TREATMENTS		Anthocyanin	Chlorophyll a	Chlorophyll b	Carotenoid
0 applications		130.78	2.29	1.06	4.14
2 applications		143.15	2.31	0.78	4.54
4 applications		141.64	2.36	0.74	4.70
6 applications		133.76	2.27	0.84	4.21
8 applications		136.55	2.44	0.76	5.01
10 applications		135.53	2.38	0.79	4.61
SIG. LEVEL	df				
Treatment	5	0.8719	0.9289	0.5416	0.8308
Contrast					
Control vs. Urea	1	0.3650	0.5145	0.0621	0.3035
Urea linear	1	0.4393	0.6035	0.8932	0.8073
Urea quadratic	1	0.7076	0.9589	0.9575	0.9525
LSD (5 %)		23.985	0.391	0.334	1.624

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Table 4. The effect of the number of application of spray urea ($100g.l^{-1}$) on the colour pigments of 'Forelle' pears ($\mu g.g^{-1}$).

TREATMENTS		Anthocyanin	Chlorophyll a	Chlorophyll b	Carotenoid
0 applications		242.07	3.80	1.29	9.61
2 applications		207.79	3.87	1.31	9.95
4 applications		214.93	3.97	1.37	10.43
6 applications		274.59	4.07	1.39	10.80
8 applications		253.95	4.38	1.77	11.78
10 applications		224.44	4.05	1.36	10.64
SIG. LEVEL	df				
Treatment	5	0.0278	0.2373	0.2910	0.3441
Contrast					
Control vs. Urea	1	0.6367	0.1396	0.3553	0.1260
Urea linear	1	0.2081	0.1187	0.2882	0.1460
Urea quadratic	1	0.0083	0.3898	0.3402	0.4796
LSD (5 %)		44.031	0.487	0.432	1.994

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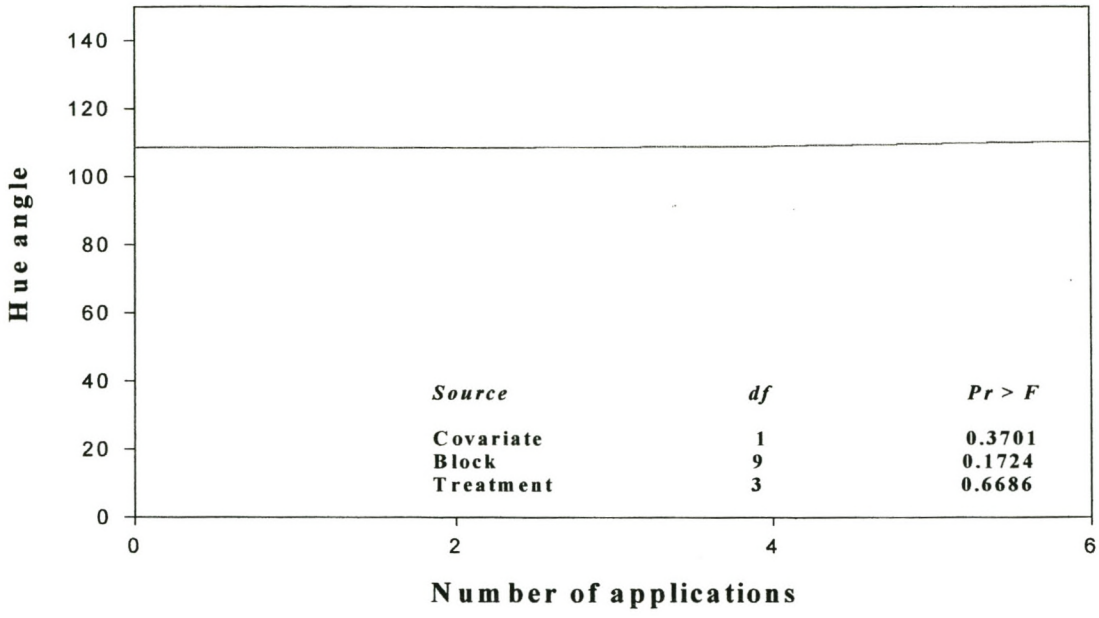

Fig. 1. The effect of the number of urea applications on the final hue angle on the best coloured side of 'Rosemarie' pears. Final hue angle values adjusted using the hue angle at the start of the trial as covariate.

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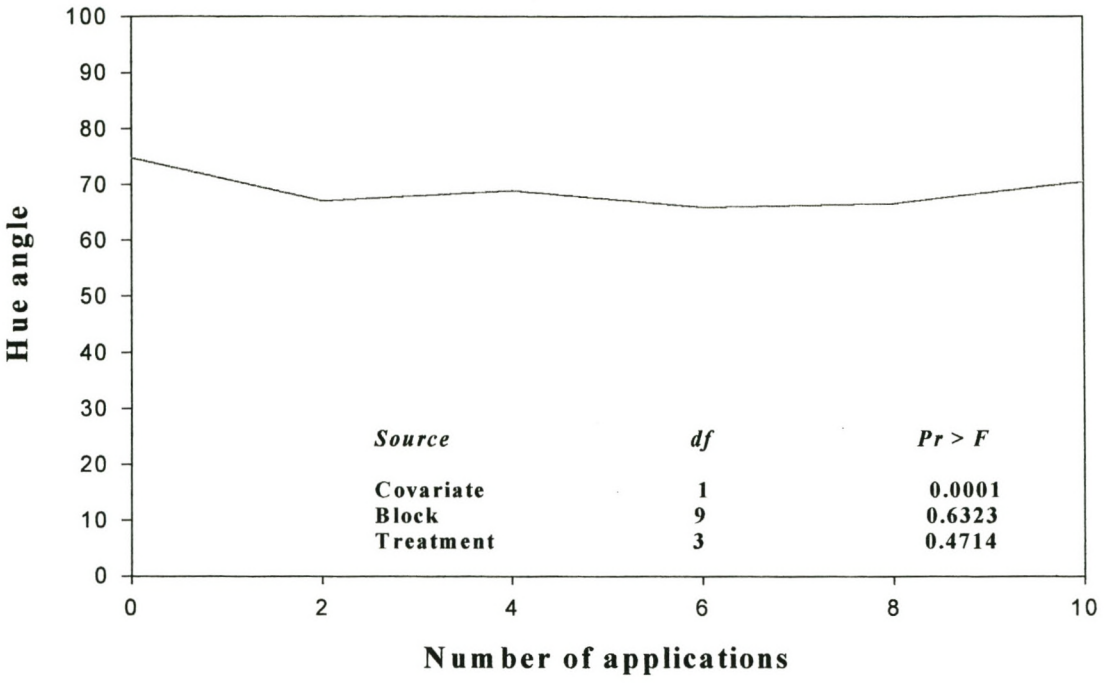

