

# CANOPY MANIPULATION PRACTICES FOR OPTIMUM COLOUR OF REDGLOBE (*V. VINIFERA* L.)

by

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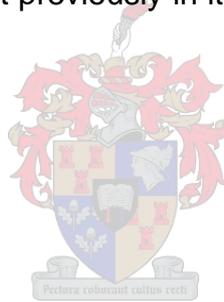
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# DECLARATION

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



Name of candidate \_\_\_\_\_

Date \_\_\_\_\_

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# PREFACE

This thesis is presented as a compilation of five chapters. Each chapter is introduced separately and is written according to the style of the South African Journal of Enology and Viticulture.

**Chapter 1: General Introduction and Project Aims**

**Chapter 2: Literature review  
Colour development of table grapes and the manipulation thereof**

**Chapter 3: Research Results  
The effect of defoliation treatments on leaf area, light environment and colour of Redglobe (*Vitis Vinifera* L.)**

**Chapter 4: Research Results  
The effect of defoliation treatments on berry composition and yield components of Redglobe (*Vitis Vinifera* L.)**

**Chapter 5: General Discussion and Conclusions**



## SUMMARY

Under certain South African conditions, Redglobe develops a colour that is too dark and thus unacceptable for the Far Eastern markets. These markets require a pink colour instead of a dark red colour. The cultivation of grapes with an acceptable colour involves amongst other, canopy management practices. This generally includes the removal of leaves and/or lateral shoots. Hereby, the leaf area and the microclimatic conditions in the canopy are altered.

The aim of this study was to test the usefulness of leaf and lateral shoot removal at different defoliation times after anthesis in order to obtain a pink coloured Redglobe crop. Other quality aspects, namely total soluble solids (TSS), total titratable acidity (TTA), berry mass and total yield, were also evaluated.

A canopy management trial was conducted on six year old Redglobe vines with moderate vigour. The treatment design was a 2 x 3 x 4 factorial and involved two leaf removal (L) levels ( $L_0$  = 0% leaf removal;  $L_{33}$  = 33% leaf removal) in combination with three lateral shoot removal (LS) levels ( $LS_0$  = 0 % lateral shoot removal;  $LS_{50}$  = 50% lateral shoot removal;  $LS_{100}$  = 100% lateral shoot removal). Four defoliation times (DT) were selected: 36 (pea berry size), 69 (véraison), 76 (one week after véraison) and 83 (two weeks after véraison) days after anthesis (DAA). A total of 24 treatment combinations, replicated in four blocks, were applied.

Generally, treatment combinations involving 33% leaf removal lowered the main shoot leaf area. Likewise, the lateral shoot leaf area was decreased by increasing levels of lateral shoot removal at any defoliation time. As expected, 33% leaf removal applied in combination with any level of lateral shoot removal, always resulted in a lower total vine leaf area compared to where 0% leaf removal was part of the treatment combination. Compensation reactions occurred and in this regard the main shoot leaf size increased due to 33% leaf removal applied at 1 week after véraison and 2 weeks after véraison. Treatment combinations involving lateral shoot removal increased the ratio of main shoot leaf area to the total leaf area. On the other hand, the main shoot leaf area percentage was lowered by the application of 33% leaf removal at 2 weeks after véraison compared to no leaf removal at the same defoliation time. It can therefore be assumed that the contribution of lateral shoot leaves to grape composition might have increased in cases where the main shoot leaf area was lowered at a later stage (e.g. 2 weeks after véraison).

The bunches were visually evaluated and divided into classes from dark (class one) to light (class nine). This visual bunch evaluation showed that the mean bunch colour was in class three (lighter than class two) due to the defoliation time. The lateral shoot removal x leaf removal interaction resulted in a mean bunch colour that was in classes 2 and 3. However, within these classes, there was a tendency that bunch colour decreased for defoliation times later than pea berry size. The lateral shoot removal x leaf removal interactions showed that bunch colour was darker when the treatment combinations involved 0% leaf removal. The percentage of bunches with the desired colour was increased by application of the treatments at véraison,

compared to the other defoliation times, and also with 50% lateral shoot removal and 100% lateral shoot removal compared to 0% lateral shoot removal. Biochemical analyses confirmed that increased levels of lateral shoot removal generally lowered the anthocyanin concentration regardless of defoliation time.

A similar effect on TSS was observed, i.e. from véraison onwards, the application of 50% lateral shoot removal and 100% lateral shoot removal tended to lower TSS. The effect of these levels of lateral shoot removal at véraison was significant. The role of the lateral shoots in colour development and sugar accumulation is therefore emphasized.

Furthermore, the special role that lateral shoots also play in berry development is illustrated in that berry mass tended to decrease when 100% lateral shoot removal in combination with 33% leaf removal and 100% lateral shoot removal in combination with 0% leaf removal were applied at véraison. This, together with the positive relationship obtained between grape colour and the lateral shoot leaf area:fruit mass ratio, accentuates the role of active leaf area during the ripening period.

The possible effect of the microclimatic light environment on colour must also be considered. However, although the light intensity increased with increased levels of LS, the colour that was obtained was probably not associated with the differences in light intensity.

It was found that it is possible to manipulate the colour of Redglobe grapes with defoliation treatments. However, the treatments that have a decreasing effect on grape colour also affected other quality parameters like TSS and berry size negatively.

Although, it is possible to reduce the colour of Redglobe through the application of leaf and lateral shoot removal at different defoliation times, the question arises whether the treatment combinations used in this study are worthwhile to pursue because the mean bunch colour that was obtained was still too dark. However, it was possible to increase the percentage of bunches with the desired colour. Therefore, if such treatments are applied, it must be approached cautiously, keeping in mind that assimilate supply has to be sustained throughout the ripening period.

## OPSOMMING

Onder sekere Suid-Afrikaanse toestande, ontwikkel Redglobe 'n donker rooi, eerder as die pienk kleur wat vir die Verre Oosterse markte aanvaarbaar is. Lowerbestuurspraktyke kan moontlik 'n rol speel ten einde die verlangde kleur te verkry. Dit sluit blaar- en sylootverwydering in. Sodoende word die blaaroppervlakte, sowel as die mikroklimaatstoestand verander.

Die doel van hierdie studie was om vas te stel of blaar- en sylootverwydering op verskillende tye na volblom 'n pienk kleur by Redglobe tot gevolg sal hê. Die ander kwaliteitsaspekte wat geëvalueer is, sluit in totale oplosbare vastestowwe (TOV), totale titreerbare suur (TTS), korrelmassa en oesmassa.

Blaar- en sylootverwyderings is uitgevoer in 'n ses jaar oue Redglobe wingerd met matige groeikrag. Die eksperimentele ontwerp was 'n 2 x 3 x 4 faktoriaal met twee vlakke van blaarverwydering (L), nl L<sub>0</sub> (0% blaarverwydering) en L<sub>33</sub> (33% blaarverwydering) in kombinasie met drie vlakke van sylootverwydering (LS), nl. LS<sub>0</sub>, (0% sylootverwydering), LS<sub>50</sub> (50% sylootverwydering) en LS<sub>100</sub> (100% sylootverwydering). Die ontblaring is by vier tye (dae) na volblom (DNVB) toegepas: Ertjekorrelstadium (36 DNVB), véraison (69 DNVB), 1 week na véraison (76 DNVB) en 2 weke na véraison (83 DNVB). 'n Totaal van 24 behandelings kombinasies, wat in vier blokke herhaal is, is toegepas.

Oor die algemeen het die behandelingskombinasies wat 33% blaarverwydering ingesluit het, die hooflootblaaroppervlakte verlaag. Sylootblaaroppervlakte is ook verlaag deur toenemende vlakke van sylootverwydering by enige ontblaringstyd. Die verlaagde totale blaaroppervlakte per stok wat verkry is, wanneer 33% blaarverwydering in kombinasie met enige vlak van sylootverwydering toegepas is, teenoor wanneer 0% blaarverwydering deel van die behandelingskombinasie was, was te verwagte. By 33% blaarverwydering het kompensasiereaksies voorgekom deurdat die hooflootblare vergroot het wanneer dit by 1 week na véraison en 2 weke na véraison toegepas is in vergelyking met die toepassing van die genoemde behandeling by ertjekorrelstadium. Behandelingskombinasies wat sylootverwydering ingesluit het, het die verhouding van hooflootblaaroppervlakte tot totale blaaroppervlakte verhoog. Hierteenoor het 33% blaarverwydering die hooflootblaaroppervlakte persentasie verlaag toe dit by 2 weke na véraison toegepas is, vergeleke met geen blaarverwydering by dieselfde behandelingstyd. Die aanname kan dus gemaak word dat die bydrae van die sylootblaaroppervlakte tot korrelsamstelling verhoog het in gevalle waar die hooflootblaaroppervlakte verlaag is by 'n later ontblaringstyd (bv. 2 weke na deurslaan).

Die trosse is visueel volgens 'n kleurkaart in klasse, van donker (klas een) na lig (klas nege), ingedeel. Hierdie visuele evaluering van trosse het getoon dat die gemiddelde troskleur wat verkry is as gevolg van die ontblaringstyd, in klas drie (ligter as klas twee) was. Die gemiddelde troskleur voortgebring deur die sylootverwydering x blaarverwydering interaksie, was in klasse twee en drie. Binne hierdie klasse was

daar egter 'n tendens dat troskleur verminder is by ontblaringstye later as ertjekorrelstadium. Troskleur was donkerder in gevalle waar die sylootverwydering x blaarverwydering interaksie 0% blaarverwydering ingesluit het. Die persentasie trosse met die verlangde kleur is vermeerder deur behandelings by deurslaan toe te pas in vergelyking met die effek van die ander ontblaringstye en ook wanneer 50% sylootverwydering en 100% sylootverwydering toegepas is vergeleke met 0% sylootverwydering. Hierdie bevinding, nl. dat sylootverwydering oor die algemeen die antosianienkonsentrasie verlaag het ondanks die ontblaringstyd, is bevestig deur die biochemiese kleuranalise.

Vir TOV is 'n soortgelyke effek waargeneem, nl. vanaf véraison en daarna het die toepassing van 50% sylootverwydering en 100% sylootverwydering dit verlaag. Die effek van hierdie vlakke van sylootverwydering by véraison was betekenisvol. Hierdie resultate beklemtoon die rol van sylote tydens kleurontwikkeling en suikerakkumulاسie.

Die spesiale rol van sylote in korrelontwikkeling word geïllustreer deur die dalende tendens vir korrelmassa wanneer 100% sylootverwydering in kombinasie met 33% blaarverwydering, asook 100% sylootverwydering in kombinasie met 0% blaarverwydering toegepas is by véraison. Hierdie resultate, tesame met die positiewe verwantskap wat tussen druifkleur en die sylootblaaroppervlak:vrugmassa verkry is, beklemtoon die rol van aktiewe blaaroppervlakte gedurende die rypwordingsperiode.

Die moontlike mikroklimaatseffek op troskleur moet ook oorweeg word. Die ligintensiteit in die trossone het toegeneem met toenemende vlakke van sylootverwydering, maar die kleurverskille wat verkry is, kan waarskynlik nie hiermee geassosieer word nie.

Daar is gevind dat dit moontlik is om kleur van Redglobe druiwe met lowerbestuurspraktyke te manipuleer. Die behandelings wat kleur verminder het, het egter ander kwaliteitsaspekte, soos TSS en korrelgrootte, negatief beïnvloed.

Hoewel dit moontlik was om die kleur van Redglobe, d.m.v. blaar- en sylootverwydering by verskillende tye, te verminder, het die vraag oor die verdienstelikheid van sulke praktyke ontstaan omdat die gemiddelde troskleur steeds te donker was om aan sekere markvereistes te voldoen. Tog was dit moontlik om die persentasie trosse met die verlangde kleur te vermeerder. Dus, die toepassing van sulke praktyke moet omsigtig benader word en die feit dat assimilaatvoorsiening deur die rypwordingsperiode volgehou moet word, moet in gedagte gehou word.

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# **GENERAL INTRODUCTION AND PROJECT AIMS**

## GENERAL INTRODUCTION AND PROJECT AIMS

To fulfill the demands of consumers worldwide, the South African table grape industry is constantly challenged to produce grapes of the best quality. The best possible quality can only be obtained if cultivation practices are applied correctly. Knowledge and understanding regarding biochemical and physiological processes in the grapevine will ensure the implementation of the correct cultivation strategies.

Although taste and nutrition play vital roles in consumer preference, appearance, and thus colour, convinces consumers to purchase fresh products like table grapes. Colour, in the case of red and black grapes, is caused by anthocyanin pigments (Winkler *et al.*, 1974). If anthocyanin biosynthesis is affected negatively, colour is impaired. Problems, in terms of insufficient colour, are common amongst table grape cultivars (Douglas, 1951; Weinberger & Harmon, 1974; Van der Merwe, 2001). However, a dark colour is not always preferable. In some cases, Redglobe develops a colour that is too dark and thus unacceptable for the Far Eastern markets. These markets require a pink berry colour.

To achieve the optimum Redglobe colour, suitable for the Far Eastern markets, a holistic approach to the employment of cultivation practices must be followed. Generally, specific quality requirements are obtained through the correct integration of long-term (Douglas, 1951; Pirie, 1979; Ough & Nagaoka, 1984; Archer, 1990; Brossaud *et al.*, 1999; Hunter & Archer, 2001a) and short-term cultivation practices (Viljoen, 1951; Cirami *et al.*, 1985; Archer & Fouché, 1987; Hunter *et al.*, 1991; Hunter & Archer, 2001b). Thus, a multidisciplinary approach is the first step towards grape quality.

Partial removal of leaves or lateral shoots have been shown to affect berry colour (Peterson & Smart, 1975; Candolfi-Vasconcelos & Koblet, 1990; Petrie *et al.*, 2000; Vasconcelos & Castagnoli, 2000), berry sugar (Koblet *et al.*, 1994; Petrie *et al.*, 2000; Vasconcelos & Castagnoli, 2000), and berry mass (Candolfi-Vasconcelos & Koblet, 1990; Koblet *et al.*, 1994; Petrie *et al.*, 2000) negatively. In some other instances berry colour is enhanced through controlled leaf removal in areas other than the bunch zone (Hunter *et al.*, 1991; Hunter *et al.*, 1995). This is ascribed to the impact that leaf thinning has on the source:sink ratio in the canopy (Carbonneau, 1996). So, the important role leaves play in colour development has raised the question whether bunch colour development can be manipulated through leaf removal.

The aims of this study were to test the effect of canopy management practices at different stages of berry development on Redglobe berry colour. It was therefore hypothesised that the colour of Redglobe berries can be reduced, to obtain the ideal pink colour through canopy management at a specific critical time. Furthermore, the effects of defoliation on other quality parameters such as total soluble solids (TSS), total titratable acidity (TTA), pH and berry size were also determined.

The usefulness of leaf and lateral shoot removal to alter grape colour and other quality aspects to meet requirements of consumers were therefore determined.

In order to achieve the abovementioned goals, the following approaches were followed:

1. The choice of a relevant Redglobe vineyard with specific canopy and production requirements;
2. Application of different levels of leaf removal on main shoots and lateral shoot removal at different times after anthesis;
3. The determination of the effect of leaf and lateral shoot removal on leaf area and light intensity;
4. The visual colour observations, as well as laboratory analyses to determine the effect of leaf and lateral shoot removal on anthocyanin concentration;
5. The determination of the effect of leaf and lateral shoot removal on TSS, TTA, pH and berry mass.

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# **LITERATURE REVIEW**

## **COLOUR DEVELOPMENT OF TABLE GRAPES AND THE MANIPULATION THEREOF**

# LITERATURE REVIEW

## 2.1 INTRODUCTION

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The table grape industry in South Africa is committed to producing grapes of an outstanding quality to meet the requirements and standards of the consumers. Good prices on the export market serve as motivation to cultivate and prepare the best possible product.

Although taste and nutrition play a role in consumer preferences, grape berry colour and size ultimately convince consumers to purchase the product. Before they ripen, the green colour of grapes is due to chlorophyll, while carotenes and xanthophylls are responsible for the yellow and orange colours in skins of ripe grapes (Winkler *et al.*, 1974). Red, purple and black grapes owe their colour to anthocyanins (Akiyoshi *et al.*, 1962; Pirie, 1979; Hrazdina & Moskowitz, 1980; Hrazdina, 1982; Ribéreau-Gayon, 1982; Mazza, 1995; Carreño *et al.*, 1997). Any factor that affects anthocyanin biosynthesis and anthocyanin content will have an impact on colour quality. Problems with poor colour development are common among table grape cultivars. Examples of this is Flame Seedless which develops insufficient colour in areas where temperatures are too high (Weinberger & Harmon, 1974; Lombard, 2003) and Barlinka that does not colour in cases of excessive crop load (Douglas, 1951). Redglobe, on the other hand, sometimes develops a colour that is too dark and thus unacceptable for the Far Eastern markets which require a pink colour.

Cultivation for optimum colour involves both long-term and short-term cultivation practices. Vine spacing, young vine training and trellising are long-term cultivation practices that have an impact on the interception and utilisation of sunlight energy (Zeeman, 1981; Kliewer *et al.*, 2000). Short-term cultivation practices on the other hand, such as pruning, suckering, shoot positioning, tipping, topping and leaf thinning, also have an impact on sunlight interception and utilisation. The way in which these practices affect sunlight interception is through their impact on the canopy. Sufficient leaf area (Kliewer & Weaver, 1971; Kingston & Van Epenhuijsen, 1989), the age composition of the canopy (Hunter, 2000) and the contribution of younger leaves (Candolfi-Vasconcelos & Koblet, 1990; Vasconcelos & Castagnoli, 2000) are also important aspects to keep in mind in the production of quality grapes.

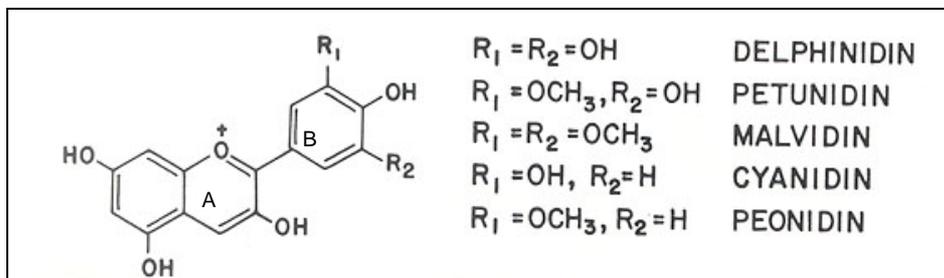
Since the expression of berry colour is largely connected to anthocyanin development, the first part of this literature review will focus on: the structure of anthocyanins, their development during berry ripening, and the anthocyanin composition of some cultivars. Thereafter, the factors that affect colour, including cultivation strategies involved in producing grapes with the required colour, will be addressed. Canopy management practices and the effect thereof on colour and grape composition via effects on the leaf area:fruit mass ratio and microclimate will also be discussed. Finally, possible strategies involving the abovementioned concepts and practices for improving the quality of berry colour will be presented.

## 2.2 THE ANTHOCYANINS OF *VITIS VINIFERA* L.

### 2.2.1 STRUCTURE OF ANTHOCYANINS

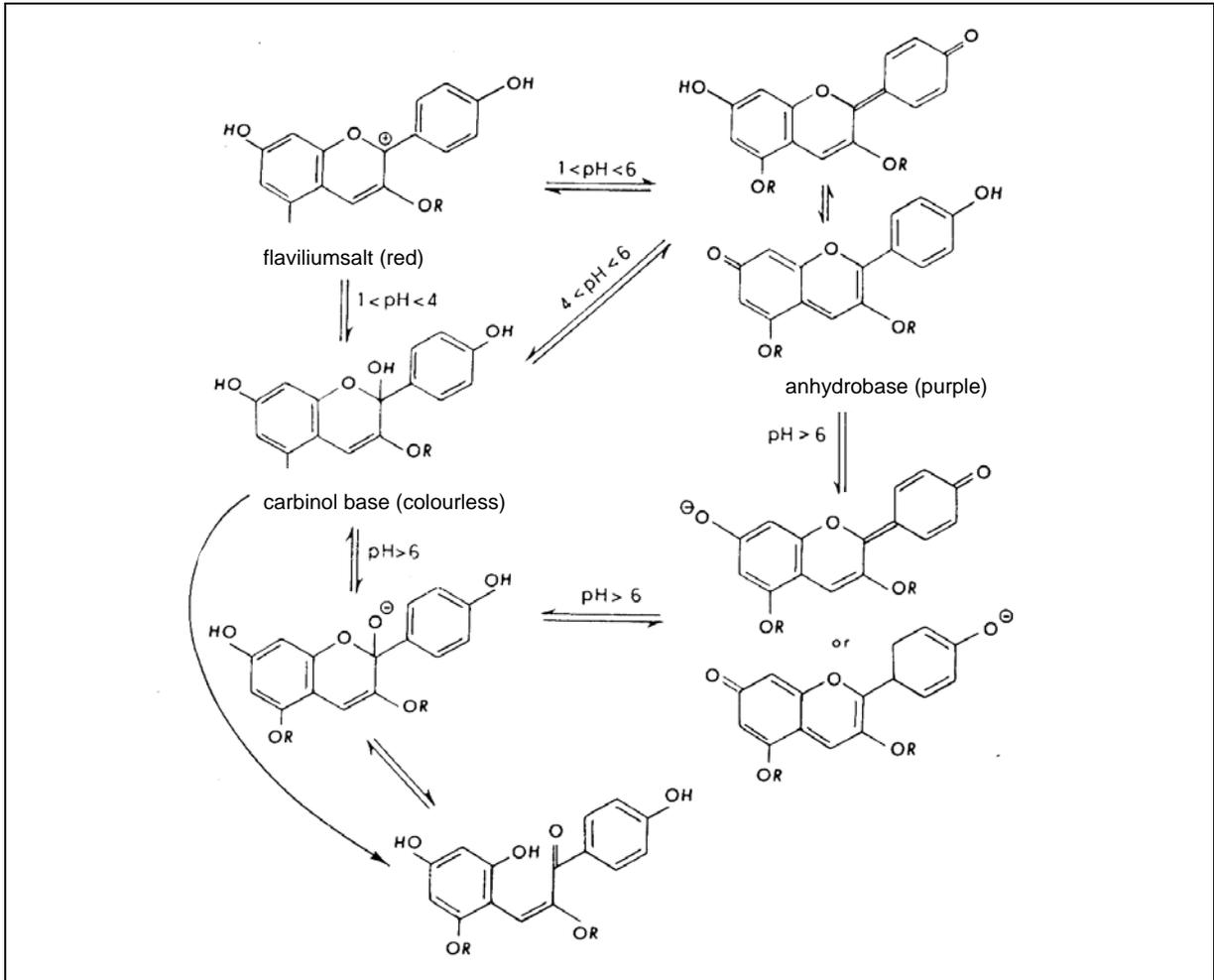
Anthocyanins are the principle phenolic compounds from which the colour of red grapes is derived (Winkler *et al.*, 1974). White grapes, on the other hand, owe their colour to proanthocyanidins (Dumazert *et al.*, 1973). These compounds all form part of the flavonoids, which, according to Mitrakos & Shropshire (1972), all have the same C<sub>15</sub> (C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub>) skeleton (Fig. 2.1).

