Effect of shading and ethephon on the anthocyanin composition of ‘Crimson Seedless’ (*Vitis vinifera* L.)

by

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DECLARATION

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Date: 25-09-2010

SUMMARY

‘Crimson Seedless’ is currently one of the most important and popular table grape cultivars produced in South Africa, and as such it is of great economic value for table grape producers. Major concerns with ‘Crimson Seedless’ is that it is prone to inadequate colouring, and with increased yields the berry size decreases. An additional difficulty is that methods used to increase berry size, further impede berry colouring. A plant growth regulator (PGR) commonly used in table grape production, to enhance colour formation, is ethephon (2-chloro-ethyl-phosphonic acid, 2-CEPA). In recent years significant research has been done on the effect of sunlight on anthocyanin production in grapes, although this has primarily been on wine grape cultivars. Currently, there is limited knowledge on the effect of sunlight on table grapes, and how this might influence their anthocyanin composition and content. The effect of ethephon on colour of grapes and other fruit have been extensively researched and well documented. However, the effect of ethephon on the anthocyanin composition of ‘Crimson Seedless’ is not well known. The current study aimed to explore the effect of sunlight (by matter of exclusion) and management practices, namely defoliation and ethephon application, on the anthocyanin profile and content of ‘Crimson Seedless’. Four different treatments were applied to two ‘Crimson Seedless’ vineyards, the first site located in Paarl, and the second in De Doorns. The treatments were: 1. Naturally exposed bunches, 2. Exposed bunches treated with ethephon, 3. Bunches kept in shade boxes, 4. Shaded bunches treated with ethephon. At the De Doorns site an additional defoliation treatment was superimposed over the above treatments. An HPLC technique was modified for the separation and detailed profiling of ‘Crimson Seedless’ anthocyanins and was used to analyse the effect of the reported treatments on the anthocyanin profile of berry skins. The predominant anthocyanin in ‘Crimson Seedless’ is peonidin-3-glucoside (Pn-gluc), and this was found to be significantly increased only by ethephon application, and was not altered by sunlight or leaf removal. The responses of the other anthocyanin types varied according to the respective treatments applied. However, a general observation was that ethephon application more consistently increased the concentration of anthocyanins in berry skins than did sunlight. Leaf removal had the least significant effect on anthocyanin concentration.

OPSOMMING

‘Crimson Seedless’ is tans een van die belangrikste en gewildste tafeldruif cultivars wat in Suid-Afrika verbou word en daarom is dit van groot ekonomiese waarde vir tafeldruif-produsente. ‘Crimson Seedless’ is egter daarvoor bekend dat dit te swak kleur (volgens uitvoer spesifikasies) en tweedens is die cultivar geneig om kleiner korrels te ontwikkel wanneer die oeslading vermeerder word. ‘n Addisionele probleem is dat die praktyke wat in die industrie gebruik word om korrels te vergroot ‘n verdere negatiewe impak op ‘Crimson Seedless’ se kleur ontwikkeling kan veroorsaak. Die plant-groei-reguleerder wat algemeen in tafeldruif verbouing gebruik word, ten einde beter gekleurde druiwe te produseer, is ethephon (*2-chloro-ethyl-phosphonic acid, 2-CEPA*). In die laaste paar jaar was daar baie navorsing gedoen oor die effek wat sonlig het op die antosianien produksie van druiwe, maar navorsing was gefokus op wyndruif cultivars. Huidiglik is daar beperkte tegniese kennis oor die effek wat sonlig op tafeldruiwe het, en hoe dit moontlik die antosianien samestelling en inhoud kan beïnvloed. Daar is ook reeds verskeie studies gedoen en data gepubliseer oor die invloed wat ethephon op die kleur het van druiwe en ander vrugte, maar die invloed wat ethephon op die antosianien samestelling van ‘Crimson Seedless’ het, is nie wel bekend nie. Die doel van hierdie studie was om die effek van sonlig (deur uitsluiting) en bestuurspraktyke (blaarverwydering en ethephon toediening) te bestudeer en hoe dit die antosianien samestelling van ‘Crimson Seedless’ beïnvloed. Vier verskillende behandelings is toegedien in twee ‘Crimson Seedless’ wingerde, die eerste proefperseel in die Paarl en die tweede proefperseel in De Doorns. Die behandelings was: 1. Natuurlik blootgestelde trosse, 2. blootgestelde trosse met ethephon, 3. Trosse met skadubokse omhul, 4. Skaduboks trosse met ethephon. By De Doorns is ‘n addisionele blaarverwydering proef bygebring. ‘n *HPLC* tegniek was aangepas om die antosianien samestelling en inhoud van ‘Crimson Seedless’ te bepaal, en om die effek van die behandelings te ondersoek. Die *HPLC* data het getoon dat peonodien-3-glukosied (Pn-gluc) die primêre antosianien in ‘Crimson Seedless’ is met die hoogste inhoud van al die antosianiene. Pn-gluc was betekenisvol beïnvloed deur ethephon toediening, terwyl die ander behandelings geen betekenisvolle effekte daarop gehad het nie. Die effekte wat die ander antosianiene gehad het, het gevarieer volgens die behandelings wat toegedien was. ‘n Algemene observasie was dat ethephon toediening die antosianien konsentrasie in ‘Crimson Seedless’ druiwe skille meer konsekwent vermeerder het as die sonlig blootstelling. Die

blaarverwydering het die minste betekenisvolle effek op die antosianien inhoud van ‘Crimson Seedless’ gehad.

Hierdie tesis is opgedra aan my pa Thys wat tydens my studies skielik na onse Hemelse Vader treuggekeer het.

Asook aan almal wat bly glo het in my vermoëns, wat my aangemoedig het om dit ook self te glo, en sonder wie hierdie nooit moontlik sou wees nie.

2008 was a trying year filled with sadness and loss

“Some of us think holding on makes us strong; but sometimes it is letting go.”

- *Hermann Hesse*

Vaarwel:

Oom Joos, Ouma, Oom Chris & Pappa

BIOGRAPHICAL SKETCH

Riaan Human was born on 11 May 1982, in Cape Town, South Africa. He matriculated from Afrikaanse Hoër Seunskool in 2000. In 2001 he enrolled as a *BScAgric* student at the University of Stellenbosch and obtained the degree in 2004, majoring in Oenology, Chemistry and Viticulture. In 2005 he enrolled for an *MScAgric* degree in Viticulture at the University of Stellenbosch.

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INTRODUCTION AND PROJECT AIMS

GENERAL INTRODUCTION AND PROJECT AIMS

1.1 INTRODUCTION

The Crimson Seedless (*Vitis vinifera* L.) grape is a late season, attractive, red seedless grape cultivar, introduced in 1989 as a seedless alternative to Emperor. 'Crimson Seedless' is the result of five generations of hybridization at the U.S. Department of Agriculture, Horticultural Field station in Fresno, California. It was received favourably by consumers due to its elongated, firm berries and crisp eating quality (Ramming *et al*. 1995). The clone C33-199, a late ripening, white seedless grape with all white grapes in its parentage, was used in the hybridization with 'Emperor' to produce 'Crimson Seedless'. The cross was made in 1979 by David Ramming and Ron Tarailo, with 85 resultant seedlings that were planted in 1980. Out of four seedlings selected, 'Crimson Seedless' was the only red seedless cultivar. 'Crimson Seedless' was selected in 1983 and tested as C102-26 (Ramming *et al*. 1995). The source of seedlessness is 'Thompson Seedless' (also known as 'Sultanina') which was used as a parent in the first generation crossing (Ramming *et al*. 1995).

Across the world 'Crimson Seedless' is currently a very popular table grape cultivar; in South Africa it is one of the most planted cultivars and is third in terms of total area of table grape vineyards in production. The popularity of 'Crimson Seedless' can be ascribed to the following; it is a late maturing, red seedless grape which is not susceptible to berry crack thus allowing for a longer ripening period; and fruit kept in cold storage tends to remain in good condition, with similar storage characteristics to 'Emperor'. Another reason for its popularity could be that 'Crimson Seedless' was released as a public cultivar, with no restrictions on its propagation.

However, some of the main problems with the production of 'Crimson Seedless' are related to its colour and size. A further problem with 'Crimson Seedless' colour is that with increased yields and practices that are used to increase berry size, colour is further decreased. Even with all of the favourable characteristics of this cultivar, the problem remains a lack of adequate colour. Thus, research on this cultivar has been driven by a search for ways in which to increase the export output by increasing colour, quantity and quality. Research has shown that 'Crimson Seedless' has one of the lowest reported average concentration of anthocyanins (mg/kg of fresh weight) in studied cultivars (Cantos *et al*. 2002). It was also shown that 'Crimson Seedless' had the highest amount and proportion of acylated anthocyanins (Cantos *et al*. 2002). Nearly 66% of the measured anthocyanin of Crimson Seedless is peonidin-3-glucoside while the total amounts of the acylated anthocyanins contribute to 8.6%.

Anthocyanins are coloured pigments, thus the manipulation of anthocyanin production in grapes, is potentially a means of influencing the visual perception of colour in the fruit. Various factors influence anthocyanins and they can be extrinsic, such as environmental conditions namely climate, light, temperature, nutrition and water status, which could have a direct effect on the anthocyanin synthesis and degradation; or indirect effects via plant growth and photosynthesis, which influences the partitioning of photo-assimilates and soluble salts to grape bunches. Also, the intrinsic factors which influence anthocyanins are the grapevine cultivar's genetic information, which is determined by species, cultivar and clone. The genetic information intrinsic to a grapevine cannot be altered, so after establishing a vineyard, it can only be accommodated by vineyard management.

This study aimed at investigating the 'Crimson Seedless' anthocyanin profile and how environmental factors such as vine light environment and bunch shading affected it, as these factors have been shown to influence the colour of other table grape cultivars significantly (Wicks 1979, Wicks & Kliewer 1983). Another part of the study aimed at determining the effect of ethephon application in combination with shading. Ethephon has been shown to improve colour in various table grape cultivars (Wicks 1979, Wicks & Kliewer 1983), and a important research output was to determine the interaction this plant growth regulator would have with other environmental conditions, potentially further enhancing 'Crimson Seedless' colour through its regulatory effect on the anthocyanin profile. An additional leaf removal experiment was also incorporated in the study to determine how this management practice might influence the anthocyanin concentration and profile of 'Crimson Seedless' in combination with the other treatments. Finally, the fruit ripeness parameters for all the treatments were measured to determine what the effects, if any, of these different treatments were on 'Crimson Seedless' fruit composition.

1.2 SPECIFIC PROJECT AIMS

The current study aimed to explore the potential effects of shading and ethephon application on the colour of 'Crimson Seedless' via the treatments effects on the anthocyanin profile and content: By determining the 'Crimson Seedless' anthocyanin concentration and profile under prevailing South African conditions and to evaluate management practices influence on the anthocyanin profile and composition of 'Crimson Seedless'.

Key issues addressed within the current study were:

1. **The effect of excessive shading on 'Crimson Seedless' anthocyanin profile.** To investigate the effect of decreased sunlight incidence on developing fruit, due to cluster shading, on the final concentration of anthocyanins in 'Crimson Seedless' skins. To determine whether there were any effects of shading on the composition of anthocyanins.

2. **The effect of defoliation on 'Crimson Seedless' anthocyanin profile.** To explore the effect of a 50% leaf removal treatment in terms of the grapevine's light microclimate, and to determine the effect on anthocyanin concentration and composition in grapes. To seek to understand the influence of leaf removal in terms of canopy microclimate and/or photoassimilate partitioning by examining the response of fruit ripening to the treatment measured in terms of sugar accumulation, juice pH and titratable acidity.

3. **The interactive effects of ethephon and management practices on 'Crimson Seedless' colour.** To explore the interaction between ethephon application at a commercial level and the treatment of leaf removal/shade to determine whether there is an enhancement/dampening of the treatments. Can ethephon application overcome possible negative effects on the anthocyanin composition that might be caused by excessive shading?

1.3 LITERATURE CITED

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Chapter 2

Literature review

A review of the biochemical and environmental control of grape colour with special reference to table grape cultivation

2.1 INTRODUCTION

The ‘Crimson Seedless’ (*Vitis vinifera* L.) grape is a late season, attractive, red seedless grape cultivar, introduced in 1989 as a seedless alternative to ‘Emperor’. ‘Crimson Seedless’ is the result of five generations of hybridization at the U.S. Department of Agriculture, Horticultural Field station in Fresno, California (Figure 2.1), it was received favourably by consumers due to its elongated, firm berries and crisp eating quality.

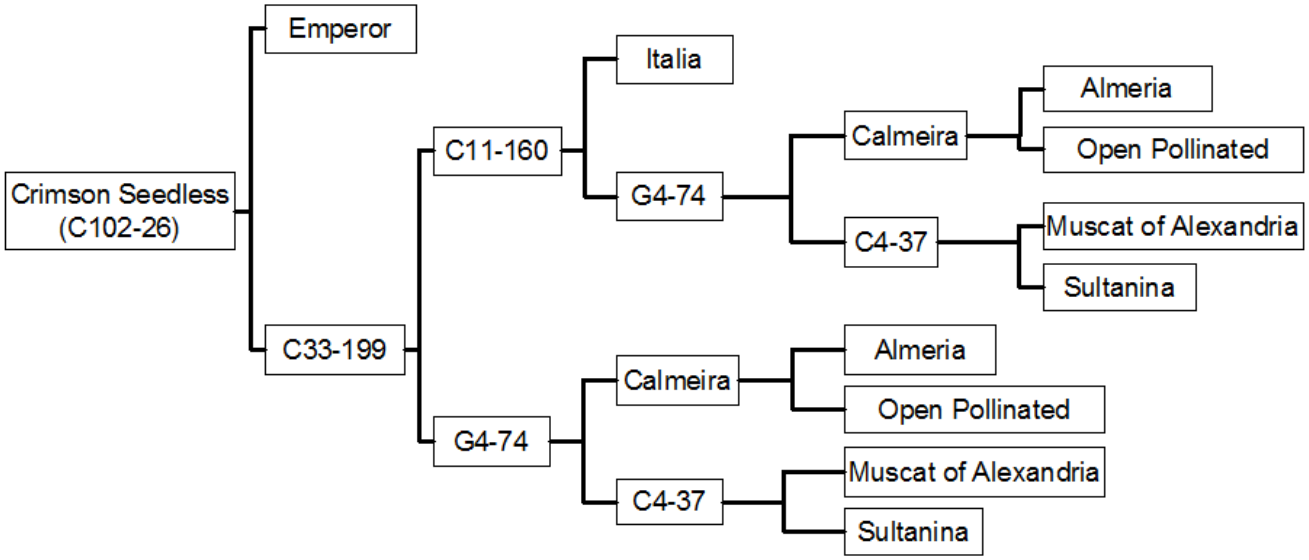


Figure 2.1 Parentage of ‘Crimson Seedless’ (Ramming *et al.* 1995).

C33-199, a late ripening, white seedless grape with all white grapes in its parentage, was used in the hybridization with ‘Emperor’ to produce ‘Crimson Seedless’. The cross was made in 1979 with 85 resultant seedlings that were planted in 1980. Out of four seedlings selected, ‘Crimson Seedless’ was the only red seedless cultivar. ‘Crimson Seedless’ was selected in 1983 and tested as C102-26. The source of seedlessness is ‘Thompson Seedless’ (also known as ‘Sultanina’) which was used as a parent in the first generation crossing.

Across the world ‘Crimson Seedless’ is currently a very popular table grape cultivar; in South Africa it is one of the most planted cultivars and is third in terms of total area of vineyards in production. The popularity of ‘Crimson Seedless’ can be ascribed to the following: It is a late maturing, red seedless grape which is not susceptible to berry crack, it can thus be kept on the vine for longer periods of time; and fruit kept in cold storage remained in a good condition, with similar storage characteristics to ‘Emperor’ (Ramming *et al*. 1995). Another reason could be that ‘Crimson Seedless’ was released as a public cultivar, with no restrictions on its propagation (Ramming *et al.* 1995). However, some of the main problems with ‘Crimson Seedless’ is a lack of colour and inadequate size; a further problem with ‘Crimson Seedless’ colour is that with increased yields and

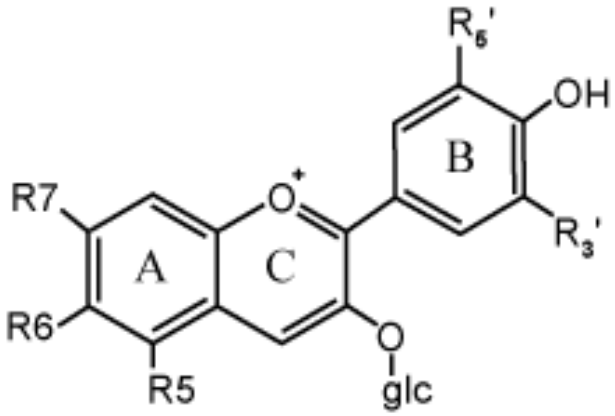
practices that increase berry size, the colour decreases even more. Thus, the table grape industry seeks ways in which to increase the export output by increasing colour, quantity and quality.

2.2 ANTHOCYANINS: THE CHEMICAL BASIS FOR GRAPE COLOUR

Anthocyanins are water-soluble, vacuolar pigments, responsible for colouration of fruits, flowers, stems and leaves in most of the higher order plants (Van Buren 1970, Ribéreau-Gayon *et* al. 2000). They are also the major pigments found in coloured grape cultivars, characterized by a diverse range of colours, hues and shades from pink to black. It has been shown that the quantity and composition of these anthocyanins influence berry skin colour in grapes (Mazza & Miniati 1993, Shiraishi & Watanabe 1994, Ribéreau-Gayon *et al*. 2000). This group of chemical compounds have been the most extensively researched of any class of phenolic substance in grapes, and in grapes more than in any other plant (Van Buren 1970), due to the importance of colour on quality aspects of plant products. In grapes, anthocyanins are localized primarily in the vacuoles of the skin cells (Timberlake 1982) and are mostly limited to the first three to six sub epidermal cell layers (Hrazdina *et al*. 1984), with a high concentration gradient increasing from the interior towards the exterior of the grape.

2.2.1 Anthocyanin structure

Anthocyanins, amongst other compounds such as flavonols and flavones, form part of the flavonoid group. The flavonoids are C15 phenolic compounds which share a common structural unit, the C6-C3-C6 flavone skeleton and are characterized by two benzene cycles connected via the C3-oxygenated heterocycle. The flavonoid molecule is thus made up of two aromatic rings; the A-ring being synthesized by head-to-tail condensation of acetate units and the B-ring from the Shikimic acid pathway via phenylalanine, and the connecting C3 heterocycle is derived either from the 2-phenyl chromone nucleus or the 2-phenyl chromanone nucleus (Ribéreau-Gayon *et al*. 2000). Flavonoids, with the exception of a few, appear in the form of glycosides, in other words they are bound to a sugar. There are also several different classes of flavonoids distinguished by the oxidation level of the bridge carbons. For instance, anthocyanins are frequently present as glycosides of anthocyanidins. The anthocyanins commonly occur as B-glucosides with sugars at the 3 and/or 5 positions and in grapes anthocyanins are primarily glucosides bound with a D-glucose, because these molecules are much more stable in glucoside form, compared to aglycone form. The 3 position, with a few exceptions, is always glycosated, and disaccharides examined so far contain at least one glucose molecule as a sugar. There are five common anthocyanins found in grapes and their structure is shown in Figure 2.2. The type of anthocyanin is determined by the substitution of the lateral nucleus, there can be two or three substituents (OH and OCH3).



Anthocyanidin	R5'	R3'	Colour
Malvidin	CH3	OCH3	purple-red
Delphinidin	H	OH	pink
Petunidin	CH3	OH	purple
Peonidin	CH3	H	purple-blue
Cyanidin	H	H	red

Figure 2.2 Chemical structure of the anthocyanin molecule (Ribéreau-Gayon *et al*. 2000).

2.2.2 The anthocyanin biosynthetic pathway

Anthocyanin biosynthesis in grape skin has been quite extensively studied; it has been determined that anthocyanins are synthesized from phenylalanine through an anthocyanin biosynthetic pathway, regulated by gene expression (Boss *et al.*, 1996a & c, Jeong *et al.* 2004, Mori *et al.* 2005) and the associated enzyme activities of expressed proteins (Hrazdina *et al.*, 1984). This anthocyanin biosynthetic pathway forms part of both the phenylpropanoid and flavonoid pathways (Figure 2.3). The biosynthesis of anthocyanins proceeds by a series of ordered chemical reactions catalyzed by enzymes produced during berry development and after the onset of ripening (véraison) (Boss *et al.* 1996a, El-Kereamy *et al.* 2003). Anthocyanin biosynthesis is developmentally triggered at véraison about 8–10 weeks after blooming and continues throughout the ripening growth phase (Boss *et al.* 1996a, Castellarin *et al*. 2006). At véraison the grape berry softens and the acid to sugar balance starts decreasing. During the véraison developmental period, intensive anthocyanin synthesis is triggered in the sub-epidermal layer of red cultivar berry skins (Hrazdina *et al.* 1984; El-Kereamy *et al.* 2003).

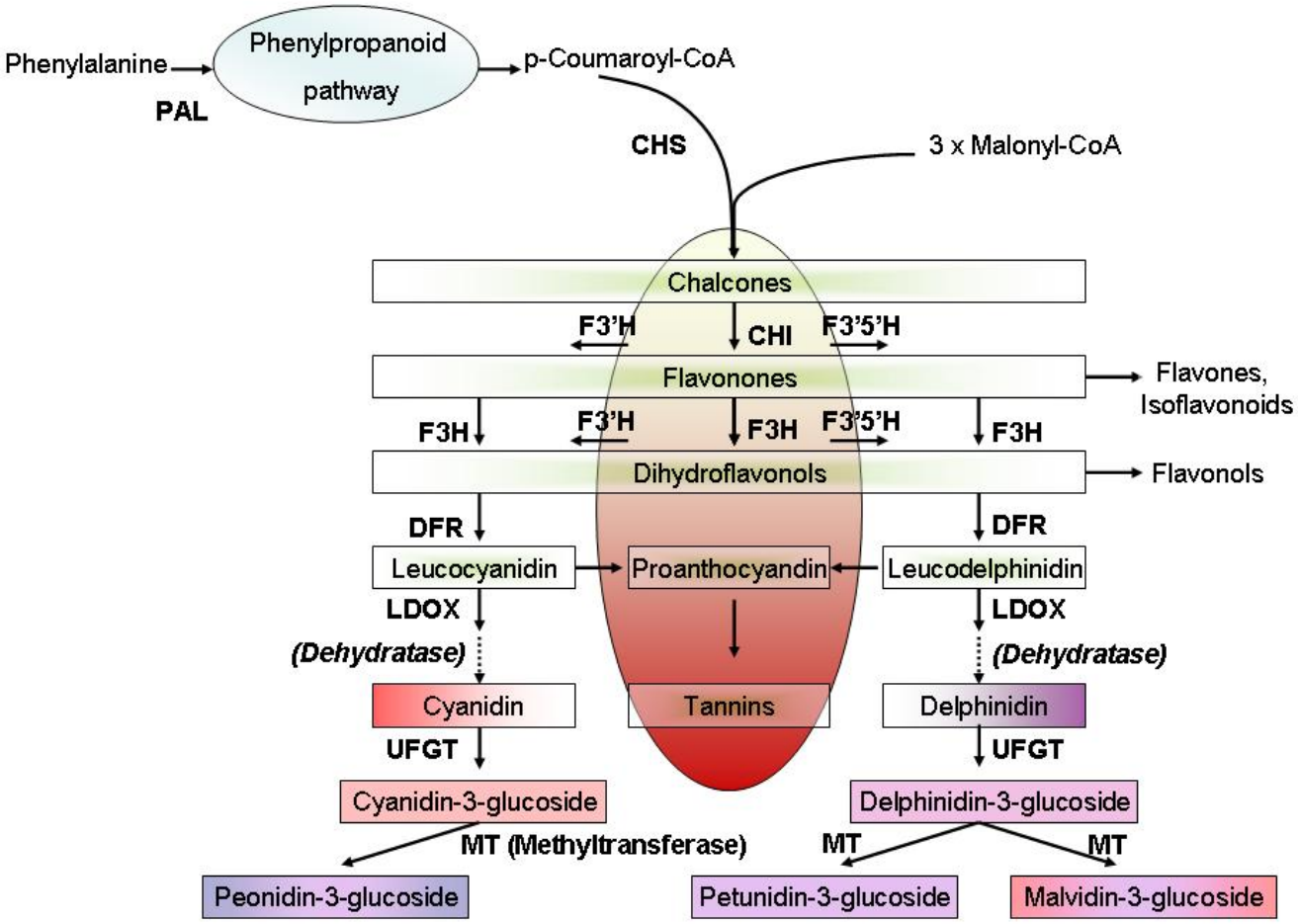


Figure 2.3 Biosynthetic pathway of anthocyanins adapted from Mattivi *et al.* (2006).

Anthocyanins are synthesized by the enzymes involved in the biosynthetic pathway, and the genes corresponding to the expressed enzyme proteins have been isolated from many plants including the berries and seedlings of grapevine (Sparvoli *et al.* 1994, Boss *et al*. 1996a, El-Kereamy *et al*. 2003, Yamane *et al*. 2006). The cDNAs derived from seven of the genes encoding these enzymes were isolated by Sparvoli *et al.* (1994): phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX) and UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT) (Boss *et al.* 1996a, El-Kereamy *et al.* 2003, Downey *et al.* 2003). This laid the foundation for later research investigating anthocyanin biosynthesis on a molecular basis at the mRNA level.