Fig. 2. The effect of the number of urea applications on the final hue angle on the best coloured side of 'Flamingo' pears. Final hue angle values adjusted using the hue angle at the start of the trial as covariate.

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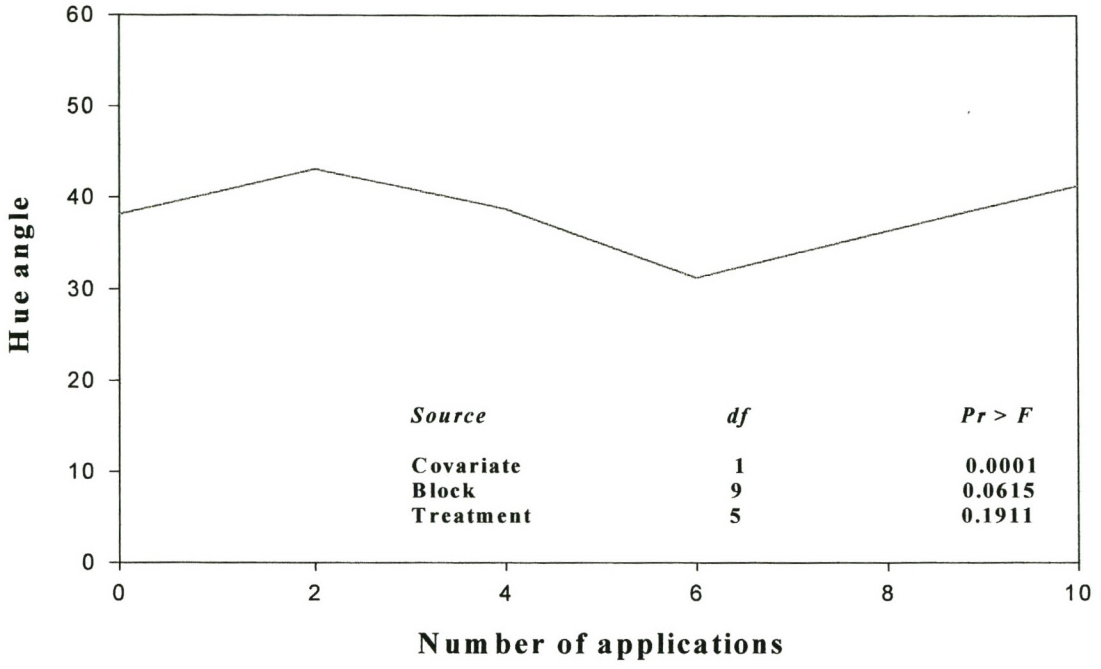

Fig. 3. The effect of the number of urea applications on the final hue angle on the best coloured side of 'Forelle' pears. Final hue angle values adjusted using the hue angle at the start of the trial as covariate.

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4. **PAPER III** - THE EFFECT OF EVAPORATIVE COOLING ON RED FRUIT COLOUR OF 'ROSEMARIE' PEARS.

Abstract

The low Class1 packout percentage due to poor colour development of bi-coloured pear cultivars in South Africa is a concern to the deciduous fruit industry. High temperatures are one of the main reasons for poor colour development. Evaporative cooling can improve red peel colour development in apples. Overhead cooling was applied with a micro-irrigation system to a commercial 'Rosemarie' pear orchard in the Ceres area of the Western Cape, South Africa (33°10'S; 19°20'E). Water was applied with pulsed irrigation for a three-week period (24 December 1998 to 14 January 1999) before harvest. Irrigation was activated when internal fruit temperature reached 24°C (day) and 19°C (night), irrigation continued until internal fruit temperature reached 21°C (day) and 16°C (night). No significant differences were found in colour, soluble solids, fruit size, firmness or yield.

INTRODUCTION

'Rosemarie', a bi-colour pear cultivar bred in South Africa, achieves high prices on the international fruit markets. The percentage of well-coloured fruit produced per tree is, however, not satisfactory (Huysamer, 1997). Pome fruit production areas in South Africa are of the world's warmest fruit producing areas and contribute to the unsatisfactory fruit colour of red- and bi-coloured apples and pears. The pigments chlorophyll and carotenoids located in plastids and the phenolic pigments (anthocyanins, flavonols and pro-anthocyanindins) located in the vacuole are responsible for fruit colour. The flavonols and pro-anthocyanidins do not contribute significantly to overall fruit colour, but may be important in enhancing and stabilising the colour of anthocyanin by co-pigmentation (Lancaster et al., 1994). Anthocyanins are glycosides of some twenty naturally occurring anthocyanidins, and are responsible for the red colour of fruit (Harborne, 1967). Anthocyanin formation in apples and pears occurs over a period of up

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to six months on the tree (Lancaster, 1992). There are two peaks of anthocyanin formation in bi-colour or red apples: a first peak during the phase of intense cell division in the fruit, and a second peak coinciding with maturation of the fruit. The latter is of prime importance for good colour development in apples (Lancaster, 1992; Saure, 1990). Bi-colour pears do not display the second peak of anthocyanin synthesis under local conditions. Young fruitlets should be exposed to direct sunlight during the early stages of fruit development (petal drop to approximately 40 days after petal drop) to ensure adequate anthocyanin formation (Viljoen, 1996) and good red colour expression at harvest.