The anthocyanin pigments occur in the berry skins and are located in the vacuoles of the first three to six sub-epidermal cell layers (Moskowitz & Hrazdina, 1981). Phenylalanine ammonia-lyase (PAL), one of the key enzymes in anthocyanin biosynthesis, also occurs in the epidermal cells (Roubelakis-Angelakis & Kliewer, 1986).



**Figure 2.1** The anthocyanidins in *Vitis* species (Wulf & Nagel, 1978).

The anthocyanins are present in the free, non-complexed form in equilibrium between flavilium salt (red), anhydrobase (purple) and the colourless carbinol base (Singleton, 1982). The first two flavonoid components lose their colour with an increase in pH. In Fig. 2.2, the structural formation of anthocyanins, as a function of pH, can be seen. It is evident that the pH of aqueous solutions plays an important role in the colour expression of anthocyanins.

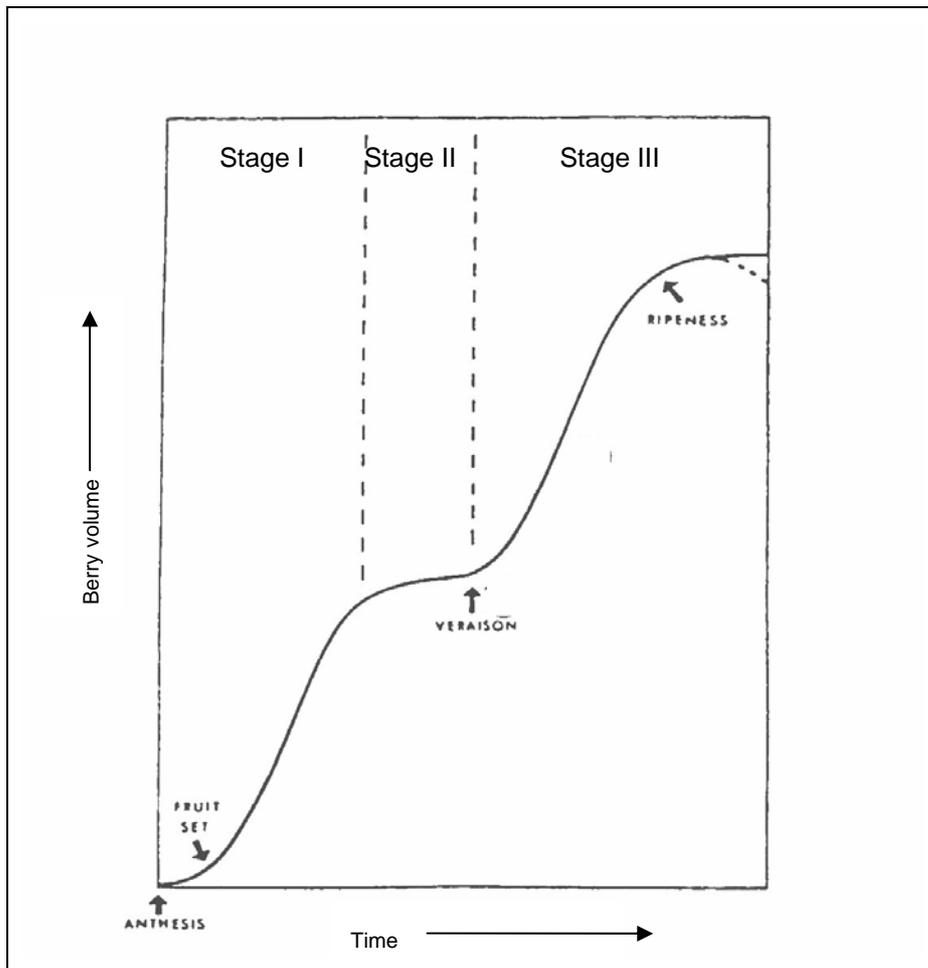


**Figure 2.2** Structural transformation reactions of anthocyanins as a function of pH in an aqueous solution (Hrazdina, 1982).

## 2.2.2 MECHANISM OF ANTHOCYANIN BIOSYNTHESIS DURING RIPENING

Grape berries develop according to a typical, double sigmoid growth pattern (Fig. 2.3), which is normally divided into three stages (Matthews *et al.*, 1987; Coombe, 1992). Stage I occurs after berry set and consists mainly of cell division, as well as some expansion of the existing cells. Stage II is known as the lag phase and depicts the onset of véraison. During stage III (ripening stage), the skin colour changes, the berries soften, the sugar concentration increases, acidity declines and cell volume increases. The anthocyanin content increases shortly after the start of sugar accumulation and continues throughout the ripening period (Pirie & Mullins, 1980; Hrazdina *et al.*, 1984; Fernández-López *et al.*, 1992; Boss *et al.*, 1996a; Hunter *et al.*, 2004; Nadal *et al.*, 2004) and then decreases during the later stages of ripening (Somers, 1976). This decrease in anthocyanin content was, however, initially ascribed to berry shrinking which adversely affects the extractability of anthocyanins (Somers, 1976), possibly due to a tighter cell wall structure caused by faster senescence and less tissue hydration (Sivilotti *et al.*, 2005). On the other hand, Hunter *et al.* (2004) attributed the inability of further anthocyanin extraction, on

a whole berry basis six to seven weeks after véraison, to a probable deterioration of anthocyanins at that stage.



**Figure 2.3** The growth pattern of the grape berry (Coombe, 1992).

The composition and amount of anthocyanins present in coloured cultivars depend on genetic properties (Ribereau-Gayon, 1982; Mazza, 1995). Production of anthocyanins depends on enzyme production and activity (Kakegawa *et al.*, 1995). A key enzyme in anthocyanin biosynthesis is UDP-glucose flavonoid-3-O-glucosyl transferase (UFGT) (Boss *et al.*, 1996a; Boss *et al.*, 1996b; Boss *et al.*, 1996c; Downey *et al.*, 2004). The UFGT gene is expressed only in coloured grapes that synthesise anthocyanins (Boss *et al.*, 1996c). The close connection between UFGT (Boss *et al.*, 1996a) and phenylalanine ammonia-lyase (PAL) activity (Hrazdina *et al.*, 1984; Kakegawa *et al.*, 1995; Hiratsuka *et al.*, 2001b) and increase in anthocyanin concentration in grape berries, seems to illustrate the important role these enzymes have in anthocyanin synthesis. For example, the role of PAL is to channel phenylalanine away from protein synthesis toward flavonoid biosynthesis (Mitrakos & Shropshire, 1972; Hrazdina *et al.*, 1984). However, due to the involvement of the products of PAL in other pathways, such as lignin synthesis, it is difficult to correlate PAL activity directly with anthocyanin production (Hrazdina, 1982). This is illustrated by the fact that Kakegawa *et al.* (1995) found anthocyanin biosynthesis to be

inhibited by restrained PAL and restrained chalcone synthase (another enzyme correlated with anthocyanin biosynthesis) activity.

Anthocyanin formation depends on the availability of phenylalanine (Fig. 2.4), which is synthesised from sugars via the shikimic acid pathway (Hrazdina *et al.*, 1984). The fact that the addition of phenylalanine to *Vitis* cell cultures initiates anthocyanin accumulation (Kakegawa *et al.*, 1995) substantiates the role of phenylalanine as precursor for colour development.

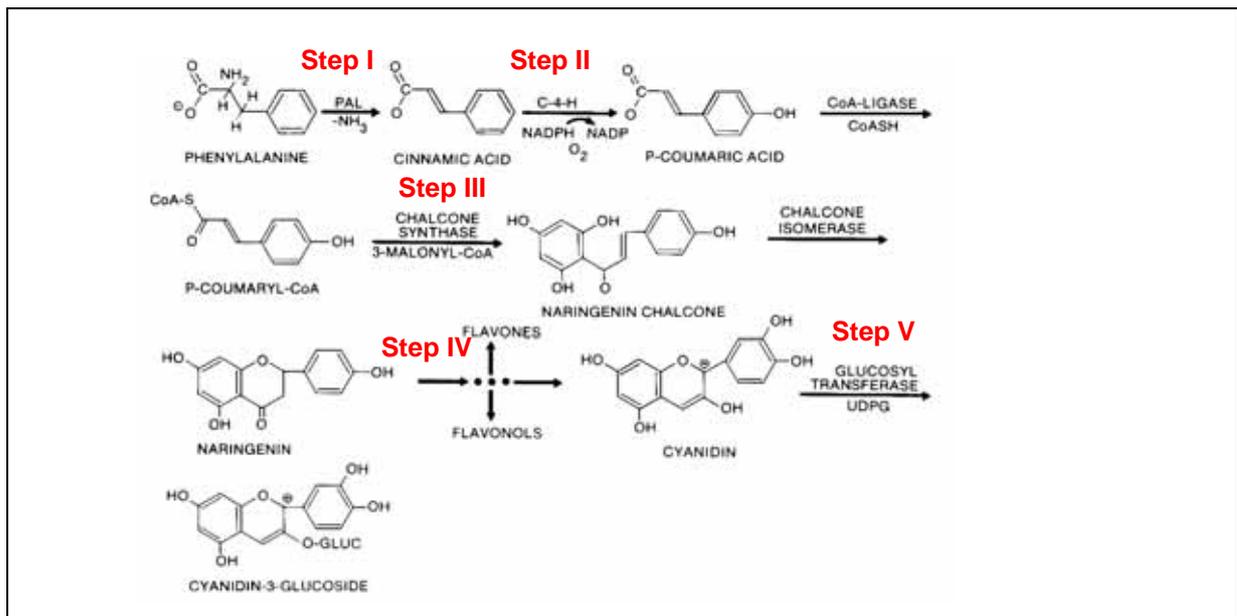
The graphic explanation of anthocyanin biosynthesis that is given in Fig. 2.4, is summarised as: Step I. PAL is deaminated to cinnamic acid (Mitrakos & Shropshire, 1972). Step II. Cinnamic acid is then hydroxylated to form p-coumaroyl-CoA which forms the basic C<sub>9</sub> unit for the B-ring (derived from eritrose-4-phosphate and phosphoenol pyruvate via the shikimic pathway) (Mitrakos & Shropshire, 1972). Step III. A decarboxylative condensation involving P-coumaroyl-CoA with three molecules of malonyl-CoA derivatives results in naringenin chalcone, which is the central C<sub>15</sub> intermediate for all flavonoids and forms the A-ring (Mitrakos & Shropshire, 1972). The latter is hydrolysed and serves as an attachment point for sugars (Mitrakos & Shropshire, 1972). Step IV. Isomeration of naringenin chalcone yield a flavanone (Roggero *et al.*, 1986). Step V. The flavanone undergoes different enzyme-catalysed reactions, leading to flavones, flavonols, isoflavones or anthocyanins (Roggero *et al.*, 1986). On the initial flavanone, a hydroxylation in the B-ring occurs which makes cyanidin the first anthocyanin pigment in grape skins (Roggero *et al.*, 1986). Although not shown in Fig. 2.4, cyanidin can be modified via hydroxylation, methylation, glycosylation and esterification reactions. For example, if methylated, cyanidin is transformed into peonidin and if cyanidin is hydroxylated, delphinidin forms.

The building blocks of anthocyanins are the anthocyanidins (Fig. 2.1), namely cyanidin, delphinidin, petunidin, peonidin and malvidin (Wulf & Nagel, 1978; Singleton, 1982; Mazza, 1995). Anthocyanidins do not occur free, but in bound form as 3-glucosides in *V. vinifera* species and as 3,5-diglucosides in other *Vitis* species, such as *Vitis rupestris*, *Vitis riparia* and *Vitis labrusca* (Wulf & Nagel, 1978; Singleton, 1982). Some anthocyanin pigments seem to be more stable than others and Roggero *et al.* (1986) divided them into three classes: (1) stable pigments (peonidin and malvidin), (2) intermediate pigments (petunidin) and (3) primitive pigments (cyanidin and delphinidin). Cyanidin is considered to be the most primitive colour pigment (Hrazdina, 1982). In advanced plant families, including the *Vitaceae*, cyanidin is transformed into peonidin or malvidin, which are more stable pigments (Roggero *et al.*, 1986).

Roggero *et al.* (1986) proved that an evolution of anthocyanin pigments takes place during the ripening of Syrah (Shiraz): delphinidin drops in concentration and the malvidin concentration gradually increases as soon as the biosynthesis of the anthocyanins ceases. Furthermore, cyanidin and delphinidin decrease rapidly after

peaking three to four weeks after véraison, whereas peonidin and malvidin form continuously.

In closing, it is important to keep in mind that the evolution of anthocyanins depends on factors such as cultivar, soil and climatic conditions, as well as the specific agricultural practices applied (Fernández-López *et al.*, 1992). Light, temperature, water, nutrients, leaf area:fruit mass ratio, as well as long-term and short-term cultivation strategies, are aspects that affect colour due to the impact they have on anthocyanin synthesis. These aspects will be discussed in sections 2.4 and 2.5 in greater detail.

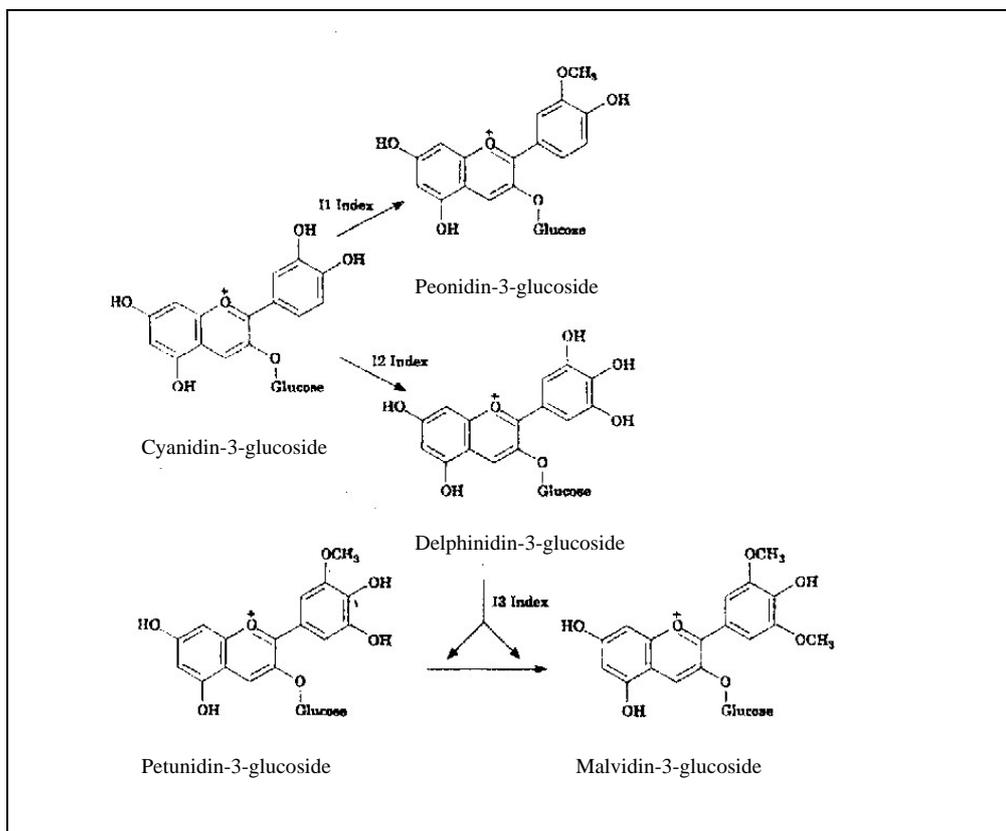


**Figure 2.4** Anthocyanin biosynthesis (Hrazdina *et al.*, 1984).

### 2.3 ANTHOCYANIN-BASED CLASSIFICATION OF TABLE GRAPES

According to Harborne (1988), the anthocyanins have no direct physiological role in primary metabolism. They do, however, contribute to sensory perception (Clydesdale, 1993), a principle criterion in table grape consumption. Anthocyanins also play an important role in taxonomy, to characterise species or cultivars (Hrazdina, 1982). According to the colour of the skins, the table grape cultivars can be classified into the following groups: green-yellow, pink, red, red-grey, red-dark violet, red-black and blue-black (OIV, 1983). Since grape skin colour is correlated with anthocyanin content, Cravero *et al.* (1994) was able to develop a colour-based grouping of red cultivars. In addition to this, Carreño *et al.* (1995) described a colour index for red grapes (CIRG), based on lightness, red-greenness and blue-yellowness. The CIRG can be applied for the objective evaluation of the skin colour of red grapes. It can serve as a way to check the degree of maturation in cases where the total soluble solids correspond to the maturity standards, but the colour is not acceptable for consumption.

Carreño *et al.* (1997) made a study of the anthocyanin composition of several red table grape cultivars. The data obtained were grouped from a physicochemical point of view to correlate with the CIRG and the colour chart of the OIV descriptor list for grapevine cultivars. By using the anthocyanin content according to the CIRG and OIV descriptor list, indices which are related with enzyme activities were calculated (Fig. 2.5). The I1 and I3 indices provide information about methylation reactions in the di-substituted and tri-substituted anthocyanins respectively. The I2 index is affected by the incorporation of a third hydroxyl group in the B-ring. The I4 and I5 indices depend on esterification with acetic and p-coumaric acid respectively. The values of these indices indicate the principle anthocyanin components which varies according to cultivar. For example, if the value for the I2 index is low, but the value for the I1 index is high, it indicates a blockage in the biosynthetic pathway of peonidin and of tri-substituted anthocyanins. Therefore, cyanidin is then the main anthocyanin component. The cultivars can be classified according to these indices, as indicated in Table 2.1. Cultivars included in group one, for example, have a low anthocyanin content, low I1 and I2 values, their skin colour is pink or red and cyanidin is the main anthocyanin component. Those in groups seven to nine have a high anthocyanin content, high I2 values and I5 shows maximum values. Their skin colour appears red-black or blue-black and malvidin is the main anthocyanin component.



**Figure 2.5** The final reactions of anthocyanin biosynthesis, where I1 = peonidin compounds/total anthocyanins; I2 = delphinidin + petunidin + malvidin/total anthocyanins; I3 = malvidin/delphinidin + petunidin + malvidin; I4 = acetic esters/total anthocyanins and I5 = p-coumaric esters/total anthocyanins (Carreño *et al.*, 1997).

According to Carreño *et al.* (1997), linear correlation between CIRG and delphinidin + petunidin + malvidin components/total anthocyanins revealed that the cultivars with a more intense colour showed the highest levels of tri-substituted anthocyanins (delphinidin, petunidin and malvidin). The dark red, violet and black cultivars contain monoglucosides of delphinidin, petunidin and malvidin (Winkler *et al.*, 1974; Wulf & Nagel, 1978; Singleton, 1982; Bakker & Timberlake, 1985; Hebrero *et al.*, 1988). On the other hand, the anthocyanin make-up of the red cultivars comprises mostly of peonidin-3-glucoside (Fong *et al.*, 1971; Carreño *et al.*, 1997; Cantos *et al.*, 2002), whereas the principle pigment in the light red cultivars is cyanidin-3-glucoside (Akiyoshi *et al.*, 1962; Winkler *et al.*, 1974).

Anthocyanidins can be acylated by p-coumaric acid, caffeic acid and acetic acid (Fong *et al.*, 1971). Evidence was obtained that the acylated anthocyanins are preferentially formed from malvidin-3-glucoside (Wulf & Nagel, 1978). In some cultivars acylated anthocyanins are present (Rankine *et al.*, 1958; Albach *et al.*, 1959; Fong *et al.*, 1971; Wulf & Nagel, 1978; Fernández-López *et al.*, 1992) and in some they are absent (Fong *et al.*, 1971; Wulf & Nagel, 1978; Cantos *et al.*, 2002).

**Table 2.1** Varietal classification according to total anthocyanins and the hydroxylation and methylation indices (Carreño *et al.*, 1997).

Group	Anthocyanin content	Hydroxylation & methylation indices	Main component	Colour	Cultivars
I	low	very low I1, I2	cyanidin	pink red	Sultanina Rosada Muscat Flame
II	low	high I1, low I2	peonidin	red	Redglobe
				red-dark violet	Queen of the Vineyard
III	low	medium-low I1, I2	cyanidin+peonidin	red	Flame Seedless
IV	low	high I1, very low I2	peonidin	red-black	Cardinal
				red-dark violet	Red Malaga
V	medium	I3>I1>I2	peonidin+malvidin	red-dark violet	Emperor
				red-black	Moscat Hamburg
VI	medium-low	I2>I3>I1	malvidin+delphinidin+petunidin	red-dark violet	Ruby Seedless
VII-IX	high	I3>I2>I1, max I5	malvidin	red-black or blue-black	La Rochelle + Alphonse Lavallée

Results obtained by Mattivi *et al.* (1990) made it possible to qualify differences linked to the synthesis of anthocyanins. The indices they used to separate

grapevines numerically were the percentage of the five monoglucosides, the summations of acetic esters (malvidin-3-caffeoate plus all five p-coumaric acids), as well as a series of relations correlated to enzyme reactions in anthocyanin biosynthesis. Calò *et al.* (1994), however, proposed that a ratio between di- and tri-hydroxy-substituted anthocyanins for classifying grape cultivars must be used.