Boss *et al.* (1996a) investigated the regulation of anthocyanin production in grape berries by utilizing cDNAs encoding the anthocyanin biosynthetic enzymes. They investigated the expression of seven pathway genes (PAL, CHS, CHI, F3H, DFR, LDOX, and UFGT; Figure 2.3) in grape berry skin tissues samples taken throughout the developmental period. Northern blot analysis indicated that anthocyanin pathway gene expression occurred in two phases. All the genes in the pathway,

except UFGT, were expressed briefly early in berry development and again after véraison, when colour development occurred. They had found that before véraison no anthocyanins could be detected in the samples. This was presumably because UFGT was missing (Boss *et al.* 1996a). Using the cDNA fragments as probes, they showed that expression of the gene for UFGT is the major control point to anthocyanin biosynthesis in grapes (Boss *et al.* 1996 a, b). They concluded that the pattern of gene expression in grape berry skins could be explained in relation to regulatory genes. This was further investigated by other researchers and they have indicated that gene expression during the initial phase of berry growth was for flavonols, flavan-3-ol monomers, and proanthocyanidin biosynthesis and the only anthocyanins were synthesized during fruit ripening (Bogs *et al.* 2006, Boss *et al.* 1996a, b, c).

The early steps in the biosynthesis of anthocyanins require the deamination of phenylalanine by PAL to cinnamic acid, which eventually leads to the production of 4-coumaroyl-CoA via the phenylpropanoid pathway (Heller & Forkmann 1988). An early committed step in flavonoid biosynthesis is the condensation of three molecules of malonyl-CoA and one molecule of 4-coumaroyl-CoA by CHS to produce a chalcone. This step is often considered to be the rate limiting step for this pathway (Mazza & Miniati 1993). The next phase of anthocyanin biosynthesis forms part of the flavonoid pathway and the change from chalcone to anthocyanin is mediated by the following enzymes: chalcone is isomerised by CHI into flavanone which is hydroxylated with F3H to form dihydroflavonols. Dihydroflavonols are converted to leucoanthocyanidins with DFR catalysis. LDOX produces anthocyanidin from leucoanthocyanidins and the final step involves the addition of a glucose molecule to anthocyanidin to form anthocyanin in a process catalyzed by UFGT (Mazza & Miniati 1993, Jeong *et al.* 2004, Mori *et al.* 2005).

In the grape berry, the coordinated expression of most of the structural genes involved in this pathway, except UFGT, suggests the involvement of two groups of regulatory factors during berry ripening (Boss *et al.* 1996a). Studies on the regulation of the genes involved in flavonoid metabolism have made it possible to identify a regulatory mechanism of the flavonoid biosynthetic pathway, which appears to be under the control of two families of transcription factors, the MYC and MYB proteins (Ageorges *et al.* 2006, Deluc *et al.* 2006). The first group of regulatory genes are proposed to control expression of PAL, CHS, CHI, F3H, DFR, LDOX and anthocyanidin synthase (ANS), while another group induces UFGT gene expression. If this were the case, the first group of regulatory genes would have to be expressed early in berry development and the second group, triggering UFGT expression, would be expressed after véraison (Boss *et al.* 1996a, Deluc *et al.* 2006). A study by Kobayashi *et al.* (2001) suggested that a regulatory gene plays a critical role in anthocyanin biosynthesis in grapes. In *Arabidopsis sp.,* various researchers have shown that each specific branch of the flavonoid pathway is regulated by a different MYB factor (Borevitz *et al.* 2000, Nesi *et al.* 2001, Mehrtens *et al.* 2005, Ageorges *et al.* 2006).

In grapevine two very similar VvMYBA genes were identified as putative regulators of anthocyanin synthesis in the grape skin of Kyoho (*Vitis labruscana: V. labrusca* x *V. vinifera*) by particle bombardment of somatic embryos with MYB gene constructs (Bogs *et al*. 2006). These *Myb*-related genes, such as *VlmybA1-1*, *VlmybA1-2*, and *VlmybA2*, regulate anthocyanin biosynthesis in Kyoho, a black-skinned cultivar (Kobayashi *et al.* 2002). Since a MYB-related gene associated with the regulation of the UFGT gene was identified in *Vitis labruscana* berries (Kobayashi *et al.* 2002), defining regulation in the second part of development could be determined by these genes; however the regulation of the earliest stages of gene expression was still unknown. Deluc *et al.* (2006) presented results from their study indicating that a single R2R3-MYB gene *VvMYB5a*, may in fact regulate expression of the genes for the whole anthocyanin biosynthetic pathway. This regulatory gene activates structural genes in the phenylpropanoid pathway, which in turn leads to the production of anthocyanins. The specific steps in the biosynthesis of precursors to the anthocyanins are not as well known as those for other flavonoid groups, but since the biochemical behaviour of anthocyanins is closely related to the other classes of flavonoids, it has been concluded that anthocyanins are synthesized through the phenylpropanoid and flavonoid pathways (Jeong *et al.* 2004). These pathways are regulated by enzyme activities (Hrazdina *et al.* 1984) and gene expression (Boss *et al.* 1996a).

2.3 THE GENETIC AND ENVIRONMENTAL CONTROL OF GRAPE COLOUR

2.3.1 Genetic factors

2.3.1.1 Genetic fingerprint

An early example of hereditary properties in terms of grape colour was when Hendricks and Anthony (1915) noted that white skinned fruit was a recessive colour in grapes, compared with red or black fruit. They found all shades of red to black to be possible in seedling vines from crosses between cultivars of different colour, and that there was no such thing as a simple heritable character for red or black fruit. As far as is known, all wild species of grapes have coloured fruit. The differences in phenolic composition among species of *Vitis*, within varieties of one species, and among intra-species or intra-varietal crosses have been of interest for a long time, but until recently, study was limited to rather gross, observable differences. Ribéreau-Gayon *et al.* (2000) found that the anthocyanins of samples of the fruit of 14 different species of *Vitis* were quite different in the relative proportion of different specific pigments. The presence of diglucoside anthocyanins in large quantities is specific to certain species in the genus *Vitis* (*V. riparia*, *V. rupestris* and *V. labruscana*) (Ribéreau-Gayon *et al.* 2000). This lack of 3,5 - diglucosides among the anthocyanins of *V. vinifera* cultivars and their general occurrence in other species commonly used for fruit production or in hybridization are now well documented. In fact, the absence of diglucoside anthocyanins is now one well-accepted test contributing to proof that a specific variety or cultivar belongs to *V. vinifera*.

Generally there are five anthocyanins found in red grapes, these include malvidin-, delphinidin-, peonidin-, cyanidin- and petunidin- as 3-glucosides. In most *V. vinifera* wine grape cultivars, malvidin-3-glucoside is the most abundant pigment, varying from 90% of total anthocyanins in 'Grenache' to just under 50% in 'Sangiovese' (Ribéreau-Gayon *et al.* 2000). A few exceptions are evident, however, for example peonidin-3-glucoside was found to be the major anthocyanin in some Spanish wine grape cultivars (Garcia-Beneytez *et al.* 2002) instead of malvidin-3-glucoside. In *V. vinifera* table grape cultivars, a different anthocyanin composition has been noted to that of *V. vinifera* wine grape cultivars. Work by Carreño *et al.* (1997) has described the total anthocyanins and the different proportions in anthocyanin profiles for 32 red table grape cultivars. Later work by Cantos *et al.* (2002), Table 2.1, gave very similar results to those originally published by Carreño *et al.* (1997) with a few differences; which could be due to factors such as light intensity, irrigation, soil composition or other agronomic factors which have effects on the phenolic composition of grapes. In these studies on the compositional differences between table grape cultivars, it was found that the main anthocyanin in all the cultivars was peonidin-3-glucoside, in contrast to most wine grape cultivars. The other most abundant anthocyanins they found in table grapes were cyanidin-3-glucoside and malvidin-3-glucoside. Interestingly, Gonzalez-Neves *et al.* (2005) found that the amount of cyanidin-3-glucoside, peonidin-3-glucoside and the acylated derivatives of these anthocyanins where higher in fresh grape skins compared to wines and crushed grapes.

Table 2.1 Anthocyanins content of table grape cultivars (Cantos *et al.* 2002).

Anthocyanin	Red Globe	Flame	Crimson	Napoleon
Delphinidin-3-glucoside	4.7	34.3	1.1	1.9
Cyanidin-3-glucoside	28.9	32.7	6.6	11.1
Petunidin-3-glucoside	2.7	17.9	0.9	1.4
Peonidin-3-glucoside	65.4	32.4	45.2	40.6
Malvidin-3-glucoside	9.3	33.4	8.8	17.8
Cyanidin-3-p-coum *	1.4	0.0	1.2	0.0
Peonidin-3-p-coum *	2.9	0.0	4.7	5.9
Total anthocyanins	**115.3**	**150.7**	**68.5**	**78.7**

Values are expressed as $mg.kg^{-1}$ of fresh weight of grape berry (skin + flesh).

*Abbreviations used: Cyanidin-3-p-coum, cyanidin-3-*p*-coumaroylglucoside and Peonidin-3-p-coum, peonidin-3-*p*-coumaroylglucoside.

2.3.1.2 Genetic regulation of anthocyanin biosynthesis

Two classes of genes are required for anthocyanin biosynthesis, the structural and regulatory genes. The structural genes encode the enzymes that directly participate in the formation and storage of anthocyanins and other flavonoids. The regulatory genes regulate the expression of the structural genes, and control the spatial and temporal accumulation of pigments (Procissi e*t al*. 1997, Nesi *et al*. 2001, Mehrtens *et al*. 2005). As previously outlined in an earlier section, Boss *et al.* (1996a) looked at the expression of seven anthocyanin biosynthetic pathway genes and their implications in 'Shiraz' berry ripening. The pathway that was elucidated is shown in Figure 2.3. The accumulation of anthocyanins at véraison coincided with the increased expression of all seven genes in the pathway, which suggests that there is a coordinated regulation of all of these genes in the developing grape berry skin (Boss *et al*. 1996a). Northern blot analysis of the expression of the genes in 'Shiraz' berry skins supported the finding that anthocyanin accumulation continues throughout ripening. Every sample taken after véraison showed that all of the genes studied were expressed. However, all the studied genes, except UFGT, were also expressed in young berry skins up to 2–4 weeks post-flowering, but no anthocyanins could be detected in these samples, presumably because UFGT was not yet expressed. This suggests that the major control point to anthocyanin biosynthesis in grape berry skins is the UFGT gene and its corresponding protein.

Kobayashi *et al*. (2002) investigated the anthocyanin biosynthesis in 'Kyoho' grape (*V. labruscana*), and found that it is controlled by two kinds of transcription regulators, which are members of the *myb* and *myc* gene families *VlmybA*s and *VlmybA*1. Kobayashi *et al.* (2002) also found that *VlmybA*s and its homologue, *VlmybA*1, are putative regulatory genes for the anthocyanin biosynthesis of grapes that are involved in the regulation of UFGT expression. In 'Shiraz' grape berries, where anthocyanins accumulate in the skin but not in the flesh, samples from 'Shiraz' flesh show the same pattern of expression to that in the berry skin, except that neither PAL nor UFGT expression was detected, and CHS was not expressed late in development (Boss *et al*. 1996a). PAL and CHS might be encoded by other gene family members, so that could explain why northern analyses did not detect their expression, but only one UFGT gene seems to be present in the grape genome and this was not expressed in the flesh (Sparvoli *et al*. 1994). The genes being expressed in the 'Shiraz' flesh could be regulating the synthesis of other flavonoid-derived molecules.

Castellarin *et al.* (2006) has shown that genes encoding flavonoid 3'- and 3', 5'-hydroxylases are expressed in the skin of ripening red berries that synthesize anthocyanins and that there is a correlation between the expressed genes and the ratio of accumulation of red (cyanidin-based) and blue (delphinidin-based) anthocyanins (Figure 2.3). This indicates that the *VvF3'H* and *VvF3'5'H* expression is consistent with the colour of the ripening bunches. In table grapes this possibly means that there is a greater expression of *VvF3'H*, since cyanidin-3-glucoside and

peonidin-3-glucoside are the major anthocyanins formed although this has not yet been shown in research.

2.3.1.3 Regulation of the biosynthetic pathway by external factors

Fujita *et al.* (2006) showed that the effects of light and plant hormones on flavonol accumulation were different from anthocyanin accumulation, although anthocyanins and flavonols share the same upstream biosynthetic pathway. Thus it seems that flavonol biosynthesis is under a different control system compared to anthocyanin biosynthesis. Downey *et al.* (2004a) reported that the expression of *VvUFGT* is correlated to ripening and anthocyanin accumulation in berry skins, which is in agreement with the original work by Boss *et al.* (1996a). Downey *et* al. (2004) also found that the level of *VvUFGT* expression was similar in shaded and sun-exposed fruit. The expression in the latter stages of ripening was consistent with anthocyanin content, which suggests that shading has little effect on gene expression involved in anthocyanin biosynthesis. However, in another study by Jeong *et al.* (2004), which looked at effects of shading on the expression of anthocyanin pathway genes in 'Cabernet Sauvignon', they found that shading suppressed anthocyanin accumulation and it affected the transcription of both UFGT and the other pathway genes. The mRNA accumulation of *VvmybA1* was affected by shading in the same manner as the mRNA accumulation of the pathway genes. It was suggested that *VvmybA1* may control the transcription of the anthocyanin biosynthesis genes, and not just UFGT. The differences found in these two studies indicate that the regulation of the gene expression in the biosynthetic pathway may therefore be cultivar dependent.

A study by Kobayashi *et al.* (2001) described an ethylene-responsive element within the UFGT gene promoter. A stimulation of UFGT activity following exposure to ethylene may therefore result in rapid accumulation of anthocyanins from the pool of precursors, and this would necessitate an increase in flux through the flavonoid biosynthetic pathway, as observed by the increased transcript accumulation of CHS and F3H, following ethylene treatment. The results described in the later work of El-Kereamy *et al.* (2003) provided additional evidence for the role of ethylene treatment in the increased transcript accumulation of genes encoding anthocyanin biosynthetic enzymes in grapes.

2.3.2 Environmental factors: light and temperature

The climatic conditions of the region (macro-climate) or site (meso-climate) in which a grapevine grows ultimately determine the environmental factors which will influence the growth and development of this plant. The major environmental factors which influence grapevines directly and indirectly are the temperature and light environments. The microclimate in turn is influenced by various factors which a producer can modify. Thus by adjusting the vine microclimate, both the light and temperature environment of an individual vine can be modified and adjusted to optimally

influence the growing conditions of the canopy and bunches. One might want to manipulate the vine microclimate with vineyard management practices, but the meso- and macro-climatic characteristics of a vineyard site are set, and therefore limit the cultivars that are ideally suited for that site. An overview of the role of these two key factors, light and temperature, as they relate to grape colour production will therefore be discussed before a detailed discussion of vineyard management practices.

2.3.2.1 Studies of bunch shading on grape colour

To determine the impact of light on grape colour, various researchers have experimented with shading, more specifically the direct shading of grapes. A significant early study by Rojas-Lara and Morrison (1989) applied a direct cluster shading treatment where they shaded bunches and surrounding leaves, leaving 80% of the canopy exposed in 'Cabernet Sauvignon' vines. In another treatment they shaded both clusters and leaves, only leaving the top 20% of shoot tips receiving sunlight. Polypropylene cloth was used as shading and it only allowed about 8% of ambient light to penetrate. The air temperature under the cloth was found to be higher than the ambient temperature, but there was no effect on the fruit temperature. They found that the anthocyanin accumulation in fruits was more affected in cluster shading treatments than in leaf shading treatments; there was also less anthocyanin in shaded fruit than there were in the exposed fruit. Morrison and Noble (1990) also found that the anthocyanin content of grapes was lower for 'Cabernet Sauvignon' berries from naturally shaded clusters compared with sun-exposed clusters. Fujita *et al.* (2006) also found that the accumulation of anthocyanins and transcription of their biosynthetic genes were suppressed by shading in the berry skins of 'Cabernet Sauvignon'.

Further research attempted to elucidate the effect of shading on anthocyanin composition. Gao and Cahoon (1994) conducted shading experiments on 'Reliance', a *Vitis* hybrid. They compared two levels of shading, 95% and 55%, with control vines. They found that with 95% shading the total anthocyanin concentration as well as concentrations of individual anthocyanins were decreased, but in comparison with the 55% shading and the sun-exposed vines, the authors found that the percentages of peonidin 3-glucoside, malvidin 3-glucoside and acylated cyanidin derivatives increased while the cyanidin 3-glucoside percentage decreased. These results showed that the level of sun exposure can alter the anthocyanin profile as well as influence the total colour. Later experiments done by Haselgrove *et al.* (2000) on the effect of shading on 'Shiraz' phenolic composition showed that there is a shift from the glucoside anthocyanins to the acylated forms.

Although these studies confirmed that light could potentially influence anthocyanin concentration and composition, the distinction between the effects of light and temperature were not achieved. In order to do this, Bergqvist *et al.* (2001) investigated the effects of sunlight exposure, measured as the amount of photosynthetically active radiation (PAR), on the berry growth and composition of

'Cabernet Sauvignon' and 'Grenache', the clusters were grown from shaded conditions (PAR < 10 $\mu mol.m^{-2}.s^{-1}$) to fully exposed (PAR > 600 $\mu mol.m^{-2}.s^{-1}$) with the treatment extended to a comparison between the afternoon shaded side (north) and afternoon exposed side (south). They found a general increase of anthocyanin concentration in the grape berries which had greater exposure to light, but their results also showed that temperature played a more significant role than light, as the differences between the north (cooler) and south (warmer) side indicated (Bergqvist *et al.* 2001). The authors generally found that at the same PAR level, the midday berry temperature was 3 – 4˚C higher for clusters exposed to afternoon sun (south side). Their results suggest that the effects of light on fruit composition is dependent on the elevation of berry temperature, thus prolonged exposure to direct sunlight should be avoided in warm regions to obtain maximum anthocyanin colour. Work done by Tarara and Spayd (2005) gave further insight into this phenomenon in 'Merlot' grapes. The effect of shade and temperature on berry composition was evaluated by creating naturally shaded conditions, training the shoots of several vines to a single side. Their results showed that light increased the total concentration of anthocyanins, but the greatest increase in anthocyanin synthesis was obtained by chilling exposed clusters. That study also compared seasonal differences, and noted that in a cooler year, the treatment effects of light exposure were greater.

For a more detailed investigation of the effect of shading on both anthocyanin biosynthesis and composition, Downey *et al.* (2004a) looked at the effect of shade on flavonoid biosynthesis in Shiraz berries throughout berry development over three successive seasons using a ventilated shade box which prevented bunch heating. In two out of the three seasons bunch exposure had no effect on anthocyanin content, in the other season studied, anthocyanin content was reduced in response to shade, but was thought to be associated with increased bunch temperature. It was suggested that there may be two systems regulating anthocyanin accumulation in grapes; a first system which synthesizes a base level of anthocyanins, and an inducible system that is light-requiring, which in response to anthocyanin degradation at high temperature can produce supplementary anthocyanin. This study showed that grapes grown in shade did accumulate anthocyanins, which indicates that light is not an absolute requirement for anthocyanin biosynthesis in 'Shiraz' berries.

Downey *et al.* (2004b) also published data about the effects of different levels of bunch exposure levels on 'Cabernet Sauvignon'. It was found that anthocyanin concentrations were generally higher in exposed fruit and as exposure to light was increased, the level of anthocyanins in the fruit also increased. They concluded that light alone might not be the greatest contributor to anthocyanin biosynthesis, but that temperature has a greater effect on anthocyanin content and composition than light. Additionally, anthocyanin composition was altered in shaded fruit compared with naturally exposed fruit and it was found that the anthocyanin composition in shaded fruit was

altered such that it had a greater proportion of deoxygenated anthocyanins, the glucosides of cyanidin and peonidin.

The reports in the literature shed light on the regulation of anthocyanin biosynthesis and maintenance in grapes at two levels. Firstly, that biosynthesis is cultivar dependent, and as such may or may not be influenced by shade. Secondly, the effect of sunlight on anthocyanin production is very closely linked to berry temperature, and it is difficult to separate the effects experimentally. It is therefore important to study temperature and sunlight as separate effects on anthocyanin biosynthesis.

2.3.2.2 Whole-canopy shading

The effects of leaf shading from a dense canopy might lead to lower bunch temperatures, lowered water tension, higher humidity and less air movement, with an eventual decrease in the metabolic rate of the grapevine, causing an unfavourable microclimate for grape production, essentially because conditions which lower the photosynthetic activity of the grapevine have been induced (Wu *et al*. 2003). This sort of microclimate created by dense canopies negatively affects the quality and composition of grapes.

Shading experiments on the whole vine canopy have shown that leaf shading has an adverse effect on the overall grape quality of the product. Shading affects the size, composition and pH of grapes, and can lead to a general delay in fruit ripening (Palliotti & Cartechini 2002, Tomasi *et al.* 2003, Andrade *et al.* 2005, Castro *et al.* 2005, Coventry *et al.* 2005). Researchers have found that leaf shading was significantly correlated to an increase in the potassium concentration which in turn led to an increase in the pH (Rojas-Lara & Morrison 1989, Morrison & Noble 1990, Hunter *et al.* 2004).

Grapevines with excessive vegetative growth often have a significant amount of leaf shading (Hunter *et al.* 1995), where interior leaves do not receive enough PAR. Beyond three leaf layers, light exposure is significantly reduced and shaded leaves are not photosynthetically active. When photosynthesis stops, no sugar is being produced, and ATP is channelled toward activation of the enzyme for potassium exchange. Thus additional potassium is pumped into the berry. Malate still may be respired under these conditions, resulting in a decrease in the organic acid pool. As a result of the utilisation of malic acid by the plant and uptake of potassium, the fruit has low titratable acid (TA) and high pH values. Smart *et al.* (1982, 1985, 1990) and Smart (1982, 1985), suggested that shaded leaves are responsible for potassium uptake in bunches of ripening fruit. This in turn, along with smaller berries caused by vigorous growth and shading, leads to a higher pH and lower glucose and fructose production (Smart 1988, Hunter & Visser 1990a, Hunter *et al.* 1991).

Furthermore, shading causes a higher production of malic acid, and a decrease in tartaric acid production.

Research done by both Rojas-Lara and Morrison (1989) and Morrison and Noble (1990) also found that leaf shading led to an increased amount of malic acid, with the lower respiration rate of malate and the amount of tartaric acid decreased, it will lead to higher pH values. Hunter *et al.* (2004) also found that leaf thinning reverses the effects of leaf shading, increasing the TA, decreasing the malic acid concentration and lowering the pH.

Coventry *et al.* (2005) found that light in the fruiting zone of 'Cabernet Franc' increased the sugar content, the total phenols, flavonols and anthocyanins, and advanced véraison (ripening). Andrade *et al.* (2005) also found that basal leaf removal had no significant effect on yield or on grape soluble solids. This is probably because the basal leaves are the oldest leaves in the vine and at this point in the season they do not contribute as much to the assimilate pool.

Morrison and Noble (1990) found that shaded vines had slower rates of berry growth and sugar accumulation due to leaf shading which reduced the berry growth and the slowed the rate of sugar accumulation, the sugar content in these grapes were lower compared with berries from exposed vines (Rojas-Lara & Morrison 1989, Tomasi *et al.* 2003). Morrison and Noble (1990) showed that anthocyanins were lower in fruit which developed in shaded canopies, while Rojas-Lara and Morrison (1989) also found that anthocyanin accumulation was affected by shade, but concluded that it was affected more by cluster shading than by leaf shading. Even though various researchers have shown that an increase in light also increases the colour intensity and anthocyanins, it does not mean that a highly exposed environment with high light incidence is the ideal microclimate for grape development, there is still an amount of shade needed to protect the bunches from sunburn and to prevent the thermal degradation of anthocyanins (Spayd *et al*. 2002, Tarara & Spayd 2005).

2.3.2.3 Temperature

Anthocyanin production is sensitive to different temperature conditions; temperature can either increase synthesis or decrease synthesis of anthocyanins. Iland (1989) showed that the ideal temperature for anthocyanin biosynthesis is between 17 and 23˚C and above 23˚C the degradation of anthocyanins take place. Hendrickson *et al.* (2004) observed that the growth rates of vines located in warmer sites were between 34 – 63% higher compared to vines in cooler sites. The photosynthesis measurements showed that the difference in carbon gain between grapevines from warmer and cooler sites were due to low temperatures restricting the photosynthetic activity of the vines located in the latter. Higher growing temperatures are associated with a lowered content of malvidin and higher content of delphinidin and petunidin (Tomasi *et al.* 2003). Keller and Hrazdina

(1998) found that cyanidin-3-glucoside was the anthocyanin most strongly influenced by prevailing environmental conditions, while malvidin-3-glucoside was the least affected.

It has already been discussed in this review that the colouration of berry skins is influenced by temperature, but since the specific details of this effect are only hypothetical, it led Yamane *et al.* (2006) to investigate the effect of temperature on anthocyanin biosynthesis in grape berry skins. This is the most comprehensive work done up to date on temperatures influence on the synthesis of anthocyanins. In their experiment they used potted 'Aki Queen' which they kept at different temperatures (20˚C and 30˚C) for periods of two weeks and compared four different stages of growth with the different temperature regimes. This was done firstly to find the temperature sensitive stages for colouration and secondly to find the mechanisms that effect anthocyanin accumulation under different temperature regimes.