Light, temperature, pathogens, soil types, rootstocks, fertilisation, growth regulators, scoring, etc. are factors that can affect red colour development in fruit. The two most important factors, however, are light and temperature. The best coloured fruit are found in parts of the canopy receiving 70-80% full sunlight (approximately 1120 $\mu mol.m^{-2}.s^{-1}$ at mid day under S.A conditions), medium coloured fruit at 40-70% and worst coloured fruit in parts receiving less than 40% full sunlight (Barrit et al., 1987; Jacyna & Soczek, 1980; Macheix et al., 1990).

Low night temperatures (15°C) promote anthocyanin synthesis in the light at moderate temperature (Creasy, 1976; Siegelman and Hendricks, 1958). High temperatures during the day or night are not conducive for anthocyanin synthesis (Arakawa, 1991; Faragher, 1983; Macheix et al., 1990; Mazza & Miniati, 1993; Saks et al., 1990; Saure, 1990; Stösser, 1999). According to Tan (1979), low temperatures (6°C) stimulate phenylalanine ammonia-lyase (PAL), which is required for anthocyanin synthesis in apple peel. The phenylalanine ammonia-lyase inactivating system (PAL-IS) is activated at high temperatures and inhibited at low temperatures. According to Faragher (1983) the regulation of PAL is due to inhibition of PAL at high temperatures rather than a direct stimulation of PAL at low temperatures.

The production of anthocyanin is dependent on the carbon products, mainly carbohydrates, formed during photosynthesis. During very hot weather, mid day

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depression of photosynthesis occurs, thus reducing carbohydrate synthesis, which may explain poor colour development at high day time temperatures. Low night temperatures reduce respiration, which lowers consumption of carbohydrates and therefore result in an increase in available carbohydrate for anthocyanin synthesis (Lancaster, 1992; Mazza & Miniati, 1993; Varga & Wertheim, 1976).

Evaporative cooling (EC) of trees by overhead irrigation improved red colour in apple fruits (Williams & Mayles, 1990). Fruit temperatures are lowered by water evaporating from the fruit surfaces and leaves. Heat removed by conduction from the fruit to water plays a minor role. The efficiency of EC depends on water application rate and climatic conditions (Evans, 1993b; Parchomchuk & Meheriuk, 1996). Apart from improved red colour development EC increased TSS and titratable acidity in 'Fuji' apples (Andrews, 1993; Warner, 1993) and delayed maturity Hinman (1993).

A further objective of EC is to maintain photosynthesis at a higher rate, by reducing mid day depression of photosynthesis (Evans, 1993a; Williams & Mayles, 1990). Net photosynthesis was about 30% higher in evaporatively cooled trees, compared to uncooled trees (Andrews, 1993) due to a 35% increase in stomatal conductance of evaporatively cooled trees.

This paper reports on the effect of overhead evaporative cooling on colour development in 'Rosemarie' pears.

MATERIAL AND METHODS

Plant material

This trial was conducted on fifth leaf 'Rosemarie' pears on BP3 rootstock in a commercial orchard in the Ceres area (Koue Bokkeveld) of the Western Cape, South Africa (33°10'S; 19°20'E). This area has a Mediterranean like climate with cool, wet winters and dry, hot summers. The trees were planted at a spacing of 4.5 m x 1.5 m

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(1481trees.ha^{-1}) in a north-south row orientation. The trees were trained to a free-standing central leader.

Evaporative cooling system

The system consisted of a Carel IR 32 WO controller with two unequal repeating timers, a stainless steel probe 4 mm in diameter and 80 mm in length, two 12 V batteries with connecting wires, a reversible solenoid (AC) and a 12 V solar panel as energy source. The probe was inserted to a depth of 35 mm in the calyx end of a pear, receiving full sunlight and located in the top of the tree canopy. Overtree micro-irrigation with micro emitters (2.7 mm/hour) was installed above every second tree. An irrigation frequency of 4.5 minute on was followed by 2.5 minutes off. Irrigation continued until the core temperature decreased below a set value. Irrigation during the day (7:00-19:00) started when the internal core temperature reached 24°C and stopped when the temperature decreased to 21°C. During the night (19h00-7h00) irrigation commenced when the internal core temperature reached 19°C and terminated when the temperature reached 16°C. The trial commenced on 24 December 1998, three weeks before harvest and continued until 14 January 1999. The overhead irrigation system was installed in three rows with fifty trees each, 10 trees of the middle row were used for data collection. A row without overhead irrigation served as control. The experimental design was thus non-statistical.

Data collected

At harvest (13 January 1999) ten fruit from each of the ten experimental trees were picked at random. Measurements recorded were: (1) fruit diameter, measured with a caliper, (2) flesh firmness, measured with an 'Effigy' penetrometer with a 8 mm plunger, on opposite, pared sides of the fruit, and (3) total soluble solids, expressed as % Brix as measured with a refractometer. The remaining fruit from both treatments were harvested separately, and total yield per tree was determined. Fruit were graded for colour, using the Capespan (P.O. Box. 505, Bellville, 7530) colour chart, P 26. The colour chart rates fruit from 1 to 12 with 1 the best and 12 the worst coloured fruit. Colour ratings 1 to 10

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are marketed as Cape export quality (Class 1) and 11 to 12 as Crown. Fruit size and other defects were not taken into account in determining the class 1 packout.