## 2.4 FACTORS THAT AFFECT BERRY COLOUR

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Apart from genetic properties, the composition and amount of anthocyanins in coloured cultivars also depend on the stage of maturity, seasonal conditions, as well as terroir and yield (Mazza, 1995). According to Pirie (1979), temperature during ripening and factors determining carbohydrate status in the vine and fruit affect grape colour. Furthermore, he also stated that the application of plant growth regulators to the vine or fruit, and berry size are responsible for variation in skin pigments. The most important factors that affect the biosynthesis of anthocyanins, and berry colour, are described below.

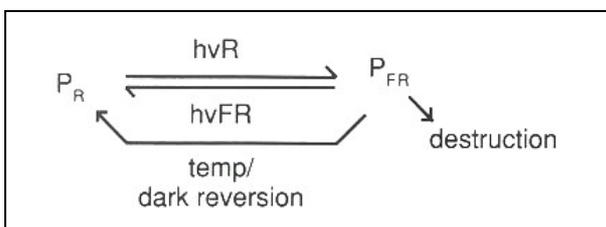
### 2.4.1 LIGHT

A favourable light environment is beneficial for photosynthesis because nitrate reductase (Hunter & Ruffner, 1997) is light dependent. Anthocyanin biosynthesis also benefits through a favourable light environment because phenylalanine ammonia-lyase (PAL) activity depends on light (Roubelakis-Angelakis & Kliewer, 1986).

Optimal photosynthesis is between 600 and 800  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Kriedeman, 1968) within the 400 to 700 nm waveband (Smart, 1987). Under South African conditions, the light intensity on a cloudless, sunny day range from 1800 to 2400  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Archer & Swanepoel, 1987). However, not all the photosynthetic photon fluence rate (PPFR) is absorbed for utilisation by the leaves. Smart (1985) found that mature Shiraz leaves absorb approximately 85% of the available PPFR. The rest is either reflected (6%) or transmitted (9%). Furthermore, the photosynthetic rate is light saturated at approximately one third of full sunlight (*ca.* 800  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and the light compensation point is at 1% of full sunlight or *ca.* 15 to 30  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Smart, 1987). Such low PPFR values prevail in dense canopies (Peacock *et al.*, 1987; Williams, 1987; Archer & Strauss, 1989; Peacock *et al.*, 1994). Douglas (1951) attributed the lack of colour of Barlinka to a dense canopy that limits the incidence of direct sunlight on most of the leaves. It is thus clear that sunlight affects the supply of energy for photosynthesis and that a favourable light environment is required for colour development (Haselgrove *et al.*, 2000; Kataoka *et al.*, 2004).

Smart (1987) explains another way in which sunlight affects grapevine physiology and thus fruit composition, i.e. the fact that radiation in the 300 to 1500 nm range has a thermal effect (tissue heating) and also a phytochrome effect (R:FR, 660:730 nm). Radiation in the red spectra (650 to 700 nm) is necessary to convert phytochrome in

plant leaves (proteinaceous pigments associated with the absorption of light) from the inactive form,  $P_r$ , to  $P_{fr}$ , the active form (Mitrakos & Shropshire, 1972).  $P_{fr}$  not only controls nitrate reductase and invertase, but also activates the genes that induce anthocyanin synthesis (Mitrakos & Shropshire, 1972). Phenylalanine ammonia-lyase (PAL) is thus activated (Smart, 1987), with a consequent enhancement in anthocyanin biosynthesis (Mitrakos & Shropshire, 1972). However,  $P_{fr}$  is unstable in the dark and progressively disappears in one of two ways. Firstly, through non-photochemical reversion to  $P_r$ , or secondly, by breakdown or transformation to a substance without photoreversibility (Mitrakos & Shropshire, 1972). Furthermore, the active form of phytochrome can either be destroyed thermally or reversed to  $P_r$  by high temperatures (Fig. 2.6) (Mitrakos & Shropshire, 1972).



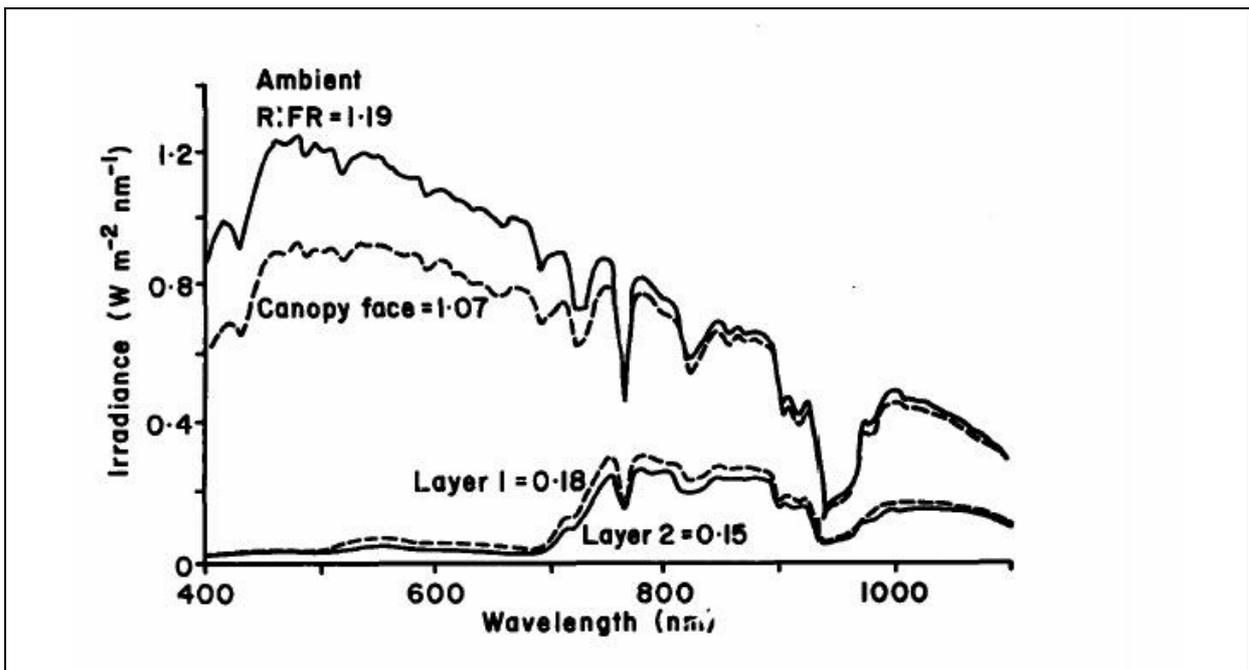
**Figure 2.6** Scheme for the inter-conversion of phytochrome forms (Mitrakos & Shropshire, 1972).

Therefore, a high red:far red (R:FR) ratio, which is typical of direct sunlight, will lead to more plant leaf phytochrome to be in the active form (Smith, 1982) and thus an enhancement in anthocyanin biosynthesis (Mitrakos & Shropshire, 1972). A low R:FR ratio, as occurs in a dense canopy, therefore, decreases the anthocyanin concentration (Kliewer & Smart, 1989). In this regard, Archer & Strauss (1989) found that skin colour of Cabernet Sauvignon grape berries in natural shade were significantly reduced. They attributed it to the inhibition of phytochrome reactions linked with anthocyanin biosynthesis. In their study, the red light was filtered out and phytochrome was converted to the inactive form (Salisbury & Ross, 1989).

According to Smith (1982), the estimated epidermal phytochrome photoequilibrium ( $P_{fr}:P_{total}$ ) in plant leaves is sensitive to R:FR ratios less than 1.15 (shade) because then the equilibrium shifts towards phytochrome being in the inactive form, whereas a high R:FR ratio ( $>1.15$ ) shifts the phytochrome photoequilibrium ( $P_{fr}:P_{total}$ ) to approximately 60%, meaning that the phytochrome is mostly in the  $P_{fr}$  form. In this regard, it was found that red light supplementation to the leaves of Cabernet Sauvignon enhances colour development (Smart *et al.*, 1988). A low R:FR ratio in a dense canopy (shade) therefore decreases anthocyanin concentration (Smart, 1987). The spectral distribution of sunlight measured above, at the canopy surface and inside the canopy, as well as the rapid loss of PPFR and change of R:FR ratio can be seen in Fig. 2.7

Furthermore, in shade, which is typical of dense canopies, potassium is loaded into the phloem instead of sugar (Giaquinta, 1983) and translocated to the berries where it forms a salt with tartaric acid (Mattick *et al.*, 1972; Storey, 1987). The

proportion of tartaric acid is therefore lowered. The change in the relative proportions and strengths of the acids present in grape juice (Boulton, 1980b) and also by the potassium and sodium concentrations in grape tissues will affect pH (Boulton, 1980a). In this case pH increases because malate is a weaker acid than tartaric acid (White *et al.*, 1968; Dawson *et al.*, 1986). The increased pH might reduce colour due to enzymatic degradation of anthocyanins and in this regard, Calderón *et al.* (1992) found that peonidin, delphinidin and cyanidin are the most favourable substrates for peroxidase at pH 4. Apart from enzymatic degradation, anthocyanins are in the colourless form between pH 4 and 6 (Fig. 2.2).



**Figure 2.7** Spectral distribution of sunlight measured above, at the canopy face and at leaf layers one and two of the kauwhata two tier trellis (Smart, 1987).

## 2.4.2 TEMPERATURE

Apart from the effect of light, temperature also contributes to berry composition and colour development (Iland, 1989; Mabrouk & Sinoquet, 1998). Both photosynthesis and anthocyanin biosynthesis depend on optimum temperatures for enzyme activity (Kriedeman, 1968; Kliewer, 1970a; Pirie, 1979; Spayd *et al.*, 2002; Carreño *et al.*, 1998).

Optimum day/night temperature combinations have been identified for the maximum colouration of grapes (Kliewer & Torres, 1972). In that study it was found that higher colouration, due to cool night temperatures, could have increased the level of sugars in the first three to six subepidermal layers of the berry skins (where anthocyanins are located). Furthermore, Kliewer & Torres (1972) attributed the high levels of skin sugar to lower respiratory losses, which could account for enhanced anthocyanin synthesis. The association of berry skin sugar levels with anthocyanin biosynthesis was confirmed by Pirie & Mullins (1977). Reduced colour under high

temperature conditions can be attributed either to a reduction in anthocyanin biosynthesis, or a degradation of pigments, or to a combination of both (Kliewer, 1973). Kliewer (1977) ascribed reduced anthocyanin biosynthesis under high temperature conditions to the apparent blockage or inactivation of the enzyme systems.

Haselgrove *et al.* (2000) found that, if bunches are heavily shaded, light was the limiting factor in anthocyanin biosynthesis, but when bunches received direct sunlight for most of the day, temperatures in excess of 35°C inhibited anthocyanin synthesis.

Sun exposure increases the solar heating of grape berries (Smart & Sinclair, 1976). Red or black grapes exposed to direct solar radiation can be 7 to 15°C warmer than ambient temperatures (Smart *et al.*, 1977; Bergqvist *et al.*, 2001; Spayd *et al.*, 2002). The heating of berries has previously been correlated with a reduction in anthocyanin biosynthesis (Kliewer, 1970a; Kliewer & Torres, 1972; Kliewer, 1977; Haselgrove *et al.*, 2000). Acidity and pH are also affected by temperature. The higher temperatures of heated berries will lead to acid degradation and a consequent rise in pH (Kliewer & Lider, 1968; Buttrose *et al.*, 1971; Ruffner *et al.*, 1976; Smart *et al.*, 1977; Reynolds *et al.*, 1986; Wolf *et al.*, 1986; Bledsoe *et al.*, 1988; Iland, 1989; Rojas-Lara & Morrison, 1989). Malic acid, in particular, is quickly lost at high temperatures (Kliewer & Lider, 1968; Buttrose *et al.*, 1971; Kliewer, 1971). This leads to an increase in pH because malate contributes to titratable acidity (Philip & Kuykendall, 1973). A decrease in colour can therefore indirectly be ascribed to the abovementioned temperature effect on the grape berry's pH, since the anthocyanidins peonidin, delphinidin and cyanidin are enzymatically degraded at pH 4 by peroxidase in the vacuoles of berry skin cells (Calderón *et al.*, 1992).

Management practices to improve colour development should therefore not only create a canopy for bunches to receive sufficient light for anthocyanin biosynthesis, but also to protect the berries from excessive heating.

### 2.4.3 WATER

Water affects almost every biological process in the plant (Mauseth, 1995) and is essential in every metabolic pathway (Salisbury & Ross, 1992). Amongst others, photosynthesis depends on an adequate water supply. Therefore, water stress reduces photosynthesis (Kriedeman & Smart, 1971; Liu *et al.*, 1978). The reduction in photosynthesis leads to a reduction in grapevine sugar production (Freeman *et al.*, 1980; Salón *et al.*, 2005), which impairs colour development (Hardie & Considine, 1976). The anthocyanin content, as well as the proportions of the different components are changed by mild water stress (Kennedy *et al.*, 2002; Bindon, 2004).

Non-irrigated or minimally irrigated vines produce grapes with a higher anthocyanin concentration than irrigated vines (Pirie & Mullins, 1977; Freeman, 1983; Freeman & Kliewer, 1983; Matthews & Anderson, 1988; Ginestar *et al.*, 1998b; Esteban *et al.*, 2001; Kennedy *et al.*, 2002; Ojeda *et al.*, 2002; Tregoat *et al.*, 2002; Deloire *et al.*, 2004). On a sandy soil, the maintenance of 40% plant available water

(PAW) depletion levels between budbreak and harvest enhanced the colour of Barlinka trained onto a 1.5 m slanting trellis, whereas 60% depletion reduced colour development (Myburgh, 1996).

Darker colour is often associated with smaller berries that develop under water stress conditions (Freeman, 1983; Matthews & Anderson, 1988; Ojeda *et al.*, 2002; Peterlunger *et al.*, 2002). Smaller berries are obtained by water stress conditions during the period after flowering due to reduced cell division (Hardie & Considine, 1976; Van Zyl, 1984; McCarthy, 1997; Peterlunger *et al.*, 2002; Myburgh, 2003; Rogiers *et al.*, 2004; Salón *et al.*, 2005) or by less cell expansion, if water stress is induced at véraison (Hardie & Considine, 1976; Matthews *et al.*, 1987; Van Zyl, 1984; Peterlunger *et al.*, 2002). Water deficits modify the structural properties of the cell components and, consequently, cell wall extensibility, thereby limiting the enlargement of the pericarp cells (Ojeda *et al.*, 2001). It is also likely that the expansive growth of the inner mesocarp is inhibited by water stress more than that of the skin tissue, thus resulting in a higher skin:pulp ratio (Roby & Matthews, 2004). Darker colour in the case of smaller berries can therefore be attributed to the higher skin:pulp ratio brought about by the water stress conditions (Ojeda *et al.*, 2002; Peterlunger *et al.*, 2002). Another reason for increased colour in smaller berries is the availability of more assimilates for berry ripening, thereby enhancing anthocyanin and sugar accumulation, which causes an enhancement in ripening (Van Leeuwen *et al.*, 2004).

On the other hand, differences in anthocyanin concentration due to water availability are probably not just related to berry size, because it plays a limited role in determining the solute concentration in fruit of different sizes. For example, it was found that the skin:pulp ratio of well watered Cabernet Sauvignon grapevines is independent of berry size (Roby & Matthews, 2004). The anthocyanin concentration in the berries of Shiraz and Cabernet Sauvignon subjected to partial rootzone drying (PRD) also increased independently of berry size and might be mediated from physiological signals within the fruit or vine (Bindon, 2004) and due to ongoing photosynthesis because stomata of non-irrigated vines are less sensitive to abscisic acid (ABA) (Freeman *et al.*, 1980). This enable vines subjected to a water deficit to assimilate CO<sub>2</sub> at lower leaf water potentials and thus to continue photosynthesis.

There are, however, conditions of water stress which reduces colour development. For example, intense water deficits between flowering and véraison limit anthocyanin biosynthesis (Ojeda *et al.*, 2001) and delay ripening (Sipiora & Gutiérrez Granda, 1998). Water stress from véraison until maturity reduces the exposed leaf area and photosynthetic activity, thereby inducing a source limited situation in terms of berry growth and accumulation of sugar (Deloire *et al.*, 2004). Therefore, a reduction in carbohydrate availability can be proposed as the reason for reduced anthocyanin biosynthesis (Hardie & Considine, 1976). Enhanced pigmentation due to the addition of sugars to *in vitro*-cultured grape cells (Larronde *et al.*, 1998; Hiratsuka *et al.*, 2001a) substantiates this finding. Pirie & Mullins (1977)

found that only the sugar content of the grape berry skin is related to the anthocyanin content, whereas Sipiora & Gutiérrez Granda (1998) suggested that total berry sugar and anthocyanin accumulation are closely related.

Excessive irrigation during ripening also impairs colour development in table grapes (Viljoen, 1951). This is attributed either to a dilution effect in larger berries (Matthews & Anderson, 1988; Esteban *et al.*, 2001), or to an excess crop load (Morris, 1980) that causes insufficient partitioning of photosynthates between bunches (Winkler, 1930; Malan, 1953; Kliewer & Weaver, 1971).

Irrigation affects vegetative growth (Myburgh, 1989; Ginestar *et al.*, 1998a; Esteban *et al.*, 1999; Nir *et al.*, 2000; Salón *et al.*, 2005). When it is limited by water stress conditions (Freeman *et al.*, 1980), a reduction in the canopy leaf area enhances fruit exposure and thus berry colour (Ginestar *et al.*, 1998b). However, shoot growth might not be the direct outcome of soil water availability but due to chemical signals originating in the drying roots (Dry & Loveys, 1998). These signals are suspected to be related to hormones. Stoll *et al.* (2000) found reductions in zeatin and zeatin-riboside in the roots, shoot tips and buds of vines under PRD irrigation. They contended that this might contribute to a reduction in shoot growth. Furthermore, chemical signals might affect stomatal control (Stoll *et al.*, 2000). Stomatal control depends on root water potential and signals from root-sourced ABA (Correia *et al.*, 1995). Du Toit *et al.* (2003), on the other hand, found that the reduction in stomatal conductance during the PRD cycle was correlated with the reduction in nitrate reductase activity.

From the abovementioned scenarios, it is clear that the effects of plant water availability on colour can either manifest in effects on the leaf area needed for sufficient ripening, or on the microclimatic conditions. However, the microclimatic effect might only account partially for differences in colour, because the response of the anthocyanin pathway may be the result of physiological signals within the fruit or vine, rather than the effect of microclimate alone (Bindon, 2004).

#### 2.4.4 NUTRIENTS

Various elements are required for grapevine growth (Carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), sulphur (S), iron (Fe), calcium (Ca), magnesium (Mg), boron (B), manganese (Mn), copper (Cu), zink (Zn), molybdenum (Mo) and chlorine (Cl)).

**Nitrogen** fertilisation impacts the vegetative growth to the largest degree (Ewart & Kliewer, 1977; Conradie & Saayman, 1989; Saayman & Lambrechts, 1995b; Choné *et al.*, 2001; Conradie, 2001a; Conradie, 2001b; Cheng & Xia, 2004). The result is that an over-supply in N, expressed as excessive growth, affects the maturation and colour development of grapes indirectly. For example, excessive shoot growth causes a delay in maturity (Christensen *et al.*, 1994; Spayd *et al.*, 1994; Conradie, 2001b) due to increased shading (Spayd *et al.*, 1994) or too many active growing points that compete with the bunches for assimilates (Keller *et al.*, 1998).

Furthermore, a reduction in colour due to increased N may also be attributed to a high crop load because of larger berries (Saayman & Lambrechts, 1995b), or a reduction in the anthocyanin concentration, irrespective of vegetative growth and crop load (Hilbert *et al.*, 2003).

Furthermore, Okamoto *et al.* (2003) detected fewer anthocyanoplasts, glucose and fructose in the skins and juice of berries of Pione vines that received 1.5 times or twice the amount of normal N supply. They suggested that anthocyanoplast development is affected by nutritional status and that both low sugar content and high levels of nitrogenous compounds reduce the formation of anthocyanoplasts. Reduced colour due to high N rates can also be the result of the breakdown of anthocyanins by glucosidase and peroxidase activities (Calderón *et al.*, 1992; Keller & Hrazdina, 1998).

However, sufficient N nutrition is required, without which proper colour development is not achieved. Ewart & Kliewer (1977) assumed that increased colour due to N application could be ascribed to the effect of N on the synthesis of anthocyanin precursors in the leaves. Nitrogen deficiency causes leaves to be small and older leaves often fall prematurely (Mills & Jones, 1996). On the other hand, Choné *et al.*, (2001) found smaller berries and increased anthocyanin content in the wines of grapes from vines subjected to N deficiency. The increased anthocyanin content can either be attributed to the skin:pulp ratio, as a result of smaller berries (Ojeda *et al.*, 2002; Peterlunger *et al.*, 2002), or to the favourable canopy microclimate created by reduced vine vigour (Spayd *et al.*, 1994; Haselgrove *et al.*, 2000).

Finally, the proportions of anthocyanin components are also changed by N fertilisation (Okamoto *et al.*, 2003). Hilbert *et al.* (2003) found that the berry skins of vines that received limited N fertilisation (1.4 mM) had lower amounts of acylated anthocyanins than the berry skins of vines that received average (3.6 mM) or excessive (7.2 mM) levels of N fertilisation. On the other hand, mean N fertilisation resulted in the lowest percentage of non-acylated anthocyanins and the highest amount of acylated anthocyanins. Keller & Hrazdina (1998) found that the malvidin component in Cabernet Sauvignon berry skins increased with high rates of N fertilisation (3.4 g per vine) and low light intensity during véraison. On the other hand, Okamoto *et al.* (2003) found that the same component in the berry skins of Pione grapes was lowered by high rates of N (120 mg/L) compared to the others.