The results of that study showed that the amount of anthocyanins accumulated for vines grown at 20˚C were significantly higher compared to the 30 ˚C treatment, and the most sensitive stage of colouration was 1-3 weeks after véraison. The grapevines with the highest anthocyanin content at harvest were also found to be those growing at 20˚C one to three weeks after véraison. The possible increase of anthocyanins could be due to the marked increase of abscisic acid (ABA) in this treatment compared to the others. The concentration of ABA in the berry skins was 1.6 times higher at 20˚C compared to 30˚C. The importance of ABA as a regulator of anthocyanin biosynthesis will be discussed at a later stage in this review.

They also found a higher expression of *VvmybA1,* a myb-related regulatory gene, and the expression of biosynthesis enzymes at 20˚C than at 30˚C. These results indicated that the high and low temperatures during ripening, especially one to three weeks after véraison, affect the production or degradation of ABA which in turn influence the expression of *VvmybA1*. The product of *VvmybA1* then controls the expression of the anthocyanin biosynthetic enzyme genes which cause the increase in anthocyanins.

2.4. VINEYARD MANAGEMENT PRACTICES

There are various types of management practices which can be applied in grapevines to facilitate its adaptation to the environment, either by modifying the micro-climate: via trellising, hedging, shoot positioning, shoot removal, shoot tipping, suckering and leaf removal; or by applying chemicals which will be beneficial for the vine, such as fertilizers and plant growth regulators (PGRs). Thus, to achieve the best quality grapes, producers have various tools at their disposal, which can either be physical (such as leaf removal) or chemical (such as PGRs).

2.4.1 Grapevine photosynthetic capacity as a function of leaf area

Factors that influence the activity of photosynthesis can be environmental or internal. Environmental factors are light intensity, temperature and moisture, while internal factors are the age of the leaf, the yield of the vine and the genetic factor (the variety and species of the vine).

2.4.1.1 Photosynthesis and source-sink relationship within the grapevine

The age of leaves is important for photosynthetic activity, since the photosynthetic capability of leaves increases until it reaches a maximum potential at full maturity and decreases thereafter. The photosynthetic activity of grapevine leaves changes as they mature and also depends on water availability and PAR that is available for that leaf (Sánchez-de-Miguel *et al.* 2005). According to their study on 'Tempranillo', photosynthesis is higher for primary shoots vs. lateral shoots, mature leaves vs. old or young leaves and higher water potential vs. lower water potential. Iland (1989) determined that leaves reached their maximum size about 30 – 40 days after unfolding, and many researchers believe that maximum photosynthetic activity is achieved with maximum leaf size, and it stays at maximum activity for 30 days, after which it starts to decline. Leaves photosynthetic activity has a positive contribution to the assimilate pool till an age of 80 – 90 days. After this period the leaves become sinks and use more photosynthetic product than they produce (Kriedemann *et al.* 1969).

Young leaves are not capable of sustaining themselves; they do not provide enough photosynthetic product until they reach about 30% of full maturity size (Kriedemann *et al.* 1969, Kliewer and Bledsoe 1987, Iland 1989). After this stage the leaves start contributing to the grapevine's net photosynthetic production, but before this stage they are strong sinks and accumulation of acids are found. When the leaves mature, they have a higher sugar to acid ratio and are also net producers, or sources. Koblet (1978) found that leaves are net photosynthetic producers after they reach respectively 50% and 75% of mature size for main shoot and lateral shoot leaves. Mature leaves are not only producers and exporters of photosynthetic products, but they are also very important for reserve accumulation later in the season.

The positions of the leaves are also very important since the position of the mature leaves determine the flow (translocation) of photosynthetic products in the grapevine and this is extremely important when it comes to making informed viticultural decisions. The position of the source changes throughout the season and moves in an upward direction on the shoot as the different leaves reach maturity. It is a prerequisite to know how the assimilation translocation pattern functions and changes throughout the grapevine's development when it comes to making decisions such as the time and application of summer canopy management treatments; consequences of applying actions at the wrong time, are reductions in photosynthesis, plant growth and grape yield (Bota *et al.* 2001, Flexas *et al.* 2002).

2.4.1.2 Leaf area

In a shoot density experiment on grapevines, Castro *et al.* (2005) found that the lowest shoot density (11 shoots per m of cordon) looked at in their experiment had the greatest colour intensity, whilst an amount of 17 shoots per m of cordon had the best canopy microclimate. To broadly define the term 'leaf area-to-fruit weight ratio', it is the amount of leaf area, exposed to sunlight, needed to optimally ripen one gram of fruit. A precise definition as to the correct ratio is a source of debate, but it appears that the leaf area:fruit ratio is largely dependent upon the cultivar and the climatic conditions where that genotype is growing.

Nuzzo (2004) found that the yield produced by a grapevine influenced the leaf area index (LAI) and LAI determines the maximum light intercepted. Thus for an increased yield, the LAI needs to increase for that vine to optimally ripen the fruit. If the LAI becomes too high, the interior leaves are shaded and this lowers the rate of photosunthesis for the total vine. Zulini *et al.* (2004) stated that in extremely vigourous vines, the practice of shoot thinning improves the light penetration, while bunch thinning was sufficient in low vigour vines; these are practices to improve the balance of the vine.

Research has shown that between 10 and 15 cm^2 of leaf area is needed to ripen one gram of fruit to optimal ripeness (Hunter and Visser 1990b, and references therein). Palliotti and Cartechini (2002) found that a leaf area:fruit ratio of approximately 6 cm^2/g resulted in good yield and optimum fruit quality for wine grapes. Below this value the density of the canopy was not capable of ensuring optimal development and maturation, while above this value the canopy size had negative effects on fruit quality. This means that dense canopies have a bigger leaf area-to-fruit ratio in theory, but the shaded leaves within the canopy does not contribute to photosynthetic products. In dense canopies they can actually become sinks, using photosynthetic product that is required for allocation to fruit development.

2.4.1.3 Photoassimilate partioning

Van den Heuvel *et al.* (2002) looked at the effect of shading on the partitioning patterns of ^{14}C photo-assimilates in 'Chardonnay' vines. After 2 hours of pulse $^{14}CO_2$ exposure the partitioning was investigated in a 22 hour chase. There were significant differences between both the light environment and the amount of shaded shoots on the vine. The light adapted shoot trans-located 26.1% and 12.7% more radioactivity to the roots and trunk, respectively, than leaves from the shaded shoots. Recovered ^{14}C in the water-soluble fraction of the fed leaf appeared to be more affected by the number of shoots than by the light environment of the fed leaf. Thus sink strength may have a greater role than light environment on the carbon partitioning; this means that a large proportion of interior leaves versus outer leaves may be costly to the carbohydrate budget of a vine.

The effect of weak light on the distribution of photo-assimilates in *Vitis vinifera* cv. Jingyu was studied by Zhan *et al.* (2002). They found that the ^{14}C-photoassimilate was mostly distributed to young leaves and stems with a little distributed to the roots. The metabolism of ^{14}C-photo-assimilates distributed to the entire vine was also changed under the weak light environment. Porro *et al.* (2001) found that 'Chardonnay' vines that were shaded by 50% provided 50% less dry matter than control vines, even though the shoot growth in shaded vines was higher. They also found that in leaves of shaded grapevines, net photosynthesis was always lower than that of exposed grapevines leaves. Zhan *et al.* (2002) also found that net photosynthetic rate for shaded vines were lower than those exposed to natural light. The opposite extreme to these findings has also been shown to occur, such that leaves with higher transpiration and light exposure are the preferred sinks over grapes of *Vitis vinifera* (Weissenbach and Ruffner 2002). This can be remedied by defoliation, removal of these sinks which in turn would route the flow back to the remaining sinks, namely the clusters.

Evidence from various sources has shown that long-wavelength light can modify the composition of grapes (Smart 1986, Smart 1988, Wolf *et al*. 1990, Bledsoe *et al*. 1988, Haselgrove *et al*. 2000, Spayd *et al*. 2002). Thus low light intensity leads to the following; grapes tend to have higher acid content with low sugars, there appears to be a delay in ripening and colour development is impacted negatively. It seems low light intensity tend to reduce the quality of affected grapes through limitation in photo-assimilate translocation. May *et al.* (1969) in field defoliation studies with 'Sultana', found that removal of one-third to two-thirds of the leaves on fruitful shoots in various combinations after all unfruitful shoots had been removed decreased berry weight, total soluable solids (TSS), and sugar per berry by 3% to 36%. They further showed that carbohydrates are readily translocated between shoots on the same cane, and to a much lesser extent between canes.

Vivin *et al.* (2002) designed a model based on source-sink relationship to simulate the seasonal carbon supply and partitioning among vegetative and reproductive plant parts of an individual vine on a daily basis. The model is based on the hypothesis that carbon allocation is primarily ruled by the sink strength of plant organs. Studies have elucidated the differences between assimilate uptake capacity of leaves that have developed in shade compared to those with good sun exposure. Poni and Intrieri (2001) have found that by measuring the single-leaf gas-exchange response, it makes it possible to model the likely responses of vines under various management regimes. Thus one can determine how to improve the microclimate and subsequent assimilate translocation for a vine with winter pruning and types of trellis systems.

2.4.2 Leaf removal (LR)

In most parts of South Africa the vineyards display excessive vegetative growth, which is mainly due to a favourable climate, especially higher temperatures (Hunter *et* al. 1995). Vigorous canopy growth can detrimentally affect the general canopy microclimate and the source:sink relationships in grapevines, since excessive growth reduces photosynthetic activity of leaves (Smart 1974, Kriedemann 1977, Smart 1985, Koblet 1984, Hunter & Visser 1988a, b, c and 1989). Excess foliage further impedes effective pest and disease control (Stapleton & Grant 1992) which would often lead to a smaller yield and lower quality fruit. High humidity and low air flow in a dense canopy-interior (Hunter & Visser 1990a), usually caused by excessive growth, promotes bunch rot (Smart *et al*. 1990).

Considering the possible negative impacts, excessive vigour is a major concern for producers striving to obtain prolonged, maximum production of quality grapes. Minimizing vegetative dominance will, therefore, require careful plant manipulation to prevent physiological imbalances and ensure that both sources and sinks function to full capacity (Hunter *et al*. 1995). Canopy manipulation is used successfully in grape production to balance the vegetative and reproductive growth of vines. With canopy manipulation one can increase colour, size and overall appearance of fruit, depending on which way the canopy is altered. This is especially important for table grape producers, as an aesthetic product is required.

One of the ways in which a producer can increase colour via canopy manipulation, is by removing leaves, also known as partial defoliation (Hunter *et al*. 1995). Partial defoliation is widely recognized as an invaluable practice to counteract the deleterious effects of excessive growth, and plays a beneficial role in grapevine production (Koblet 1984, Koblet 1987, Kliewer & Smart 1989, Smart *et al*. 1990). For example, work done by Gubler *et al.* (1987) has shown that basal leaf removal was extremely effective in reducing the incidence and severity of bunch rot caused by *Botrytis cinerea*, thus improving grape and vine quality. Partial defoliation as a canopy management practice has already been widely used by viticulturists in search of superior grape quality (Hunter *et al.* 1991), however, although some investigators reported improvements in grape coloration with leaf removal (Koblet 1987, Koblet 1988, Marquis *et al.* 1989, Ezzahouani & Williams 2003), no specific and extensive study on the effect of partial defoliation on pigment accumulation in the grape skin has been done. Leaf removal could influence colour in various ways; one possibility being that it could directly affect photosynthesis by an altered canopy light environment (Smart 1974, Kriedemann 1977, Smart 1985, Koblet 1984, Hunter & Visser 1988a, b, c, 1989 and 1990a, Archer 2002), which could influence the amount of photosynthetic product and/or precursor molecules available for grape colour development. Secondly, it could have an effect on the actual microclimate of the bunch (Buttrose & Hale 1971, Hunter *et al.* 2004, Ezzahouani & Williams 2003, Andrade *et al.* 2005, Castro *et al.* 2005, Poni *et al.* 2006). For this reason, colour could be affected

by light and/or temperature; or by affecting potassium uptake and transport, consequently influencing the pH of the berry and hence grape colour.

2.4.2.1 Applying defoliation

Defoliation is the removal of leaves during the growth period, which could be done either manually or mechanically. The amount of, and position on the shoot of leaves that will be removed depends upon what process the producer wants to influence; whether the action will be used to improve light penetration, pest management, photosynthetic activity, photo-assimilates translocation or improve colour (Buttrose and Hale 1971, Smart 1974, Kriedemann 1977, Pirie and Mullins 1977, 1980, Smart 1982, Smart *et al.* 1982, Smart 1985, Koblet 1984, 1988, Bledsoe *et al*. 1988, Hunter and Visser 1988a, b, c, 1989 and 1990a, Hunter *et al.* 1995, Archer 2002, Poni *et al.* 2003, Hunter *et al.* 2004, Ezzahouani & Williams 2003, Andrade *et al.* 2005, Castro *et al.* 2005, Poni *et al.* 2006). Usually defoliation is the removal of yellow, shaded and photosynthetically inactive leaves from the canopy and the usual time of application is between the pea-size berry developmental stage and véraison. However, in many experiments with partial defoliation, leaves were indiscriminately removed and plants severely stressed (Hunter *et al*. 1995). It is thus necessary to know what the function of the leaves are and what role they play during the different developmental stages, before defoliation is applied.

While focusing on a single problem in research, short- and long-term effects on leaf, fruit, and root physiology have frequently been neglected. Therefore, the effects of different degrees of partial defoliation (33 and 66%) over the whole canopy, commencing at different developmental stages of the vine (budburst, berry set, pea-size, and véraison), on various physiological aspects were examined extensively (Pirie & Mullins 1977, 1980, Moskowitz & Hrazdina 1981, Ribéreau-Gayon & Glories 1982, Singleton 1982, Roubelakis-Angelakis & Kliewer 1986, Koblet 1988, Marquis *et al*. 1989). Poni *et al.* (2003) determined the degree of correlation between total canopy light interception and that of whole-canopy net CO_2 exchange. For a given training system, a method of measuring the total canopy light interception was sufficiently precise to predict the seasonal increase of canopy net CO_2 exchange rate as well as the total leaf area needed for maximum activity. This gives a value above which the additional leaf area will result in shading without enhancing the carbon assimilation. The method of Poni *et al.* (2003) can therefore be used to determine that amount of leaves one can safely remove from the canopy.

2.4.2.2 Effects of defoliation

Defoliation is successfully applied as a method of improving light penetration, such that the amount of diffuse solar radiation reaching interior canopy leaves and fruit decreases geometrically as the number of leaf layers increase (Smart, 1982). Thus, by removing leaves the amount of radiation that reaches the interior canopy should increase (Bledsoe *et al*. 1988). However, in addition to

altering light interception, defoliation may also affect other aspects of vine physiology such as bud fertility, fruit composition and yield (Smart *et al*. 1982, Bledsoe *et al*. 1988, Morrison & Noble 1990). Ezzahouani and Williams (2003) found that the average light measured in the fruiting zone of defoliated leaves ranged from 52 –969 $\mu mol.m^{-2}.s^{-1}$ compared to the average light of 13 – 59 $\mu mol.m^{-2}.s^{-1}$ measured for the un-defoliated vines of 'Ruby Seedless', in Morocco. Thus, the extra leaves removed with defoliation produce a better light and a cooler canopy microclimate (Hunter *et al.* 2004, Poni *et al.* 2006).

Palliotti and Cartechini (2001) found that mean leaf blade inclination varied from 81.4˚ on sun-exposed vines to 15.4˚ for shaded vines, this change in leaf angle is a way for the vine to control the amount of light intercepted as the vine cannot utilise light with an intensity of more than ~800 $\mu mol.m^{-2}.s^{-1}$, while at 28 $\mu mol.m^{-2}.s^{-1}$ the amount of carbohydrate produced by photosynthesis in a leaf, is approximately equal to, if not lower than the amount consumed by respiration. This is known as the leaf's photosynthetic compensation point, and a light level below this intensity causes leaves to turn yellow (Archer & Strauss 1989, Ashton & Admiraal 1990, Archer 2002).

The effect of improving the grapevine microclimate can improve amongst other things, the grape colour. Andrade *et al.* (2005) found that the removal of the basal leaves had a favourable effect on light microclimate in the cluster zone with positive consequences on polyphenol synthesis. Castro *et al.* (2005) found that the removal of basal leaves, from 'Touriga Naçional', improved the canopy microclimate by having a lower leaf layer number in the fruiting zone. This had positive effects on the penetration of radiation in the fruit zone with a lower percentage of interior leaves and clusters. Leaf removal significantly improved the colour intensity and had no significant effects on the yield of the vine. Ezzahouani and Williams (2003) also found that there was a slight colour increase in defoliated vines and an additional average berry weight increase. Changes in fruit composition and wine sensory properties were also reported when the light environment was altered by viticultural practices such as summer pruning, trellising (Smart 1985) or leaf removal (Buttrose & Hale 1971).

Correct application of defoliation insures an optimal microclimate within the canopy, which will ensure that the remaining leaves have optimal photosynthetic capability (Hunter & Visser 1990a). As a compensation mechanism, photosynthetic activity of individual leaves increase with a decrease in leaf area (Koblet *et al*. 1994). Thus, the removal of leaves in unfavourable positions, such as the interior of vine canopies, will cause the other remaining leaves to increase photosynthetic production. Andrade *et al.* (2005) also found that the removal of basal leaves in a canopy had no effect on the grape TSS or yield. They concluded that the remaining leaves were sufficient for ripening. This is in agreement with the findings of Palliotti and Cartechini (2002) in 'Sangiovese'. Effective leaf area, i.e., the amount of leaf area which receives PAR, is the main factor that determines the photosynthetic capacity of a vine and any unfavourable conditions which

stop the stomata guard cells from opening and closing could lower the photosynthetic capacity. In some cases light could be such a factor. Costanza and Charbonneau (2004) have determined that leaf water potential is affected by changes in light interception caused by canopy manipulation. With an increase in light, there is an increase in transpiration and this will cause the closure of stomata and a decrease in photosynthesis. High humidity caused by an unfavourable microclimate is also non-conducive to photosynthesis. In fact, Wu *et al*. (2003) found that high humidity and low light intensity (thus a poor micro-climate), led to a decrease in net photosynthetic rate, stomatal conductance, intra-cellular concentrations of CO_2, as well as less Rubisco, chlorophyll a and chlorophyll b. When they tried to reverse the negative effects of the poor micro-climate by using low humidity and higher light intensity, they found that the effects could not be reversed.

2.4.3 Trellis system

Gladstone and Dokoozlian (2003) looked at the influence that a trellis system has on the light microclimate within grapevine canopies by comparing the influence of leaf area density and canopy configuration within 6 trellis systems commonly used in California. They found that the leaf layer number was greater in non-divided systems compared to divided systems, while shoot positioned systems achieved well exposed cluster zones on higher leaf area densities and lower leaf layer numbers compared to non-positioned canopies. They also found that the fruit zone photosynthetic flux was more than 10% of ambient sunlight in low density canopies and less than 5% in high density canopies. This has a direct effect on photosynthesis and production of assimilates, as well as fruit ripening. In trellis systems with low-light environment due to excessive growth, the trellis system can be expanded to accommodate the limitations on leaf photosynthesis (Schultz 2003). Using a pliable lyre system can be advantageous to any viticulturist since this system allows one to manage both the water and light absorption by the vineyard without any costly add-ons (Carbonneau *et al.* 2004).

The effects of five different training systems were evaluated on ‘Shiraz’ in the Barossa Valley. The different training systems all have different effects on the canopy microclimate, and amount of light received. Berry anthocyanins and total phenolics exhibited a negative relationship with crop load per metre of canopy, while there was a slight positive relationship with bunch exposure, when evaluated over all training systems (Wolf *et al.* 2003). Not only does the training system determine what the light interception will be, but it also affects the source-sink balance (Mattii & Orlandini 2005), which ultimately determines whether the crop will be ripened sufficiently. That is why viticultural practices, especially canopy management, are widely accepted as a means of controlling grape and wine composition, including phenols, by alternating microclimate to influence the vine physiology. As previously discussed, the leaf area-to-fruit ratio is a critical factor in determining grape ripeness and anthocyanin content.

2.4.4 Plant growth regulators (PGRs)

Chemicals commonly used to manipulate the vine and fruits in grape production, which could influence colouration either positively or negatively (Blommaert & Steenkamp 1977), are PGRs. In the grapevine there are five types of PGRs or plant hormones that occur naturally: abscisic acid (ABA), auxins, cytokinins, ethylene and gibberelic acid (GA_3). These hormones all have different functions and peak at different stages during vine and berry development as they are responsible for the regulation of growth and ripening. The function of GA_3, in table grape production, is to increase the size of grape berries which produce an easier eating grape with a more attractive appearance (Dokoozlian & Peacock 2001). Alleweldt (1977) suggested that auxins and GA_3 could synergistically enhance berry growth by inducing attraction sites for assimilates from the vine leaf. However, the problem with GA_3 is that it has an inhibiting effect on PAL activity and ethylene production, and as such significantly retards the accumulation of anthocyanins (Boo *et al.* 1997, Awad & De Jager 2002). ABA and ethylene are known as the maturity hormones (Han *et al.* 1996, Kim *et al.* 1998, Ferrer & Gonzalez-Neves 2002, Delgado *et al.* 2004). ABA plays a role in the ripening of grapes through the stimulation of gluconeogenesis causing an increase of sugar accumulation and content in grapes, and ethylene is a hormone related to ripening by increasing sugar, degrading chlorophyll and increasing anthocyanin production (Szyjewics *et* al. 1984, Giovanni 2001, Corrales-Garcia & Gonzalez-Martinez 2001, Antolin *et al.* 2003, Blakenship & Dole 2003, Mohammed & Abu-Goukh 2003).

Ethylene-releasing compounds like ethephon (2-chloro-ethyl-phosphonic acid, 2-CEPA), applied at véraison, have been used successfully in many cultivars to improve the colour of red grapes (Powers *et al.* 1980, Szyjewics *et al.* 1984, Dokoozlian *et al.* 1993, Fitzgerald & Patterson 1994, Delgado *et al.* 2004, Gallegos *et al*. 2006). Ethephon has been demonstrated to hasten colouration in tomato, pepper, and other plants (Weaver & Montgomery 1974). 'Pinot noir' grown in central Washington often produces poorly coloured fruit and consequently red wines lacking in colour (Powers *et al.* 1980). Ethephon application to grapevines has been shown to accelerate ripening, increase colour, and reduce vegetative growth. Results have been variable depending on time of application, rate of application, cultivar, and location (Weaver & Pool 1971, Coombe & Hale 1974, Eynard *et al.* 1975, Johnson & Nagel 1976, Steenkamp *et al.* 1977, Dokoozlian *et al.* 1993, 1994).

2.4.4.1. Abscisic acid

ABA has been found to increase the anthocyanin concentration and hasten the maturation of grapes when it is applied at the onset of ripening (Boo *et al.* 1997, Ban *et al.* 2003, Jeong *et al.* 2004). Endogenous ABA concentrations in the skins of grape berries are closely correlated to the accumulation of anthocyanins (Inaba *et al.* 1976, Kataoka *et al.* 1983, Pirie & Mullins 1976). This could be due to the stimulating effect of ABA on PAL activity in the grapes skin, or due to the increase in the expression of genes involved in the anthocyanin biosynthetic pathway in response

to ABA. Generally, anthocyanin synthesis and PAL activity have been shown to have a close physiological relationship and when ABA increases PAL activity, anthocyanins levels were also increased (Boo *et al.* 1997). Recent studies have reported that ABA treatment of 'Kyoho' grapes at véraison enhanced the accumulation of anthocyanins and the expression of *PAL*, *CHS*, *CHI*, *DFR*, *LDOX*, and *UFGT* in berry skins (Ban *et al.* 2003, Yamane *et al.* 2006). Jeong *et al.* (2004) also showed that ABA treatment of 'Cabernet Sauvignon' grapes enhanced the expression of *VvmybA1*, a putative regulatory gene of anthocyanin biosynthesis in grapes, and thus with ABA application, the accumulation of anthocyanins were enhanced. In their study Fujita *et al.* (2006) found that ABA had enhanced the accumulation of anthocyanins and the transcription of their biosynthetic genes. Interestingly, Tomana *et al*. (1979) found that high temperatures (30°C and above) inhibited anthocyanin accumulation and reduced endogenous ABA concentration. However, spraying ABA to the clusters restored the level of anthocyanin accumulation in high-temperature-treated grapes (Kataoka *et al.* 1984, Yamane *et al.* 2006). These results suggest that ABA plays a key role in anthocyanin biosynthesis in grapes (Yamane *et al.* 2006).

2.4.4.2. Ethylene (Ethephon, 2-CEPA)

Ethylene regulates many aspects of fruit ripening (Szyjewicz *et al.* 1984, Abeles *et al.* 1992), and is considered to be the hormone of fruit maturation and senescence because it promotes degradation of chlorophyll (Hartmann 1992) with changes in texture and flavour (Worku *et al.* 1975, Lopez *et al.* 2000). For a considerable time, grape ripening was thought to have been ethylene-independent given its classification as a non-climacteric fruit (Coombe & Hale 1973, Abeles *et al.* 1992, El-Kereamy *et al*. 2003, Chervin *et al*. 2005, Chervin *et al*. 2006). However, the grape industry has been using ethephon with some success to enhance berry anthocyanin accumulation and increasing TA/TSS ratio (Weaver & Montgomery 1974, Szyjewicz *et al*. 1984, Shulman *et al.* 1985). The classification of grapes as non-climacteric fruit was mainly due to a set of data showing only weak changes in endogenous ethylene levels around véraison (Coombe & Hale 1973), a development stage often considered the beginning of ripening in grape berries when sugar accumulation increases, acid decreases, the berry softens and pigmentation occurs (El-Kereamy *et al*. 2003). At véraison, intensive anthocyanin synthesis is triggered in the sub-epidermal layer in the berries of red cultivars (Hrazdina *et al.* 1984). However, small increases in respiration rate and internal ethylene concentration have been observed by other researchers, taking into account the variations in gases dissolved in grape tissues at véraison (Allewaldt & Koch 1977). More recent work has indicated that some aspects of non-climacteric fruit ripening may be associated with ethylene responses (Giovanni 2001, Chervin *et al*. 2005), and it is now well established that ethylene is involved during the ripening of non-climacteric fruits such as grape and strawberry (Trainotti *et al.* 2005, Chervin *et al*. 2006).