RESULTS AND DISCUSSION

The class1 packout for cooled trees (61.27 ± 9.69) was 5% higher than control trees (56.29 ± 8.14) (Table 1). The small improvement in Class 1 packout in 'Rosemarie' pears may be explained by the following reasons.

The trial started on 24 December 1998, three weeks before harvest and continued until 14 January 1999. However, average maximum temperatures were above 24°C (the upper set value) from 22 November 1998 (Figure 1). If cooling had been installed earlier in the season a better effect on colour would have been possible, probably due to less degradation of anthocyanin caused by high temperatures.

There were short breakdowns in the overhead irrigation–cycle, due to interruption in electric power supply resulting in poor temperature control. According to Williams & Mayles (1990), the decision to cool is a season–long commitment. A breakdown in the system or short break in cooling can lead to increased culling of apples.

According to Williams & Mayles (1990) both ambient and core temperature should be monitored to asses when to switch on the cooling as the relationship between the two is not always the same. In apples the current recommendation in Washington State is that cooling should commence when ambient air temperatures reach 29.4°C to 32.2°C and internal fruit temperatures reach 32.2°C to 33.6°C (Evans, 1993a; Williams & Mayles, 1990). In 'Delicious' apples, Unrath (1975) used overtree sprinklers to apply water at 2.5 $mm.h^{-1}$ when air temperature exceeded 28°C and fruit peel temperature exceeded 35°C, and reported improvements in red colour, soluble solids and size (Parchomchuk & Meheriuk, 1996). On 'Red Delicious' apples intermittent cooling, usually 15 minutes on and 45 minutes off, is preferable to continuous cooling, which does not allow the water to

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evaporate. Application rates of approximately 450-560 litres.min^{-1}.ha^{-1} should be used in orchards. However, poor water quality can lead to mineral deposits on the fruit and leaves (Williams & Mayles, 1990). The temperatures we used to activate the overhead irrigation system were lower than generally recommended for apples. This could have resulted in too much water being applied and not allowing for evaporation to cool the fruit. In effect, therefore, hydrocooling occurred, which is less effective.

Flesh firmness of cooled 'Rosemarie' did not differ from the control fruit (Table 1), which is in contrast to what Andrews (1993), Hinman (1993) and Warner (1993) found on apple where cooled fruit were firmer. Flesh firmness of cooled 'Bartlett' pears decreased faster than flesh firmness in noncooled trees, but not in 'd' Anjou' pears (Lombard et al., 1966). There were no significant differences in total soluble solids (Table 1), which is in contrast with apples where higher soluble solids and titratable acids were found with cooling (Andrews, 1993; Hinman, 1993; Parchomchuk & Meheriuk 1996; Warner, 1993).

Fruit size and yield (Table 1) were not affected by evaporative cooling, which is in contrast to work done on apples where fruit size was improved with cooling (Andrews, 1993; Hinman, 1993; Parchomchuk & Meheriuk 1996; Warner, 1993).

Water quality and reliability of the system are important factors when overhead cooling is considered. Further research on evaporative cooling is needed to assess its value in improving red colour development in pear fruit.

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Table 1. The effect of evaporative cooling on fruit diameter, firmness, soluble solids, Class 1 percentage and yield/tree of 'Rosemarie' pears.

Treatment	Avg. Class 1*	Avg. flesh firmness (kg)	Avg. soluble solids (% Brix)	Avg. yield/tree (kg)	Avg. fruit diameter (mm)
Control	56.29 ± 8.14	15.15 ± 0.55	13.40 ± 0.62	28.35 ± 8.17	66.57 ± 3.29
Cooled	61.27 ± 9.69	15.61 ± 0.59	13.18 ± 0.40	26.90 ± 6.59	64.42 ± 1.93

* Fruit were graded individually for colour, using the Capespan (P.O. Box. 505, Bellville, 7530) colour chart (P 26). The colour chart rates fruit from 1 to 12 with 1 the best and 12 the worst coloured fruit. Colour ratings 1 to 10 are marketed as Cape export quality (Class 1) and 11 to 12 as Crown. Fruit size and other defects were not taken into account in determining the Class 1 packout.