**Phosphorus** contributes 0.1 to 0.3% of grapevine dry matter (Robinson, 1999) and plays a vital role in photosynthesis as it is part of the ADP/ATP energy system (Mauseth, 1995). Excessive P may inhibit the induction of phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS), with a consequent decrease in anthocyanin content (Kakegawa *et al.*, 1995). Phosphorus deficits, on the other hand, typically result in reduced shoot growth and basal leaves that turn yellow and fall before flowering (Robinson, 1999). Photosynthesis is therefore affected and has implications for sugar production as well as yield (Conradie & Saayman, 1989).

**Potassium** makes up about 3% of vine dry weight (Robinson, 1999) and supplementation increases yield (Conradie & Saayman, 1989). It plays a role in fruit development, as, together with sugars, malate and tartrate, it contributes to volume increase during cell expansion (Mpelasoka *et al.*, 2003). Inside the grape berry, it plays a vital role in the internal vacuole, providing electrical balance for organic and inorganic anions (Robinson, 1999), i.e. to maintain the proton balance (Iland & Coombe, 1988) through the role it plays in phloem loading and unloading. Regarding this, Walker *et al.* (2000) found a significant correlation between  $K^+$  concentration and sugar accumulation in developing grape berries. Due to the osmoregulatory function of K (Giaquinta, 1983), it is loaded into the phloem under conditions of limited sugar supply and is translocated to the berry, where it affects grape composition. It forms a salt with tartaric acid and consequently leads to a lower acid concentration (Mattick *et al.*, 1972; Storey, 1987; Jackson & Lombard, 1993; Mpelasoka *et al.*, 2003) and a higher juice pH (Morris *et al.*, 1980). The cell vacuole's pH affects the structural formation of the anthocyanin pigment, resulting in colour loss or a shift from a red-purple colour toward a more blue colour (Timberlake & Bridle, 1967; Morris *et al.*, 1980). Excessive K fertilisation can lead to higher potassium uptake by the vine roots, resulting in higher K concentration in the berry juice (Morris *et al.*, 1980). Excessive potassium is deemed to be more detrimental in the case of wine grapes, since the potassium ends up in the juice, with negative implications for wine colour and microbial stability (personal communication, Dr. W.J. Conradie, ARC Infruitec-Nietvoorbij, Soil Science Division, Klapmuts Road, Stellenbosch, 7600): for example, 900kg per ha increased the pH of Concord grape juice and reduced titratable acidity in a study by Morris *et al.* (1980).

Furthermore, a potassium deficiency manifests in the leaves and the fruit, in that leaves show chlorosis and necrosis and fall prematurely (Saayman, 1981b). In this way, a deficiency will negatively impact colour development through reduced photosynthate production rates. Vines severely deficient in K, have fewer and tight bunches with smaller, unevenly coloured berries (Peacock & Christensen, 1996).

The roles of the rest of the macronutrients are as follows: Calcium plays no direct role with regard to colour development. However, it plays an important role in N metabolism, carbohydrate translocation and protein synthesis (Saayman, 1981b; Mills & Jones, 1996; Robinson, 1999) resulting in an indirect impact on the vine's ability to properly mature the berries. Likewise, Mg, through its essential role in photosynthesis (Mills & Jones, 1996; Stassen *et al.*, 1999), will have an indirect affect on colour development. This also holds true for micronutrients like Mo, Cu and Fe, each being involved in chlorophyll synthesis (Robinson, 1999; Stassen *et al.*, 1999; Chen *et al.*, 2004). Boron, on the other hand, plays a direct role in the translocation of sugars (Stassen *et al.*, 1999), thereby affecting the pool of precursors available for anthocyanin synthesis.

Thus, it is clear that grape colour on account of nutrition can be affected directly via effects on the key enzymes involved in anthocyanin biosynthesis (Kakegawa *et*

*al.*, 1995; Okamoto *et al.*, 2003) and indirectly via effects on photosynthesis or plant cell structures (Mills & Jones, 1996; Robinson, 1999; Stassen *et al.*, 1999). Sugar is an important prerequisite for anthocyanin biosynthesis (Hrazdina *et al.*, 1984) and its availability affects colouration (Hardie & Considine, 1976; Pirie & Mullins, 1977). Therefore, malnutrition (Ewart & Kliewer, 1977; Saayman, 1981b; Mills & Jones, 1996; Robinson, 1999) could thus impair grape colour.

#### **2.4.5 LEAF AREA:FRUIT MASS RATIO**

Several authors reported a delay in maturity and a decrease in colour due to overcropping (Viljoen, 1951; Weaver *et al.*, 1957; Weaver, 1963; Weaver & McCune, 1960b; Lider *et al.*, 1973; Bravdo *et al.*, 1984; Bravdo *et al.*, 1985a; Bravdo *et al.*, 1985b; Hepner & Bravdo, 1985; Kingston & Van Epenhuijsen, 1989; Miller & Howell, 1996; Naor *et al.*, 2002). Therefore, growth and leaf area must be considered before allocating a crop load to a vine (Viljoen, 1951; Saayman & Lambrechts, 1995a). Likewise, an increased budload impairs colour (Weaver & McCune, 1960b; Morris & Cawthon, 1980; Cirami *et al.*, 1985; Morris *et al.*, 1985; Archer & Fouché, 1987; Hunter & De La Harpe, 1987). These consequences of an excessive crop load can be explained by the insufficient partitioning of photosynthates between bunches (Winkler, 1930; Malan, 1953; Kliewer & Weaver, 1971). Since anthocyanin biosynthesis is dependent on glucose and phenylalanine for anthocyanin formation in the berry skin (Pirie & Mullins, 1980; Hunter *et al.*, 1991), the effective leaf area must be enlarged according to the crop load.

The leaf area:fruit mass ratio necessary to produce grapes with improved size and composition (colour, sugar content) has been investigated several times (Winkler, 1930; May *et al.*, 1969; Kliewer, 1970b; Kliewer & Antcliff, 1970; Kliewer & Weaver, 1971; Winkler *et al.*, 1974; Smart, 1980; Jackson, 1986; Kingston & Van Epenhuijsen, 1989; Dokoozlian & Hirschfelt, 1995; Hunter, 2000). Jackson (1986) noted that a large leaf area after stage I of berry development and a high leaf area:fruit mass ratio promote the early development of colour. Thus, during stage I of berry development, when the grape berry acts as a strong sink, sugars produced by the leaves contribute largely to anthocyanin biosynthesis.

In table grape production, cluster thinning is normally applied in order to increase the leaf area:fruit mass ratio. Kliewer & Weaver (1971) showed that pruning and cluster thinning resulted in significantly better colouration due to the higher leaf area:fruit mass ratio. The reduction of bunches (sinks) means that more assimilates can be allocated to the remaining bunches (Naor *et al.*, 2002). Cluster thinning increases the amount of anthocyanins in the grapes (Kliewer & Weaver, 1971; Mazza *et al.*, 1999; Guidoni *et al.*, 2002). Dokoozlian & Hirschfelt (1995) found that berry colour was more sensitive than berry weight or soluble solids to crop load and that the berry skin anthocyanin content at harvest was 50% higher for vines that were cluster thinned at berry set compared to vines that were not. The thinning of clusters four weeks after berry set resulted in a more rapid accumulation of colour than when

thinning was done at other stages of berry development (Dokoozlian & Hirschfelt, 1995). In the same study, the similar growth rate of berries of both cluster-thinned vines and vines that were not thinned prior to fruit softening indicates that the latter were not source-limited prior to softening. Sufficient leaf area at the initial stages of berry development was the reason that cluster thinning had little effect on fruit development at this stage. Differences in berry fresh weight and soluble solids between cluster-thinned vines, where thinning took place at different stages between pre-bloom and six weeks after berry set, and unthinned vines were observed only after fruit softening. It is thus at the stage of rapid sugar accumulation that the unthinned vines become source-limited (Dokoozlian & Hirschfelt, 1995). Winkler (1958) found that berry thinning after set resulted in a more uniform colour. This could be explained by the larger leaf area per unit mass of fruit at that stage. However, intensive vegetative growth prompts sink competition between growing tips and developing berries and therefore limits assimilates for bunches, which may account for insufficient colour associated with strong vegetative growth (Bravdo *et al.*, 1985b; Keller *et al.*, 1998).

Hunter (2000) recommends that rather than considering the leaf area alone, the age composition of the leaf area should also be taken into account, because young leaves (on lateral shoots) and older leaves (middle and basal leaves) contribute differently to grape composition. Leaves on the lateral shoots, being the younger leaves in the canopy, seem to play a major role in metabolic processes during fruit ripening. Hale & Weaver (1962) reported that lateral shoots behave as young leaves, but become net exporters as soon as they have two fully expanded leaves. According to Koblet (1977), lateral shoots without grapes export their carbohydrates to the clusters on the main shoot. Candolfi-Vasconcelos & Koblet (1990) showed that fruit from canopies that were composed only of lateral shoots had higher colouration. Vasconcelos & Castagnoli (2000) confirmed that a higher proportion of leaves from lateral shoots per unit leaf area improved skin anthocyanin content per berry and per mass of fruit. Hunter (1999) found that younger leaves produced more tartaric acid than malic acid for a supplementary irrigated Sauvignon blanc/R110 vineyard. However, this acid is not translocated to the berries and therefore, the acid content of the grape berry and the proportions of the acids are determined by localised synthesis within the berry, from carbohydrate precursors (Ruffner, 1982). Shoot tipping and selective leaf removal at appropriate growth stages can improve the ratio of young:old leaves.

## 2.5 CULTIVATION STRATEGIES TO MANIPULATE THE COLOUR QUALITY OF TABLE GRAPE CULTIVARS

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### 2.5.1 LONG-TERM CULTIVATION STRATEGIES

#### 2.5.1.1 Site selection - Terroir

Terroir is the soil, climate and landscape that are managed through combinations of cultivation practices and cultivars for the production of quality grapes (Saayman, 1992a). Of the environmental aspects, climate- and soil-related factors are regarded as the most important for vineyard site selection (Saayman, 1977; Saayman, 1981a, Saayman, 1992a; Saayman, 1992b). Viljoen (1951) attributed the colour differences of table grapes to climatic and regional factors and Brossaud *et al.* (1999) proposed that the anthocyanin content of Cabernet Franc largely depends on the vine environment. Furthermore, the ripening time of a cultivar is determined by the geographic location of the site (Le Roux, 1948). Therefore, it is recommended that a thorough study of the soil and climatic conditions must be done before any decision is made regarding the table grape cultivar to be established (Le Roux, 1957). The correct choice of cultivar would thus contribute to cultivation success, because it has implications for the difference in the time of marketing in different regions.

Much value is often attached to soil as a factor determining quality. Soil modifies the effect of the climate and plays an important role in determining grape quality. It has been proved that root growth determines aboveground growth (Buttrose & Mullins, 1968; Saayman & Van Huysteen, 1980; Saayman, 1982; Richards, 1983; Archer *et al.*, 1988; Archer & Hunter, 2005). Therefore, the aboveground performance of the vine will be determined partly by soil factors affecting root growth.

The physical properties of a soil determine its water-holding capacity (Hillel, 1980), nutrient status (Campbell & Souster, 1982; Hassink *et al.*, 1993), as well as accompanying soil conditions, such as temperature (Hillel, 1980). In the Western Cape Province of South Africa, soils are subjected to excess moisture and thus cold soil temperature at the beginning of the growth season, although the soils dry out during the ripening period (Saayman, 1977). Conradie *et al.* (2002) found that budburst occurs earlier on a drier and thus a warmer soil than on a wetter, cooler soil. In general, higher root temperatures are more beneficial for enhanced and earlier budbreak (Kliwer, 1975; Zelleke & Kliwer, 1979; Graham *et al.*, 2002). This can probably be attributed to the effect of soil temperature on cytokinin production in the roots (Skene & Kerridge, 1967; Zelleke & Kliwer, 1981). Weaver *et al.* (1968) stated that cytokinins affect budbreak and have a positive effect on cell division (Coombe, 1973; Alleweldt, 1977), and thus on shoot elongation (Skene & Kerridge, 1967; Kliwer, 1975). On the other hand, Lombard (2003) proposed that cytokinins are not directly involved in budbreak, but is needed for the growing process following budbreak. Therefore, vegetative growth can be enhanced via the soil/root

temperature (Kliwer, 1975). Furthermore, fertile soils, i.e. soils with excessive nitrogen, stimulate excessive shoot growth, resulting in fruit shading (Spayd *et al.*, 1994) which has implications for colour development (Smith, 1982; Smart, 1987; Dokoozlian & Kliwer, 1995). In this regard, heavily textured clay soils have more nitrogen than sandy soils (Campbell & Souster, 1982; Hassink *et al.*, 1993) and might therefore result in inferior colour (Le Roux, 1957). Le Roux (1948) reported that Barlinka was subjected to poor colour development when grown on a fertile soil under irrigation because irrigation results in denser canopies (Esteban *et al.*, 1999) due to enhanced vegetative growth (Myburgh, 1989). It has already been said that conditions in dense canopies reduce skin colour (Archer & Strauss, 1989).

A soil pH within the range of 5.0 to 7.5 is usually not limiting to nutrition and growth (Saayman, 1981b) but acidic soil conditions impede root growth (Conradie, 1988; Bates *et al.*, 2002). Soil pH determines the uptake of nutrients necessary for growth. Under acidic conditions (pH<5.5), P may become unavailable, whereas micronutrients are readily available (Robinson, 1999). In alkaline soils (pH>8), P also becomes insoluble and most of the micronutrients are converted to unavailable forms (Robinson, 1999).

Since the enzymes involved in anthocyanin synthesis are regulated by temperature (Kliwer, 1977), the colour of grapes can be affected by the reflection of solar energy from the soil towards the bunch zone. Darker coloured soils absorb solar radiation, whereas solar radiation is reflected by light coloured soils (Fregoni, 1977; Hillel, 1980). The direction of a sloping surface also affects the amount of solar radiation that is intercepted. Slopes facing the sun are warmer than those that face away from the sun (Hillel, 1980). In South Africa, the northern and north-western slopes are warmer and drier than the southern and eastern slopes (Van der Westhuizen, 1981; Bonnardot *et al.*, 2002). However, according to Van der Westhuizen (1981), the direction of the slope is of much less importance in South Africa, a country of sufficient sunshine, than in the colder European countries.

Vegetative growth is also affected by the mean temperature of a given site. High temperatures of 20°C up to 35°C result in longer main shoots, longer lateral shoots, and higher dry weight production (Buttrose, 1969; Buttrose, 1978). Similarly, Pratt & Coombe (1978) found less internodes per shoot in areas where winter temperatures tended to be below freezing point. Therefore, shoot crowding, and thus shade, might be experienced in vines grown under warm temperatures (20 to 35°C). Furthermore, the leaf area per vine increases at higher temperatures (Kliwer, 1975). Shade and high temperatures both would have consequences for colour development (Robinson, 1988; Archer & Strauss, 1989).

Temperature also affects table grape quality by affecting metabolism of organic acid (Kliwer & Lider, 1968; Buttrose *et al.*, 1971; Kliwer, 1973; Ruffner *et al.*, 1976; Smart *et al.*, 1977; Reynolds *et al.*, 1986; Wolf *et al.*, 1986; Bledsoe *et al.*, 1988; Iland, 1989; Rojas-Lara & Morrison, 1989), sugars (Kliwer, 1973; Smart *et al.*, 1977; Reynolds *et al.*, 1986; Kliwer *et al.*, 1988) and anthocyanins (Kliwer, 1970a;

Kliewer, 1973; Kliewer, 1977; Iland, 1989; Mabrouk & Sinoquet, 1998; Haselgrove *et al.*, 2000). A prerequisite for the synthesis of anthocyanins is the availability of sugar (Hardie & Considine, 1976). Considering that temperatures above 35°C cause stomatal closure, with a consequent reduction in photosynthesis and thus a decrease in sugar (Kriedeman, 1968; Kriedeman & Smart, 1971; Kriedeman, 1977; Farquhar & Sharkey, 1982), has implications for colour development. Therefore, it is recommended by Iland (1989) that the temperature conditions at a site must be such that the largest part of the vine's growing season falls within the range for 90 to 100% photosynthetic efficiency. This will ensure optimal carbohydrate accumulation and thus carbohydrate availability for anthocyanin biosynthesis (Hardie & Considine, 1976). Apart from the indirect impairing effect of high temperatures on colour via a reduction in photosynthesis, it was shown that very high day/night temperatures (37/32°C) inactivate or destroy the enzymes involved in anthocyanin synthesis in the grape berry (Kliewer, 1977). Kliewer & Torres (1972) found that when the difference between day and night temperatures exceeds 10°C, colouring of Pinot Noir and Cabernet Sauvignon berries were inhibited. In the light of this, Hunter & Archer (2001a) recommended that conditions that fall within the optimum temperature ranges for the different physiological processes, such as colour development, should always be selected or created.

The availability of water is another consideration in site selection. Water availability affects soil temperature (Hillel, 1980; Myburgh, 1989), photosynthesis (Archer & Strauss, 1990) and shoot growth (Myburgh, 1996). Inhibited canopy growth will result in more favourable light and temperature conditions for colour biosynthesis (Haselgrove *et al.*, 2000). The adequacy of seasonal rainfall is also a criterion when selecting the vineyard site in cases where the vines are solely dependent on rain for their water supply or in cases where supplementary water is limited. The seasonal distribution of rainfall is also of great importance. A water deficit during the pea-size stage of berry development can be beneficial for colour formation (Matthews & Anderson, 1988; Esteban *et al.*, 2001; Ojeda *et al.*, 2002). However, the enhancement in colour due to the higher skin:pulp ratio (Ojeda *et al.*, 2002; Peterlunger *et al.*, 2002) obtained with smaller berries (Van Zyl, 1984; McCarthy, 1997; Ginestar *et al.*, 1998b; Myburgh, 2003; Deloire *et al.*, 2004) is only of importance to wine grapes. Large berries are favoured by consumers in the case of table grapes (Le Roux & Meynhardt, 1954). Therefore, sufficient water supply during the cell division phase is recommended.

Apart from the effect of high relative humidity (RH) on the occurrence of fungal diseases (English *et al.*, 1989), RH can affect colour development via its effect on photosynthesis. When the difference between air vapour content and intercellular vapour content exceeds a critical level, stomata close and photosynthesis stops (Salisbury & Ross, 1992). Consequently, insufficient carbohydrate availability would result in insufficient colour (Hardie & Considine, 1976) in areas of high humidity.

Moderate winds have a cooling effect on grapes and favour grape quality (Hunter & Archer, 2001a). Apart from physical damage, wind-exposed vines have been reported to show reduced growth and yield compared to sheltered vines (Ewart *et al.*, 1987; Hamilton, 1989). Moderate to strong wind (3.6 to 10.7 m.s<sup>-1</sup>) reduces stomatal conduction (Kobriger *et al.*, 1984). Photosynthesis is decreased as a result of stomatal closure (Kriedeman & Smart, 1971; Raschke, 1975; Freeman *et al.*, 1982; Salisbury & Ross, 1992). Therefore, a decrease in colour is expected due to carbohydrate deficiency (Hardie & Considine, 1976). Wind causes water loss through increased rates of transpiration and evapotranspiration, with implications for grapevine water use and thus irrigation scheduling (Ewart *et al.*, 1987; Campbell-Clause, 1994) and the possible effects thereof on grape colour (Viljoen, 1951; Freeman, 1983; Esteban *et al.*, 2001; Deloire *et al.*, 2004).

### 2.5.1.2 Choice of rootstock

Southey & Archer (1988) found that the distribution of roots is determined more by the soil conditions, whereas the root density is determined mainly by the rootstock cultivar. Root growth and distribution have implications for aboveground growth (Buttrose & Mullins, 1968; Saayman & Van Huysteen, 1980; Saayman, 1982; Richards, 1983; Archer *et al.*, 1988; Swanepoel & Southey, 1989; Hunter, 1998; Archer & Hunter, 2005). Subterranean and top growth balance is a prerequisite for vine balance (Archer & Hunter, 2004).

The choice of rootstock is important in terms of the vigour it induces in the scion cultivar. Strong vigour causes a leaf area:crop imbalance (Bravdo *et al.*, 1985b) and excessive shading (Smart *et al.*, 1985) which affects the photosynthetic performance of the vine (Vanden Heuvel *et al.*, 2004) and consequently anthocyanin biosynthesis (Hardie & Considine, 1976).

It was found by Malan (1960a) that the rootstock Jaquez did not only induce the strongest vigour in Alphonse Lavalée, but also increased yield. Rootstocks increase yield as a result of more vegetative growth and thus a greater production of photosynthates (May *et al.*, 1973). The greater production of photosynthates can be ascribed to the enhanced effect of rootstocks on assimilation rates (Candolfi-Vasconcelos *et al.*, 1994; Koblet *et al.*, 1996). Increased yields can be ascribed to increases in the number of bunches and/or berries per cluster (May *et al.*, 1973; Reynolds & Wardle, 2001). This affects the leaf area:fruit mass ratio and might cause overcropping, which results in a delay in maturity (Main *et al.*, 2002) and less colour (Winkler, 1958). Therefore, diminished colour from the use of different rootstocks can be ascribed to a leaf area:fruit mass ratio imbalance (Gawel *et al.*, 2000; Walker *et al.*, 2000). Ezzahouani & Williams (1995) reported that, under non-irrigated conditions, grapevines on Rupestris du Lot, R110, 140 Ruggeri and 41B showed a lighter skin colour of Ruby Seedless compared to vines on R99, SO4, 101-14 Mgt and 1103 Paulsen. Grapes of scions grafted on Rupestris du Lot had the least berry colour. They attributed this to the fact that the scions grafted on Rupestris

du Lot had a lower leaf water potential than those on other rootstocks. Low leaf water potential causes stomatal closure with the effect of decreasing photosynthesis (Kriedeman & Smart, 1971) and thus carbohydrate manufacturing. Since the availability of the latter has been proven to influence berry colour (Hardie & Considine, 1976), rootstock might thus affect berry colour through its effect on carbohydrate availability.