When ethephon, a strongly acidic water-soluble formulation, is in solution above a pH of 5, the molecule starts to spontaneously hydrolyze and it releases ethylene (Corrales-Garcia & Gonzalez-Martinez 2001). It is the ethylene that is released from ethephon that stimulates the production of endogenous ethylene (Hartmann 1992, Shibli *et al.* 1997, El-Kereamy *et al.* 2003), which increases fruit sugar, acidity and colour, thus accelerating the ripening process (Powers *et al*. 1980, Gomez-Cordoves *et al.* 1996, Lopez *et al.* 2000, Awad & De Jager 2002). In cool growing regions, where heat units are frequently insufficient for grape maturation, it may be appropriate to use growth regulators to accelerate fruit maturity and enhance the colour of grapes (Powers *et al*. 1980, Delgado *et al.* 2004).

Ethylene researchers believe that the response of grapes to its application is a type of wounding response (Reid 1992, El-Kereamy *et al.* 2003). The period between the treatment and the harvest is important, and analyses need to be taken at various intervals because the effects of ethephon on berry composition can vary with time (Gallegos *et al*. 2006). It was recently shown that ethylene synthesis is active immediately before inception of berry ripening (Chervin *et al*. 2006) and that treatment with 1-methyl-cyclopropene (1-MCP), a specific inhibitor of ethylene receptors (Blakenship & Dole 2003), partially blocked berry growth, acidity drop, and anthocyanin accumulation (Chervin *et al.* 2004, Chervin *et al*. 2005). In a previous report, Chervin *et al.* (2004) showed that 1-MCP was unlikely to have unspecific toxic effects since 1-MCP treatment before the inception of ripening did not produce any effect on variables such as berry diameter, skin anthocyanin accumulation, and juice acidity.

The application of ethephon on grapes during véraison results in the highest contents of total polyphenols, anthocyanins, flavanols and proanthocyanidins, which translates into higher visual colour intensities for grapes (Nikolaou *et al.* 2003, Lombard *et al.* 2004). Other researchers have demonstrated that while anthocyanin accumulation in grapes was stimulated, other flavonoid compounds did not increase (Kim *et al.* 1998, Awad & De Jager 2002, Dokoozlian 2002, Peppi & Dokoozlian 2003). This could be due to the fact that anthocyanin synthesis and PAL activity have a close physiological relationship (Boo *et al.* 1997) and when ethephon stimulates PAL activity, the precursors in the biosynthetic pathway is produced which in turn is converted into anthocyanins since UFGT expression is also increased by ethylene (El-Kereamy *et al.* 2003). In a recent paper, Kobayashi *et al.* (2001) showed the existence of an ethylene-responsive element in the sequence of the UFGT gene promoter. El-Kereamy *et al.* (2000) reported that ethephon treatments enhance gene expression of some enzymes CHS, F3H, DFR, LDOX and UFGT that promote the synthesis of phenolic compounds following the application of the product. The application of ethephon on ‘Cabernet Sauvignon’ led to a 6-fold increase of internal ethylene in the 24 hours following application. This rise of internal ethylene was associated with increased levels of CHS and F3H transcripts. The increased gene expression persisted for 20 days before returning to normal levels.

The transcript levels of LDOX and UFGT were similarly enhanced by ethephon. El-Kereamy *et al.* (2003) showed that the levels of anthocyanin were higher in ethylene-treated grapes compared to non-treated grapes. This was the first evidence found that ethylene does indeed trigger gene expression related to anthocyanin biosynthesis. This means that ethylene could induce colour increase by activating the expression of genes involved in the anthocyanin biosynthetic pathway (Gallegos *et al*. 2006). Steenkamp *et al.* (1977) found increases in the anthocyanin concentration 9 days after application, and other researchers have also found that the use of ethephon hastens the accumulation of total anthocyanin content in grape berry skins (Hale *et al.* 1970, Roubelakis-Angelakis & Kliewer 1986, Kyu *et al.* 1998, El Kereamy *et al*. 2003, Gallegos *et al*. 2006).

Table grapes may reach all minimum maturity standards, but attaining adequate colour remains a problem (Jensen *et al*. 1975). A possible solution for the lack of colour is to use ethephon, as it has shown potential in colouring grapes (Szyjewics *et al*. 1984). Ethephon is employed to increase berry coloration; however, being a senescence promoter, ethylene can also induce fruit drop and berry softening at maturity and during storage (Yahuaca *et al*. 2006). Weaver and Pool (1971) applied ethephon to the table grape cultivars Tokay and Emperor. They reported no colour enhancement of 'Tokay', but some concentrations for some sampling dates increased colour in 'Emperor'. Jensen *et al.* (1973) obtained colour enhancement by ethephon applied at 300 and 600 ppm in both 'Tokay' and 'Emperor'. Results were generally best for applications made shortly after véraison. Ethephon reduced berry firmness (Weaver & Montgomery 1974). Application of ethephon at 200 to 1000 ppm has been shown to increase anthocyanin synthesis in 'Carignane' and 'Emperor' grapes (Weaver & Pool 1971). The optimum time for treatment was about 2 weeks after véraison, but only a few ethephon-related reports have studied the changes in the main berry compounds during ripening, to determine the best moment to apply the product (Powers *et al.* 1980, Wolf *et al.* 1990) and the findings seem to indicate that there are cultivar differences (Szyjewics *et al.* 1984, Avenant & Avenant 2006, Gallegos *et al.* 2006, Yahuaca *et al.* 2006).
In further experiments done with ethephon, it was found that the colour enhancing effect of ethephon was increased by addition of ethanol to the application solution (Farag *et al.* 1992, Dokoozlian 2002, El-Kereamy 2002, Nikolaou *et al.* 2003). The reason for this enhanced effect is that ethanol could have enabled the ethephon molecule to penetrate the cuticle (wax) layer and reach the fruit's skin (Farag *et al*. 1992). This synergistic effect may have enhanced colour, but it negatively affected wax deposition on the berry surface and this affects the overall fruit appearance.

Application of ethephon also influenced other parameters of the ripening process and did not only have an effect on colour. In experiments done to compare the effect of different chemicals on grape maturation, ethephon was found to have the greatest effect on sugar levels and pH values, thus hastening the grape's level of ripeness (Han *et al.* 1996, Nikolaou *et al.* 2003, Delgado *et al.*

2004). Chervin *et al*. (2006) found the presence of four ethylene *cis*-elements in the promoter of *VvSUT1*, which is a functionally validated sucrose transporter of grape berry (Ageorges *et al.* 2000). The study of Chervin *et al*. (2006) focused on the role of 1-MCP in sucrose accumulation and in the relative abundance of transcripts coding for two sucrose transporters, *VvSUC11* (also known as *VvSUT1*) and *VvSUC12*, that show increased expression around véraison (Davies *et al.* 1999) and that have been functionally validated as sucrose transporters (Manning *et al.* 2001). Sugar transport may be one target of ethylene action in rice (Ishizawa & Esashi 1988) and in sugar beet (Saftner 1986). However, ethephon-treated grapes, according to the findings of some authors (Steenkamp *et al.* 1977), may appear more mature than they really are, because the colour development of the skin is not closely linked to the increments of the sugar content in the flesh. Therefore, contradictory results have been noted in the effects of ethephon on TSS, pH, K and TA of must, and these have mainly been ascribed to differences in cultivar tested, timing, concentration and application method (Szyjewics *et al.* 1984). If the regulatory role is a causal one, the modification of the anthocyanin or total phenolic levels in the skin should be a reflection of a change in skin sugars (Wicks & Kliewer 1983). The use of light (Jensen 1953, le Roux 1953) and ethephon (Jensen *et al.* 1975), which are known to modify coloration in table grapes, are ways to test this idea.

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Chapter 3

GENERAL MATERIALS AND METHODS

GENERAL MATERIALS AND METHODS

3.1 SITE DESCRIPTION

Experiments were conducted over a single season, 2005/2006, at two sites located in Paarl (33°08′ S, 18°59′ E and Alt. 138 m), and De Doorns (33°47′ S, 19°67′ E and Alt. 457 m). The trials were conducted on 5-year-old commercial *V. vinifera* L. cv. Crimson Seedless (C102-26) vineyards, grafted on 'Richter 110' (*V. Berlandieri* x *V. rupestris* var. 'Martin') rootstocks. For the Paarl site, vine spacing was 1.5 m in east/west orientated rows, with 3.5 m between rows (~1905 vines/ha), and for the De Doorns site vine spacing was 1.8 m in east/west orientated rows, with 2.8 m between rows (~1985 vines/ha). At both sites, the Gable trellising system with split cordon was used.

3.2 EXPERIMENTAL DESIGN

For both the Paarl and De Doorns experimental sites, a single vine row was selected within each experimental block and treatments were assigned in a randomised experimental design. A single vine was used per treatment replicate with two adjacent vines in-row, between replicates, as buffer vines. At the Paarl and De Doorns sites, four and eight treatment replicates were used respectively for the application of ethephon and shading experiments. At the De Doorns site an additional eight treatment replicates were used for the application of an additional defoliation experiment, thus 16 treatment replicates were used at De Doorns, split into eight plots with two treatment replicates per plot. The defoliation treatment was randomly assigned to one of the vines in each plot, while the other remained as a control. Thus at De Doorns eight experimental blocks were selected and the two main treatments were assigned to two single vines in each block; a defoliation treatment was applied to one vine, whilst the other vine was kept as a control. The experimental layout for De Doorns is shown (Figure 3.1) and the treatments used for each replicate (Figure 3.2), was applied at both locations on both sides of the vine, due to the split cordon trellis system used; thus eight bunches were selected per vine. For the De Doorns site, bunches from buffer vines were used to monitor ripening throughout the season (Figure 3.3).

27	X	Block 4	51	X	Block 8
26	Treatment		50	Treatment	
25	X		49	X	
24	X		48	X	
23	Treatment		47	Treatment	
22	X		46	X	
21	X	Block 3	45	X	Block 7
20	Treatment		44	Treatment	
19	X		43	X	
18	X		42	X	
17	Treatment		41	Treatment	
16	X		40	X	
15	X	Block 2	39	X	Block 6
14	Treatment		38	Treatment	
13	X		37	X	
12	X		36	X	
11	Treatment		35	Treatment	
10	X		34	X	
9	X	Block 1	33	X	Block 5
8	Treatment		32	Treatment	
7	X		31	X	
6	X		30	X	
5	Treatment		29	Treatment	
4	X		28	X	
3	Buffer			Continued	
2					
1					
Vine	Row 39		Vine	Row 39	

Canopy management treatment	
50% Leaf Removal	Blue
No Leaf Removal	Orange

Figure 3.1 The randomised-block design at De Doorns for the defoliation experiment. Each block contains six vines with X denoting the buffer vines. A canopy management treatment was assigned to each of the treatment vines.

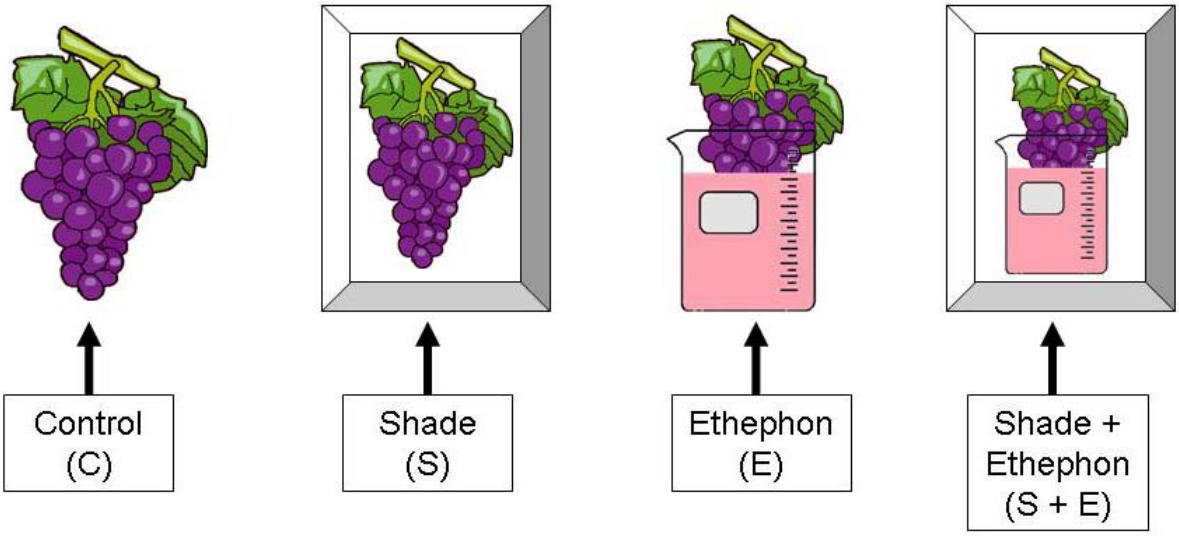


Figure 3.2 Sub-treatments applied to four randomly selected bunches on each side of the vine.

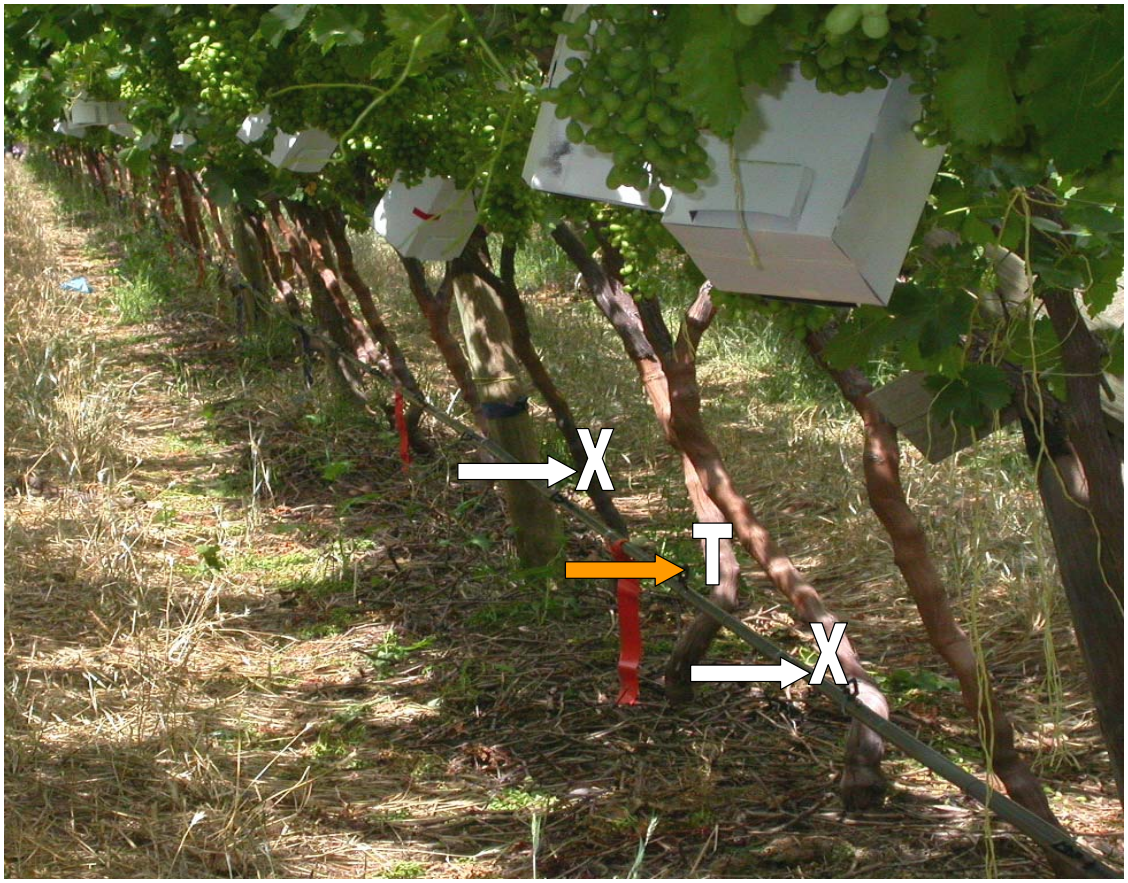


Figure 3.3 The experimental plot lay-out, each block is made up of six vines, two treatment vines (T) and four buffer vines (X). Grapes on the buffer vines were sampled throughout the season to monitor ripeness levels.

3.3 TREATMENTS

3.3.1 Ethephon and shade treatments

For each treatment vine, four similar bunches were selected on alternate sides of the vine for the treatments and numbers were randomly assigned to the different treatments. Treatments were applied on single bunches within a single vine for each replicate: control (no treatment); E (ethephon application only); S (shade application only) and E + S (ethephon and shade application) (Figure 3.2). Shade (S) treatments were applied with the use of shade boxes (Figure 3.4) which were used to cover bunches immediately after berry set (November 2005) when berry diameter was ~2 mm. All bunches were trimmed to a length of ~13 cm before the shade boxes were put into place, and secured to the shoots with cable ties, over the randomly selected bunches. The shaded bunches remained enclosed until harvest. Ethephon (E) treatments were applied, one week after véraison (10 January 2006), by dipping bunches for 20 s into a plant growth regulator solution (200 ppm Ethrel®; 48% w/v Ethephon, Bayer CropScience, USA) with a standard buffering wetting agent (Break-Thru® S240; 75% w/v Polyether-modified polysiloxane, Evonik Industries, Germany), while control bunches were immersed in a water and Break-Thru® (at 40 mL/100L H_2O) solution for 20 s. To ensure that the shaded bunches were not exposed to direct sunlight they were dipped while retained inside the shade boxes. The ethephon concentration used was equivalent to that used for commercial production of 'Crimson Seedless' in the South African table grape industry.

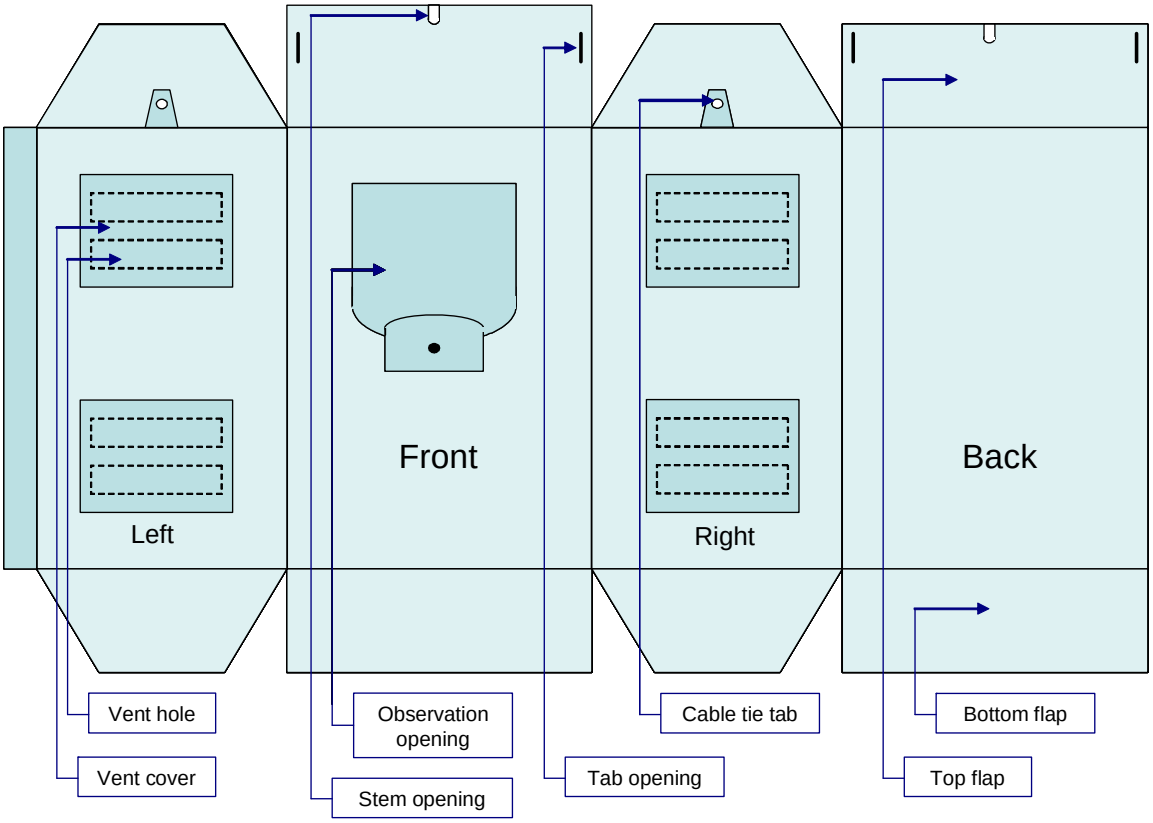
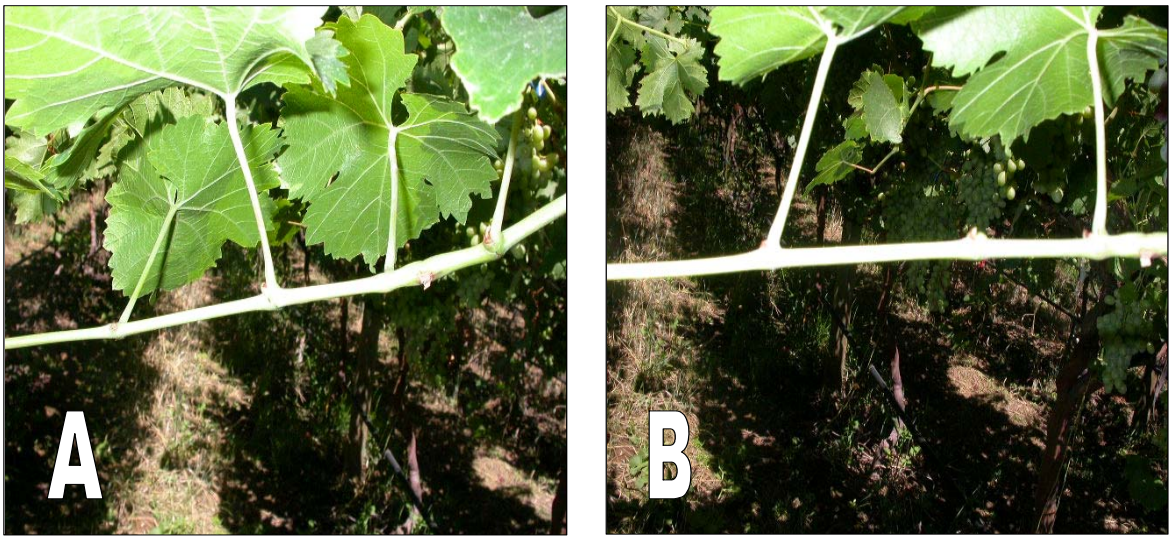


Figure 3.4 The polypropylene shade box designed, by Professor Jeff Bindon, to prevent bunch heating and exclude all light.

3.3.2 Defoliation treatment

The defoliation treatment was applied to determine the effect of 50% leaf removal, applied at véraison, on the light environment of the vine and subsequent anthocyanin content and composition of the grapes. Leaf removal is a common practice in wine grape production, but the effects that it may have on 'Crimson Seedless' anthocyanins have not been examined previously. For the treatment, all of the main shoots were vertically positioned and hedged 5 cm above the uppermost canopy wire as standard practice. Following this, every second leaf on the main shoots of treatment vines were removed (Figure 3.5); starting at the base of the shoot and moving up to the tip of the shoot. None of the lateral shoots were defoliated and there was also no follow-up leaf removal action performed. The shoots were hedged three times during the growing season (14 December 2005, 11 January 2006 and 15 February 2006) to maintain an open canopy.



Figures 3.5 A. Shoot before leaf removal and **B,** after leaf removal.

3.4. Field measurements (De Doorns defoliation experiment)

3.4.1. Light measurements

Light measurements were taken on a weekly basis to compare the light interception between defoliated vines and control vines. The measurements were taken throughout the growth season for both the control vines and the treatment vines from véraison, when the first leaves were removed (11 January 2006), up and till one week before harvest (8 March 2006). The light measurements were taken in the vineyard rows by placing a ceptometer (Sunfleck PAR ceptometer, Decagon Devices, Inc., USA) beneath the third canopy wire in a parallel position to the fruiting zone (Figure 3.6). These measurements were taken when the sun was at its zenith. Light quantity was assessed in $\mu mol.m^{-2}.s^{-1}$ using the ceptometer and expressed as the percentage of the ambient photosynthetically active radiation (PAR).

Figures 3.6 Light penetration into the vineyard canopy was measured with a ceptometer on a weekly basis.

3.4.2. Leaf measurements

Where the defoliation treatment was applied, leaf measurements were taken post harvest. These measurements were done by taking four shoot samples per vine, two shoots from each side of the vine. Shoots were randomly selected, but were representative for each vine. All of the leaves were removed from the four shoots taken per vine and separated into main shoot leaves and lateral shoot leaves. The lengths of the main shoots and the lateral shoots were then measured before the shoots were discarded. The leaves however were kept at -20 ˚C until their leaf areas were measured with a Li-3000 portable area meter (LI-COR Biosciences, USA). Before winter pruning, the shoots per vine were counted and following pruning, the pruning weight was taken for each treatment vine.