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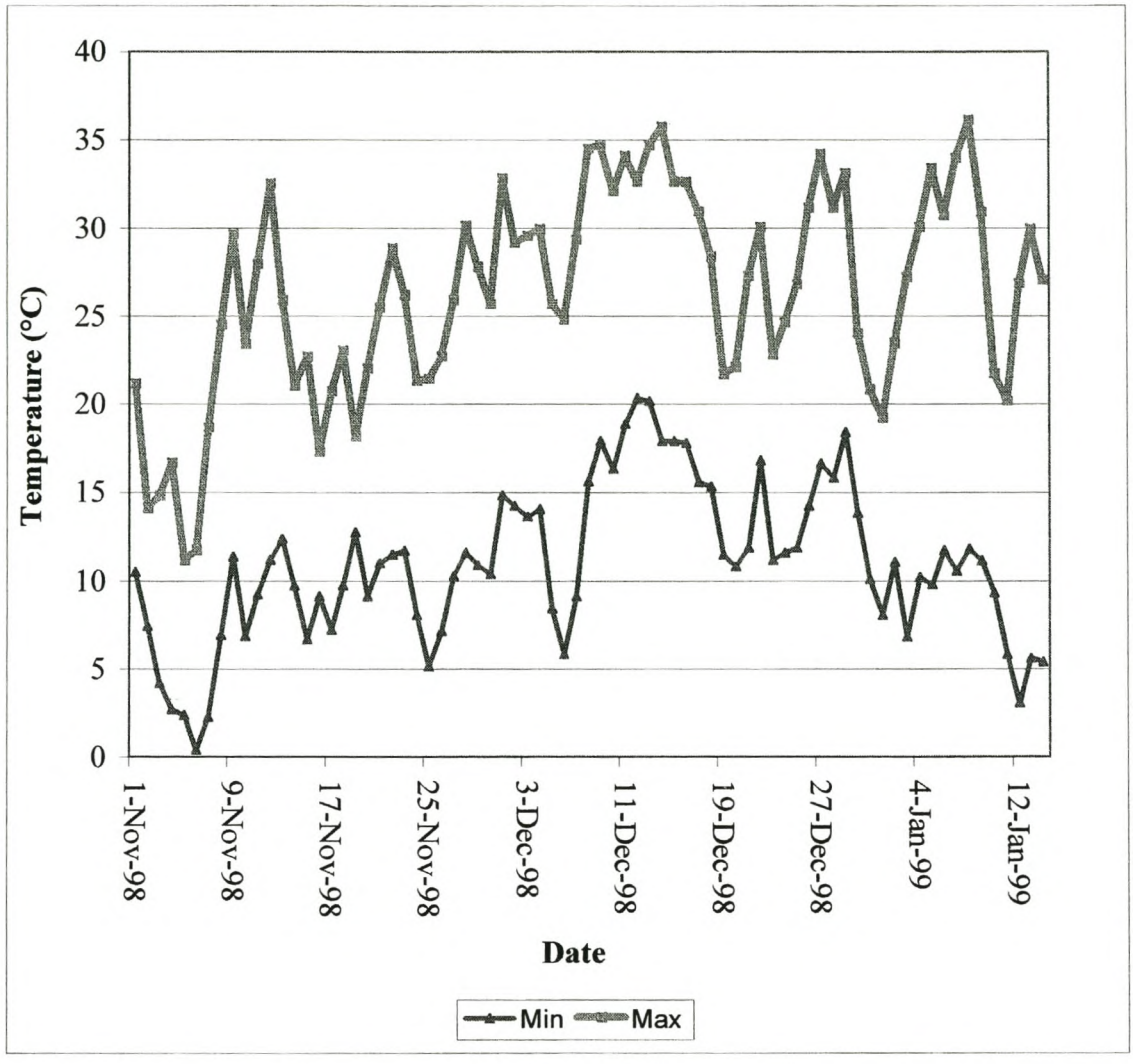

Figure 1. Minimum and maximum temperatures (12h00-24h00) as measured for Langrivier (1 November 1998 – 14 January 1999).

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5. PAPER IV - THE INFLUENCE OF COLD STORAGE AND RIPENING ON PEEL PIGMENT CONCENTRATIONS OF 'FORELLE', 'BON ROUGE' AND 'RED D' ANJOU' PEARS.

Abstract

Little is known of the changes in pigment concentrations of the peel of pear fruit during storage. The aim was therefore to determine the effect of storage period for 6 and 8 weeks at -0.5°C and ripening at 21°C for 1 week on anthocyanins, carotenoids, chlorophyll a and chlorophyll b on fruit of three pear cultivars. This trial was conducted in three commercial orchards in the Ceres area of the Western Cape, South Africa (l33°10'S; 19°20'E). There were no significant differences in the anthocyanin concentration in 'Bon Rouge' after cold storage compared to fruit at harvest, whilst for 'Red d' Anjou' anthocyanin concentration increased significantly after cold storage. Results with 'Forelle' were inconsistent. During ripening anthocyanin of 'Red d' Anjou' did not change, whereas the results for 'Bon Rouge' and 'Red d' Anjou were inconsistent. However, cold storage had no effect on the anthocyanin concentrations of 'Bon Rouge' and 'Forelle'. Cold storage significantly decreased the carotenoid concentrations of 'Bon Rouge', but not in 'Red d' Anjou' and 'Forelle'. The carotenoids of Bon Rouge', 'Red d' Anjou' and 'Forelle' decreased significantly more during ripening at 21°C. The chlorophyll a and chlorophyll b concentrations of 'Bon Rouge' decreased significantly during storage at -0.5°C, compared to fruit at harvest, but not in 'Red d' Anjou' and 'Forelle'. During ripening at 21°C chlorophyll a and chlorophyll b decreased significantly in 'Bon Rouge', 'Red d' Anjou' and 'Forelle'.

INTRODUCTION

In order to achieve optimum prices for red and bi-coloured cultivars, it is essential to maintain the red colour throughout the storage life of the fruit. Very little work has been done on the changes in the pigments of apple and pear cultivars, viz., anthocyanins,

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carotenoids, chlorophyll a and chlorophyll b, during commercial storage. Viljoen (1996) concluded that ripening decreased the concentrations of the carotenoids and chlorophyll, which unmasked the red colour. Viljoen (1996) also found that in certain cultivars anthocyanin decreased during cold storage. The main anthocyanin in pear peel is cyanidin-3-galactoside (Dussi et al., 1995; Francis, 1970), while cyanidin 3-arabinoside (Francis, 1970; Macheix et al., 1990; Mazza & Miniati, 1993; Timberlake & Bridle, 1971) and peonidin 3-galactoside (Dussi et al., 1995; Redelinghuys, 1969) make a minor contribution.

The aim of this study was to determine the effect of cold storage at –0.5°C and ripening at 21°C on the anthocyanin, carotenoid, chlorophyll a and chlorophyll b concentrations of bi-colour or red pear cultivars.

MATERIAL AND METHODS

Plant material

Fruit were harvested from three commercial orchards in the Ceres area of the Western Cape, South Africa (33°10'S; 19°20'E. This area is characterised by a Mediterranean climate with cold, wet winters and warm, dry summers. 'Forelle' trees in their eleventh leaf were planted at a spacing of 4.57 m x 2 m (1094 trees.ha^{-1}). 'Bon Rouge' trees in their fifth leaf and 'Red d' Anjou' trees in their eighth leaf were planted at a spacing of 4.5 m x 1.5 m (1481 trees.ha^{-1}). The trees were trained to free-standing, central leader systems.