Finally, the rootstock affects nutrient uptake (Nikolic *et al.*, 2000; Garcia *et al.*, 2001a; Garcia *et al.*, 2001b). As discussed in chapter 2.4.4, nutrients contribute to plant growth and structure, as well as to grape colour.

### **2.5.1.3 Row orientation**

For vertical trellis systems, row direction is critical for sunlight interception (Van der Westhuizen, 1981) and it determines the duration of sunlight exposure to the bunches. Smart (1973) found that vertically trained vines with an east-west row orientation in the Southern hemisphere intercepted less light than north-south rows due to the rectangular shape of the canopy with only a small part of the canopy subjected to direct incident sunlight. Considering this, if vines are trained horizontally, a larger part of the canopy will therefore be exposed to direct sunlight if oriented in an east-west direction. This also ensures that bunches are protected from direct sunlight.

### **2.5.1.4 Vine spacing**

The purpose of the correct vine spacing is to obtain optimal soil utilisation (Archer *et al.*, 1988; Archer, 1990; Hunter, 1998), sunlight interception (Archer, 1990; Archer & Strauss, 1990), physiological processes (Archer, 1990; Archer & Strauss, 1990) and overall performance (Archer & Strauss, 1991). Burger (1990) found that narrow inter-vine spacing (1.23 m) of Sultanina on a fertile alluvial soil resulted in compact foliage and reduced sunlight penetration in the canopy. Similarly, Hunter *et al.* (1996) found a poor canopy microclimate with close spacing (1.0 m x 1.0 m and 1.0 m x 0.5 m). The conditions accompanying dense canopies therefore have implications for bud fertility (May, 1965; Smart *et al.*, 1982a, Smart *et al.*, 1982b), photosynthesis (Archer & Strauss, 1989; Archer & Strauss, 1990; Hunter & Ruffner, 1997) and anthocyanin biosynthesis (Roubelakis-Angelakis & Kliewer, 1986; Archer & Strauss, 1989).

Although Kliewer *et al.* (2000) found a less dense canopy and improved light conditions in the bunch zone of widely spaced vines (3.0 m), maturity was delayed compared to that of grapes from closely spaced vines. Archer (1990) attributed reduced colour in widely spaced vines to insufficient leaf area available for bunch nutrition. In this regard, Kliewer *et al.* (2000) found that the leaf area:fruit mass ratio, as well as the percentage lateral shoot leaf area as a proportion of the total leaf area, decreased. It was shown by Mannini *et al.* (2003) that the wider spacing (1.5 m x 2.7 m) of vigorous “Nebbiolo Michet” in a cool climate area resulted in a

lower leaf layer number and a lower total leaf area:sun-exposed leaf area ratio, and thus a higher anthocyanin content. Therefore, the correct vine spacing for a specific cultivar, climate and soil type prevents problems associated with poor microclimate, and wide spacing is recommended for fertile soils (Archer & Strauss, 1990), especially for vigorous cultivars, such as Sultanina (Burger, 1990).

#### **2.5.1.5 Trellis system**

The correct choice of trellis system is essential to accommodate vegetative growth in a way that ensures sufficient utilisation of resources (Zeeman, 1981), without limiting the leaf area of vigorously growing cultivars (Viljoen, 1951). The choice of a trellis system is not only determined by soil potential, rootstock and scion varieties, and the purpose of cultivation (Zeeman, 1967a), but also by the plant spacing, cultivar and vine vigour (Zeeman, 1971; Cirami *et al.*, 1999). Uys (1976) stated that the trellis system is inseparable from vine spacing. He added that there should be a balance between surface area and the surface of the vine on the trellis. This will contribute to obtaining maximum production of the best quality. The trellis should prevent shoot crowding (Shaulis & May, 1971; Van den Ende, 1984; Valentini *et al.*, 1996) and reduce canopy shade (Shaulis & May, 1971; Reynolds *et al.*, 1995; Reynolds *et al.*, 1996; Kliwer *et al.*, 2000). The trellis system determines the light environment within the canopy (Douglas, 1951; May *et al.*, 1973; Peacock *et al.*, 1994; Moreno & Pavez, 2000) and this enhanced light conditions stimulate leaves photosynthetically (Ezzahouani & Williams, 2003).

In addition to reducing canopy density (Reynolds *et al.*, 1995; Reynolds, *et al.*, 1996), wide trellises increase sunlight interception and utilisation (Zeeman, 1971; Swanepoel *et al.*, 1990; Peacock *et al.*, 1994). The use of a wide trellis system induces earlier ripening and a better colour quality of Alphonse Lavalée (Olivier, 1957). Le Roux (1959) confirmed these results and found earlier ripening of Alphonse Lavalée on a roof trellis system than on a slanting trellis system.

Due to the importance of light in colour development (Douglas, 1951; Kliwer *et al.*, 1967; Roubelakis-Angelakis & Kliwer, 1986; Hunter *et al.*, 1995b), it is not strange that Le Roux (1960) found that wider trellis systems favour colour development in Barlinka grapes. More colour due to larger/wider trellis systems can be attributed to a larger leaf area intercepting light, resulting in a greater production of photosynthates (Ezzahouani & Williams, 2003).

### **2.5.2 SHORT-TERM CULTIVATION STRATEGIES**

#### **2.5.2.1 Pruning**

The correct pruning method is determined by the grape cultivar and the location of cultivation. Early ripening cultivars, such as Flame Seedless and Prime, in early areas, such as the Lower Orange River region, are pruned earlier. The time of

pruning affects the time of ripening (Malan, 1956; Malan, 1961b). The pruning method, i.e. spur or cane pruning, is determined by bud fertility (Malan, 1959b; Malan, 1960b; Malan, 1961a).

Zeeman & Archer (1981) emphasised four purposes of pruning: developing young, balanced vines with a favourable structure, obtaining a balance between vegetative and reproductive growth, producing yields that often have good quality, as well as positioning bearers in a favourable position as close as possible to the permanent structure of the vine. Maintaining the vine structure through pruning ensures good spreading of the summer foliage (Malan, 1958). In turn, this leads to optimal light interception for photosynthesis and further biological processes that contribute to colour development. Archer & Hunter (2004) say that, in order to obtain the balance between vegetative and reproductive growth, spur spacing must be such that shoot crowding does not occur. Secondly, they state that spurs must have a length (not more than two buds) that ensures fertile shoots to reach the appropriate length for a correct leaf surface:fruit mass ratio in order to ensure complete ripening.

The budload left at pruning determines the yield and vegetative growth (Lider *et al.*, 1973; Lider *et al.*, 1975; May *et al.*, 1976; Byrne & Howell, 1978; Freeman *et al.*, 1979; Jackson *et al.*, 1984; Morris *et al.*, 1984; Intrieri *et al.*, 1999). Lighter colour and reduced sugar concentration due to light pruning can be ascribed to overcropping (Morris & Cawton, 1980; Morris *et al.*, 1984; Cirami *et al.*, 1985; Morris *et al.*, 1985; Archer & Fouché, 1987; Hunter & De la Harpe, 1987). These consequences of excess cropload can be explained by the insufficient partitioning of photosynthates between bunches (Winkler, 1930; Malan, 1953; Kliwer & Weaver, 1971). On the other hand, light pruning might also enhance colour due to a smaller berry size that accompanies the increased yields (Freeman, 1983). Another reason for enhanced ripening as a result of light pruning is the increased number of carbohydrate sinks that stimulates photosynthesis (Chandler & Heinicke, 1925; Neales & Incoll, 1968; Byrne & Howell, 1978). A combination of crop load, carbohydrate distribution, movement of plant hormones to and from the growing points, and light interception, can also be put forward as a reason for darker colour due to minimal pruning (Clingleffer, 1989). This, however, is not applicable to table grapes.

### **2.5.2.2 Suckering**

The benefits of suckering, as emphasised by Zeeman (1967b) and Zeeman (1983), are: enhanced growth of the remaining shoots and decreased canopy density. During suckering, excess, unnecessary shoots (sinks) that do not contribute to bunch quality are removed, and the use of reserve compounds during the first part of the season is restricted (Archer & Beukes, 1983; Zeeman, 1983). Viljoen (1951) states that shoot removal prevents the occurrence of a thick leaf sheath on top of the trellis system and thus improves the light environment within the canopy, resulting in better colour development.

### 2.5.2.3 Shoot positioning

To obtain full benefit from suckering, and in order to take a decision on any further canopy management, it is important that shoots are positioned and tightened down. The positioning of shoots ensures the even distribution and sufficient exposure of leaves to sunlight (Malan & Carstens, 1971). Mabrouk & Sinoquet (1998) consider shoot orientation as the main determinant for bunch exposure. Shoot positioning decreases shoot crowding (Morris *et al.*, 1985). It also improves light interception and distribution (Smart, 1988; Moreno & Pavez, 2000; Volschenk & Hunter, 2001), and thus ensures sufficient colour development (Morris *et al.*, 1984). Cirami *et al.* (1985) found that shoot positioning resulted in a darker colour of the berries. Hunter & Archer (2001b) stated that increased airflow, due to shoot positioning, also leads to lower berry temperatures, which contribute to grape quality.

### 2.5.2.4 Tipping/Topping

Several authors reported on the contribution of lateral shoot leaves to grape composition (Koblet, 1977; Candolfi-Vasconcelos & Koblet, 1990; Avenant, 1994; Hunter, 2000; Vasconcelos & Castagnoli, 2000). Candolfi-Vasconcelos *et al.* (1994) found that leaves from lateral shoots and the younger leaves at the top of the canopy had similar photosynthetic rates, which were higher than those of the older leaves lower down in the canopy. Therefore, the lack of younger leaves for efficient photosynthesis might also be a reason for lower sugar concentration in the berries of widely spaced vines.

The removal of the shoot tip changes the direction of nutrient translocation, away from the shoot tip towards the bunches (Quinlan & Weaver, 1970; Zeeman & Archer, 1981). Tipping involves the removal of 2 to 5 cm of the shoot tip (Zeeman & Archer, 1981) and, during bloom, it ensures improved berry set (Coombe, 1959; Malan, 1959a; Coombe, 1962). Topping involves the removal of 15 to 25 cm of a young growing shoot (Zeeman & Archer, 1981) and reduces vigour (Reynolds & Wardle, 1989a; Reynolds & Wardle, 1989b). Removal of the shoot tip also stimulates the development of lateral shoots (Koblet, 1987; Reynolds & Wardle, 1989a; Reynolds & Wardle, 1989b; Wolf *et al.*, 1990; Poni & Giachino, 2000; Vasconcelos & Castagnoli, 2000). This stimulation of the development of more lateral shoots and the associated younger leaves in the canopy will increase the photosynthetic capacity of the canopy during ripening (Coombe, 1959; Poni & Giachino, 2000; Hunter & Archer, 2001b). These young mature leaves have a higher photosynthetic capacity than basal leaves (Iacono & Sommer, 2000). It is thus evident that young, fully expanded leaves are an important source of carbohydrates during berry ripening. Therefore, more assimilates are exported to the bunches (Koblet, 1977).

Furthermore, topping prevents drooping of shoots over the trellis top and therefore increases the percentage of the available PPFR (Reynolds & Wardle, 1989a; Wolf *et al.*, 1990). As a result, fruit composition is enhanced provided that source reduction does not outweigh the benefit of the enhanced light environment.

On the other hand, severe topping removes the photosynthetic leaf area and thus the reduction in leaf area which is often the reason for a reduction in colour (Le Roux & Malan, 1945; Reynolds & Wardle, 1989b).

The timing of the topping action is also crucial for optimal colour development. Viljoen (1951) stated that the topping of shoots during véraison results in poor colour development. This can be attributed to the appearance of actively growing shoot tips, acting as sinks (Bravdo *et al.*, 1985b; Keller *et al.*, 1998), which allocate growth substances away from the bunches. Therefore, shoot tip removal must be done at the beginning of flowering to ensure well developed lateral shoot leaves, which contribute to berry ripening, at véraison (Poni & Giachino, 2000).

### 2.5.2.5 Leaf thinning

The removal of leaves and lateral shoots in the bunch zone increases bunch temperature, PPFR and the R:FR ratio. (Viljoen, 1951; Kliwer & Smart, 1989; Peacock, 1996; Dry, 2000). It also ensures exposure of the remaining leaves and grapes to uniform and filtered sunlight, as well as homogenous ripening (Smith *et al.*, 1988; Hunter *et al.*, 1995b; Koblet *et al.*, 1996). Because leaf thinning changes the source:sink ratio (Carbonneau, 1996), leaf thinning increases the photosynthetic activity of the remaining leaves (source) (Koblet *et al.*, 1996). Hunter *et al.* (1995b) found that the photosynthetic activity of the remaining leaves and the metabolic activity of the bunches increased with leaf removal in combination with suckering and shoot positioning. The export of photoassimilates is thus increased through a lower source:sink ratio (Hunter & Visser, 1988b). Mansfield & Howell (1981) even found that bunches on completely defoliated Concord vines were powerful sinks that mobilised carbohydrates from parts of the vine other than the leaves.

Removal of the basal leaves is a very common practice during seasonal table grape canopy management and it is usually done at the beginning of véraison. Leaf removal in the bunch zone improves anthocyanin biosynthesis as a result of better bunch exposure (Koblet, 1987; Iland, 1988; Smith *et al.*, 1988). A well-exposed bunch zone during the pré- and post-véraison periods ensures maximum sink metabolism, resulting in maximum sucrose attraction, low pH, maximum anthocyanin synthesis, and maximum organic acid and flavour compound synthesis (Hunter & Archer, 2001b).

Although removal of the basal leaves is beneficial in terms of microclimate, it should be kept in mind that the basal leaves continuously nourish the bunches. The basal leaves contribute to maintenance metabolism (Hunter *et al.*, 1995a) because of their stable and ongoing photosynthetic activity, as well as their sustained nitrate reductase activity up until harvest and thereafter (Hunter & Visser, 1988a; Hunter & Archer 2001b). Hunter *et al.* (1991) found a tendency towards higher anthocyanin concentrations in the skins of partially defoliated Cabernet Sauvignon grapes. This was attributed to the fact that partial defoliation caused photosynthetic stimulation of older leaves. This increase in photosynthetic activity resulted in the accumulation of

phenylalanine and sucrose, the precursors of anthocyanin biosynthesis, in the bunches and a stimulation of the activity of phenylalanine ammonia-lyase, the enzyme that channels phenylalanine towards anthocyanin biosynthesis.

## **2.6 THE EFFECT OF PLANT BIOREGULATORS ON GRAPE COLOUR**

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The five types of plant growth regulators that occur naturally in the grapevine are auxins, cytokinins, gibberellin (GA), abscisic acid (ABA) and ethylene (Seymour *et al.*, 1993). Inside the vine and the berry, these growth regulators peak at different stages of grapevine and berry development (Coombe, 1960; Coombe, 1973; Coombe & Hale, 1973; Düring *et al.*, 1978; Scienza *et al.*, 1978).

Growth regulators might not have a direct impact on grape quality, especially colour. However, through the role auxins and GA play in berry growth (Coombe, 1960; Alleweldt, 1977; Lavee & Nir, 1986) it might impact on berry colour due to the fact that it induce attraction sites for assimilates (Weaver *et al.*, 1969; Alleweldt, 1977).

Through its role in budbreak (Weaver *et al.*, 1968; Mauseth, 1995) and cell division in grape berries (Coombe, 1973; Alleweldt, 1977), cytokinins might have an effect on the canopy and thus the photosynthetic capacity and carbohydrate accumulation in the bunches (Hunter, 2000). Due to the fact that cytokinins are involved in the regulation of flower initiation (Palma & Jackson, 1989), it might have an effect on the potential crop load (and thus the partitioning of assimilates) (Winkler, 1930; Malan, 1953; Kliewer & Weaver, 1971) which affects berry colour (Hepner & Bravdo, 1985; Miller & Howell, 1996; Naor *et al.*, 2002).

ABA plays a role in the ripening (increase in sugar content) of grapes through the stimulation of gluconeogenesis (Palejwala *et al.*, 1985). ABA treatment at the beginning of ripening enhances anthocyanin accumulation in the grape skin (Kataoka *et al.*, 1982; Ban *et al.*, 2000) probably because it increases PAL activity and therefore anthocyanin concentration in berry skins (Kondu *et al.*, 1998). It also increases the metabolic flow rate of the phenylpropanoid pathway in the grape skin and enhances biosynthesis of the phenylpropanoid metabolites (Ban *et al.*, 2000).

Ethylene plays a role in ripening and enhances colour development (Coombe & Hale, 1973; Peacock *et al.*, 1977; Fitzgerald & Patterson, 1994; Nikolaou *et al.*, 2003; Lombard *et al.*, 2004). A possible mechanism involved in the response of exogenous ethylene is as follows: stimulation of the long-term expression of the chalcone synthase (*CHS*), flavanone 3-hydroxylase (*F3H*) transcripts and 3-O-glucosyl transferase (*UFGT*) genes, which relates directly to anthocyanin biosynthesis in grape berries (El-Kereamy *et al.*, 2003).

## 2.7 THE EFFECT OF GIRDLING ON GRAPE COLOUR

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Girdling (cincturing) can be described as the removal of a 3 to 6 mm ring of bark down to the cambium in a complete circle around the vine trunk or arms (Cirami *et al.*, 1999) and is implicated in the interruption of the basipetal movement of assimilates (Winkler *et al.*, 1974; Roper & Williams, 1989). As a result of phloem disruption, assimilates, such as sucrose, increase in the parts of the vine (bunches) above the girdle (Hunter & Ruffner, 2001). Not only carbohydrate assimilates, but also endogenous hormones, increase in grape berries as a result of phloem disruption. Vines can be girdled to improve berry set (Coombe, 1959), increase berry size (Dokoozlian *et al.*, 1994; Ezzahouani & Williams, 2001; Orth *et al.*, 1989; Williams *et al.*, 2000), advance ripening and thus improve grape colour (Peacock *et al.*, 1977; Carreño *et al.*, 1998; Ezzahouani & Williams, 2001; Nikolaou *et al.*, 2003). Timing of this action is crucial. Shoot girdling of Shiraz vines at véraison increases anthocyanin levels in the bunches above the incision (Gholami, 2004), whereas ripening measured in terms of sugar:acid ratio and colour intensity of Bien Donn e and Dan-ben-Hannah is delayed if girdling is applied before bloom or after fruit set (Orth *et al.*, 1989). The latter was attributed to an excessive crop load. Similarly, Dokoozlian *et al.* (1994) attributed delayed ripening of Crimson Seedless grapes to overcropping as a result of larger berries, which in turn increased the yield of girdled vines. The optimum time for girdling to effectively enhance colour and thus ripening rate seems to be at the onset of véraison (Peacock *et al.*, 1977; Dokoozlian *et al.*, 1993; Carreño *et al.*, 1998; Nikolaou *et al.*, 2003; Gholami, 2004).

## 2.8 STRATEGY FOR GRAPE COLOUR MANAGEMENT

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As described above, various factors affect the extent to which grapes will colour. By incorporating management strategies whereby these factors are addressed, the table grape producer can potentially improve the vineyard's potential for optimum colour development. A comprehensive approach, including irrigation, fertilisation, vine spacing, vine training, the trellis system, pruning, and foliage management practices must be implemented to ensure healthy, well shaped and strong growing vines. Factors that affect the table grape industry with associated cultivation approaches for optimal colour development are given below.

**Suboptimal photosynthesis** and the accompanied conditions, such as shade, should be avoided. A light intensity between 600 and 800  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  is optimal for photosynthesis (Kriedeman, 1968). Anthocyanin biosynthesis takes place at light intensities between 60.6 to 90  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Dokoozlian & Kliewer, 1995; Kataoka *et al.*, 2004). This, however, seems to be cultivar dependent. Some cultivars, like Tokay and Emperor have an inability to form anthocyanins under suboptimal light conditions (Weaver & McCune, 1960a). Cultivars with anthocyanin components lacking an -OH or -OCH<sub>3</sub> group in the 5' position of the B-ring is the most sensitive to unfavourable

light and temperature conditions (Kliewer, 1977). Thus, for more colour, bunches of cultivars in this category, i.e cultivars with cyanidin and peonidin, have to be well exposed at all times. Therefore, to address the problem of excessive colour in cultivars such as Redglobe, it can be proposed that this cultivar, with peonidin as main component, would develop a lighter colour when exposure to direct sunlight is limited.

**Optimum day/night temperature combinations** benefit colouration for a given cultivar (Kliewer & Torres, 1972). The optimum day temperature range for colouration appears to be between 17.5 and 35°C (Pirie, 1979; Spayd *et al.*, 2002). Temperature contributes to grape colouration via the effect it has on photosynthesis, as well as anthocyanin biosynthesis. Therefore, site selection must be done according to the optimum temperature range for biological processes. Coloured cultivars must therefore be produced in areas where most of the ripening period falls within the optimum temperature range for anthocyanin biosynthesis. It can be assumed that cultivation of cultivars, such as Redglobe, that tend to develop excessive colour would inhibit the mentioned biological processes and might therefore reduce the grape skin colour. However, suboptimal temperatures might also affect the time of ripening.

**The application of growth regulators**, as well as the timing of application can either have an enhancing or a delaying effect on colour development. To prevent poor colour development of Flame Seedless, the best time to apply gibberellic acid (GA) for the enlargement of Flame Seedless berries is at 7 to 9 mm berry diameter (Wolf *et al.*, 1996). The use of synthetic cytokinins, such as N-(2-chloro-4-pyridil)-N'-phenylurea (CPPU) and N<sup>1</sup>-p-hehyl-N'-1,2,3-thiadiazol-5-yl urea (TDZ) in combination with gibberellic acid (GA) causes a decrease in anthocyanins (Reynolds *et al.*, 1992). Ethepon, again, enhances colour development (Weaver & Montgomery, 1974; Fitzgerald & Patterson, 1994; El-Kereamy *et al.*, 2003). However, timing of the application is crucial. Blommaert *et al.* (1975) stated that the most appropriate time for the application of ethepon to Barlinka seems to be related to ethylene production in the berries themselves, which is approximately two to three weeks before harvest, whereas ripening might be delayed if 2-chlorethylphosphonic acid (ethep) is applied at the second half of phase I or the beginning of phase II of berry development (Hale *et al.*, 1970).