3.4.3. Temperature measurements

Tinytag (TGP-4017, Gemini Technologies, UK) data loggers were used to compare ambient temperature with shade-box temperature at 5 min intervals for a period of 10 days (7 - 17 January). Two data loggers were placed in a defoliated canopy, one inside a shade box and the other within the fruiting zone. Two more data loggers were placed in a control canopy similarly with one data logger in a shade box and the other within the fruiting zone.

3.5. Analysis of grape berry composition

3.5.1 Monitoring of berry ripening (De Doorns)

To monitor fruit development during the season, buffer vines at the De Doorns site were used as reference vines. Bunches were randomly selected on each of the buffer vines and assigned a sub-treatment, bunches were either dipped in the ethephon or the control solution. Berry sampling was performed on a weekly basis from véraison till harvest (11 January 2006 – 15 March 2006). Twenty berries were randomly sampled each time and sampling was done from the top, middle and bottom of bunches in order to get a representative sample of each treatment.

The twenty berries sampled for each treatment replicate, were weighed and the average berry weight determined. Following this, the berries were crushed by hand in plastic bags, and the juice was used to determine sugar as total soluble solids (TSS), measured with a refractometer and expressed as degrees Brix (˚Bx); the hydrogen ion concentration (pH) and the titratable acidity (TA) was determined with an automated titrator (Metrohm 785 DMP Titrino with a Metrohm 760 Sample Changer, Metrohm AG, Switzerland). The maturity index (MI) was also calculated, by using the TA/TSS ratio (Boulton *et al*. 1996).

3.5.2. Fruit analysis at harvest

The harvest dates for the two sites differed, Paarl was on 24 February 2006 and De Doorns on 15 March 2006. Treatment bunches were collected and weighed separately. For analyses, 40 berries were collected at random from each bunch, 20 were used to determine TSS, TA, pH and MI as described in 3.5.1, and the remaining 20 berries were frozen at -20 ºC for later analysis of anthocyanins.

3.5.3 Extraction and quantification of anthocyanins

Twenty berries were collected at harvest, weighed and kept frozen at -20 ºC for the analysis of anthocyanins by reverse phase-HPLC, which was performed within 6 months following harvest. The skins of the berries were removed with a scalpel and freeze-dried. The freeze dried skins were finely ground in liquid nitrogen using a mortar and pestle. Ten mL of an acidified hydro-alcoholic solution (50% methanol:water, pH 2 with HCl) was added to 500 mg of the ground berry skins. The mixture was kept at room temperature shaking for 2 hours. The mixture was centrifuged (13000 rpm, 5 min.) and the supernatant retained.

An aliquot of the supernatant was transferred to HPLC vials. Extracts were separated and quantified by HPLC (HP Agilent 1100, Hewlett-Packard, Agilent Technologies, USA) using a Supelcosil guard column with a Supelcosil LC-18-DB (15 x 4.6mm, 3µm) column (Sigma-Aldrich Corporation, Supelco, USA). A ramped gradient of 10% formic acid (Solvent A) and 80% acetonitrile (Solvent B) was used. The solvent gradients and ramping procedure for the method

shown in Table 3.1. The final run time was 55 min. All anthocyanins were quantified according to a malvidin-3-monoglucoside (Mv-gluc, Extrasynthese, France) standard curve and identified by their elution order in comparison to the Mv-gluc standard. Chemstation software (Hewlett-Packard, Agilent Technologies, USA) was used for chromatographic integration and analysis. For spectrophotometric analysis of the extracts, 4 mL of 1M HCl was added to 1 mL of the supernatant and was left to stand at room temperature for 3 h (Iland *et al*. 2000). The absorbance of the solution was measured at 520 nm and 280 nm for the determination of total anthocyanins and total phenolics, respectively. The anthocyanins were identified by their order of elution relative to a standard of Mv-gluc according to the pattern described by Wulf and Nagel (1978). All anthocyanins were quantified according to a Mv-gluc standard curve which had a linear response within the range of concentrations injected onto the column (0.1-1 mg/mL), giving an R^2 value of 0.9927.

Table 3.1 The modified HPLC method: gradient and run-times.

Time (min)	Solvent A	Solvent B	Flow
0.00	7.30%	92.70%	1.00ml/min
15.00	21.40%	78.60%	1.00ml/min
30.00	33.10%	66.90%	1.00ml/min
36.00	80.00%	20.00%	1.00ml/min
40.00	80.00%	20.00%	1.00ml/min
45.00	7.30%	92.70%	1.00ml/min

3.6 Berry measurement results

Table 3.2 Split-plot analysis of fruit composition for 'Crimson Seedless' with blocks split into leaf removal vs. no leaf removal and selected bunches were treated with either shade boxes, ethephon (200 ppm) or a combination of both in 2005-2006. Significance (P) determined for seven factors. P was calculated using repeated measures ANOVA where * indicates (P < 0.05); ** (P < 0.01) and ns is not significant (n = 8).

Parameters	**No leaf removal treatment**				**Leaf removal treatment**			
Sub treatments	**Control**	**Ethephon**	**Shade**	**E + S[a]**	**Control**	**Ethephon**	**Shade**	**E + S[a]**
Bunch weight (g)	556.38	595.19	555.88	536.63	564.38	601.19	541.75	532.75
Berry weight (g)	3.99	4.20	4.16	4.32	4.21	4.40	4.26	4.34
TSS (°Brix)	19.86	20.26	18.86	18.93	20.13	20.26	18.51	19.29
TA (g/L)	4.38	4.20	4.03	4.07	4.24	4.09	4.22	4.19
pH	3.80	3.80	3.86	3.84	3.84	3.84	3.88	3.87

Significance (P)	**A[b]**	**B[b]**	**C[b]**	**A x B[b]**	**A x C[b]**	**B x C[b]**	**A x B x C[b]**
Bunch weight (g)	0.980	0.651	0.117	0.937	0.727	0.403	0.920
Berry weight (g)	0.643	0.062	0.482	0.669	0.406	0.585	0.744
TSS (°Brix)	0.880	0.099	0.002**	0.583	0.853	0.580	0.099
TA (g/L)	0.953	0.382	0.235	0.925	0.235	0.107	0.159
pH	0.511	0.545	0.014*	0.914	0.547	0.878	0.944

[a]Bunches were treated with ethephon and enclosed in a shade box.
[b]A = canopy management treatment (NLR vs. LR); B = ethephon sub-treatment (0ppm vs. 200ppm) and C = shade sub-treatment (control vs. shade box) with the various treatment interactions.

3.7 Anthocyanin measurement results

Table 3.3 Split-plot analysis of anthocyanin composition in skins of 'Crimson Seedless' with blocks split into leaf removal vs. no leaf removal and selected bunches treated with either shade boxes, ethephon (200 ppm) or a combination of both in 2005-2006. Significance (P) determined for seven factors. P was calculated using repeated measures ANOVA where * indicates (P < 0.05); ** (P < 0.01); *** (P < 0.001) and ns is not significant (n = 8).

Parameters	**No leaf removal treatment**				**Leaf removal treatment**			
Sub treatments	**Control**	**Ethephon**	**Shade**	**E + S[a]**	**Control**	**Ethephon**	**Shade**	**E + S[a]**
Total anthocyanincs (mg/kg)	0.633	0.723	0.470	0.656	0.557	0.882	0.342	0.464
Total phenolics (A280 units)	1.559	1.719	1.603	1.593	1.446	1.653	1.589	1.522
3-monoglucoside anthocyanins (mg/kg)	0.423	0.515	0.310	0.466	0.359	0.616	0.206	0.309
Delphinidin	0.032	0.032	0.012	0.023	0.030	0.040	0.008	0.014
Cyanidin	0.061	0.050	0.032	0.029	0.076	0.081	0.023	0.026
Petunidin	0.033	0.034	0.029	0.026	0.033	0.042	0.016	0.019
Peonidin	0.207	0.284	0.176	0.311	0.160	0.325	0.117	0.199
Malvidin	0.091	0.115	0.061	0.077	0.061	0.127	0.041	0.051
3-p-coumaryl anthocyanins (mg/kg)	0.171	0.170	0.134	0.160	0.151	0.202	0.116	0.129
Delphinidin	0.022	0.022	0.017	0.022	0.013	0.025	0.008	0.015
Cyanidin	0.035	0.033	0.028	0.032	0.034	0.041	0.027	0.029
Petunidin	0.031	0.028	0.025	0.026	0.030	0.032	0.023	0.022
Peonidin	0.047	0.051	0.035	0.049	0.042	0.062	0.032	0.038
Malvidin	0.036	0.036	0.029	0.032	0.037	0.041	0.026	0.025

Table 3.3 (continues…)

Table 3.3 (continued)

Significance (P)	A[b]	B[b]	C[b]	A x B[b]	A x C[b]	B x C[b]	A x B x C[b]
Total anthocyanincs (mg/kg)	0.427	0.000***	0.040*	0.702	0.136	0.311	0.333
Total phenolics (A280 units)	0.227	0.187	0.656	0.963	0.570	0.099	0.484
3-monoglucoside anthocyanins (mg/kg)							
Delphinidin	0.549	0.000***	0.000***	0.533	0.204	0.388	0.272
Cyanidin	0.229	0.030*	0.000***	0.818	0.009**	0.280	0.937
Petunidin	0.498	0.000***	0.002**	0.831	0.096	0.094	0.899
Peonidin	0.253	0.000***	0.621	0.504	0.158	0.751	0.279
Malvidin	0.437	0.000***	0.035*	0.880	0.425	0.090	0.304
3-p-coumaryl anthocyanins (mg/kg)							
Delphinidin	0.199	0.000***	0.162	0.926	0.401	0.432	0.482
Cyanidin	0.751	0.000***	0.027*	0.597	0.297	0.552	0.788
Petunidin	0.481	0.000***	0.016*	0.332	0.268	0.480	0.844
Peonidin	0.575	0.000***	0.060	0.525	0.208	0.340	0.253
Malvidin	0.466	0.000***	0.048*	0.446	0.297	0.279	0.533

[a] Bunches were treated with ethephon and enclosed in a shade box.
[b] A = canopy management treatment (NLR vs. LR); B = ethephon sub-treatment (0ppm vs. 200ppm) and C = shade sub-treatment (control vs. shade box) with the various treatment interactions.

3.8 Results and discussions

As there is statistically no interactive effects observed between the main treatment and the shading sub-treatment results (tables 3.2 and 3.3), the canopy management (defoliation) treatment will be discussed in chapter 4 and in chapter 5 the ethephon and shading treatments will be discussed. *Anthocyanin concentrations in Table 3.3 are expressed as mg per g of fresh skin weight.

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3.9 LITERATURE CITED

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Chapter 4

RESEARCH RESULTS

4.1 A study of the interactive effect of defoliation and ethephon on the anthocyanin composition of (*Vitis vinifera* L. cv.) Crimson Seedless

4.1.1 Abstract

This study compared a defoliation treatment in combination with a standard ethephon application and explored the effects on the anthocyanin profile of *Vitis vinifera* 'Crimson Seedless'. Applying a partial defoliation treatment, removal of 50% of the leaves on the main shoots, at véraison was sufficient to significantly increase the photosynthetically active radiation (PAR) in the fruiting zone of 'Crimson Seedless' on a horizontal trellis system, such that there was an increase of 77% compared to control vines. There was no significant difference observed between the ripening rates for the defoliated vines compared to control vines, neither was there any significant difference in the parameters measured at harvest. Except for cyanidin-3-glucoside which was significantly increased by the leaf removal treatment, the leaf removal treatment had generally led to slight decreased concentrations of all anthocyanins quantified. The results indicate that only ethephon treatment had significantly influenced the anthocyanin composition of 'Crimson Seedless', even though leaf removal had significantly increased light interception.

Key words: Crimson Seedless, table grape, *Vitis vinifera*, anthocyanin, cyanidin, defoliation, leaf removal, canopy management, ethephon, Ethrel.

4.1.2 Introduction

'Crimson Seedless' is one of the most important table grape cultivars in South Africa and grown in every table grape producing area (Human & Bindon 2008). However, this cultivar is prone to inadequate colouring (Carreno *et al*. 1997, Cantos *et al*. 2002, Peppi & Dokoozlian 2003, Avenant & Avenant 2006, Peppi *et al*. 2006, Yahuaca *et al*. 2006) which is detrimental to the fruit quality. Various reasons for inferior colour development in grapes have been reported for the conditions prevalent in South Africa, such as high temperatures (Kliewer & Torres 1972, Kliewer 1977, Mori *et al*. 2005, Yahuaca *et al*. 2006) and vigorous growth with dense, shaded canopies (Smart *et al*. 1988, Hunter & Visser 1990, Hunter *et al*. 1991). It is also common practice for 'Crimson Seedless' producers to apply plant growth regulators (Avenant & Avenant 2006, Peppi *et al*. 2006); commonly ethylene releasing compounds like ethephon, applied at véraison, have been used successfully in many *Vitis vinifera* L. cultivars to improve the colour of red grapes (Szyjewics *et al*. 1984, Fitzgerald & Patterson 1994, Delgado *et al*. 2004, Gallegos *et al*. 2006, Yahuaca *et al*. 2006).

Ethephon use have also been shown to negatively effect grape quality (Jensen *et al*. 1975, Yahuaca *et al*. 2006) and the effect on fruit composition varies between cultivars. Contradictory results have been noted in the effect ethephon has on soluble solids, titratable acidity and pH (Szyjewicz *et al*. 1984). This is mainly dependant on the timing, concentration and method of application. Due to the variability of results for ethephon application, alternative methods of colour

enhancement, such as leaf removal, should be investigated. Leaf removal has been extensively researched on wine grapes (Kliewer & Antcliff 1970, Bledsoe *et al*. 1988, Hunter *et al*. 1991, Poni *et al*. 2006). Hunter *et al*. (1991) found that anthocyanin concentration tended to be higher following partial defoliation and to increase the later defoliation was applied, resulting in the highest concentration with defoliation from véraison. Bledsoe *et al*. (1988) found that leaf removal significantly increased the photon fluence rate in the fruiting region of the canopy throughout the season. This could lead to increased cluster exposure, a subject which has also been extensively documented (Kliewer & Antcliff 1970, Crippen & Morrison 1986a and b, Bledsoe *et al*. 1988, Smart *et al*. 1988, Morrison & Noble 1990, Price *et al*. 1995, Bureau *et al*. 2000, Hasselgrove *et al*. 2000, Bergqvist *et al*. 2001, Downey *et al*. 2004, Cortell & Kennedy 2006). The effect of cluster exposure on colour and anthocyanins could be positive (Morrison and Noble 1990, Hunter *et al*. 1991, Price *et al*. 1995) or negative (Kliewer 1977, Crippen & Morrison 1986b, Fitzgerald & Patterson 1994).

In most parts of South Africa the vineyards display excessive vegetative growth; this is mainly due to a favourable climate, especially high temperature, which contributes to this vigorous growth (Hunter *et al*. 1995). The vigorous growth can detrimentally affect the general canopy microclimate and the source:sink relationships in grapevines, since excessive growth reduces photosynthetic activity of leaves (Hunter & Visser 1988a, b and 1989, Koblet 1984, Kriedemann 1977, Smart 1974, 1985a and b). Excess foliage further impedes effective pest and disease control (Stapleton and Grant 1992) which would often lead to a smaller yield and lower quality fruit. High humidity and low air flow in a dense canopy-interior (Hunter and Visser 1990), usually caused by excessive growth, promotes bunch rot (Smart *et al.* 1990). High vigour vines also have too much canopy shading (Smart 1985 a and b, Smart *et al*. 1985) which is detrimental to the light microclimate and has also been shown to increase berry pH and potassium uptake.

Considering the possible negative impacts on production, excessive vigour is a major concern for producers striving to maintain long-lasting and maximum production of quality grapes. Minimizing vegetative dominance will, therefore, require careful plant manipulation to prevent physiological imbalances and ensure that both sources and sinks function to full capacity (Hunter *et al*. 1995). Canopy manipulation is used successfully in grape production to balance the vegetative and reproductive growth of vines. With canopy manipulation one can increase colour, size and overall appearance of fruit, depending on which way the canopy is altered. This is especially important for table grape producers, as an aesthetic product is required. One of the ways in which a producer can increase colour via canopy manipulation, is by removing leaves, also known as partial defoliation (Hunter *et al*. 1995).

Partial defoliation is widely recognized as an invaluable practice to counteract the deleterious effects of excessive growth, and plays a beneficial role in grapevine production (Koblet 1984, 1987,

Kliewer & Smart 1989, Smart *et al.* 1990). Partial defoliation as canopy management practice has already been widely used by viticulturists in search of superior grape quality (Hunter *et al.* 1991); however, although some investigators reported improvements in grape coloration with leaf removal (Koblet 1987, Hunter *et al*. 2004 and references therein), no specific and extensive study on the effect of partial defoliation on pigment accumulation in table grape skin has been done. There are various ways in which leaf removal could influence the colour of grapes, it could be as a result of directly affecting photosynthesis (Hunter & Visser 1988a, b and 1989, Koblet 1984, Kriedemann 1977, Smart 1974, 1985b), thus the photosynthetic product and/or precursor accumulation; or leaf removal could have an affect on the microclimate of the bunch (Buttrose & Hale 1971, Hunter *et al*., 2004), thus affecting the light and temperature environment of the bunch and for this reason grape colour could be affected by light and/or temperature. The present investigation was conducted primarily to answer the following questions: How does leaf removal, applied at véraison, affect grape development, fruit and anthocyanin composition and whether there is an interactive effect between ethephon application and leaf removal on anthocyanin and fruit composition of 'Crimson Seedless'?

4.1.3 Materials and methods

4.1.3.1 Site description

This defoliation trial was conducted in a single season, 2005/2006, as an additional treatment in the study of Human and Bindon (2008). The site was located at De Doorns in the Hex River Valley (33°47′S, 19°67′E) (Western Cape). The Hex River Valley is one of the major table grape growing regions of South Africa with 37.33% of the total amount of table grape vines in South Africa.

The experimental site was a 5-year-old commercial *V. vinifera* L. cv. Crimson Seedless vineyard, grafted on 'Richter 110' (*V. Berlandieri* x *V. rupestris* var. Martin) rootstock. The vine spacing was 1.8 m in east/west orientated rows, with 2.8 m between rows with about 1985 vines/ha. A Gable trellis system with split cordon was used, as described by Avenant (1991). The vineyard management and fertilisation for the site was described by Avenant and Avenant (2006).

4.1.3.2 Treatments

A single vine was used per treatment replicate with two adjacent vines in-row, between replicates, used as buffer vines. There were eight treatment plots with two treatment vines per plot (Chapter 3, Figure 3.1), thus 8 replicates were used per treatment. The main treatment was a defoliation treatment with two levels applied, 0% leaf removal (NLR) and 50% leaf removal (LR). All of the main shoots were vertically positioned and hedged ~3 – 5 cm above the uppermost canopy wire. Following this, the defoliation treatment (LR) was randomly assigned to one of the vines in each plot and was applied one week pre-véraison, whereby every second leaf on the main shoots of treatment vines were removed starting at the base of the shoot and moving up to the tip of the

shoot. This manner of leaf removal was done without bias as both mature and immature leaves were removed (Chapter 3, Figure 3.5). The reason for applying the 50% LR in this way, instead of just removing basal leaves, was because of the horizontal trellis system used in this study. None of the lateral shoots were defoliated and there was also no follow-up leaf removal action performed. The shoots were hedged three times during the growing season (14 December 2005, 11 January 2006 (véraison) and 15 February 2006) as part of standard canopy management to maintain an open canopy.

There was also a sub-treatment applied to each treatment vine. An ethephon application which consisted of a control treatment (C; 0 ppm ethephon) and a standard ethephon application (E; 200 ppm ethephon) applied post-véraison. Four grape bunches were randomly selected for each data vine, two on each side of the vine. A completely randomized design was applied and random numbers were used to assign treatments to each bunch. Treatments were applied on single bunches within a single vine for each replicate. Ethephon (E) treatments were applied, one week after véraison (18 January 2006), by dipping bunches for 20 s into a plant growth regulator solution (200 ppm Ethrel®; 48% w/v Ethephon, Bayer CropScience, USA) with a standard buffering wetting agent (40 mL/100L H_2O Break-Thru® S240; 75% w/v Polyether-modified polysiloxane, Evonik Industries, Germany). There were no follow-up ethephon treatments and each bunch was only dipped once. The buffer vines received the same treatments as the experimental vines and were used to monitor the ripening progress of the grapes for the different treatments by sampling berries throughout the season.

4.1.3.3 Grape sampling

To monitor fruit development during the season, bunches on the buffer vines were used as reference samples. On a weekly basis, from véraison (11 January 2006) till a week before harvest (8 March 2006), 20 berries were randomly collected from the buffer vines.

The twenty berries sampled for each treatment replicate, were weighed, and the average berry weight determined. Following this, the berries were crushed by hand in plastic bags, and the juice was used to determine the sugar as total soluble solids (TSS), measured with a refractometer and expressed as degrees Brix (˚Bx); the pH and the titratable acidity (TA), was determined with a automated titrator (Metrohm 785 DMP Titrino with a Metrohm 760 Sample Changer, Metrohm AG, Switzerland). The maturity index (MI) was also calculated, defined as the ratio of TSS:TA (Boulton *et al*. 1996).

At harvest (15 March 2006) only the treatment bunches from the data vines were collected and weighed separately. For analyses, 40 berries were collected at random from each bunch, 20 berries were used for fruit analysis by determining the berry weight, TSS, TA, pH and MI; and the remaining 20 berries were weighed and frozen at -20ºC for later analysis of anthocyanins.

4.1.3.4 Light measurements

Light measurements were taken on a weekly basis to compare the light penetration of defoliated vines and control vines. The measurements were taken on a weekly basis from véraison (11 January 2006), when the first leaves were removed. The light measurements were taken in the vineyard rows by placing a ceptometer (Sunfleck PAR ceptometer, Decagon Devices, Inc., USA) beneath the third canopy wire in a parallel position to the fruiting zone (Chapter 3, Figure 3.6) when the sun was at its zenith. Light quantity was assessed in $\mu mol.m^{-2}.s^{-1}$ using the ceptometer and expressed as the percentage of the ambient photosynthetically active radiation (PAR).

4.1.3.5 Determination of leaf area

At the end of the season (30 March 2006) shoots were collected to measure leaf area (LA) and shoot lengths. Vines were still in a good condition with mostly mature leaves, there were also no visible signs of sickness, disease or viral symptoms. As the method used for LA and shoot measurements is a destructive technique, only four shoots per vine were sampled and the process was only done once, representative shoots on each vine were randomly selected. The leaves on the shoots were crisp, green and still typical of the majority of the vineyard. All of the leaves were removed from the shoots collected and separated into main shoot leaves and lateral shoot leaves. The lengths of the main shoots and the lateral shoots were then measured before the shoots were discarded. The leaves however were kept at -20°C until their leaf areas were measured by a Li-3000 portable area meter (Li-Cor Biosciences, Lincoln, Nebraska, USA). Before winter pruning, the shoots per vine were counted and following pruning, the pruning weight for each treatment vine was recorded.

4.1.3.6 Extraction and quantification of anthocyanins

The berries collected at harvest were weighed and kept frozen at -20°C for reverse phase-HPLC analysis of anthocyanins, which was done within 3 months of harvesting the grapes. The skins of these berries were removed from the flesh with a scalpel, after which it was freeze-dried and then finely ground in liquid nitrogen using a mortar and pestle. Ten mL of an acidified hydro-alcoholic solution (50% methanol:water; pH 2 with HCl) was added to 500 mg of the ground skins. The skins were extracted at room temperature (18°C), shaking for 2 hours. The extract was centrifuged (13000 rpm, 5 min.) and the supernatant retained. Total phenolics and total anthocyanins in the berry skins were determined according to Iland *et al*. (2000). One mL of the supernatant was acidified with 4 mL of 1M HCl, and left to stand for three hours. The absorbance of the acidified methanolic skin extracts were measured at 280 and 520 nm respectively. The 280 nm measure represents the phenolic compounds present in the grape skin, which includes total anthocyanins, flavonols, flavan-3-ol monomers, proanthocyanidins (tannins) and other simple phenolics; whereas the 520 nm measure represents anthocyanins alone. Both are therefore a rough representation of

total levels within these groups of compounds. Another aliquot of the centrifuged supernatant was transferred to HPLC vials.

Extracts were separated and quantified by reverse phase-HPLC (HP Agilent model 1100, Hewlett-Packard, Agilent Technologies, USA) using a Supelcosil 3µm Opti-guard column with a Supelcosil LC-18-DB (15 x 4.6mm, 3µm) column (Sigma-Aldrich Corporation, Supelco, USA). A ramped gradient of 10% formic acid and 80% acetonitrile was used. The final run time was 55 minutes. All anthocyanins were quantified with a malvidin-3-monoglucoside (Mv-gluc, Extrasynthese, France) standard curve, which had a linear response within the range of concentrations injected onto the column (0.1-1 mg/mL), giving an R^2 value of 0.9927, and identified by their elution order in comparison to the Mv-gluc standard, according to the pattern described by Wulf and Nagel (1978). Chemstation software (Hewlett-Packard, Agilent Technologies, USA) was used for the chromatographic analysis and integration.

4.1.3.7 Calculating an estimate of enzyme activity

The ratios of the different anthocyanin derivatives were calculated according to the method described by Mattivi *et al.* (2006).