Experimental design and treatments

A complete randomised design was used with five treatments, and ten replicates, consisting of seven fruit each. The fruit were harvested at their optimum harvest dates in Ceres and subsequently cold stored at -0.5°C for 6 or 8 weeks. At harvest, 70 fruit were selected at random for uniformity of size and analysed for anthocyanins, carotenoids and

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chlorophylls. After storage for 6 or 8 weeks, 140 fruit were selected randomly, 70 analysed immediately and another 70 analysed after ripening at 21°C for a week.

Laboratory procedures for spectrophotometric analysis

Fruit were peeled with a potato peeler. The peel of seven fruit was pooled and the total fresh mass was determined. The peel was frozen at –80°C, and then lyophilised. Dry mass was then determined, and the peel was ground to a fine powder with a 'Maulinex type 684' grinder.

Pigment extraction

Anthocyanins. Anthocyanin extraction was based on the procedure of Siegelman & Hendricks (1958). Fifteen ml of a 1 % HCL:MeOH solution was added to 500 mg of sample and left for 1 hour at 5°C in the dark. Following the extraction period, the samples were centrifuged for 5 min. at 550 g. Absorbance of the supernatant was measured at 530 nm on a 'Beckman DU - 64' spectrophotometer.

Anthocyanin concentration was expressed as $\mu g.g^{-1}$ cyanidin 3-galactoside (cy 3-gal) equivalents in dry peel, as cy 3-gal is the major anthocyanin pigment in pear peel (Dussi et al., 1995; Francis, 1970). A standard curve was obtained with idaein chloride (cy 3-gal) purchased from Carl Roth and Company, Germany.

Carotenoids and chlorophylls a and b. Carotenoid and chlorophyll extractions were based on the procedures of Goodwin (1958) and Harborne (1973), respectively. Fifteen ml of an 80 % acetone: H_2O solution was added to 500 mg of sample and left for 18 hours at 5°C in the dark. Following the extraction period, samples were centrifuged for 5 min. at 550 g. The supernatant was transferred to separation funnels, and 15 ml diethyl ether was added. The lower layer was discarded. The ether layer was washed free of acetone with water (3 x 15 ml) and dried by standing for 30 min. over $MgCO_3$ pentahydrate.

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Absorbance of the carotenoids was measured at 436 nm, and chlorophyll at 660 nm and 642 nm on a Beckman DU-64 spectrophotometer with diethyl ether as the blank.

A standard curve was obtained with trans-B-carotene purchased from Sigma. Concentration was expressed as $\mu g.g^{-1}$ B-carotene equivalents in dry peel, with the assumption that B-carotene is the major carotenoid in pear peel, as is the case in apples (Knee, 1972). The following equations were used to determine chlorophyll a and b concentrations (Strain et al., 1971).

Chl a (μg/g dry mass) = 9.93 (A660) - 0.777 (A642)
Chl b (μg/g dry mass) = 17.6 (A642) - 2.81 (A660)

Data analysis

Data were analysed using the GLM (General Linear Means) Procedure in the SAS (Statistical Analysis Systems) Program (SAS Inc., 1990).

RESULTS AND DISCUSSION

Anthocyanin concentration ($\mu g.g^{-1}$)*:* During cold storage anthocyanin concentration did not change in 'Bon Rouge', but increased in 'Red d' Anjou', while in the case of 'Forelle' the concentration did not change during 6 weeks of cold storage, but increased after 8 weeks of cold storage. During ripening anthocyanin concentration increased in fruit cold stored for 6, weeks in 'Bon Rouge'. In 'Red d' Anjou' anthocyanin did not change during ripening whereas for 'Forelle' anthocyanin increased during ripening in fruit cold stored for 6 weeks, but decreased in fruit cold stored for 8 weeks. Similar inconsistencies were reported by Viljoen (1996) e.g. in 'Bon Rouge' anthocyanin did not change during ripening for fruit cold stored for 8 weeks. The inconsistency is likely due to variation in anthocyanin concentration of fruit at harvest

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Anthocyanin synthesis in apples in light can continue for a further 48 hours when placed in the dark (Lancaster 1992; Saure, 1990; Siegelman & Hendricks, 1958). In 'Jonathan' apples, Diener (1982) also found that anthocyanin synthesis could occur in the dark at low temperatures. In contrast, Lin et al (1989) and Bishop and Klein (1975) found a decrease in anthocyanin concentration in 'Starkrimson' and 'McIntosh' apples, during storage.

Carotenoid concentration ($\mu g.g^{-1}$): The carotenoid concentration of 'Bon Rouge' decreased significantly during storage at –0.5°C, but not in 'Forelle' or 'Red d' Anjou' (Table 2). The carotenoid concentrations of 'Bon Rouge', 'Red d' Anjou' and 'Forelle' decreased significantly during ripening. During ripening carotenoid concentration decreased more in 'Bon Rouge' than in 'Forelle', while the smallest decrease occurred in 'Red d' Anjou'. This is in agreement with Viljoen (1996) who found the largest decrease in carotenoid concentrations in 'Bon Rouge' followed by 'Forelle', with the smallest decrease in 'Red d'Anjou'.

Chlorophyll a ($\mu g.g^{-1}$): Chlorophyll decreased during storage in 'Bon Rouge', but not in 'Red d' Anjou' or 'Forelle' (Table 3). The loss in chlorophyll a of 'Bon Rouge', 'Red d'Anjou' and 'Forelle' occurred during ripening at 21°C. The decrease in chlorophyll a concentration was more in 'Bon Rouge', than in 'Forelle', followed by 'Red d' Anjou' (Table 3).

This is in agreement with Viljoen (1996) who reported a decrease in the chlorophyll a concentrations of 'Bon Rouge', 'Forelle' and 'Rosemarie' during storage. However, Viljoen (1996) found no decrease in chlorophyll a concentration of 'Red d' Anjou' pears during storage. Loss of chlorophyll during ripening has previously been reported in apples, which is associated with the respiratory climacteric (Workman, 1963; Rhodes, 1970; Wooltorton, 1967) and an increase of chorophyllase activity (Looney and Paterson, 1967; Rhodes, 1970; Wooltorton, 1967).