**Girdling**, conducted at the onset of véraison, advances ripening and improve grape colour (Carreño *et al.*, 1998). Thus, girdling would not form part of the seasonal cultivation practices where a lighter colour is required.

**The induction of water stress** between berry set and pea size enhances colour development. This is due to an increase the skin:pulp ratio (Ojeda *et al.*, 2002). However, since this practice results in smaller berries (Salón *et al.*, 2005), it is not practical for the table grape industry which aims towards production of large berries.

Obtaining the darkest possible colour is, however, not always the goal. Under certain conditions, Redglobe is dark red, while some markets require a pink colour.

The dark colour of the berry skin can be attributed to the amount of anthocyanins present, because the type of anthocyanin is genetically determined (Boss *et al.*, 1996c; Ribéreau-Gayon, 1982). Conditions that inhibit anthocyanin accumulation must therefore be created. Possible solutions seem to be the removal of the source of anthocyanin precursors and ABA (Davies *et al.*, 1986; Düring *et al.*, 1978; Jensen, 1986). Partial defoliation increases anthocyanin concentration (Hunter *et al.*, 1991). On the other hand, excessive leaf removal might result in a delay in ripening or even an inability to reach the required sugar levels (Koblet *et al.*, 1994). Since there is proof for the contribution of lateral shoots to anthocyanin accumulation, the removal thereof will result in reduced colour (Candolfi-Vasconcelos & Koblet, 1990; Avenant, 1994; Vasconcelos & Castagnoli, 2000). Lateral shoot removal must be done with great caution, since lateral shoots also contribute to sugar accumulation (Vasconcelos & Castagnoli, 2000) in the fruit during ripening, and to starch accumulation in the vine (Candolfi-Vasconcelos & Koblet, 1990).

Light limiting conditions in the bunch zone may result in a lower anthocyanin concentration. It must be kept in mind that the leaf area that is critical for adequate ripening depends on effective illumination (Jackson, 1986). Shaded leaves and berries can cause decreased berry anthocyanin production, because reactions dependent on light and temperature within the optimum ranges for anthocyanin biosynthesis are inhibited.

## 2.9 CONCLUSIONS

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Only a comprehensive strategy will result in the best colour quality. Site, cultivar, vine training, trellising system and vine spacing are interdependent and need to be considered to reach the particular goal.

The aim of cultivation strategies such as vine spacing, training, pruning and trellising is to create homogenous vines. A good vine structure, as well as the support of the trellis system, ensures that leaves are oriented for maximal sunlight interception for photosynthesis. Such vines are characterised by a balance between root volume and top growth, a balance between left and right cordon arms, as well as a balance between leaf area and crop load.

Vines are spaced and trained according to the vigour of both the rootstock and scion cultivar as well as soil potential. Incorrect vine spacing and training and the choice of an unsuitable trellis system (i.e. a trellis system that cannot accommodate the vigour of a given cultivar efficiently) might result in vegetative growth that either limits berry ripening and colour development through effects on microclimate, or via effects on the leaf area:fruit mass ratio.

Canopy management starts at pruning. The degree of pruning determines the extent of shoot crowding and hence shade. Apart from the effects on microclimate, pruning also affects the leaf area:fruit mass ratio. Pruning determines crop level. Therefore, sufficient leaf area ensures the partitioning of carbohydrates, as well as of

precursors of anthocyanin biosynthesis, to the grape berries. Suckering and positioning of shoots ensures that more light in the optimum activity range reaches the inner leaf layers and the bunches for photosynthesis and anthocyanin biosynthesis. Leaf removal forms part of a synergistic strategy and therefore mistakes made in terms of site selection, spacing and training cannot be corrected by leaf removal.

A knowledge and understanding of physiological interactions in the grapevine canopy are required to apply the practice of leaf removal correctly. Knowledge of the plant's reaction to manipulation is also necessary to reach quality objectives. When leaves are removed, the source of some hormones is removed and anthocyanin biosynthesis may be affected. Removal of the basal leaves is very beneficial for colour development, because of the enhanced light environment, but since there is proof that these leaves are capable of stable and ongoing photosynthetic activity, it is recommended that the leaves are not removed or only minimally removed. Leaf thinning ensures that sufficient light reaches these older leaves for photosynthesis and they are therefore still able to contribute to carbohydrate accumulation. Correct leaf thinning facilitates uniform sun exposure (diffused sunlight) of the leaves and bunches. Sufficient light in the bunch zone is necessary for the activation of phenylalanine ammonia-lyase, and thus anthocyanin biosynthesis. Therefore, the absence of light in the bunch zone might result in a lighter berry colour. Although light in the bunch zone is important, it must be kept in mind that leaf thinning causes levels of bunch exposure which may increase berry temperature. Taking into consideration, the effect of temperature on colour pigment synthesis, the conclusion can be drawn that the bunches must not be overly sun-exposed in cases where it is difficult to obtain the optimal colour. This conclusion is based on the premise that high temperatures inhibit the activity of enzymes involved in anthocyanin biosynthesis.

The removal of shoot tips results in the development of lateral shoots and thus younger leaves that contribute to carbohydrate accumulation, but only if done timeously. Leaf thinning and tipping or topping also increase the ratio of young:old leaves, if done timeously. Young leaves, such as those on lateral shoots contribute significantly to grape colour. Therefore, the removal thereof will reduce colour. Since leaves on the lateral shoots also contribute to carbohydrate accumulation in the grape berry, the removal must be done with caution. If done indiscriminately, it can result in delayed ripening.

Because of the physiological importance of light, the aim of canopy management should be to prevent shade and should be structured towards obtaining a desired colour in grape berries. The removal of foliage to create a better light environment must be done judiciously. Excessive removal of leaves, especially younger leaves, may counteract the positive effect of the better light environment and will result in grapes that do not ripen properly decreasing overall grape quality. Sufficient leaf

area is critical for providing adequate photoassimilates (sucrose and phenylalanine) to the bunches during ripening.

Water stress results in smaller berries with an increased skin:pulp ratio, which has implications for the production of a darker colour. Enhanced colour is not necessarily negative in cases where a darker colour is preferred, but smaller table grape berries are not accepted by consumers. Given the additional consequences of water stress on shoot physiology, and thus photosynthesis, due to hormonal action, this practice is not recommended in table grape production.

A decrease in colour development by application of gibberellic acid and cytokinin application is not recommended, due to the fact that it can be detrimental for overall grape quality. Ethepon enhances colour development. Since there is evidence that application at any stage might cause a reduction in acidity, this practice must be applied with great caution.

In South Africa, canopy management for wine grapes on vertical trellis systems has received a lot of attention during the past 15 years. There are, however, only a few guidelines regarding canopy management for table grapes in this context (horizontal trellis systems). Basal leaf removal is recommended at véraison, but the importance of the contribution of the basal leaf to the maintenance of metabolism is not accentuated enough. Literature regarding canopy management to obtain lighter colour only explores the effects of bud load on colour development. The effect of canopy management on the colour of table grapes has also not been investigated under South African conditions. The problems regarding colour development of Redglobe and Crimson Seedless are currently addressed by the ARC project WW1116. One of the aims of this project is to manipulate grape colour by means of canopy management. The potential to manipulate colour through skillfull canopy management, where the microclimate and the leaf area:fruit mass ratio is controlled, is therefore, a relevant research direction for the South African industry.

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# **RESEARCH RESULTS**

**THE EFFECT OF DEFOLIATION  
TREATMENTS ON LEAF AREA, LIGHT  
ENVIRONMENT AND COLOUR OF  
REDGLOBE  
(*VITIS VINIFERA* L.)**

# THE EFFECT OF DEFOLIATION TREATMENTS ON LEAF AREA, LIGHT ENVIRONMENT AND COLOUR OF REDGLOBE (*VITIS VINIFERA* L.)

## ABSTRACT

A defoliation trial was conducted on Redglobe vines in order to obtain the pink colour desired by Far Eastern markets. Six year old Redglobe vines with moderate vigour were used. The experiment involved two leaf removal (L) levels ( $L_0$  = 0% leaf removal;  $L_{33}$  = 33% leaf removal) in combination with three lateral shoot removal (LS) levels ( $LS_0$  = 0% lateral shoot removal;  $LS_{50}$  = 50% lateral shoot removal;  $LS_{100}$  = 100% lateral shoot removal). The defoliation treatment combinations were done at four different times (DT): 36 (pea berry size), 69 (véraison), 76 (one week after véraison) and 83 (two weeks after véraison) days after anthesis (DAA), resulting in 24 treatments, replicated in four blocks.

Treatment combinations involving  $L_{33}$  tended to lower the main shoot leaf area. Likewise, the lateral shoot leaf area was decreased by increasing levels of LS at any DT. When  $L_{33}$  was applied in combination with any LS level, the total vine leaf area was always lower compared to where  $L_0$  was part of the treatment combination. The main shoot leaf size was increased by  $L_{33}$  applied at  $DT_{76}$  and  $DT_{83}$  compared to  $L_{33}$  applied at  $DT_{36}$ . The ratios of main and lateral shoot leaf area to total leaf area were subjected to significant two-factor interactions. Treatment combinations involving LS (DT x LS and LS x L) increased the percentage main shoot leaf area. It was, however, lowered by the application of  $L_{33}$  at  $DT_{69}$  and  $DT_{83}$  compared to  $L_0$ .

Visual bunch evaluation showed that the mean bunch colour was in class three due to any DT. The LS x L interaction resulted in a mean bunch colour that was in classes two and three. However, within these classes, there was a tendency that bunch colour decreased with DT's later than  $DT_{36}$ . The LS x L interactions showed that bunch colour was darker when the treatment combinations involved  $L_0$ . The percentage of bunches with the acceptable colour was increased by  $DT_{69}$  compared to the other DT's, and also with  $LS_{50}$  and  $LS_{100}$  compared to  $LS_0$ . These findings, together with the positive relationship obtained between grape colour and the lateral shoot leaf area:fruit mass ratio, accentuates the role of active leaf area during the period shortly after  $DT_{69}$  (véraison). The effect of light intensity in the bunch zone also could have contributed to the final grape colour, since the light intensity in the bunch zone was increased by increased levels of LS applied at any DT. Furthermore, there was a negative relationship between light intensity in the bunch zone and grape colour. However, although the light intensity increased with increased levels of LS, the colour that was obtained was probably not associated with the differences in light intensity.

It is important to note that it is possible to manipulate the colour of Redglobe grapes with defoliation treatments. However, the treatments that decrease grape colour can also affect other quality parameters negatively.

### 3.1 INTRODUCTION

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Under certain South African conditions, Redglobe develops a colour that is too dark and thus unacceptable for the Far Eastern markets. These markets require a light pink colour instead of a dark red colour. Cultivation to obtain the ideal colour involves long-term (Douglas, 1951; Pirie, 1979; Ough & Nagaoka, 1984; Archer, 1990; Brossaud *et al.*, 1999; Hunter & Archer, 2001a) and short-term cultivation practices (Viljoen, 1951; Ciriaco *et al.*, 1985; Archer & Fouché, 1987; Hunter *et al.*, 1991; Hunter & Archer, 2001b).

Amongst other, short-term practices consist of leaf and lateral shoot removal. Removal of the basal leaves is a common practice during table grape canopy management and is usually done at the beginning of véraison (Zeeman, 1983; Wagener *et al.*, 1985). Leaf removal prevents abrading of bunches (Viljoen, 1951; Peacock, 1996), improves airflow (English *et al.*, 1989; Hunter & Visser, 1990a) and reduces relative humidity (Kliwer & Smart, 1989; Hunter & Visser, 1990a) to prevent diseases such as *Botrytis cinerea*/sour rot (Volschenk & Hunter, 2001). Labour practices, such as bunch preparation and harvest, are also simplified (Peacock, 1996). Furthermore, colour development is impacted through the different effects of leaf and lateral shoot removal on plant metabolism. They are discussed below.

Leaf removal increases the photosynthetic photon fluence rate (PPFR) (Kliwer & Smart, 1989; Hunter *et al.*, 1995; Dry, 2000) in the canopy. The increased PPFR affects photosynthesis and anthocyanin biosynthesis. A favourable light environment is beneficial for photosynthesis and anthocyanin biosynthesis in the vine because both nitrate reductase (Hunter & Ruffner, 1997) and phenylalanine ammonia-lyase (PAL) activity (Roubelakis-Angelakis & Kliwer, 1986) are light dependent. In dense canopies, PPFR in the interior of the grapevine can be as little as 1% of the ambient PPFR (Peacock *et al.*, 1987; Williams, 1987; Peacock *et al.*, 1994). Smart (1987) states that PPFR values in shade are  $<10 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . In this regard, it has been found that a reduction of light intensity adversely affects berry colouration (Douglas, 1951; Kliwer, 1970a; Kliwer & Lider, 1970; Avenant, 1994; Downey *et al.*, 2004; Kataoka *et al.*, 2004). Therefore canopy management, in the form of leaf removal, is important to increase light penetration and enhance the associated physiological processes.

According to Smart (1987), light has, apart from energy supply and tissue heating, a phytochrome effect (R:FR, 660:730 nm). Light in the red spectra (650 – 700 nm) is necessary to convert phytochrome from the inactive form,  $P_r$ , to  $P_{fr}$  the active form (Mitrakos & Shropshire, 1972). The latter activates the enzymes that affect fruit composition (Smart, 1987). According to Mitrakos & Shropshire (1972),  $P_{fr}$  not only controls nitrate reductase and invertase, but it also activates the genes that induce

anthocyanin synthesis. Phenylalanine ammonia-lyase (PAL) also is activated by  $P_{fr}$  with a consequent enhancement in anthocyanin biosynthesis (Mitrakos & Shropshire, 1972). The R:FR ratio is increased through leaf removal (Kliewer & Smart, 1989; Dry, 2000). According to Smith (1982), the estimated epidermal phytochrome photoequilibrium ( $P_{fr}:P_{total}$ ) in plant leaves is sensitive to R:FR ratios that are less than 1.15 (shade) and the equilibrium decreases under such conditions. Furthermore, it was stated that photoequilibrium is insensitive to R:FR ratios larger than 1.5. Last mentioned ratio, can therefore be regarded as optimal. The R:FR ratio at the canopy surface is 1:1, whereas it decreases to less than 0.1 in shaded canopies (Smart *et al.*, 1982; Smart, 1987). A low R:FR ratio (0.45 to 0.36) in a dense canopy (shade) decrease anthocyanin concentration (Smart, 1987), while red light supplementation increases the R:FR ratio and thus enhances colour development (Smart *et al.*, 1988). A high R:FR ratio (1.1 to 1.2) shifts the phytochrome photoequilibrium ( $P_{fr}:P_{total}$ ) to approximately 60% in the  $P_{fr}$  form (Smith, 1982). Archer & Strauss (1989) found reduced skin colour and sugar concentration in shaded Cabernet Sauvignon berries. These authors attributed it to the inhibition of phytochrome driven enzyme reactions as result of the fact that the shorter wavelengths are filtered out and phytochrome is thus converted to the inactive form ( $P_r$ ).

Increased bunch zone temperature: Leaf thinning increases the temperature in the bunch zone (Haselgrove *et al.*, 2000). Despite positive effects of increased fruit temperature on berry composition (Kliewer *et al.*, 1988), increased berry temperature, due to exposure to direct sunlight, may decrease anthocyanin concentration (Bergqvist *et al.*, 2001). Furthermore, Redglobe is very susceptible to sunburn (Van der Merwe, 2001), meaning that leaf removal strategies must ensure the maintenance of shading on the bunches.

Increased photosynthetic activity: Leaf thinning changes the source:sink ratio in the canopy (Carbonneau, 1996) in that it increases the photosynthetic activity of the remaining leaves, i.e. the source (Hunter *et al.*, 1991; Koblet *et al.*, 1996). The removal of leaves in the bunch zone improves anthocyanin biosynthesis as a result of better bunch (sink) exposure (Koblet, 1987; Iland, 1988; Smith *et al.*, 1988) and thus higher metabolic activity (Hunter *et al.*, 1995). For winegrapes, trained on a 1.5 m slanting trellis, Hunter *et al.* (1995) found that following leaf removal, the photosynthetic activity of the remainder of the leaves on the vine and the metabolic activity of bunches increased in combination with suckering and shoot positioning. The export of photoassimilates is thus increased through a lower source:sink ratio (Hunter & Visser, 1988). The stimulated photosynthetic activity of the remaining leaves also delays their senescence (Koblet *et al.*, 1996). Hunter *et al.* (1991) attributed the higher anthocyanin concentration in the grape skins of partially defoliated Cabernet Sauvignon vines to the photosynthetic stimulation of the remaining older leaves. It was therefore recommended that, apart from the leaf area, the age composition of the leaf area should also be taken into account because young (leaves on lateral shoots) and older leaves (middle and basal leaves) contribute differently to grape composition

(Hunter, 2000). Although improved light environment within the canopy improves metabolic activity of the leaves and bunches (Hunter *et al.*, 1995), severe leaf removal can counteract the positive effect of light. Severe restriction of effective leaf area is detrimental to fruit quality and delay ripening (Weaver, 1963; Kingston & Van Epenhuijsen, 1989).

The leaf area:fruit mass ratio necessary to produce grapes of improved size and composition (colour, total soluble solids) have been investigated several times. Records of ratios varying from 6.2 to 17.2 cm<sup>2</sup>.g<sup>-1</sup> are available (Winkler, 1930; May *et al.*, 1969; Kliewer, 1970b; Kliewer & Antcliff, 1970; Kliewer & Weaver, 1971; Winkler *et al.*, 1974; Smart, 1980; Jackson, 1986; Kingston & Van Epenhuijsen, 1989; Dokoozlian & Hirschfeldt, 1995; Hunter, 2000).

Finally, leaves on lateral shoots seem to play a major role in metabolic processes during fruit ripening. Candolfi-Vasconcelos & Koblet (1990) showed that wine grapes from canopies that were composed only of lateral shoots had higher colouration. Vasconcelos & Castagnoli (2000) confirmed that where more lateral shoot leaves are present, improved skin anthocyanin content per berry and per mass of fruit is obtained. The leaves on lateral shoots, being the younger leaves in the canopy, might have played a major role in the metabolic processes occurring during fruit ripening. The leaves on lateral shoots are the younger leaves in the canopy and play an important role in the metabolic processes occurring during fruit ripening because their gas exchange rates are comparable to the main shoot leaves at the top of the canopy (Candolfi-Vasconcelos *et al.*, 1994).

It is therefore clear that the environmental conditions within the canopy, and the physiological and biochemical functioning of the vine at different phenological stages, can be affected by cultural practices, namely canopy manipulation techniques. From this point of view, the contribution of main shoot and lateral shoot leaves to berry colour has been investigated in the current study, in particular their contribution to berry colour.

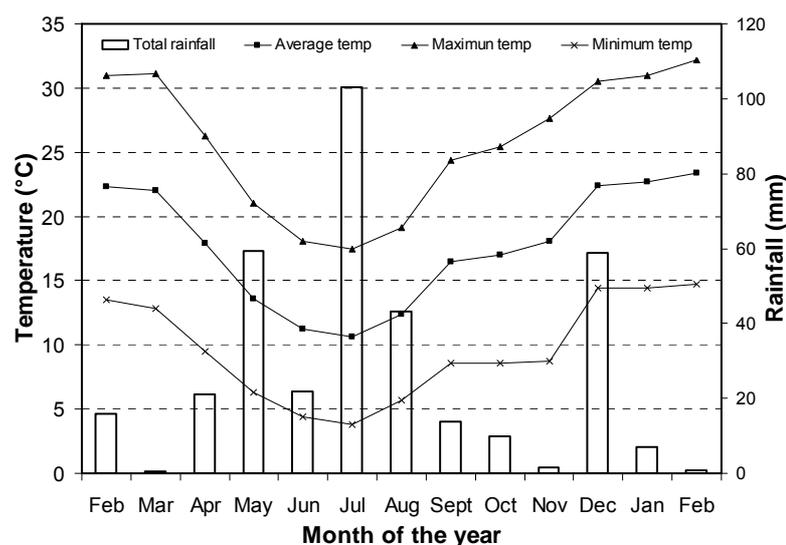
## **3.2 MATERIALS AND METHODS**

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### **3.2.1 EXPERIMENTAL VINEYARD**

The experiment was conducted on a virus-free six-year-old *Vitis vinifera* L. cv Redglobe grafted onto Ramsey (*Vitis Champinii*) vineyard with moderate vigour. It is situated on the farm Grandview (33° 30' 23" S; 19° 35' 43" E) in the Hex River Valley, De Doorns. The vines were spaced 2.74 m x 1.83 m on a sandy-loam soil and trained onto a gable trellis system as described by Zeeman (1981), with the rows orientated in an east-west direction. The vines were split into two double split cordons at the same height above ground level. Each vine was pruned to 20 spurs per vine, spaced evenly (15 cm apart) on four cordon arms.

Figure 3.1 shows the monthly temperature and rainfall for the De Doorns experimental farm mechanical weather station for the 2002/2003 season, which is the period during which the trial was conducted. The mean February temperature (month during which the berries ripen) from 1963 to 2002 was 21.8 °C and the Winkler index for the area is 1906. This area is thus classified into region III, which means that the climate of the Hex River valley is moderately warm and suitable for the production of red and black grapes (Winkler *et al.*, 1974). The small differences between day and night temperatures further contribute to sufficient grape colour (Kliewer & Torres, 1972).



**Figure 3.1** Monthly temperature and rainfall for the De Doorns Experimental Farm (2002/2003) Hex River Valley, South Africa (Source: ARC-ISCW).