Estimate of F3′5′H activity:

$$\text{Ratio 1: } \frac{\text{3′, 5′-dihydroxy}}{\text{3′-hydroxy}} = \frac{\text{sum of delphinidin-, petunidin-, and malvidin-3-glucosides}}{\text{sum of cyanidin- and peonidin-3-glucosides}}$$

Estimate of 3′OMT activity:

$$\text{Ratio 2: } \frac{\text{3′-methoxy}}{\text{3′-hydroxy}} = \frac{\text{peonidin-3-glucosides}}{\text{cyanidin-3-glucosides}}$$

Estimate of 5′OMT activity:

$$\text{Ratio 3: } \frac{\text{3′, 5′-methoxy}}{\text{3′, 5′-hydroxy}} = \frac{\text{malvidin-3-glucosides}}{\text{delphinidin-3-glucosides}}$$

4.1.3.8 Statistical analysis

Statistical analysis was carried out using STATISTICA software (data analysis software system), version 7.1 (StatSoft, Tulsa, OK). A Repeated Measure ANOVA (RMA) technique was applied to the data and the mean values were separated using Duncan's range test for significant differences. The RMA is used to analyze designs in which responses on multiple dependent variables correspond to measurements at the different levels of one or more varying factor, as each vine served as the replicate for all treatments the RMA was able to separate treatment effects statistically and also discern the interactive effects between treatments. This analysis allowed for the comparison of the treatment means at three levels: Canopy management (Defoliation) treatment (C); Ethephon treatment (E); and the interactive effect between defoliation and ethephon treatments (C x E).

4.1.4 Results

4.1.4.1 Light penetration and leaf measurements

The light data collected throughout vine development, as measured from véraison with the ceptometer, shows that there was a significant difference between the leaf removal (LR) and control (NLR) vines for the amount of light intercepted in the fruiting zone (Table 4.1). These values were expressed as a percentage of the ambient PAR measured and the data indicates that the LR treatment had significantly increased the amount of light let into the fruiting zone throughout the entire season, with an average increase of 77% to 192 μmol. $m^{-2}.s^{-1}$ compared to the 109 μmol.$m^{-2}.s^{-1}$ of the NLR vines. This indicates that from véraison there was an increased exposure for the berries and bunches on the LR vines. The inner canopy leaves of the LR vines potentially received an increased amount of PAR. Leaf area (LA) expressed per cm of shoot length showed significant difference between vine treatments. The LR treatment vines had an average of ~12 cm^2/cm of total shoot length compared to the ~15.5 cm^2/cm of total shoot length for the NLR vines. There was no significant difference observed between the shoot lengths or the number of shoots for either canopy treatment due to the continuous canopy management applied during the season (Table 4.2).

Table 4.3 Comparison of sunlight incidence as a percentage of ambient PAR in the canopies of defoliated (LR) and control (NLR) vines over the developmental season 11 January 2006 – 8 March 2006

Date	No leaf removal	Leaf removal	p-value[a]	Minimum PAR[b]	Optimum PAR[c]	Ambient PAR
11 Jan '06	7.45%	13.63%	*	1.61%	40.22%	1988.9
18 Jan '06	6.29%	12.04%	*	1.52%	37.93%	2109.0
27 Jan '06	5.25%	10.62%	*	1.44%	35.89%	2229.0
01 Feb '06	3.99%	7.97%	ns	1.59%	39.87%	2006.5
08 Feb '06	3.28%	5.34%	*	1.72%	42.98%	1861.5
15 Feb '06	3.88%	7.89%	**	1.47%	36.85%	2171.0
22 Feb '06	5.23%	8.78%	*	1.61%	40.26%	1987.0
01 Mar '06	6.23%	9.67%	*	1.71%	42.77%	1870.5
08 Mar '06	7.34%	10.66%	ns	1.82%	45.61%	1754.0
Average	5.44%	9.62%	*	1.61%	40.26%	1997.5

[a] Significance level indicated by * ($p < 0.05$); ** ($p < 0.01$) and ns for non significant.
[b] light compensation point (32 μmol.$m^{-2}.s^{-1}$) expressed as percentage of the ambient PAR.
[c] optimum PAR (800 μmol.$m^{-2}.s^{-1}$) for photosynthesis expressed as a percentage of the ambient PAR.

Table 4.4 Comparison of canopy measurements in defoliated (LR) and control (NLR) vines post harvest (30 March 2006).

Parameter	No leaf removal treatment	Leaf removal treatment	Significance (P)[e]
Number of shoots	82.5	83.25	ns
Shoot length (cm)	434.57	441.74	ns
Leaf area (LA) (cm^2)[a]	6716.41	5562.08	ns
Main shoot (MS) LA (cm^2)[b]	3872.42	3358.4	ns
Lateral shoot (LS) LA (cm^2)[c]	2843.98	2203.67	ns
LA.cm^{-1} of shoot length (cm^2)[d]	15.49	12.12	**
LA.cm^{-1} of MS length (cm^2)[d]	13.02	9.97	ns
LA.cm^{-1} of LS length (cm^2)[d]	22.11	20.59	ns

[a] LA for each shoot including main and lateral shoots.
[b] LA meassured for the main shoot only.
[c] LA meassured for all of the lateral shoots.
[d] An expression of the average LA per cm of MS length, LS length and for total shoot length.
[e] Significance level indicated by ** ($p < 0.01$) and ns for non significant.

4.1.4.2 Grape ripening

During the 10 weeks, from véraison (11 January 2006) until harvest (15 March 2006), the data indicates (Table 4.5) that the canopy management (50% leaf removal) treatment had no significant effect on any of the parameters measured. The ethephon treatment only had a significant effect on the sugar and as expected time significantly influenced all of the parameters measured (Figure 4.1). From the data there was a three way interactive effect on the TA. Berries from the NLR vines control bunches had a significantly higher acid compared to the control bunches of the LR vines, whilst the ethephon bunches acid levels were similar for both NLR and LR vines. The ethephon treated grapes showed had higher pH values from week 6 onward, which explains the significant interactive effect observed for T x E, the same can be said for the TSS where the ethephon treated grapes had significantly higher °Brix readings through weeks 6, 7 and 8. Initially, during the first six weeks, the NLR vines had heavier berries compared to the berries from LR vines, however as the season progressed the differences in average berry weights between the canopy management treatments became minimal and there were no significant difference between the average berry weights of the treatments.

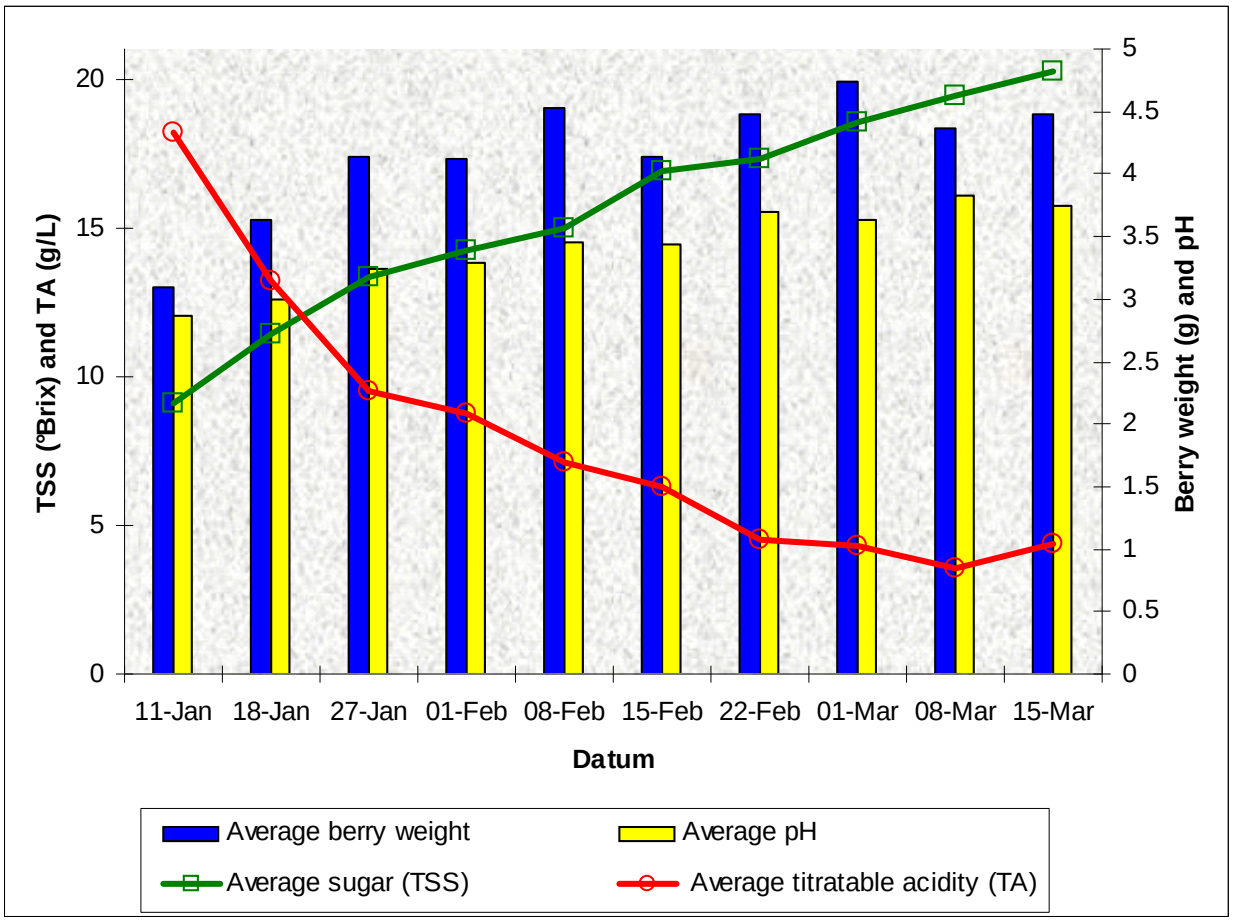

Figure 4.1 Four parameters measured to observe the ripening of 'Crimson Seedless' during the season, from véraison (11 January 2006) until harvest (15 March 2006).

Table 4.5 The effect of defoliation and ethephon on grape ripening parameters (*Vitis vinifera* L.cv. Crimson Seedless).

Date	Berry weight (g)	TSS (°Bx)	TA (g/L)	pH	Berry weight (g)	TSS (°Bx)	TA (g/L)	pH
Treatment		**No leaf removal**				**Leaf removal**		
Sub-treatment				**Etephon**				
11 Jan '06	3.30	9.44	17.26	2.87	3.00	9.28	17.93	2.86
18 Jan '06	3.81	11.26	13.22	2.99	3.62	11.32	13.27	2.99
27 Jan '06	4.35	13.06	9.51	3.24	4.11	13.78	9.19	3.26
01 Feb '06	4.37	14.06	8.85	3.30	3.99	14.46	8.60	3.31
08 Feb '06	4.84	14.92	7.02	3.45	4.46	15.26	6.84	3.42
15 Feb '06	4.45	16.96	6.00	3.45	3.98	17.36	5.85	3.45
22 Feb '06	4.61	17.78	4.37	3.71	4.49	17.64	4.10	3.75
01 Mar '06	4.98	18.44	4.18	3.64	4.65	19.30	4.24	3.68
08 Mar '06	4.60	19.46	3.60	3.82	4.31	19.66	3.49	3.85
15 Mar '06	4.56	20.24	4.27	3.76	4.45	20.58	4.34	3.76
Sub-treatment				**Control**				
11 Jan '06	3.29	9.12	20.33	2.87	2.80	8.64	17.33	2.84
18 Jan '06	3.76	11.76	13.54	3.00	3.34	11.28	12.88	3.00
27 Jan '06	4.17	13.34	9.48	3.26	3.95	13.16	9.82	3.23
01 Feb '06	4.29	14.18	8.73	3.29	3.80	14.24	8.97	3.29
08 Feb '06	4.61	14.90	7.42	3.46	4.22	14.72	7.12	3.45
15 Feb '06	4.27	16.34	6.64	3.43	3.87	16.94	6.67	3.42
22 Feb '06	4.57	16.98	4.70	3.68	4.22	16.88	4.84	3.66
01 Mar '06	4.81	18.24	4.46	3.60	4.55	18.24	4.28	3.60
08 Mar '06	4.46	19.36	3.51	3.82	4.07	19.34	3.67	3.82
15 Mar '06	4.47	20.16	4.42	3.75	4.41	20.14	4.47	3.73

Significance (P)[a]	**Berry weight**	**TSS**	**TA**	**pH**
Treatment (C)[b]	0.064	0.348	0.192	0.962
Sub-treatment (E)[b]	0.357	0.014*	0.118	0.104
Time (T)[b]	0.000***	0.000***	0.000***	0.000***
T x C	0.627	0.108	0.412	0.605
T x E	0.998	0.040*	0.773	0.010**
T x C x E	0.990	0.707	0.025*	0.870

[a] Significance level indicated by * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$) and ns = non significant.
[b] C = canopy management treatment (NLR vs. LR); E = ethephon sub-treatment (0ppm vs. 200ppm) and T = measurements taken from véraison till harvest.

4.1.4.3 Yield components

At the time of harvest there were no significant differences for any of the parameters measured for both of the canopy management treatments applied (Table 4.6). At harvest there were also no significant differences observed for the ethephon sub-treatment that was applied, even though during the ripening period the TSS was significantly increased for ethephon treated bunches compared to the TSS of the control bunches.

Table 4.6 Fruit composition for 'Crimson Seedless' treated either with leaf removal (50%), ethephon (200 ppm) or a combination of both in 2005-2006. Significance (P) determined for three factors and was calculated using repeated measures ANOVA (n = 8).

Parameter	No leaf removal treatment		Leaf removal treatment		Significance (P)[a]		
	Control	Ethephon	Control	Ethephon	C[b]	E[b]	C x E[b]
Bunch weight (g)	556.38	595.19	564.38	601.19	ns	ns	ns
Average berry weight (g)	3.99	4.20	4.21	4.40	ns	ns	ns
TSS (°Brix)	19.86	20.26	20.13	20.26	ns	ns	ns
TA (g/L)	4.38	4.20	4.24	4.09	ns	ns	ns
pH at 20°C	3.80	3.80	3.84	3.84	ns	ns	ns
Maturity index (MI)	4.53	4.82	4.75	4.96	ns	ns	ns

[a] Significance level indicated by ns = non significant.
[b] C = canopy management treatment (NLR vs. LR); E = ethephon sub-treatment (0ppm vs. 200ppm) and C x E is for the interaction between treatments.

4.1.4.4 Anthocyanin composition

The HPLC analysis with the concentration of the various anthocyanins shows (Table 4.8) that only cyanidin-3-glucoside (Cn-gluc) was significantly influenced by the canopy treatment, with the grapes from the defoliated (LR) vines having higher concentrations of Cn-gluc. Generally the LR treatment had led to small decreases in the amounts of all other anthocyanins measured. There were also no significant interactive effect observed between the canopy treatment and ethephon sub-treatment. To calculate an estimate of the enzyme activity for the different treatments, the ratios were calculated according to the methods of Mattivi *et al*. (2006) and given (Table 4.7). The canopy management led to greater flavonoid-3-hydroxylase (F3'H) activity as indicated by the reduced value of ratio 1 in grapes from LR vines and also less methyl transferase (3' OMT) activity (ratio 2). Ethephon on the other hand led to an increase of ratio 2, indicating an increase in 3'OMT activity.

Table 4.7 Comparison of ethephon treatment in defoliated (LR) and control (NLR) vines and the effect on the ratios of anthocyanin classes in 'Crimson Seedless' berry skins (n = 8).

Parameters	No leaf removal treatment		Leaf removal treatment		Significance (P)		
	Control	Ethephon	Control	Ethephon	C[b]	E[b]	C x E[b]
(mg/kg fresh weight)							
3'4'-OH	22.94	52.43	21.20	52.85	ns	***	ns
3'4'5'-OH	13.34	27.77	10.40	27.14	ns	***	ns
Ratios							
3'4'5'-OH / 3'4'-OH	0.63	0.54	0.47	0.50	*	ns	ns
Pn / Cn	3.35	6.48	2.73	4.21	*	**	ns
Mv / Dn[+]	2.83	3.93	2.47	3.22	ns	ns	ns

[+] Dn was not quantifiable for some treatments, thus: NLR (Control) n=5; NLR (Ethephon) n=8; LR (Control) n=4; and LR (Ethephon) n=6
[a] significance level indicated by * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$) and ns = non significant.
[b] C = canopy management treatment (NLR vs. LR); E = ethephon sub-treatment (0ppm vs. 200ppm) and C x E is for the interaction between treatments.
[c] 3'4'-OH: Cyanidin & Peonidin and 3'4'5'-OH: Delphinidin, Petunidin & Mavidin

Table 4.8 Comparison of ethephon treatment in defoliated (LR) and control (NLR) 'Crimson Seedless' vines anthocyanin composition. Significance (P) determined for three factors and was calculated using repeated measures ANOVA (n = 8).

Parameter	No leaf removal treatment		Leaf removal treatment		Significance (P)[a]		
	Control	Ethephon	Control	Ethephon	C[b]	E[b]	C x E[b]
Average berry weight (g)	4.025	4.231	4.173	4.291	ns	ns	ns
Skins of 20 berries (g) Fresh weight	7.387	11.666	8.019	9.747	***	ns	ns
Freeze dried weight	2.570	3.937	2.902	3.473	**	ns	ns
Total anthocyanin (mg/kg)[c]	1607.004	2317.339	1480.428	2341.772	ns	**	ns
Total phenolics (A280 units)	153.800	171.863	144.563	165.338	*	ns	ns
3-monoglucoside anthocyanins (mg/kg)[c]	1078.187	1682.684	978.990	1652.324	ns	**	ns
Delphinidin	82.161	98.563	68.304	103.197	ns	*	ns
Cyanidin	158.260	162.418	189.682	212.921	*	ns	ns
Petunidin	78.301	108.239	83.664	104.962	ns	*	ns
Peonidin	523.440	933.375	477.168	878.489	ns	***	ns
Malvidin	230.662	383.366	165.535	349.479	ns	**	ns
3-p-coumarylglucoside anthocyanins (mg/kg)[c]	436.991	521.743	393.331	527.326	ns	**	ns
Delphinidin	53.016	66.759	33.552	63.585	ns	*	ns
Cyanidin	90.713	100.808	98.781	107.833	ns	ns	ns
Petunidin	78.387	84.117	70.312	83.473	ns	*	ns
Peonidin	122.090	159.731	113.027	164.118	ns	**	ns
Malvidin	92.784	110.329	77.658	108.316	ns	**	ns

[a] Significance level indicated by * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$) and ns = non significant.

[b] C = canopy management treatment (NLR vs. LR); E = ethephon sub-treatment (0ppm vs. 200ppm) and C x E is for the interaction between treatments.

[c] Anthocyanin concentration as expressed in mg per kg of fresh fruit.

4.1.5 Discussion

4.1.5.1 Light penetration and leaf measurements

The differences in sunlight penetration observed between the canopies of the LR and NLR vines can be accounted to the effects of the 50% defoliation applied, since neither the average number of shoots per vine nor the shoot length differed statistically between the treatment vines. However, the total LA did not differ significantly between the LR and NLR treatments, with the LR vines only having 15% smaller LA compared to the NLR vines, even though 50% of the leaves were removed from the main shoots in the LR treatment. The reason for this could be that the remaining leaves on the LR vines could have increased more in size than the control vines leaves, to compensate for the loss of the removed leaves, or it could be due to the hedging applied. Since the shoots were topped three times during the growing season which limited the shoot length and the effect of the 50% LR. The 50% defoliation treatment had a definite effect as was found by the significant increase in light exposure in the LR vines compared to the NLR vines. Nonetheless, the LA as expressed per cm of shoot length indicates a significant difference between treatments, which could account for the difference in sunlight interception observed.

4.1.5.2 Grape ripening

Initially in the beginning of the ripening stage, 2 weeks after defoliation till the 6th week, the average berry weights of LR vines were significantly heavier compared to the berries of LR vines. It seems that increased berry exposure, caused by the LR treatment, led to the smaller berries observed in the LR vines, but if one considers the literature, there should be an increase in the TSS which was not observed. Another possible reason could be that less photo-assimilates were available for cell growth and development after the LR treatment was applied initially, thus this led to smaller berries forming on the LR vines, which obtained normal size at the end of ripening, before harvest, when more leaves became photosynthetically active to produce more photo-assimilates. Powers *et al*. (1980) showed that ethephon accelerated ripening, increased colour and reduced vegetative growth in ‘Pinot noir’ grapes and vines, but results have been variable as to the effects of ethephon on grapes (Szyjewicz *et al*. 1984). For the ethephon treatment, there were points at which the ethephon treated grapes differed in composition from the control grapes, the ethephon treated grapes had a lower TA, with higher TSS and pH values compared to those of the grapes from the control treatment. This is in agreement with Gallegos *et al*. (2006). However, these differences in composition during ripening did not persist, and at the time of harvest, there were no significant difference between treatments. This is in agreement with the finding of Powers *et al*. (1980) who showed that initial acceleration in TSS accumulation was not maintained and there was no significant difference between ethephon treated and control grapes at harvest.

4.1.5.3 Yield components

The data indicates that there was no significant difference for any of the treatments investigated in this study. The isolated effect of ethephon was discussed in the other part of this study, outlined in Chapter 5 (Human & Bindon 2008). Generally, the effect of ethephon on fruit maturity and composition is well documented but the results vary between cultivars. In numerous cultivars ethephon had no effect on TSS or TA levels. No or little change has been the usual finding by researchers examining the effect ethephon has on pH. Total yield and weight per berry were also generally unaffected by ethephon application (Szyjewics *et al*. 1984).

Various researchers (Kliewer & Lider 1968, Smart *et al*. 1988) have shown that clusters that are more exposed to solar radiation have lower malate concentrations than those from shaded treatments. In this regard we would have expected the LR vines to have had lower TA values and higher pH values, as was the case in this study, however the differences were very small. Presumably, this decrease in malate is due to the higher temperatures experienced by the more exposed fruit, as the respiration of malate is increased under these conditions (Bledsoe *et al*. 1988). Bledsoe *et al*. (1988) found that a reduction in malate leads to lower concentrations of potassium and this will lead to decrease in pH. They also found the TSS to increase in sun exposed fruit without any berry weight reduction whilst Poni *et al*. (2006) also noted that leaf removal increased the TSS for two cultivars examined. The slight increase observed in LR vines TSS values are in agreement with these authors, but due to the method of defoliation applied in this experiment there were no significant differences observed. Kliewer and Antcliff (1970) found that leaf removal decreased berry weight and increased TSS. The data from the literature clearly shows that the way in which defoliation is applied can influence the parameters measured at harvest, all of the studies from literature investigated basal leaf removal (BLR). With BLR one improves canopy microclimate and also remove older leaves, that do not contribute to photosynthesis later in the season. The LR as applied in this study, however, removes both old leaves and young leaves which could have contributed to photosynthesis later in the season.

4.1.5.4 Anthocyanin composition

The data reveals that ethephon was the only treatment that had any influence on the skin weights and spectrophotometer measures, and this was supported by the HPLC data, which also indicated that only ethephon treatment had significantly influenced the anthocyanin concentration of the grape skins. Generally, the LR treatment had led to small decreases in anthocyanin concentration with LR vines having ~21% less concentration of anthocyanin compared to NLR vines. However, Cn-gluc was significantly increased by the partial defoliation treatment. Comparing the effect of LR to NLR, data showed that only the anthocyanins of Cn and its derivatives were increased by LR, while all other anthocyanins were slightly decreased

by the LR treatment. Other researchers have observed that anthocyanin content may increase with defoliation treatments. Poni *et al*. (2006) observed greater concentrations of anthocyanins in ‘Sangiovese’ with early defoliation treatments. The effect of ethephon application on the anthocyanin concentrations and anthocyanin profile of ‘Crimson Seedless’ is discussed in detail in the other part of this study (Chapter 5, Human & Bindon 2008).

Since changes in the composition of anthocyanin derivatives can be associated with the activity of the favonoid hydroxylases (F3’H & F3’5’H) and methyl transferases (3’OMT & 5’OMT) (Ageorges *et al.* 2006, Bogs *et al.* 2006, Jeong *et al.* 2006) it is a useful indicator of where the applied treatments may have influenced the flux within the anthocyanin pathway. The results indicate that the F3’5’H activity (Ratio 1) was significantly reduced by the defoliation treatment, this was due to the fact that LR significantly increased the concentration of Cn-gluc and all other anthocyanin concentrations were decreased. The 3’OMT activity (Ratio 2) was significantly decreased by LR, as LR increased the amount of Cn. The lack of any change in the observed 5′OMT activity (Ratio 3), was probably due to the fact that Dn-gluc was not quantifiable in some of the treatment bunches, prohibiting an expression of Ratio 3. Castellarin *et al*. (2006) have shown that genes encoding F3’H and F3’5’H are expressed in the skin of ripening red berries that synthesize anthocyanins; and that there is a correlation between the expressed genes and the ratio of accumulation of red (cyanidin-based) and blue (delphinidin-based) anthocyanins. This indicated that the *VvF3'H* and *VvF3'5'H* expression is consistent with the colour of the ripening bunches. In table grapes, specifically ‘Crimson Seedless’ this means that there is a greater expression of *VvF3'H* with ethephon application since cyanidin-based anthocyanins, specifically the methoxylated form, peonidin-3-monoglucoside, are the major anthocyanins formed. It was also observed that ethephon applications promote the accumulation of peonidin-3-glucoside and malvidin-3-glucoside (highly methoxylated monoglucosides) in the berry skin during ripening (Powers *et al*. 1980, Gallegos *et al*. 2006).