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Chlorophyll b concentration ($\mu g.g^{-1}$): The degradation of chlorophyll b occurred during the 8 week storage period (Table 4). 'Bon Rouge' lost more than 'Forelle', followed by 'Red d' Anjou'. The degradation of the chlorophyll b of 'Bon Rouge' and 'Forelle' was accelerated during ripening at 21°C. In contrast, there was no degradation of chlorophyll b of 'Red d'Anjou' during ripening (Table 4).

Chlorophyll b levels are substantially lower in higher plants than chlorophyll a, apart from slight differences in structure, absorption spectra and solubility properties (Devlin & Barker, 1971; Gregory, 1971).

The rate of synthesis and degradation of anthocyanin and the ratio between this pigment and carotenoids and chlorophylls determine the expression of red colour in apples and pears. It is clear that carotenoids and chlorophylls decrease during storage and ripening, which should reduce the masking of the red colour.

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Table 1. The effect of storage at –0.5°C and ripening at 21°C on the anthocyanin concentration ($\mu g.g^{-1}$) in peel of 'Bon Rouge', 'Red 'd Anjou' and 'Forelle' pears.

		Anthocyanin concentration ($\mu g.g^{-1}$)		
TREATMENT	df	'Bon Rouge'	'Red 'd Anjou'	'Forelle'
Harvest date		604.37 b	1044.00 b	188.64 bc
6 weeks (-0.5°C)		619.50 b	1317.45 a	168.38 c
6 weeks (-0.5°C + 1week (21°C)		711.13 a	1311.92 a	206.85 b
8 weeks (-0.5°C)		615.73 b	1248.55 a	247.01 a
8 weeks (-0.5°C + 1week (21°C)		576.97 b	1340.86 a	200.25 bc
SIG. LEVEL				
Treatment	4	0.0622	0.0023	0.0002
Contrasts				
Harvest vs Storage	1	0.4553	0.0001	0.1735
Week 6 vs Week 8	1	0.0354	0.6607	0.0030
Storage vs Shelf	1	0.4098	0.3426	0.7185
Interaction	1	0.0461	0.2854	0.0006
LSD (5 %)		*90.29*	*163.91*	*32.29*

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Table 2. The effect of storage at –0.5°C and ripening at 21°C on the carotenoid concentration (μg.g^{-1}) in peel of 'Bon Rouge', 'Red 'd Anjou' and 'Forelle' pears.

		Carotenoid concentration (μg.g^{-1})		
TREATMENT	df	'Bon Rouge'	Red 'd Anjou'	'Forelle'
Harvest date		13.04 a	13.31 a	10.13 a
6 weeks (-0.5°C)		10.67 b	12.42 ab	9.69 a
6 weeks (-0.5°C + 1week (21°C)		3.67 c	10.77 c	6.99 b
8 weeks (-0.5°C)		11.40 b	13.64 a	11.49 a
8 weeks (-0.5°C) + 1week (21°C)		3.46 c	11.31 bc	6.08 b
SIG. LEVEL				
Treatment	4	0.0001	0.0002	0.0001
Contrasts				
Harvest vs Storage	1	0.0001	0.0476	0.0278
Week 6 vs Week 8	1	0.5589	0.0577	0.4940
Storage vs Shelf	1	0.0001	0.0001	0.0001
Interaction	1	0.2839	0.4545	0.0414
LSD (5 %)		*1.23*	*1.37*	*1.81*

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Table 3. The effect of storage at –0.5°C and ripening at 21°C on the chlorophyll a concentration ($\mu g.g^{-1}$) in peel of 'Bon Rouge', 'Red 'd Anjou' and 'Forelle' pears.

		Chlorophyll a concentration ($\mu g.g^{-1}$)		
TREATMENT	df	'Bon Rouge'	Red 'd Anjou'	'Forelle'
Harvest date		4.21 a	4.28 a	3.43 a
6 weeks (-0.5°C)		3.37 b	3.90 ab	3.23 a
6 weeks (-0.5°C + 1week (21°C)		0.10 c	3.36 c	2.12 b
8 weeks (-0.5°C)		3.46 b	4.28 a	3.61 a
8 weeks (-0.5°C) + 1week (21°C)		0.16 c	3.69 bc	1.63 b
SIG. LEVEL				
Treatment	4	0.0001	0.0004	0.0001
Contrasts				
Harvest vs Storage	1	0.0001	0.0245	0.0014
Week 6 vs Week 8	1	0.5911	0.0205	0.7926
Storage vs Shelf	1	0.0001	0.0004	0.0001
Interaction	1	0.8891	0.8596	0.0451
LSD (5 %)		*0.41*	*0.44*	*0.601*

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Table 4. The effect of storage at –0.5°C and ripening at 21°C on the chlorophyll b concentration ($\mu g.g^{-1}$) of 'Bon Rouge', 'Red 'd Anjou' and 'Forelle' pears.

		Chlorophyll b concentration ($\mu g.g^{-1}$)		
TREATMENT	df	'Bon Rouge'	Red 'd Anjou'	'Forelle'
Harvest date		1.32 a	1.29 a	1.11 a
6 weeks (-0.5°C)		0.88 b	1.07 ab	1.07 a
6 weeks (-0.5°C + 1week (21°C)		0.07 c	1.07 b	0.79 b
8 weeks (-0.5°C)		0.83 b	1.23 a	1.17 a
8 weeks (-0.5°C) + 1week (21°C)		0.17 c	1.09 bc	0.57 b
SIG. LEVEL				
Treatment	4	0.0001	0.0468	0.0001
Contrasts				
Harvest vs Storage	1	0.0001	0.0317	0.0137
Week 6 vs Week 8	1	0.6333	0.1087	0.4294
Storage vs Shelf	1	0.0001	0.2595	0.0001
Interaction	1	0.1899	0.2059	0.0480
LSD (5 %)		*0.14*	*0.17*	*0.22*

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GENERAL DISCUSSION

Anthocyanin synthesis in 'Rosemarie' occurs predominantly during the 40 days after full bloom. During this period it is essential that fruit be well exposed to sunlight. To improve the light exposure of fruit, growers remove upright shoot growth of the current season, and in some instances leaves that shade the young fruitlets.