Suckering, crop control and bunch preparation took place as part of standard seasonal canopy management practices. Suckering involved the removal of infertile shoots and water shoots. Crop control involved the reduction of the potential yield to approximately 25 bunches per vine after berry set. During bunch preparation, the bunches were shortened to an approximate length of 12 cm. Berries that were smaller than the average berry size, as well as poorly coloured berries, were removed just before harvest. Gibberellic acid was applied at 10 ppm at 12 mm berry size for berry enlargement. No ethrel was applied to enhance colour development. The vineyard was irrigated by means of scheduled micro-irrigation involving water application at 30 mm per week during the active shoot-growth period and 18 mm per week from véraison to harvest. For the rest of the year, the water requirements were supplemented by rainfall. To prevent heat damage during the ripening phase, an irrigation of 3 mm was applied every time the temperature was in excess of 30°C. Fertilisation was applied at three different growth stages: budbreak (N, P and K), berry set (K), 16 mm berry size (N and K) and after harvest (N, P and K). Fertilisation applications were done on the basis of information obtained from soil and leaf analyses to maintain optimal vegetative growth.

### 3.2.2 EXPERIMENTAL DESIGN AND TREATMENTS

A randomised complete block design was used, with 24 treatment combinations replicated in four blocks with a single vine as experimental unit. The treatment design was a 2 x 3 x 4 factorial. The factors were two leaf removal (L) levels ( $L_0$  = 0% leaf removal and  $L_{33}$  = 33% leaf removal, only on the main shoots), three lateral shoot removal (LS) levels ( $LS_0$  = 0% lateral shoot removal,  $LS_{50}$  = 50% lateral shoot removal and  $LS_{100}$  = 100% lateral shoot removal) and four defoliation times (DT): 36 (pea berry size), 69 (véraison), 76 (one week after véraison) and 83 (two weeks after véraison) days after anthesis (DAA). Treatment combinations were applied evenly, only on the main shoots and from side to side in the canopy. Only the results of the 2002/2003 season are presented due to the occurrence of Bacterial blight (*Xylophilus ampelinus*) at Clovelly in the 2001/2002 season.

### 3.2.3 CANOPY MEASUREMENTS AND SAMPLING

The photosynthetic photon fluence rate (PPFR) reaching the bunch zone of the canopy was measured immediately after each defoliation, and then weekly until harvest. Readings were obtained parallel to the cordons on both sides of the row and in the bunch zone of the canopy. This was done during mid-morning, when the sky was clear. A LI-COR Model LI-250 Line Quantum Sensor was used and the average light intensity values ( $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) for the north and south facing sides of the row were calculated.

Two shoots per vine were sampled one month after harvest in order to determine the number of leaves, main and lateral shoot leaf area, number of lateral shoots, primary and lateral shoot lengths, as well as internode length on both primary and lateral shoots. During the month after harvest, re-growth and/or senescence, and thus leaf fall, probably occurred. Measuring the leaf area at harvest would be more representative of the leaf area that contributed to grape composition. A LI-COR Model LI-3100 leaf area meter was used to determine the leaf area of the two sample shoots. The mean leaf area of these shoots, together with the number of shoots per vine, was used to calculate total grapevine leaf area. This leaf area included the leaves on lateral shoots.

At harvest (16 °B), 50 berries per vine were sampled randomly. The berry samples were cold stored at  $-0.5^\circ\text{C}$  for two days prior to sensory colour evaluation (See section 3.2.4) and biochemical analyses.

### 3.2.4 BERRY MEASUREMENTS, EVALUATION AND ANALYSES

The berry samples (50 berries per sample) were used fresh for berry mass, colour and biochemical juice analyses. At harvest, bunches were also subjected to sensory (visual) colour evaluation according to the D.35 colour chart of the Deciduous Fruit

Producers' Trust (DFPT) and were classified into nine different colour classes. The ideal bunch colour is between classes four and five.

The colour of the sampled berries was also assayed by means of a biochemical method to obtain an objective colour value. The modified method of Pirie & Mullins (1976), as described by Hunter *et al.* (1991), was used to determine grape skin anthocyanins. The absorbance of total anthocyanins was determined at 520 nm and expressed as mg of a mixture of acylated and non-acylated anthocyanins ( $E_{1\%}^{10\text{mm}} = 500$ ) on the basis of Somers & Evans (1977). Anthocyanins were expressed as concentration (mg/g skin dry mass) and as total amount per berry skin (mg/berry). At harvest (19 and 20 February 2003), the bunches on each vine were counted and visually evaluated for colour. Each vine's bunches were counted and the total yield per vine was weighed (kg/vine).

### 3.2.5 STATISTICAL ANALYSES

Data obtained were subjected to statistical analysis by means of the SAS program, version 8.2 (SAS Institute Inc., 1999). The colour classes evaluated were observed at different frequencies. The frequencies of observations made within the nine classes on the D.35 chart were subjected to a general linear model (GLM) technique with a logistic link function. The maximum likelihood estimators (X-beta's) were calculated on an underlying scale (McCullagh & Nelder, 1989). These estimators, which are on an interval scale, were subjected to standard two-way analysis of variance. The cut-off points for the respective classes were given as intercepts.

The acceptable coloured bunches at harvest were calculated by expressing the sum of bunches in classes four and five as a percentage of the total number of bunches evaluated. The data was then subjected to a logit transformation before being subjected to analysis of variance. An analysis of variance was performed using SAS version 8.2 (SAS Institute Inc., 1999). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). Student's t-Least Significant Differences (LSD) were calculated at a 5% significance level to compare the treatment means.

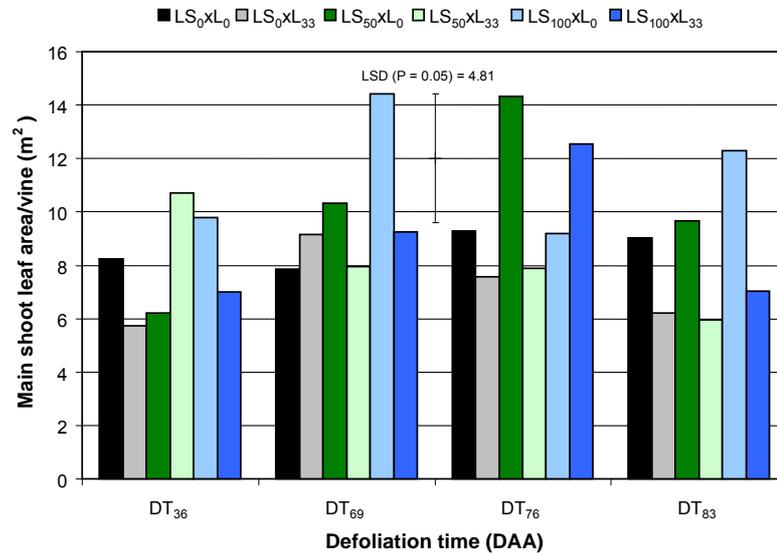
## 3.3 RESULTS AND DISCUSSION

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### 3.3.1 LEAF AREA AND LEAF AREA:FRUIT MASS RATIO

Significant three-factor interaction was evident for the main shoot leaf area, and for the number of main shoot leaves per vine (Table 3.1). It was expected that the main shoot leaf area per vine would be lower for all the treatment combinations that include  $L_{33}$  compared to those including  $L_0$ . This, however, was not the case for  $DT_{36} \times LS_{50} \times L_0$  vs  $DT_{36} \times LS_{50} \times L_{33}$ ,  $DT_{69} \times LS_0 \times L_0$  vs  $DT_{69} \times LS_0 \times L_{33}$  and  $DT_{76} \times LS_{100} \times L_0$  vs  $DT_{76} \times LS_{100} \times L_{33}$  (Fig. 3.2). The first two might be explained in the light of these treatment combinations showing the same pattern regarding the number of main shoot leaves (Fig. 3.3), which might point to an irregular main shoot length. Another

explanation could be the improved light environment due to the treatment combinations involving  $L_{33}$  which probably delayed senescence and abscission of retained leaves, resulting in a larger main shoot leaf area compared to treatment combinations involving  $L_0$ . Delayed senescence of the retained leaves was also found by Hunter *et al.* (1991) and Koblet *et al.* (1996). As expected, for  $DT_{83}$ , all the treatment combinations containing  $L_{33}$  showed a lower main shoot leaf area and leaf number than treatment combinations including  $L_0$ .



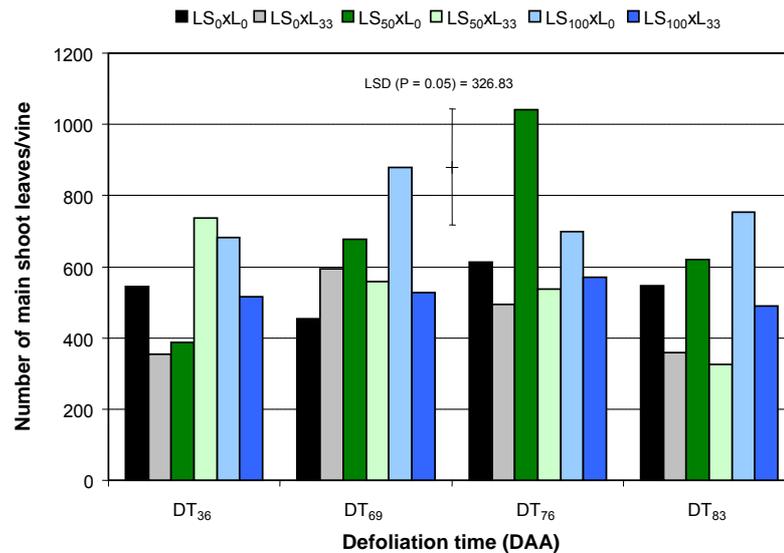
**Figure 3.2** The effect of DT x LS x L on the main shoot leaf area per vine as measured after harvest.

DT = defoliation time;  $DT_{36}$  = 36 DAA;  $DT_{69}$  = 69 DAA;  $DT_{76}$  = 76 DAA;  $DT_{83}$  = 83 DAA; DAA = Days after anthesis;  $LS_0 \times L_0$  = 0% lateral shoot removal and 0% leaf removal;  $LS_0 \times L_{33}$  = 0% lateral shoot removal and 33% leaf removal;  $LS_{50} \times L_0$  = 50% lateral shoot removal and 0% leaf removal;  $LS_{50} \times L_{33}$  = 50% lateral shoot removal and 33% leaf removal;  $LS_{100} \times L_0$  = 100% lateral shoot removal and 0% leaf removal;  $LS_{100} \times L_{33}$  = 100% lateral shoot removal and 33% leaf removal

The DT x L interaction was significant for the main shoot leaf size (Table 3.1). Regardless of DT,  $L_{33}$  did not significantly affect the size of the main shoot leaves compared to the effect of  $L_0$  (Fig. 3.4). However, application of  $L_{33}$  at  $DT_{76}$  and  $DT_{83}$  significantly enlarged the main shoot leaves compared to  $L_{33}$  applied at  $DT_{36}$ . The reason why the leaf size obtained by  $L_{33}$  applied at  $DT_{36}$  was smaller, might be the photosynthetic stimulation of the retained leaves before véraison which ensured assimilate supply to the bunches. Thus, the vines subjected to this treatment did not need to compensate by means of leaf expansion, other than when  $L_{33}$  was applied shortly after véraison ( $DT_{76}$  and  $DT_{83}$ ), during which the bunch demand for photosynthates was strong. Petrie *et al.* (2000) reported similar results. The size of the main shoot leaves was not affected by LS (Table 3.4). This differs from results obtained by Candolfi-Vasconcelos & Koblet (1990) and Koblet (1987).

With regard to the lateral shoot leaf area, a DT x LS interaction, that was significant, was observed. The same was true for the number of lateral shoot leaves and the size of the lateral shoot leaves (Table 3.1). The means for these three variables are presented in Table 3.2. Except for  $DT_{69}$ ,  $LS_{100}$  lowered the lateral shoot

leaf area per vine significantly compared to  $LS_0$ . This decrease can be ascribed to the number of lateral shoot leaves per vine which followed the same pattern as the lateral shoot leaf area per vine. Although not always significant, the same treatment combinations that resulted in a decrease of the lateral shoot leaf area per vine or the number of lateral shoot leaves per vine also reduced the size of lateral shoot leaves. Therefore, the size of the lateral shoot leaves affected the lateral shoot leaf area per vine. Generally, as expected, with increasing LS, at any DT, the lateral shoot leaf area per vine, the number of lateral shoot leaves, as well as the size of lateral shoot leaves was decreased.



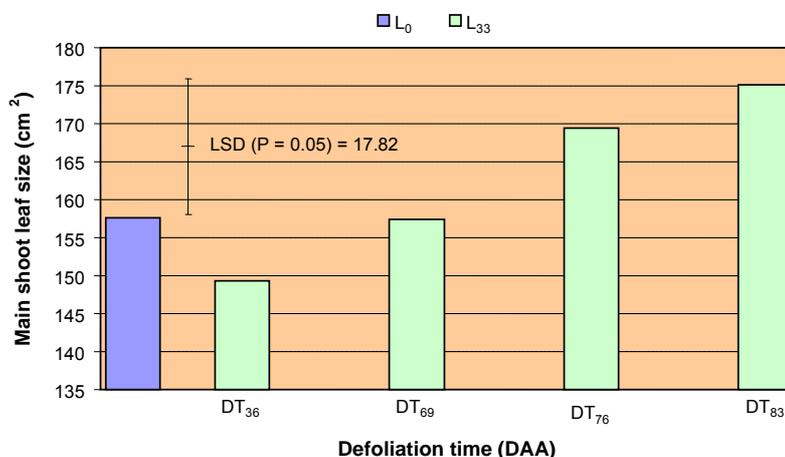
**Figure 3.3** The effect of DT x LS x L on the number of main shoot leaves per vine as measured after harvest.

DT = defoliation time; DT<sub>36</sub> = 36 DAA; DT<sub>69</sub> = 69 DAA; DT<sub>76</sub> = 76 DAA; DT<sub>83</sub> = 83 DAA; DAA = Days after anthesis;  $LS_0 \times L_0$  = 0% lateral shoot removal and 0% leaf removal;  $LS_0 \times L_{33}$  = 0% lateral shoot removal and 33% leaf removal;  $LS_{50} \times L_0$  = 50% lateral shoot removal and 0% leaf removal;  $LS_{50} \times L_{33}$  = 50% lateral shoot removal and 33% leaf removal;  $LS_{100} \times L_0$  = 100% lateral shoot removal and 0% leaf removal;  $LS_{100} \times L_{33}$  = 100% lateral shoot removal and 33% leaf removal

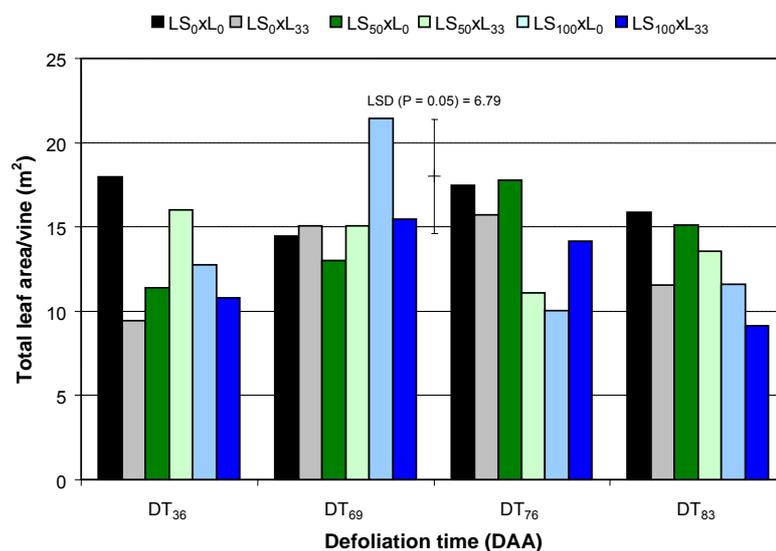
Although, there was significant three-factor interaction for the total leaf area per vine (Table 3.3), no meaningful pattern was observed between the treatments. Neither LS, nor L and also not the LS x L interaction seemed to significantly affect the total leaf area per vine according to a particular pattern. For DT<sub>83</sub>, a clear tendency existed where treatment combinations that included  $L_{33}$  reduced the total leaf area (Fig. 3.5). This is ascribed to the fact that the leaves were removed late and as a result had a limited period of compensated growth. There were no significant treatment effects for the total number of leaves per vine (Table 3.3). However, a tendency that the total number of leaves per vine decreased when increased levels of LS and L were applied (Table 3.4) was observed. Table 3.4 also shows that the latest DT (DT<sub>83</sub>) tended to decrease the total number of leaves the most compared to other DT's.

There were significant two-factor interactions (DT x LS, DT x L and LS x L) for the main and lateral shoot leaf areas as percentage of the total leaf area per vine

(Table 3.3). As expected, treatment combinations involving LS increased the main shoot leaf area compared to the lateral shoot leaf area, as percentage of the total vine leaf area (Fig. 3.6). The application of  $LS_{50}$  at  $DT_{76}$ , as well as the application of  $LS_{100}$  at  $DT_{36}$ ,  $DT_{76}$  and  $DT_{83}$  significantly increased the percentage main shoot leaf area compared to the effect of  $LS_0$ .



**Figure 3.4** The effect of DT x L on the main shoot leaf size as measured after harvest. DT = defoliation time; DT<sub>36</sub> = 36 DAA; DT<sub>69</sub> = 69 DAA; DT<sub>76</sub> = 76 DAA; DT<sub>83</sub> = 83 DAA; DAA = Days after anthesis; L<sub>0</sub> = 0% leaf removal; L<sub>33</sub> = 33% leaf removal



**Figure 3.5** The effect of DT x LS x L on the total leaf area per vine as measured after harvest.

DT = defoliation time; DT<sub>36</sub> = 36 DAA; DT<sub>69</sub> = 69 DAA; DT<sub>76</sub> = 76 DAA; DT<sub>83</sub> = 83 DAA; DAA = Days after anthesis; LS<sub>0</sub> x L<sub>0</sub> = 0% lateral shoot removal and 0% leaf removal; LS<sub>0</sub> x L<sub>33</sub> = 0% lateral shoot removal and 33% leaf removal; LS<sub>50</sub> x L<sub>0</sub> = 50% lateral shoot removal and 0% leaf removal; LS<sub>50</sub> x L<sub>33</sub> = 50% lateral shoot removal and 33% leaf removal; LS<sub>100</sub> x L<sub>0</sub> = 100% lateral shoot removal and 0% leaf removal; LS<sub>100</sub> x L<sub>33</sub> = 100% lateral shoot removal and 33% leaf removal

**Table 3.1** Analysis of variance to test treatment and interaction effects of canopy management practices for different variables of Redglobe grapevines in the Hex River Valley (Grandview), South Africa, 2002/2003.

Source	Main shoot leaf area/vine (m <sup>2</sup> )			Number of main shoot leaves/vine			Main shoot leaf size (cm <sup>2</sup> )			Lateral shoot leaf area/vine (m <sup>2</sup> )			Number of lateral shoot leaves/vine			Lateral shoot leaf size (cm <sup>2</sup> )		
	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Block	3	49.92	<0.01	3	385305.8	<0.01	3	1692.56	0.02	3	0.6553	0.96	3	17918.2	0.95	3	374.89	0.02
Defoliation time (DT)	3	24.51	0.10	3	111629.6	0.09	3	822.46	0.17	3	12.7562	0.14	3	206589.4	0.24	3	567.31	<0.01
Lateral shoots = LS	2	38.62	0.04	2	186527.7	0.03	2	464.57	0.38	2	106.7464	<0.01	2	1322128.9	<0.01	2	1463.20	<0.01
Leaves = L	1	87.83	0.01	1	455743.4	<0.01	1	653.54	0.25	1	0.5019	0.79	1	118304.6	0.37	1	23.02	0.64
DT x LS	6	5.49	0.82	6	30302.8	0.73	6	488.62	0.42	6	22.7586	0.01	6	401713.3	0.02	6	231.48	0.05
DT x L	3	15.73	0.25	3	95071.1	0.14	3	1789.04	0.02	3	5.9787	0.45	3	56134.2	0.76	3	178.68	0.16
LS x L	2	3.27	0.75	2	51585.2	0.36	2	255.00	0.59	2	19.4259	0.06	2	244790.2	0.19	2	119.99	0.31
DT x LS x L	6	33.67	0.01	6	119822.3	0.04	6	935.99	0.08	6	7.4206	0.37	6	189015.5	0.27	6	60.53	0.73
<b>Error</b>	<b>67</b>	<b>11.30</b>		<b>65</b>	<b>50082.7</b>		<b>69</b>	<b>478.51</b>		<b>67</b>	<b>6.7058</b>		<b>68</b>	<b>144707.4</b>		<b>65</b>	<b>101.27</b>	
Corrected Total	93	1433.82		91	6864115.7		95	56569.44		93	941.3800		94	17478542.4		91	14886.56	

Non-Normality (P<W)

0.59

0.45

0.71

0.67

0.28

0.66

DF = Degrees of freedom. MS = Mean Square.

P = Probability of F-ratio test

**Table 3.2** The effect of defoliation on certain canopy characteristics of Redglobe vines, Hex River Valley, South Africa, 2002/2003.