4.1.6 Conclusion

The defoliation treatment applied in this study increased bunch exposure significantly. However, the increase in bunch exposure had little effect on the anthocyanin profile and composition of ‘Crimson Seedless’. The results obtained in this study showed that ethephon had the greatest effect on anthocyanins and that the 50% leaf removal, as applied in this study, had no significant effect on fruit quality, neither anthocyanin concentration nor any other of the quality parameters measured. Due to standard hedging practices applied in the vineyard, the length of the main shoots were restricted, and this could have been why the results of the defoliation treatment were not significantly different from that of the control. In future studies, it would be interesting to note whether a 50% removal of all leaves, main shoot leaves as well as all lateral

shoot leaves, have any impact on the quality of table grapes. Producers will always strive to find practices that can reduce costs and at the same time increase the quality of their products.

4.1.7 Literature cited

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Chapter 5

RESEARCH RESULTS

INTERACTIVE EFFECT OF ETHEPHON AND SHADING ON THE ANTHOCYANIN COMPOSITION OF *VITIS VINIFERA* L. CV. CRIMSON SEEDLESS

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Interactive Effect of Ethephon and Shading on the Anthocyanin Composition of *Vitis vinifera* L. cv. Crimson Seedless

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Abbreviations: Peonidin – Pn; Malvidin – Mv; Cyanidin – Cn; Delphinidin – Dn; Petunidin – Pt; glucoside – gluc

The *Vitis vinifera* cultivar Crimson Seedless primarily accumulates the anthocyanin peonidin-3-glucoside. The research undertook the study of two factors which could influence the accumulation of anthocyanin in grape berry skins: ethephon application and shade. Ethephon treatment at 200ppm applied one week post-véraison significantly increased the concentration of all anthocyanins in berry skins. Peonidin-3-glucoside was found to increase most significantly in response to ethephon application, and was increased 150% compared with an untreated control. The proportion of 3-monoglucoside anthocyanins increased in response to ethephon application. A shading treatment did not affect total anthocyanin concentration in berry skins, but the anthocyanin cyanidin-3-glucoside was decreased significantly by shade. Its content was 50% of a sun-exposed control. The observed effects were found to occur at two sites at which the experiment was performed in the Hex River and Paarl regions. Colour development in the *Vitis vinifera* cultivar Crimson Seedless does not appear to be influenced significantly by bunch shading. The use of commercial growth regulators like ethephon exert a strong influence on anthocyanin production in grape skins of this cultivar, and are therefore a more likely solution to overcome poor colour development in its production.

Vitis vinifera L. cv. Crimson Seedless is a late ripening, red seedless cultivar which can be highly profitable as it fills a niche gap in the market, as it is a seedless alternative for the red seeded grape, 'Emperor'. It is one of the most important table grape cultivars currently produced in South Africa and is widely cultivated in table grape producing regions, such as the Berg River and Hex River Valleys. However, a concern in the commercial production of this cultivar is that it has been observed to lack adequate size and colour required for export, and that practices which improve size, such as girdling and gibberellic acid application, reduce the colour even more (Jensen *et al.,* 1975; Carreno *et al*., 1997; Cantos *et al*., 2002; Peppi & Dokoozlian, 2003; Avenant & Avenant, 2006; Peppi *et al*., 2006; Yahuaca *et al*., 2006; Cantín *et al*., 2007; Peppi *et al*., 2007). Various reasons for inferior colour development in wine and table grapes have been reported for the conditions prevalent in South Africa, such as high temperatures (Kliewer & Torres, 1972; Kliewer 1977; Mori *et al*., 2005; Yahuaca *et al*., 2006) and vigorous growth with dense, shaded canopies (Smart *et al*. 1988; Hunter *et al*., 1991).

Apart from environmental factors which influence colour development in grapes, genetic factors also pre-dispose certain cultivars to accumulate lower levels of anthocyanin. Cantos *et al*. (2002) investigated the polyphenol profiles of seven table grape cultivars, and of the four red cultivars examined Crimson Seedless was found to have the lowest anthocyanin content. The most abundant anthocyanin in most table grape varieties studied was peonidin-3-glucoside (Pn-gluc), followed by cyanidin-3-glucoside (Cn-gluc), which contrasts with *V. vinifera* winegrape cultivars in which the most abundant anthocyanin has been reported to be malvidin-3-glucoside (Mv-gluc) (Mazza, 1995; Cantos *et al*., 2002; Peppi & Dokoozlian, 2003).

In an effort to increase colour and colour uniformity of Crimson Seedless, it has become common practice for producers to apply plant bio-regulators (Avenant & Avenant, 2006; Cantin *et al*., 2007). Ethylene-releasing compounds like ethephon, applied at véraison, have been used successfully in many *Vitis vinifera* L. cultivars to improve the colour of red grapes (Jensen *et al.,* 1975; Szyjewicz *et al*., 1984; Roubelakis-Angelakis & Kliewer, 1986; Fitzgerald & Patterson, 1994; El-Kereamy *et al*., 2000; Delgado *et al*., 2004; Gallegos *et al*., 2006; Yahuaca *et al*., 2006). Earlier work by Steenkamp *et al*. (1977) also showed that ethephon increased phenylalanine-ammonia-lyase (PAL) activity in table grapes which was accompanied by increased colour development. Ethephon treatments have also been shown to enhance gene expression for enzymes involved in anthocyanin biosynthesis such as UDP glucose-flavonoid 3-o-glucosyl transferase (UFGT) with concomitant increases in anthocyanin accumulation in *Vitis vinifera* cv. Cabernet Sauvignon (El-Kereamy *et al*., 2002; El-Kereamy *et al*., 2003). Higher anthocyanin levels at harvest in ethylene-treated Cabernet Sauvignon grapes were due to increased synthesis of anthocyanins, namely Mv-gluc (El-Kereamy *et al*., 2002; El-Kereamy *et al*., 2003).

The effect of cluster shading and/or exposure to sunlight is a subject which has been extensively documented for both table grapes (Kliewer & Antcliff, 1970; Wicks & Kliewer, 1983) and wine grapes (Crippen & Morrison, 1986a, b, Bledsoe *et al*.,

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1988; Smart *et al*., 1988; Morrison & Noble, 1990; Price *et al*., 1995; Bureau *et al*., 2000; Haselgrove *et al*., 2000; Bergqvist *et al*., 2001; Downey *et al*., 2004; Cortell & Kennedy, 2006). The effect of cluster exposure on anthocyanin accumulation is variable, and has been shown to either enhance (Morrison & Noble, 1990; Hunter *et al*., 1991; Price *et al*., 1995), maintain (Haselgrove *et al*., 2000; Downey *et al*., 2004; Ristic *et al*., 2007), or reduce (Kliewer 1977; Crippen & Morrison, 1986b; Fitzgerald & Patterson, 1994) anthocyanin concentration in grapes. The interactive effect of increased solar radiation resulting in increased temperature in sun-exposed clusters may account for this variability, in that increased temperature decreases anthocyanin synthesis (Bergqvist *et al*., 2001; Spayd *et al*., 2002; Downey *et al*., 2004; Mori *et al*., 2005).

A single study exists for table grape cultivars where the combined effect of ethephon application and variation in bunch-exposure on anthocyanin accumulation was studied (Wicks & Kliewer, 1983). In that study, variable responses were found for two table grape cultivars, Ribier and Emperor. In Emperor, shading significantly reduced anthocyanin concentration in grapes, and ethephon application only minimally enhanced anthocyanin accumulation, but under sun-exposed conditions the effect of ethephon was marked, increasing to 350% of the concentration found in the sun-exposed control. Conversely, the same treatment in Ribier was found to have a negligible effect on anthocyanin concentration. This early work may indicate that the response of anthocyanin accumulation to either shade or ethephon application is highly cultivar-specific. Hence, the study aimed to address two key questions: firstly whether shading affects the anthocyanin composition of Crimson Seedless and secondly to observe an interactive effect, if any, between ethephon application and bunch shading on anthocyanin composition. For the production of Crimson Seedless in South Africa, neither the timing nor concentration of ethephon application has been shown to significantly influence colour accumulation (Avenant & Avenant, 2006). Also, the effect of ethephon on fruit composition varies between cultivars; as well as timing, concentration and method of application, as contradictory results have been noted in its effects on soluble solids, titratable acidity and pH (Szyjewicz *et al*., 1984). Thus, for the purpose of the current study, ethephon was applied at one time point and concentration, a single application of 200 ppm ethephon at véraison.

MATERIALS AND METHODS

Site description

Experiments were conducted over a single season, 2005/2006, at two sites located in Paarl (33°08'S, 18°59'E, January-February temperature min. 20°C max. 32°C, Alt. 138 m), in the Berg River Valley and De Doorns (33°47'S, 19°67'E, January-February temperature min. 15°C max. 30°C, Alt. 457 m) in the Hex River Valley. Vineyards were selected for their comparability, since the experiments were performed in a single season. Both sites were located in 5-year-old commercial *V. vinifera* L. cv. Crimson Seedless (C102-26) vineyards, grafted on 'Richter 110' (*V. Berlandieri* x *V. rupestris* var. 'Martin') rootstock. For the Paarl site, vine spacing was 1.5 m in east/west orientated rows, with 3.5 m between rows (~1905vines/ha); and for the De Doorns site, vine spacing was 1.8 m in east/west orientated rows, with 2.8 m between rows (~1985vines/ha). For both sites, a Gable trellis system with split cordon was used, as described by Avenant (1991). Vineyard management and fertilisation for the sites was similar to the practices described by Avenant & Avenant (2006). Climatic data for both locations of the study were obtained from weather stations located close to the experimental site. This data was provided by ARC Infruitec Nietvoorbij.

Treatments

For both of the experimental sites, a single vine was used per treatment replicate with two adjacent vines in-row, between replicates, used as buffer vines. At the Paarl and De Doorns sites, there were four and eight treatment replicates respectively. A completely randomized design was applied. Four similar bunches were selected on alternate sides of each treatment vine and random numbers were used to assign the different treatments to each bunch. Treatments were applied on single bunches within a single vine for each replicate: control (no treatment); E (ethephon application only); S (shade application only) and E + S (ethephon and shade application). E and E + S treatments were applied, one week after véraison (January 2006), by dipping bunches for 20 s into a plant bio-regulator solution (200ppm Ethrel; 48% w/v ethephon) with a standard buffering wetting agent (Breakthru; at 40 mL/100L H_2O). The S and E + S treatments were applied through use of shade boxes which were modelled on the design used by Downey *et al*. (2004), to cover bunches immediately after berry set (November 2005) when berry diameter was ~2 mm. All bunches were trimmed to a length of ~13 cm before the shade boxes were put into place, and secured to the shoots with cable ties, over the selected bunches. The shaded bunches remained enclosed until harvest. Temperature within the shade boxes was compared with ambient conditions within the canopy by insertion of Tinytag (TGP-4017, Gemini Technologies, UK) data-loggers, both with and without shade boxes, in the vineyard canopy. Temperature measurements were logged at 5 minute intervals. Comparison of temperature showed no significant differences between air temperature within the shade box interior and ambient temperature in the canopy.

Grape sampling

Treatment bunches were collected and weighed separately at harvest. For analysis, 40 berries were collected at random from each bunch, 20 berries were frozen at -20ºC for later analysis of anthocyanins and the remaining 20 berries were weighed and then crushed by hand to extract the juice. The juice was used to determine the total soluble solids (TSS), the titratable acidity (TA), the pH and the maturity index (MI) defined as the ratio of TSS:TA (Boulton *et al*. 1996).

Extraction and quantification of anthocyanins

The berries collected at harvest were weighed and kept frozen at -20ºC for reverse phase-HPLC analysis of anthocyanins, which was done within 3 months of harvesting the grapes. The skins of these berries were removed from the flesh with a scalpel, after which it was freeze-dried and then finely ground under liquid nitrogen using a mortar and pestle. Ten mL of an acidified hydro-alcoholic solution (50% methanol:water; pH 2 with HCl) was added to 500 mg of the ground skins. The skins were extracted at room temperature (18°C), shaking for 2 hours. The extract was centrifuged (13000 rpm, 5 min) and the supernatant retained. Total phenolics in the berry skins was determined according to Iland *et al*. (2000). One mL of the supernatant was acidified with

4 mL of 1M HCl, and left to stand for 3 hours. The absorbance of the acidified methanolic skin extracts were measured at 280 nm. Another aliquot of the centrifuged supernatant was transferred to HPLC vials.

Extracts were separated and quantified by reverse phase-HPLC (Agilent model 1100) using a Supelcosil 3 µm Opti-guard column with a Supelcosil LC-18-DB (15 x 4.6 mm, 3 µm) column. A ramped gradient of 10% formic acid and 80% acetonitrile was used. The final run time was 55 min. All anthocyanins were quantified at 520 nm against a malvidin-3-monoglucoside (Extrasynthase, Germany) standard curve, which had a linear response within the range of concentrations injected onto the column. Anthocyanins were identified by their elution order in comparison to the Mv-gluc standard, according to the pattern described by Wulf and Nagel (1978). HP Chemstation software was used for the chromatographic analysis and integration.

Anthocyanin ratios

The ratios of the different anthocyanin derivatives were calculated according to the equations described by Mattivi *et al.* (2006). These values do not account for degradation of anthocyanins or removal of precursors to form other products, but broadly reflect enzyme activity at branch points within the anthocyanin pathway.

$$\text{Ratio 1: } \frac{\text{3', 5'-dihydroxy}}{\text{3'-hydroxy}} = \frac{\text{sum of Dn-, Pt-, and Mv-3-glucosides}}{\text{sum of Cn- and Pn-3-glucosides}}$$

$$\text{Ratio 2: } \frac{\text{3'-methoxy}}{\text{3'-hydroxy}} = \frac{\text{Pn-3-glucosides}}{\text{Cn-3-glucosides}}$$

$$\text{Ratio 3: } \frac{\text{3', 5'-methoxy}}{\text{3', 5'-hydroxy}} = \frac{\text{Mv-3-glucosides}}{\text{Dn-3-glucosides}}$$

Statistical analysis

Statistical analysis was carried out using STATISTICA software (data analysis software system), version 7.1 (StatSoft, Tulsa, OK). A repeated measures ANOVA (RMA) technique was applied to the data and the mean values were separated using Duncan's range test for significant differences. The RMA is used to analyze designs in which responses on multiple dependent variables correspond to measurements at the different levels of one or more varying factor, as each vine served as the replicate for all treatments the RMA was able to separate treatment effects statistically and also discern the interactive effects between treatments. This analysis allowed for the comparison of the treatment means at three levels: E, S and the interactive effect of ethephon in conjunction with shading E x S.

RESULTS

Regional temperature

For the season of the study, the mean, maximum and minimum monthly averages for temperature together with monthly rainfall averages for De Doorns and Paarl are shown in Tables 1 and 2 respectively. For the 2005-2006 growing season, from September to March, De Doorns had a cooler average temperature than Paarl, approximately 6% cooler. However, when the mean minimum and maximum temperatures are compared, it is evident that the cooler average temperature for De Doorns is largely due to cooler overnight temperatures, with the minimum temperature at De Doorns for the growing season being 20 – 37% cooler than Paarl. On the other hand, daytime maximum temperatures at De Doorns were on average 5% higher than at Paarl for the growing season.

Fruit analysis

In the two regions where this study was conducted, Paarl was the earlier ripening region compared to De Doorns, and in the 2005-2006 season this was evident as the grapes were harvested on the 24th of February in Paarl and on the 15th of March in De Doorns**.** At both sites where the experiment was performed, the data indicate that the E-treatment did not significantly influence any of the ripeness parameters measured (Table 3). For Paarl, the S-treatment influenced the average berry weight and maturity index significantly, decreasing the average berry weight and the both skin fresh and dry weights by ~20%. For Paarl, the maturity index was 10% greater for shaded berries compared to the sun-exposed control. At De Doorns the S-treatment did not influence the average berry weight, skin weight or the maturity index significantly. Conversely, the E-treatment, significantly increased the skin weights of treated grapes compared to the control treatment. There was an average increase of ~48% in the skin weights of

TABLE 1

Mean monthly average, minimum and maximum temperatures and rainfall for the experimental site at De Doorns (33°47'S, 19°67'E, Altitude 138 m) for the growing season in 2005-2006.

Year	Month	Average (°C)	Maximum (°C)	Minimum (°C)	Rain (mm)
2005	July	13.3	21.4	5.1	44.2
	August	11.4	17.7	5.1	69.6
	September	15.7	23.6	7.7	16.3
	October	17.6	25.9	9.2	0.0
	November	19.8	27.8	11.8	20.4
	December	20.3	29.7	10.8	0.0
2006	January	23.2	32.0	14.3	1.4
	February	24.1	32.6	15.6	0.4
	March	20.0	29.1	10.8	1.9
	April	17.8	25.5	10.0	49.2
	May	13.2	20.2	6.2	72.7
	June	12.6	20.6	4.6	55.3

TABLE 2

Mean monthly average, minimum and maximum temperatures and rainfall for the experimental site at Paarl (33°08'S, 18°59'E, Altitude 457 m) for the growing season in 2005-2006.

Year	Month	Average (°C)	Maximum (°C)	Minimum (°C)	Rain (mm)
	July	15.0	21.0	9.0	62.0
	August	12.0	16.1	7.9	141.0
2005	September	15.7	21.3	10.2	39.6
	October	17.5	23.1	12.0	17.5
	November	20.6	26.3	14.9	32.0
	December	21.4	28.2	14.6	0.0
	January	24.0	30.6	17.3	0.0
	February	26.1	30.8	20.7	8.1
2006	March	21.5	28.4	14.8	1.9
	April	19.4	25.3	13.6	52.6
	May	13.7	18.6	9.1	243.4
	June	14.5	20.6	8.5	76.4

TABLE 3

Fruit composition for Crimson Seedless treated with either shade boxes, ethephon (200 ppm) or a combination of both in 2005-2006. Significance (P) determined for three factors S = shade treatment; E = ethephon application and S x E = interactive effect of shade and ethephon application. P was calculated using repeated measures ANOVA where * indicates P<0.05; ** P<0.01; *** P<0.001 and ns is not significant (De Doorns: n = 8; Paarl: n = 4).

De Doorns

Parameter	Sun-exposed clusters		Shaded clusters		Significance (P)		
	Control	Ethephon	Control	Ethephon	S	E	S x E
Bunch weight (g)	356.4	595.2	555.9	536.6	ns	ns	ns
Berry weight (g)	4.0	4.2	4.2	4.3	ns	ns	ns
Skin fresh weight (g/berry)	0.37	0.58	0.41	0.58	ns	***	ns
Skin dry weight (g/berry)	0.13	0.20	0.13	0.17	ns	**	ns
TSS (°Brix)	19.9	20.3	18.9	18.9	***	ns	ns
pH (20 °C)	3.8	3.8	3.9	3.8	**	ns	ns
TA (g/L)	4.4	4.2	4.0	4.1	*	ns	ns
Maturity index	3.5	3.7	3.6	3.6	ns	ns	ns

Paarl

Parameter	Sun-exposed clusters		Shaded clusters		Significance (P)		
	Control	Ethephon	Control	Ethephon	S	E	S x E
Bunch weight (g)	706.5	756.3	645.5	621.0	ns	ns	ns
Berry weight (g)	7.2	6.6	5.0	5.8	**	ns	ns
Skin fresh weight (g/berry)	0.73	0.72	0.56	0.61	**	ns	ns
Skin dry weight (g/berry)	0.22	0.21	0.15	0.18	*	ns	ns
TSS (°Brix)	20.1	20.1	20.6	20.3	ns	ns	ns
pH (20 °C)	3.7	3.7	3.8	3.7	ns	ns	ns
TA (g/L)	3.6	3.4	3.1	3.2	ns	ns	ns
Maturity index	4.2	4.4	4.7	4.9	**	ns	ns

the ethephon-treated grape berries compared to the control berries. At De Doorns, the S-treatment decreased TSS and TA by approximately 6%, with a small increase in pH relative to the sun-exposed clusters.

Response of the anthocyanin profile to viticultural treatments

The major anthocyanin types detected were the 3-monoglucosides (gluc) and 3-p-coumarylglucosides (coum), which were represented by the five anthocyanidins commonly found in *Vitis vinifera* grape species. By proportion, the most abundant anthocyanin group in Crimson Seedless was the 3-monoglucosides, followed by the 3-p-coumarylglucosides. The 3-acetylglucosides of Crimson Seedless were also distinguished, but depending on the treatment, were not present in sufficiently quantifiable amounts to report using HPLC analysis. The most abundant anthocyanin present in Crimson Seedless grapes at both sites of the study,

based on the quantity present in control treatment berries, was Pn-gluc (Tables 4 and 5). For the experiment De Doorns Mv-gluc followed Pn-glc in order of abundance, but for the Paarl experiment, this was Cn-gluc. Between the two sites, there were found to be small differences in anthocyanin composition, but statistically these differences were not significant.

For both sites the E-treatment had the most significant effect on total anthocyanin concentration, and was 160 and 105% greater than the control treatment for Paarl and De Doorns respectively. Ethephon was found to increase the concentration of all anthocyanin types quantified. At Paarl, Pn-gluc was the anthocyanin type most significantly increased by the ethephon application (~240%), followed by Cn-gluc (~200%). The result at De Doorns was similar where Pn-gluc was increased ~160% by the E treatment, but was followed by Mv-gluc (~110%). Overall, changes in the anthocyanin profile were observed in response to ethephon application, such that the ethephon treatment increased the proportion of the 3-monoglucosides to total anthocyanins by ~70-80% in Paarl and ~70% at De Doorns relative to the control treatment. Thus, the primary fraction of anthocyanins affected by ethephon were the monoglucoside anthocyanins.

The S treatment was found to have a negligible effect on total anthocyanin concentration for both of the experimental sites. However, the individual anthocyanins were differentially affected by shade at the different sites. The only anthocyanin that was significantly influenced by the shade treatment at both sites, was Cn-gluc, being reduced ~50% and ~34% relative to the sun-exposed control, at De Doorns and Paarl respectively. At De Doorns, Dn-gluc and Pt-gluc were also significantly reduced by the S treatment. A significant interactive effect E x S was observed between the treatments at Paarl for Dn-, Cn- and Pt-gluc, such that sun-exposed clusters with the ethephon application had significantly higher concentrations of these anthocyanins compared to the other treatments. However, no significant interactive effect was observed for total anthocyanins or the most abundant anthocyanin, Pn-gluc.

Ratios of methylated and hydroxylated forms of anthocyanins

The ratios of the different derivatives of methylated and hydroxylated forms of the anthocyanins have been used as an estimate of the degree of enzyme activity of the enzymes flavonoid-3’**,** 5’-hydroxylase (F3’5’H), 3’O-methyltrans-ferase (3’OMT) and 5’O-methyltransferase (5’OMT) (Mattivi *et al*., 2006). Since changes in the composition of anthocyanin derivatives can be associated with the activity of these enzymes (Ageorges *et al.,* 2006; Bogs *et al.,* 2006; Jeong *et al.,* 2006) it is a useful indicator of where the applied treatments may have influenced flux within the anthocyanin pathway, but this technique does not account for the possible degradation of anthocyanins. The ratios for the anthocyanin derivatives are given in Table 6. At the De Doorns site, F3’5’H activity as estimated by the ratio of 3’5’-dihydroxy/3’-hydroxy anthocyanins was lowered in response to the ethephon treatment, thus indicating a potentially greater flux within the anthocyanin pathway was towards the F3’H branch. However, for the Paarl site, there was no significant effect of ethephon application on this ratio. For both sites, the ratio of 3’methoxy/3’-hydroxy anthocyanins was increased in response to both E and S. This ratio gives an indication of the potential 3’OMT activity within the pathway, i.e. the methylation of cyanidin to form peonidin. The value of ratio of 3’5’methoxy/3’5’-hydroxy anthocyanins was not significantly affected by either of the treatments, which may indicate that the conversion of delphinidin to malvidin was not altered in this study.

TABLE 4

Anthocyanin composition in skins of Crimson Seedless treated with either shade boxes, ethephon (200 ppm) or a combination of both in 2005-2006 at De Doorns. Significance (P) determined for three factors S = shade treatment; E = ethephon application and S x E = interactive effect of shade and ethephon application. P was calculated using repeated measures ANOVA where * indicates P<0.05; ** P<0.01 and ns is not significant (n = 8).

Parameter	Sun-exposed clusters		Shaded clusters		Significance (P)		
	Control	Ethephon	Control	Ethephon	S	E	S x E
Total anthocyanin (mg/kg)	1354.6	3614.4	1752.7	3139.3	ns	**	ns
Total phenolics (A280 units)	192.4	200.0	166.3	190.6	*	*	ns
3-monoglucoside anthcyanins (mg/kg)	955.4	2873.7	1136.8	2576.7	ns	**	ns
Delphinidin	65.7	127.1	73.3	64.7	ns	*	*
Cyanidin	149.0	483.0	98.6	148.6	*	*	*
Petunidin	68.2	129.9	75.6	75.3	ns	*	*
Peonidin	586.1	1853.0	777.6	2105.7	ns	**	ns
Malvidin	104.4	280.7	111.5	182.4	ns	**	ns
3-p-coumaroyl anthocyanins (mg/kg)	315.4	590.7	362.8	489.2	ns	*	ns
Delphinidin	22.1	74.5	18.2	41.2	ns	ns	ns
Cyanidin	75.2	148.3	87.9	100.4	ns	**	*
Petunidin	61.9	65.0	55.8	62.3	ns	ns	ns
Peonidin	92.6	207.7	121.7	206.4	ns	**	ns
Malvidin	63.5	95.3	79.2	79.0	ns	ns	ns

TABLE 5

Anthocyanin composition in skins of Crimson Seedless treated with either shade boxes, ethephon (200 ppm) or a combination of both in 2005-2006 at Paarl. Significance (P) determined for three factors S = shade treatment; E = ethephon application and S x E = interactive effect of shade and ethephon application. P was calculated using repeated measures ANOVA where * indicates P<0.05; ** P<0.01; *** P<0.001 and ns is not significant (n = 4).