To assess whether these manipulations result in increased packouts that would warrant the expense, a series of trials was conducted where the upright growth of the current season were removed, bourse shoots were pinched and the spur leaves removed. In a study over two years on the same trees the effect of summer pruning of upright growth was evaluated. Results of the first season's data showed that early summer pruning, 14 November or earlier, or repeated summer pruning improved the fruit colour and Class 1 packout. The same results were not achieved in the second season. The Class 1 packout was high in the second year, ca. 75%, which indicated that the light exposure of the fruit was better. It appears that early removal of current season's upright growth in vigorous growing 'Rosemarie' trees will improve red colour development and Class 1 packout. For trees of moderate and low vigour this manipulation appears unnecessary. Repeated removal of new growth is not recommended. The temperature of fruit exposed to direct sunlight can be 15°C higher than ambient temperatures. Colour loss in 'Rosemarie' during high temperatures of December and January remains a serious problem. A degree of shading of the fruit may benefit colour retention of the fruit due to lower fruit temperatures.

Another perception regarding red colour formation commercially is that N fertilisation causes poor colour development; therefore producers are cutting on fertilisation programs. A number of urea applications were made on to the peel of pear fruit, thus elevating the N concentration of the fruit, without stimulating shoot growth.

The N concentration of the fruit increased with urea applications, however colour was not affected. This phenomenon may be due to high temperatures (above 26°C), resulting in the loss of red colour. The anthocyanin concentration of 'Flamingo' and 'Rosemarie' were not affected, nor were the hue angles. In 'Forelle' there was a

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quadratic relationship between the number of urea applications and anthocyanin concentration, which could not be explained.

Urea applications increased the chlorophyll a concentration of 'Rosemarie'. In contrast it did not affect the chlorophyll b concentration or the chlorophyll a and b concentrations of 'Flamingo' and 'Forelle'. In 'Rosemarie' urea applications increased the carotenoid concentration. However, there were no differences in the carotenoid concentration of 'Flamingo' and 'Forelle'.

It is clear that although fruit nitrogen content increased with urea applications, no differences were found in red colour of fruit. It appears that poor colour development in fruit in response to high N fertilisation is indirect e.g. stimulation of vegetative growth, resulting in shading of fruit by leaves or higher gibberellin levels in the plant, which inhibit anthocyanin synthesis.

South Africa is one of the world's warmest apple and pear-producing areas, therefore the warm summer temperatures contribute to the loss in red fruit colour of especially bi-coloured pears. Therefore, evaporative cooling (EC) was used on 'Rosemarie' pears to manipulate the high summer temperatures, ensuring better red fruit colour at harvest. EC is practised on apples with good results, improving red fruit colour, and fruit size, and decreasing sunburn. However, little work with EC has been done on pears.

EC did not affect fruit size and TSS, which is in contrast with work done on apples. The percentage class 1 fruit of EC treated trees was higher, although not dramatically so. Possible reasons for these results include: EC was started relatively late in the season and periodic breakdowns of the system compromised the data.

However, EC may be the only practical manipulation to improve red fruit colour at harvest. Further research has to be done with EC. It could be of great economic value for the producers and industry in terms of better coloured fruit, less sunburn and larger fruit due to less stress. Water quality and reliability of the system are important factors when EC is considered.

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From a marketing point of view is it important to evaluate the effect of commercial cold storage and ripening practices on the pigment concentrations of bi-coloured fruit. Therefore, pigment analyses were made on 'Bon Rouge', 'Forelle' and 'Red d' Anjou' stored under commercial conditions.

During cold storage anthocyanin concentration did not change in 'Bon Rouge', but increased in 'Red d' Anjou', while in the case of 'Forelle' the concentration did not change during 6 weeks of cold storage but increased after 8 weeks of cold storage. During ripening anthocyanin concentration increased for fruit cold stored for 8 weeks in 'Bon Rouge'. In 'Red d' Anjou' anthocyanin did not change during ripening whereas for 'Forelle' anthocyanin increased during ripening in fruit cold stored for 6 weeks but decreased in fruit cold stored for 8 weeks. Similar inconsistencies were reported by Viljoen (1996) e.g. in 'Bon Rouge' anthocyanin did not change during ripening for fruit cold stored for 8 weeks. The inconcistency is most likely due to variation in anthocyanin concentration of fruit at harvest.

The carotenoid concentration of 'Bon Rouge' decreased during storage, but not in 'Forelle' or 'Red d' Anjou'. During ripening the carotenoid concentration decreased in all three cultivars. Chlorophyll a and b decreased in 'Bon Rouge' during cold storage but not in 'Forelle' or Red d' Anjou'. Ripening decreased chlorophyll a concentration in all three cultivars. However, ripening decreased chlorophyll b concentration in 'Bon Rouge' and 'Forelle', but not in 'Red d' Anjou'.

The expression of red colour in apples and pears is determined by the rate of synthesis and degradation of anthocyanin, and the ratio between this pigment and carotenoid and chlorophyll content. It is clear that carotenoids and chlorophylls decreased during storage and ripening, which reduced the masking effect on red colour. High temperatures appear to be the main factor causing poor red colour development in 'Rosemarie' pear fruit. Future research should focus on climate modification by overhead irrigation.