Parameter	Sun-exposed clusters		Shaded clusters		Significance (P)		
	Control	Ethephon	Control	Ethephon	S	E	S x E
Total anthocyanin (mg/kg)	1607.0	2317.3	1414.6	2154.2	ns	***	ns
Total phenolics (A280 units)	153.8	171.9	160.3	159.3	ns	ns	ns
3-monoglucoside anthcyanins (mg/kg)	1078.2	1682.7	901.9	1526.6	ns	***	ns
Delphinidin	82.2	98.6	39.5	74.4	***	*	ns
Cyanidin	158.3	162.4	75.7	94.8	**	ns	ns
Petunidin	83.7	105.0	65.4	84.1	*	***	ns
Peonidin	523.4	933.4	538.2	1028.8	ns	***	ns
Malvidin	230.7	383.4	192.1	244.5	ns	***	ns
3-p-coumaroyl anthocyanins (mg/kg)	437.0	521.7	424.7	530.7	ns	***	ns
Delphinidin	53.0	66.8	53.2	71.2	ns	*	ns
Cyanidin	90.7	100.8	88.1	105.4	ns	**	ns
Petunidin	78.4	84.1	80.2	84.4	ns	***	ns
Peonidin	122.1	159.7	110.5	164.5	ns	**	ns
Malvidin	92.8	110.7	92.7	105.1	ns	***	ns

TABLE 6

Ratios of anthocyanin classes in skins of Crimson Seedless treated with either shade boxes, ethephon (200 ppm) or a combination of both in 2005-2006. Significance (P) determined for three factors S = shade treatment; E = ethephon application and S x E = interactive effect of shade and ethephon application. P was calculated using repeated measures ANOVA where * indicates P<0.05; ** P<0.01 and ns is not significant (De Doorns: n = 8; Paarl: n=4).

Ratio	Sun-exposed clusters		Shaded clusters		Significance (P)		
	Control	Ethephon	Control	Ethephon	S	E	S x E
De Doorns							
3'5'-dihydroxy / 3'-hydroxy	0.63	0.54	0.51	0.38	**	**	ns
3'-methoxy / 3'-hydroxy	3.35	6.48	6.96	10.74	**	*	ns
3'5'-methoxy / 3'5'-hydroxy	2.83	3.93	3.69	3.42	ns	ns	ns
Paarl							
3'5'-dihydroxy / 3'-hydroxy	0.42	0.26	0.34	0.15	ns	ns	ns
3'-methoxy / 3'-hydroxy	3.75	4.38	7.76	14.13	**	*	*
3'5'-methoxy / 3'5'-hydroxy	1.85	2.26	1.52	3.10	ns	ns	ns

DISCUSSION

Effect of shade and ethephon on fruit composition

The effects of ethephon on fruit maturity and composition are well documented in literature but the results are variable. For numerous cultivars generally no changes in TSS or acidity have been noted, and no or little change in pH, as well as total yield and weight per berry have been found due to its application (Szyjewicz *et al.,* 1984). The ripening response observed in this study is therefore in agreement with literature to date. On the other hand, grape developmental responses to natural or artificial shading are variable. Natural cluster shading has been noted to increase berry weight and either increased or maintained berry TA with negligible differences in TSS accumulation (Kliewer & Antcliff, 1970; Reynolds *et al*., 1986; Crippen & Morrison, 1986a; Morrison & Noble, 1990; Price *et al*., 1995). Increased TA was proposed to be due to reduced malate respiration under shaded conditions (Kliewer & Lider, 1968; Bledsoe *et al*., 1988; Smart *et al*., 1988; Price *et al*., 1995). Artificial shading of grape clusters from flowering or berry set has been shown to produce either no change in fruit composition, or decreased berry weight, increased pH due to accumulation of K^+ and increased TA due to increased malate while TSS was unchanged (Bindon, 2004; Downey *et al*., 2004;

Cortell & Kennedy, 2006; Ristic *et al*., 2007). The response to artificial shading in the experiments was variable between sites. In the case of the De Doorns experiment it delayed ripening. The reduced TSS and TA associated with this experiment was therefore probably not due to increased respiration of malic acid in the berries, but rather delayed maturity. However, in the case of the Paarl experiment, the results are in agreement with Ristic *et al*. (2007), where shade decreased berry weight while not altering TSS accumulation in Shiraz grapes. The reason for this reduced berry weight under artificial shade conditions has not been ascertained through research, but may be due to reduced dry weight accumulation pre-véraison, where the berry is unable to directly fix carbon via photosynthesis due to extreme darkened conditions and berry chlorosis (Downey *et al*., 2004).

Anthocyanin profile

Various researchers have shown that low light environments reduced the colour of grapes (Crippen & Morrison, 1986b; Smart *et al*., 1988; Morrison & Noble, 1990; Price *et al*., 1995; Bergqvist *et al*., 2001). However, as investigations into the effects of exposure on colour continued, a growing body of contradictory data began to appear (Downey *et al*., 2006). It was found in some studies that no change in total anthocyanins was observed with artificial shading (Downey *et al*., 2004; Ristic *et al*., 2007), while others have reported that increased exposure to sunlight resulted in decreased anthocyanin levels in berries, most likely due to decreased anthocyanin synthesis at the higher berry temperatures under these conditions (Hunter *et al*., 1995; Bergqvist *et al*., 2001; Spayd *et al*., 2002). In some cases, there was no difference in total anthocyanin levels, but alteration in anthocyanin composition was observed in response to altered light conditions within the bunch zone (Price *et al*., 1995; Haselgrove *et al*., 2000; Spayd *et al*., 2002; Downey *et al*., 2004). In studies on Shiraz grapes, shaded fruit generally did not have altered total anthocyanin levels, but was shown to have an increased proportion of dioxygenated anthocyanins, namely glucosides of Cn and Pn (Downey *et al*., 2004; Ristic *et al*., 2007). In another study, Keller & Hrazdina (1998) also found Cn to be the most strongly influenced by prevailing environmental conditions, while Mv was the least affected. In the current study the anthocyanin Cn in Crimson Seedless reflected the sensitivity to the shade treatment shown in other literature, and was the anthocyanin which responded most significantly to shaded conditions. However, it was decreased as a proportion of total anthocyanins in both sites where the experiment was performed rather than increased as shown in other studies (Downey *et al*., 2004; Ristic *et al*., 2007). In this research, Crimson Seedless was shown to accumulate primarily glucosides of Pn, in agreement with Cantos *et al.* (2002). This indicates that the biosynthetic pathway for anthocyanin in this cultivar is genetically pre-disposed to favour the F3'H branch of pathway toward Pn, rather than the F3'5'H branch of the pathway toward Mv as for winegrapes (Boss *et al*., 1996; Castellarin *et al*., 2006). Unlike the studies on Shiraz (Downey *et al*., 2004; Ristic *et al*., 2007) shading of Crimson Seedless berries did not alter the proportion of Pn, most likely because synthesis of this anthocyanin from Cn is already favoured in this cultivar at a genetic level. Analysis of the ratios of anthocyanins showed that conversion of Cn to Pn via the enzyme 3'OMT was most likely affected by the shade treatment, such that synthesis of Pn was favoured under shade conditions. However, only gene expression studies or radiolabelling experiments will verify this hypothesis.

The overriding effect of ethephon application to increase anthocyanin production in Crimson Seedless was an expected result of the research. Ethephon is well-known to increase the red colour of grapes of multiple cultivars (Takeda & Badr, 1977; Wicks, 1979; Powers *et al*., 1980; Wicks & Kliewer, 1983; Keller & Hrazdina, 1998; El-Kereamy *et al*., 2003; Lombard *et al*., 2004; Gallegos *et al*., 2006). There is speculation that the increase in anthocyanin is associated with increases in the presence of the monoglucoside pigments Pn and Mv (Takeda & Badr, 1977; Wicks, 1979; Powers *et al*., 1980; Wicks & Kliewer, 1983; El-Kereamy *et al*., 2003) indicating increased production of terminal anthocyanins within the biosynthetic pathway. This was confirmed through gene expression studies on Cabernet Sauvignon, which showed upregulation of the gene for UFGT (El Kereamy *et al*., 2002; El-Kereamy *et al*., 2003). In the current study, it is interesting to note the synergistic enhancement of Dn, Cn and Pt by ethephon in sun-exposed fruit at the Paarl trial site. This is unexpected, since ethephon application was generally found to promote the accumulation of highly methoxylated monoglucosides of Pn and Mv in the berry skin during ripening (Takeda & Badr, 1977; Wicks, 1979; Powers *et al*., 1980; Wicks & Kliewer, 1983; El-Kereamy *et al*., 2003; Gallegos *et al*., 2006). This may reflect a synergistic decrease in methoxylation of anthocyanins synthesised in response to ethephon under higher light conditions. The significant interactive effect of light and ethephon on the ratio of Pn/Cn may point to the involvement of a methyltransferase in the observed response.

CONCLUSIONS

This research has shown a strong cultivar-dependent effect on the response of anthocyanin accumulation to environmental conditions, in this case shading. For red table grapes, the response of anthocyanin accumulation have been shown to be either highly sensitive or insensitive to bunch shading. Crimson Seedless was shown to be insensitive to shade in terms of accumulation of its primary anthocyanin, Pn-glucoside. On the other hand, Crimson Seedless showed a strong positive response to ethephon application in terms of anthocyanin accumulation. This indicates that it is sensitive to the application of growth regulators, such as ethephon and potentially ABA (Cantín *et al.,* 2007) which can be used for the commercial enhancement of skin colour properties.

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Chapter 6

General discussions and conclusion

6.1 CONCLUSIONS

Crimson Seedless is an important table grape cultivar for the South African industry as it is a late market, red seedless grape which fills a niche gap. Competing for international market share against other southern hemisphere countries, such as Australia and Chile, producers must deliver the best possible product. For table grapes, aesthetics is a crucial part of the final perceived quality, for 'Crimson Seedless' it is thus very important to have an adequate size as well as the correct colour.

Anthocyanins are the colour pigments of grapes and the aim of the current study was to explore the possible effects of ethephon application and shading on the colour of 'Crimson Seedless' through the treatments effects on the anthocyanin composition, profile and concentration. Firstly the 'Crimson Seedless' anthocyanin concentration and profile under prevailing South African conditions needed to determined and secondly the influence of management practices (i.e., ethephon application and leaf removal) on the anthocyanin profile and composition of 'Crimson Seedless' was evaluated. This was done by addressing three key issues: Firstly, what was the effect of excessive bunch shading on 'Crimson Seedless', secondly what was the effect of partial defoliation on 'Crimson Seedless' and finally were there any interactive effects of ethephon, bunch shading and partial defoliation on 'Crimson Seedless' anthocyanin profile.

It has been shown by various researchers that low light environments reduce the colour of grapes (Crippen & Morrison, 1986; Smart *et al.* 1988; Morrison & Noble, 1990; Price *et al.* 1995; Bergqvist *et al.* 2001), but as research continued into the effect of bunch exposure on colour, contradictory data began to appear (Downey *et al.* 2006). It was found in some studies that no change in total anthocyanins was observed with artificial shading (Downey *et al*. 2004; Ristic *et al.* 2007), while others have reported that increased exposure to sunlight resulted in decreased anthocyanin levels in berries (Hunter *et al*. 1995; Bergqvist *et al*. 2001; Spayd *et al*. 2002), most likely due to decreased anthocyanin synthesis at the higher berry temperatures occurring under these conditions. At high temperatures anthocyanin degradation have also been found to occur (Spayd *et al.* 2002, Downey *et al.* 2004, Avenant & Avenant 2006).

In some cases, there was no difference in total anthocyanin levels, but alteration in anthocyanin composition was observed in response to altered light conditions within the bunch zone (Price *et al.* 1995; Haselgrove *et al.* 2000; Spayd *et al.* 2002; Downey *et al.* 2004). In the current study to investigate the effect of excessive shading on 'Crimson Seedless', artificial cluster shading was applied. This was to determine whether there were any effects of shading on the composition of anthocyanins. The cyanidin in 'Crimson Seedless' reflected the sensitivity to the shade treatment

shown in other literature (Keller & Hrazdina 1998), and was the anthocyanin which responded most significantly to shaded conditions. However, it was decreased as a proportion of total anthocyanins in both sites where the experiment was performed rather than increased as shown in other studies (Downey *et al.* 2004; Ristic *et al.* 2007). In this research, 'Crimson Seedless' was shown to accumulate primarily glucosides of peonidin, in agreement with Cantos *et al.* (2002). Unlike the studies on 'Shiraz' (Downey *et al.* 2004; Ristic *et al.* 2007) shading of 'Crimson Seedless' berries did not alter the proportion of peonidin, most likely because synthesis of this anthocyanin from cyanidin is already favoured in this cultivar at a genetic level. Analysis of the ratios of anthocyanins showed that conversion of cyanidin to peonidin via the enzyme 3'O-methyltrans-ferase was most likely affected by the shade treatment, such that synthesis of peonidin was favoured under shade conditions. However, this hypothesis can only be verified by performing gene expression studies or radio-labelling experiments. The responses of anthocyanin accumulation, for red table grapes, have been shown to be either highly sensitive or insensitive to bunch shading (Wicks 1979, Wicks & Kliewer 1983). This research has shown that 'Crimson Seedless' is insensitive to shade in accumulation of its primary anthocyanin, peonidin-3-glucoside.

The ripening response observed in this study is in agreement with literature to date, even if the grape developmental responses to natural and artificial shading do vary. Natural cluster shading has been noted to increase berry weight and either increased or maintained berry TA with negligible differences in TSS accumulation (Kliewer & Antcliff, 1970; Reynolds *et al.* 1986; Crippen & Morrison, 1986; Morrison & Noble, 1990; Price *et al.* 1995). Increased TA was proposed to be due to reduced malate respiration under shaded conditions (Kliewer & Lider, 1968; Bledsoe *et al.* 1988; Smart *et al.* 1988; Price *et al.* 1995). Artificial shading of grape clusters from flowering or berry set has been shown to produce either no change in fruit composition, or decreased berry weight, increased pH due to accumulation of K+ and increased TA due to increased malate while TSS was unchanged (Downey *et al.* 2004; Cortell & Kennedy 2006; Ristic *et al.* 2007). The response to artificial shading in this study was variable between trial sites. In the case of the De Doorns experiment it delayed ripening. The reduced TSS and TA associated with this experiment was therefore probably not due to increased respiration of malic acid in the berries, but rather delayed maturity. However, in the case of the Paarl experiment, the results are in agreement with Ristic *et al*. (2007), where shade decreased berry weight while not altering TSS accumulation in 'Shiraz' grapes. The reason for this reduced berry weight under artificial shade conditions has not been ascertained through research, but may be due to reduced dry weight accumulation pre-véraison, where the berry is unable to directly fix carbon via photosynthesis due to extreme darkened conditions and berry chlorosis (Downey *et al.* 2004).

A 50% leaf removal treatment was applied to explore the effects of defoliation on 'Crimson Seedless' anthocyanin profile. In terms of the grapevine's light microclimate, the leaf removal

treatment (LR) significantly increased the amount of PAR measured in the bunch zone by ~77%, thus the defoliation treatment applied in this study was sufficient enough to have increased bunch exposure significantly. However, even having such an increase in bunch exposure had little effect on the anthocyanin profile and composition of 'Crimson Seedless'. Generally the LR treatment led to small decreases in the concentrations of the various anthocyanins. Grapes from the LR vines anthocyanin concentration was ~21% lower compared to grapes on NLR vines. This was true for almost all of anthocyanins measured, except for cyanidin-3-glucoside which was significantly increased by the partial defoliation treatment. Poni *et al*. (2006) have observed higher concentration of anthocyanins in 'Sangiovese' vines with defoliation treatments applied early in the season. Castellarin *et al*. (2006) have shown that genes encoding flavonoid 3'- and 3', 5'-hydroxylases are expressed in the skin of ripening red berries that synthesize anthocyanins and that there is a correlation between the expressed genes and the ratio of accumulation of red (cyanidin-based) and blue (delphinidin-based) anthocyanins. The results in this study indicate that the F3'5'H activity was significantly decreased by LR. This could be due to the fact that the accumulation of anthocyanins in 'Crimson Seedless' naturally favours the F3'H side of the pathway, which synthesize the derivatives of cyanidin (peonidin and cyanidin).

The data in this study indicates that LR did not significantly influence any of the 'Crimson Seedless' composition parameters measured. The LR vines had lower TA values and higher pH values compared to NLR vines, however differences were very small. Presumably this was because of a decrease in malate due to the higher temperatures experienced by the more exposed fruit, as the respiration of malate is increased under these conditions (Bledsoe *et al*. 1988, Smart *et al*. 1988). Bledsoe *et al*. (1988) found that a reduction in malate leads to lower concentrations of potassium and this will lead to decrease in pH. They also found the TSS to increase in sun exposed fruit without any berry weight reduction whilst Poni *et al*. (2006) also noted that leaf removal increased the TSS for two cultivars examined. The slight increase observed in LR vines TSS values are in agreement with these authors, but due to the method of defoliation applied in this experiment, there were no significant differences observed. The way in which defoliation is applied can influence the parameters measured at harvest. For instance, the severity of the defoliation process, as Hunter *et al*. (1991) showed that 66% defoliation of the canopy, even though the practice showed favourable results for some parameters, was too severe with regards to the fruit composition as well as the vegetative and reproductive growth. Hunter *et al.* (1991) showed that 33% defoliation did not markedly affect the parameters measured, but it demonstrated high metabolic activity and increased vine performance.

All of the literature studies discussed application of basal leaf removal (BLR) which can be very advantageous when used on vines growing on a vertical trellis system. With BLR one improves canopy microclimate and also removes older leaves, which do not contribute to photosynthesis

later in the season, the LR as applied in this study however removes both old leaves and young leaves which could have contributed to photosynthesis later in the season. The defoliation method used in this study had no significant effect on fruit quality, neither anthocyanin content nor any other of the quality parameters measured. This is most likely due to the hedging that was applied, which limited the length of the main shoot, probably limiting the effect of the 50% leaf removal. Two weeks after the defoliation treatment was applied the average berry weight of the NLR vines were significantly heavier compared to the berries from the LR vines. This difference was observed for 4 weeks, after which there were no longer a significant difference between the treatments berry weights. The smaller berries on the LR vines could have been due to increased exposure of the berries, but according to the literature there should also have been an increase in TSS in the LR berries, but this was not the case. Another possible reason could be that less photo-assimilates were available for cell growth and development after the partial defoliation treatment was applied initially, thus this led to smaller berries forming on the LR vines which obtained normal size at the end of ripening the period, just before harvest. The LR vines might have recovered from the initial loss in photosynthetic capability as more of the new leaves on the lateral shoots became photosynthetically active to produce the needed photo-assimilates.

Ethephon was applied in conjunction with the shading and defoliation treatments, to determine whether there was an enhancement or weakening of the treatments effects on ‘Crimson Seedless’. The effects of ethephon on fruit maturity and composition are well documented in literature but the results are variable. For numerous cultivars generally no changes in TSS or acidity have been noted, with no or little change in pH, as well as total yield and weight per berry has been found due to its application (Szyjewicz *et al.* 1984). The TSS for the ethephon-treated grapes were significantly higher during weeks 6, 7 and 8 compared to the control grapes in agreement with other authors (Powers *et al*. 1980, Delgado *et al.* 2004) who found that ethephon accelerated berry ripening. However, these differences in composition during ripening did not persist, and at the time of harvest, there were no significant difference between treatments. This is in agreement with the finding of Powers *et al*. (1980) which showed that initial acceleration in TSS accumulation was not maintained and there was no significant difference between ethephon treated and control grapes at harvest. Gallegos *et al*. (2006) found that the effect of ethephon on berry composition can vary depending on meteorological conditions and variable yields. During two of the three seasons of their study ethephon had no significant effect on the TSS, but during the first year of their study the ethephon treated grapes had lower TSS over the last four weeks of the growth period compared to the control grapes. In most cases ethephon application either increased the TSS or did not affect it at all (Szyjewics *et al.* 1984).

‘Crimson Seedless’ showed a strong positive response to ethephon application in terms of anthocyanin accumulation. This indicates that ‘Crimson Seedless’ is sensitive to the application of

growth regulators, such as ethephon and potentially ABA (Cantín *et al.* 2007) which can be used for the commercial enhancement of skin colour. The overriding effect of ethephon application to increase anthocyanin production in 'Crimson Seedless' was an expected result of the research as ethephon is well-known to increase the red colour of grapes of various cultivars (Takeda & Badr, 1977; Wicks, 1979; Powers *et al.* 1980; Wicks & Kliewer, 1983; Keller & Hrazdina, 1998; El-Kereamy *et al.* 2003; Lombard *et al.* 2004; Gallegos *et al.* 2006). There is speculation that the increase in anthocyanin is associated with increases in the presence of the monoglucosides of peonidin and malvidin (Takeda & Badr, 1977; Wicks, 1979; Powers *et al.* 1980; Wicks & Kliewer, 1983; El-Kereamy *et al.* 2003) indicating increased production of terminal anthocyanins within the biosynthetic pathway. This was confirmed through gene expression studies on 'Cabernet Sauvignon', which showed up regulation of the gene for UFGT (El-Kereamy *et al.* 2002; El-Kereamy *et al.* 2003).

In the current study there was no significant interactive effect observed between ethephon application and the defoliation treatment. However, it is interesting to note that there was a significant interaction for the shade and ethephon treatment, with a synergistic enhancement of delphinidin, cyanidin and petunidin by ethephon in sun-exposed fruit at the De Doorns trial site. This is unexpected, since ethephon application was generally found to promote the accumulation of highly methoxylated monoglucosides of peonidin and malvidin in the berry skin during ripening (Takeda & Badr, 1977; Wicks, 1979; Powers *et al.* 1980; Wicks & Kliewer, 1983; El-Kereamy *et al.* 2003; Gallegos *et al.* 2006). This may reflect a possible synergistic decrease in methoxylation of anthocyanins synthesised in response to ethephon under higher light conditions.

The potential for the formation of anthocyanin in plant tissues is determined by hereditary factors as well as the more general observation that the amount of pigment formed is affected by numerous environmental factors, such as nutritional and water conditions, wounding, infections, age, temperature and light (Wicks 1979, Boss *et al.* 1996, Kobayashi *et al.* 2001, El-Kereamy *et al.* 2003, Downey *et al.* 2004, Jeong *et al.* 2004). Climatic variations can be very significant, for example, the anthocyanin content will be greater for a particular cultivar grown in a cool region compared to that same variety grown in a warm region (Spayd *et al.* 2002, Hendrickson *et al.* 2004, Yamane *et al.* 2006). The relative ratio of pigments will most likely remain the same due to genetic limitations in gene expression and biosynthesis (Boss *et al.* 1996, Procissi e*t al*. 1997, Nesi *et al*. 2001, Kobayashi *et al*. 2002, Mehrtens *et al*. 2005, Castellarin *et al.* 2006, Jeong *et al.* 2006, Avenant 2010), but differences in the total anthocyanin and total phenol content may differ due to environmental influences (Carreño *et al.* 1997, Keller & Hrazdina 1998, Cantos *et al.* 2002, Tomasi *et* al. 2003).

Two important characteristics of ‘Crimson Seedless’ with regards to anthocyanin accumulation were highlighted by this study. Firstly ‘Crimson Seedless’ is insensitive to bunch shading, and secondly, that ‘Crimson Seedless’ is sensitive to the effects of ethephon. Future research into the shading/bunch shading and ‘Crimson Seedless’ can lead to the investigation of the effects of decreased bunch exposure, by using natural canopy shading to lower bunch and berry temperatures in an attempt to improve berry colour. With regards to defoliation and table grapes, future studies can look into applying a more radical defoliation treatment, removing 50% of all leaves, including leaves from the lateral shoots. Another possibility could be removing every second shoot (only removing the non-bearing shoots). In these extreme defoliation cases one can possibly determine how table grapes will be affected by greater sunlight exposure and with fewer photo assimilate sources. To determine whether the difference between the LR vines and NLR were due to loss of photosynthetic capability or due to increased sunlight penetration, a study could be conducted where 50% of the leaves are covered in a reflective material, thus removing the photosynthetic capability of those leaves, without improving sunlight penetration. This area of research could still be of interest to the table grape industry as little is known on the effects of defoliation within a horizontal trellis system. Defoliation has proven to be a very useful to the wine grape grower seeking superior quality grapes. Another possibility is to focus on how ‘Crimson Seedless’ colour will be affected by ABA application and possibly comparing ABA vs. ethephon treatments, determining the most economical method of producing ‘Crimson Seedless’. ‘Crimson Seedless’ responds well to ethephon and with more economical ways of producing ABA, this plant growth regulator is becoming a feasible option for producers struggling with inadequate colour in their grapes. Continuing with the anthocyanin investigations and the effect each anthocyanin have on colour, it would be sensible to compare the colour measurements of a chromameter with the composition of the anthocyanins. A producer should get to know their vineyard and apply the correct management practices at their disposal be it physical actions or the use of growth regulators. If there is a continuous problem season after season, it might be possible that the genetic material of the vines are responsible and no management will have the desired effect. Recent research by Avenant (2010) have shown that selection of genetic material in the vineyard as well as in nurseries can be crucial in the long-term solution to problems affecting ‘Crimson Seedless’.

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