	Lateral shoot leaf area/vine (m <sup>2</sup> )	Number of lateral shoot leaves/vine	Lateral shoot leaf size (cm <sup>2</sup> )
<b>LS<sub>0</sub></b>	6.94 a	1083.26 a	59.32 a
<b>DT<sub>36</sub> x LS<sub>50</sub></b>	5.25 ab	1008.94 ab	51.83 abc
<b>DT<sub>69</sub> x LS<sub>50</sub></b>	4.90 ab	865.63 abc	54.84 ab
<b>DT<sub>76</sub> x LS<sub>50</sub></b>	3.51 bc	715.13 abcd	47.46 bc
<b>DT<sub>83</sub> x LS<sub>50</sub></b>	5.51 ab	1063.63 a	57.57 ab
<b>DT<sub>36</sub> x LS<sub>100</sub></b>	3.36 bc	647.88 bcd	51.60 abc
<b>DT<sub>69</sub> x LS<sub>100</sub></b>	6.59 a	1085.31 a	54.35 abc
<b>DT<sub>76</sub> x LS<sub>100</sub></b>	1.22 c	405.06 d	30.83 d
<b>DT<sub>83</sub> x LS<sub>100</sub></b>	1.85 c	528.88 cd	44.48 c
<b>LSD (P = 0.05)</b>	<b>2.61</b>	<b>381.80</b>	<b>10.30</b>

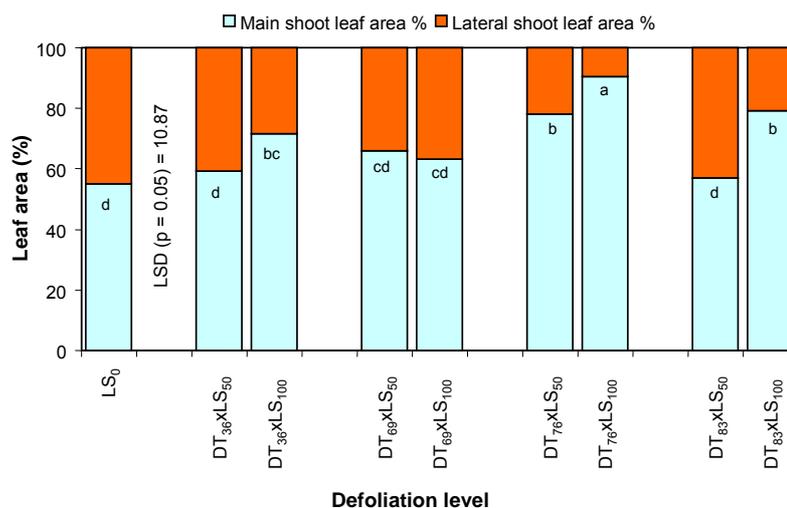
Values with the same letter do not differ significantly from each other at the 5% significance level

DT = defoliation time (DT<sub>36</sub> = 36 DAA; DT<sub>69</sub> = 69 DAA; DT<sub>76</sub> = 76 DAA and DT<sub>83</sub> = 83 DAA)

DAA = Days after anthesis

LS = lateral shoot removal (LS<sub>0</sub> = 0% lateral shoot removal; LS<sub>50</sub> = 50% lateral shoot removal; LS<sub>100</sub> = 100% lateral shoot removal)

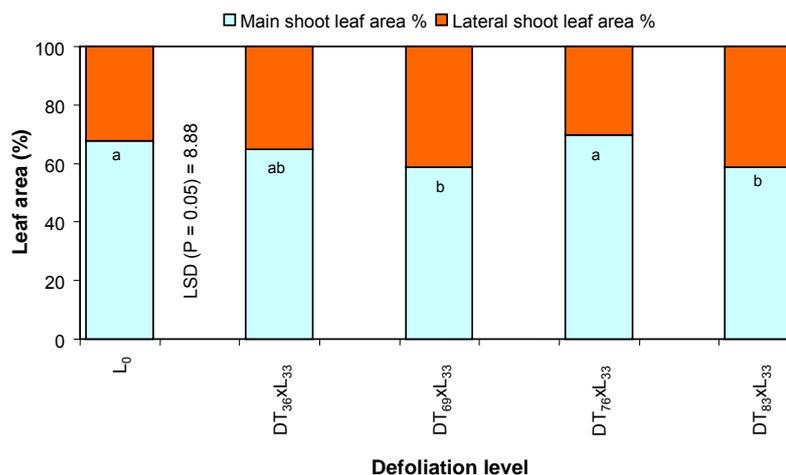
LSD= Least significant difference



**Figure 3.6** The effect of DT x LS on the main and lateral shoot leaf area as percentage of the total leaf area per vine as measured after harvest.

DT = defoliation time; DT<sub>36</sub> = 36 DAA; DT<sub>69</sub> = 69 DAA; DT<sub>76</sub> = 76 DAA; DT<sub>83</sub> = 83 DAA; DAA = Days after anthesis; LS<sub>0</sub> = 0% lateral shoot removal; LS<sub>50</sub> = 50% lateral shoot removal; LS<sub>100</sub> = 100% lateral shoot removal

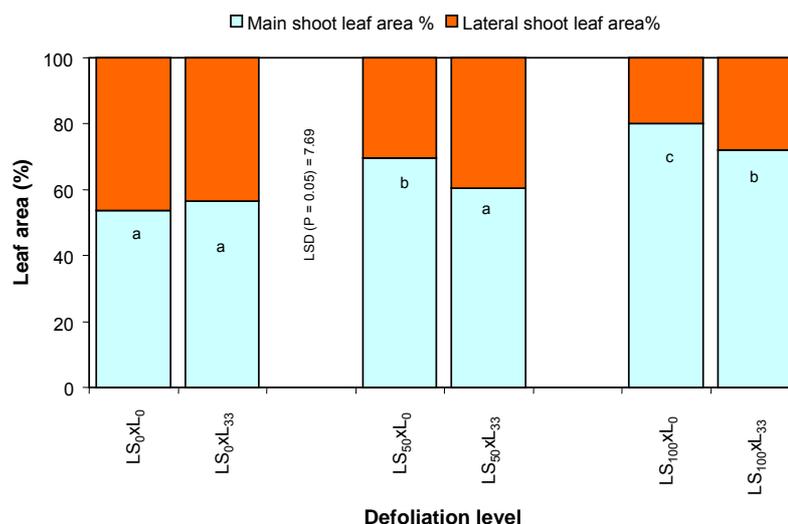
The main shoot leaf area as percentage of the total leaf area per vine was lowered significantly by DT<sub>83</sub> x L<sub>33</sub> and DT<sub>69</sub> x L<sub>33</sub> compared to L<sub>0</sub> (Fig. 3.7). Compensation in leaf growth, where less leaves were left, explains why the L<sub>33</sub> treatment at the earlier DT (DT<sub>36</sub>) had no effect on the main shoot leaf area in relation to the total vine leaf area.



**Figure 3.7** The effect of DT x L on the main and lateral shoot leaf area as percentage of the total leaf area per vine as measured after harvest.

DT = defoliation time; DT<sub>36</sub> = 36 DAA; DT<sub>69</sub> = 69 DAA; DT<sub>76</sub> = 76 DAA; DT<sub>83</sub> = 83 DAA; DAA = Days after anthesis; L<sub>0</sub> = 0% leaf removal; L<sub>33</sub> = 33% leaf removal

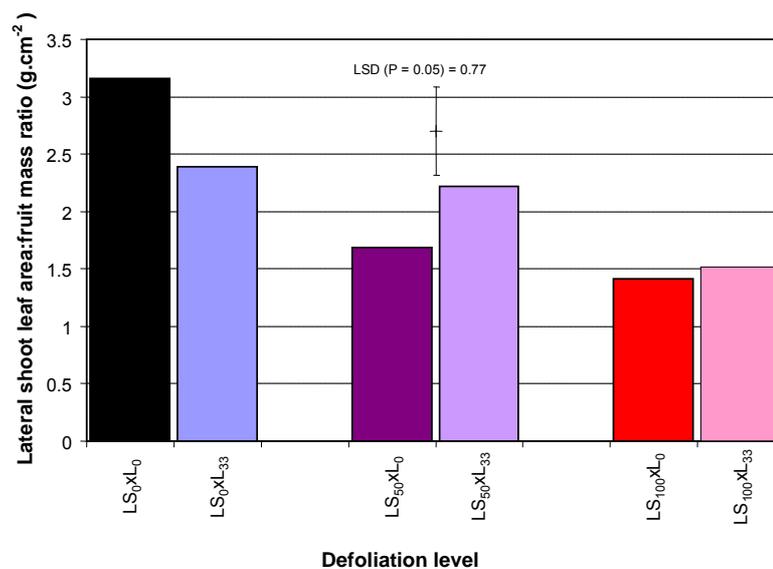
In Fig. 3.8 it can be observed that the main shoot leaf area, as percentage of the total vine leaf area, increased with increased levels of LS, regardless of the level of L. It is obvious that LS would impact the distribution of the leaves in terms of their positioning. Regardless of the level of L, this increase was significant between lateral shoot removal levels of LS<sub>0</sub> and LS<sub>100</sub>. Compared to LS<sub>0</sub>, LS<sub>50</sub> in combination with L<sub>0</sub>, significantly increased the main shoot leaf area in relation to the lateral shoot leaf area, but not in combination with L<sub>33</sub>. This implies that increased levels of LS will cause a larger percentage of aged leaves compared to young ones. It can be assumed that the contribution of lateral shoot leaves to grape composition might increase in cases where the main shoot leaf area was lowered and vice versa.



**Figure 3.8** The effect of LS x L on the main and lateral shoot leaf area as percentage of the total leaf area per vine as measured after harvest.

LS<sub>0</sub> x L<sub>0</sub> = 0% lateral shoot removal and 0% leaf removal; LS<sub>0</sub> x L<sub>33</sub> = 0% lateral shoot removal and 33% leaf removal; LS<sub>50</sub> x L<sub>0</sub> = 50% lateral shoot removal and 0% leaf removal; LS<sub>50</sub> x L<sub>33</sub> = 50% lateral shoot removal and 33% leaf removal; LS<sub>100</sub> x L<sub>0</sub> = 100% lateral shoot removal and 0% leaf removal; LS<sub>100</sub> x L<sub>33</sub> = 100% lateral shoot removal and 33% leaf removal

There were no significant differences for the total leaf area:fruit mass ratio (Tables 3.3 & 3.4). However, it seems as if this ratio decreased with later DT's. The yield was mainly affected by the number of bunches allocated to the vines. Therefore, if there is any relevance in the decrease of this ratio due to later DT's, it is solely connected to the abovementioned leaf area patterns. Increased levels of LS and L also tended to lower the abovementioned ratio, for the same reason. Likewise, L and LS affected the *main* shoot leaf area:fruit mass ratio significantly (Tables 3.3 & 3.4) for reasons connected to the effect of these treatments on main shoot leaf area (as discussed above). For the *lateral* shoot leaf area:fruit mass ratio there were significant DT x LS and LS x L interaction (Table 3.3). The effect of the DT x LS interaction can be seen in Table 3.5. The lateral shoot leaf area:fruit mass ratio was decreased significantly by DT<sub>69</sub> x LS<sub>100</sub>, DT<sub>76</sub> x LS<sub>100</sub> and DT<sub>83</sub> x LS<sub>100</sub> in comparison to LS<sub>0</sub>. DT<sub>76</sub> x LS<sub>100</sub> and DT<sub>83</sub> x LS<sub>100</sub> lowered the lateral shoot leaf area:fruit mass ratio significantly compared to DT<sub>36</sub> x LS<sub>100</sub> and DT<sub>69</sub> x LS<sub>100</sub>. This is ascribed to less time for lateral shoot leaf expansion between the last two DT's and the date of leaf area measurement. Likewise, where high levels of LS, particularly, LS<sub>100</sub>, was applied, the expected response in terms of a *lateral* shoot leaf area:fruit mass ratio decrease, was observed. Furthermore, the tendency for the *lateral* shoot leaf area:fruit mass ratio to decrease with an increased levels of LS, regardless of DT (Table 3.5) was expected. Compared to LS<sub>0</sub> x L<sub>0</sub>, the treatment combinations of LS<sub>50</sub> x L<sub>0</sub>, LS<sub>50</sub> x L<sub>33</sub>, LS<sub>100</sub> x L<sub>0</sub> and LS<sub>100</sub> x L<sub>33</sub> significantly lowered the lateral shoot leaf area:fruit mass ratio (Fig. 3.9). This is in accordance with the findings of Hunter & Visser (1990b) who found that later (i.e. between véraison and ripeness) and more severe defoliation, most significantly reduced the leaf area per g of fresh mass.



**Figure 3.9** The effect of LS x L on the lateral shoot leaf area:fruit mass ratio. LS<sub>0</sub> x L<sub>0</sub> = 0% lateral shoot removal and 0% leaf removal; LS<sub>0</sub> x L<sub>33</sub> = 0% lateral shoot removal and 33% leaf removal; LS<sub>50</sub> x L<sub>0</sub> = 50% lateral shoot removal and 0% leaf removal; LS<sub>50</sub> x L<sub>33</sub> = 50% lateral shoot removal and 33% leaf removal; LS<sub>100</sub> x L<sub>0</sub> = 100% lateral shoot removal and 0% leaf removal; LS<sub>100</sub> x L<sub>33</sub> = 100% lateral shoot removal and 33% leaf removal

**Table 3.3** Analysis of variance to test treatment and interaction effects of canopy management practices for different variables of Redglobe grapevines in the Hex River Valley (Grandview), South Africa, 2002/2003.

Source	Total leaf area/vine (m <sup>2</sup> )			Total number of leaves/vine			Ratio's of main and lateral shoot leaf area as % of the total leaf area			Total leaf area:fruit mass ratio (g.cm <sup>-2</sup> )			Main shoot leaf area:fruit mass ratio (g.cm <sup>-2</sup> )			Lateral shoot leaf area:fruit mass ratio (g.cm <sup>-2</sup> )		
	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Block	3	45.64	0.11	3	145105.0	0.65	3	382.6	0.03	3	2.76	0.68	3	6.52	0.03	3	0.62	0.65
Defoliation time (DT)	3	39.31	0.16	3	327190.8	0.30	3	630.5	<0.01	3	5.89	0.36	3	0.73	0.79	3	2.18	0.13
Lateral shoots = LS	2	23.42	0.35	2	716546.4	0.07	2	3386.8	<0.01	2	6.03	0.33	2	4.21	0.14	2	13.31	<0.01
Leaves = L	1	66.98	0.09	1	8807.3	0.86	1	539.0	0.04	1	14.77	0.10	1	12.30	0.02	1	0.10	0.77
DT x LS	6	37.80	0.13	6	404868.1	0.18	6	633.5	<0.01	6	3.89	0.63	6	0.36	0.98	6	3.89	<0.01
DT x L	3	3.34	0.93	3	69774.7	0.85	3	336.3	0.04	3	0.88	0.92	3	2.18	0.37	3	0.28	0.86
LS x L	2	16.55	0.48	2	208562.3	0.46	2	370.6	0.05	2	4.90	0.41	2	0.01	1.00	2	3.64	0.05
DT x LS x L	6	52.23	0.04	6	487542.4	0.10	6	197.5	0.14	6	2.55	0.83	6	3.59	0.12	6	0.43	0.89
<b>Error</b>	<b>66</b>	<b>22.22</b>		<b>66</b>	<b>263880.4</b>		<b>68</b>	<b>117.4</b>		<b>66</b>	<b>5.39</b>		<b>66</b>	<b>2.05</b>		<b>64</b>	<b>1.13</b>	
Corrected Total	92	2418.75		92	26255805.2		94	25069.9		92	459.30		92	208.17		90	141.39	

Non-Normality (P<W)

0.56

0.05

0.12

0.08

0.23

0.83

DF = Degrees of freedom.

MS = Mean Square.

P = Probability of F-ratio test

**Table 3.4** The effect of defoliation on certain canopy characteristics of Redglobe vines, Hex River Valley, South Africa, 2002/2003.

	Main shoot leaf size (cm <sup>2</sup> )	Lateral shoot leaf area/vine (m <sup>2</sup> )	number of lateral shoot leaves/ vine	Lateral shoot leaf size (cm <sup>2</sup> )	Total number of leaves/ vine	Total leaf area:fruit mass ratio (g.cm <sup>-2</sup> )	Main shoot leaf area:fruit mass ratio (g.cm <sup>-2</sup> )	Lateral shoot leaf area:fruit mass ratio (g.cm <sup>-2</sup> )	Average light intensity (μE.m <sup>-2</sup> .s <sup>-1</sup> )	Bunches with acceptable colour for Far East (%)	Anthoc. conc. in berry skins (mg/g)	Anthoc. content/ berry skin (mg)
<b>DT<sub>36</sub></b>	154.81	5.28	888.90	53.92	1416.72 a	6.50 a	3.92 a	2.24	124.2	6.50 b	5.37	1.28 a
<b>DT<sub>69</sub></b>	161.09	5.91	1000.96	56.20	1616.15 a	6.16 a	3.79 a	2.36	166.87	12.67 a	4.75	0.95 c
<b>DT<sub>76</sub></b>	156.86	4.30	774.39	45.33	1475.33 a	5.87 a	4.01 a	1.68	213.77	9.29 b	4.71	1.03 bc
<b>DT<sub>83</sub></b>	168.06	4.56	866.50	54.64	1336.02 a	5.29 a	3.57 a	1.96	249.93	9.08 b	5.30	1.10 b
<b>LSD (P = 0.05)</b>	-	-	-	-	<b>300.86</b>	<b>1.36</b>	<b>0.84</b>	-	-	<b>3.22</b>	-	0.144
<b>LS<sub>0</sub></b>	163.55 a	6.93	1077.42	59.29	1550.68 a	6.37 a	3.47 b	2.76	107.72	6.84 b	5.60	1.24 a
<b>LS<sub>50</sub></b>	156.06 a	4.77	913.33	52.93	1545.14 a	5.98 a	3.78 ab	1.94	162.56	10.19 a	4.99	1.08 b
<b>LS<sub>100</sub></b>	161.00 a	3.30	666.78	45.04	1292.52 a	5.51 a	4.22 a	1.47	271.88	11.13 a	4.49	0.95 c
<b>LSD (P = 0.05)</b>	<b>10.91</b>	-	-	-	<b>260.60</b>	<b>1.18</b>	<b>0.73</b>	-	-	<b>2.79</b>	-	0.12
<b>L<sub>0</sub></b>	157.60	5.11 a	847.56 a	52.78 a	1475.47 a	6.37 a	4.17 a	2.09 a	167.43 a	9.33 a	4.97 a	1.09 a
<b>L<sub>33</sub></b>	162.81	4.93 a	919.33 a	52.09 a	1450.24 a	5.56 a	3.47 b	2.03 a	193.43 a	9.44 a	5.07 a	1.08 a
<b>LSD (P = 0.05)</b>	-	<b>1.07</b>	<b>155.77</b>	<b>4.19</b>	<b>212.72</b>	<b>0.96</b>	<b>0.59</b>	<b>0.45</b>	<b>29.83</b>	<b>2.28</b>	<b>0.46</b>	<b>0.10</b>

Values with the same letter do not differ significantly from each other at the 5% significance level

“-“ = not discussed due to interaction

DT = defoliation time (DT<sub>36</sub> = 36 DAA; DT<sub>69</sub> = 69 DAA; DT<sub>76</sub> = 76 DAA and DT<sub>83</sub> = 83 DAA)

DAA = Days after anthesis

LS = lateral shoot removal (LS<sub>0</sub> = 0% lateral shoot removal; LS<sub>50</sub> = 50% lateral shoot removal; LS<sub>100</sub> = 100% lateral shoot removal)

L = leaf removal (L<sub>0</sub> = 0% leaf removal; L<sub>33</sub> = 33% leaf removal)

LSD= Least significant difference

**Table 3.5** The effect of defoliation on certain canopy characteristics of Redglobe vines, Hex River Valley, South Africa, 2002/2003.

	Average light intensity ( $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	Lateral shoot leaf area:fruit mass ratio ( $\text{g}\cdot\text{cm}^{-2}$ )
<b>LS<sub>0</sub></b>	109.65 f	2.75 a
<b>DT<sub>36</sub> x LS<sub>50</sub></b>	101.47 a	2.36 ab
<b>DT<sub>69</sub> x LS<sub>50</sub></b>	161.75 d	1.83 abc
<b>DT<sub>76</sub> x LS<sub>50</sub></b>	204.28 e	1.29 bcd
<b>DT<sub>83</sub> x LS<sub>50</sub></b>	211.39 f	2.47 a
<b>DT<sub>36</sub> x LS<sub>100</sub></b>	170.84 b	1.94 ab
<b>DT<sub>69</sub> x LS<sub>100</sub></b>	254.74 c	2.51 a
<b>DT<sub>76</sub> x LS<sub>100</sub></b>	302.08 d	0.51 d
<b>DT<sub>83</sub> x LS<sub>100</sub></b>	422.83 e	0.83 cd
<b>LSD (P = 0.05)</b>	<b>26.02</b>	<b>1.09</b>

Values with the same letter do not differ significantly from each other at the 5% significance level

DT = defoliation time (DT<sub>36</sub> = 36 DAA; DT<sub>69</sub> = 69 DAA; DT<sub>76</sub> = 76 DAA and DT<sub>83</sub> = 83 DAA)

DAA = Days after anthesis

LS = lateral shoot removal (LS<sub>0</sub> = 0% lateral shoot removal; LS<sub>50</sub> = 50% lateral shoot removal; LS<sub>100</sub> = 100% lateral shoot removal)

LSD= Least significant difference

### 3.3.2 LIGHT INTENSITY

There was significant DT x LS interaction for the average light intensity in the bunch zone (Table 3.6). Compared to the effect of LS<sub>0</sub>, the light intensity in the bunch zone was increased significantly through increased levels of LS, applied at any DT, except for LS<sub>50</sub> applied at DT<sub>36</sub>. The effect of this interaction can be seen in Table 3.5. The application of LS<sub>100</sub> at any DT significantly increased the light intensity compared to the effect of LS<sub>50</sub> applied at the same DT. An increase in light intensity as a result of lateral shoot removal was also found by Avenant (1994). For LS<sub>50</sub> and LS<sub>100</sub>, the effect of the light intensity increased due to later DT. Such increases in light intensity can be beneficial for colour development because increases in light intensity cause increases in anthocyanins (Kataoka *et al.*, 2004). However, in cases where a lighter colour is required, an increase in light intensity might lead to a colour that is darker than the colour required for a specific cultivar. Kataoka *et al.* (2004) determined that the saturation point for anthocyanin accumulation in Gros Colman grapes is 60 ( $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) under diffuse sunlight. Thus, if a lighter colour for a specific cultivar such as Redglobe is required, limiting the light intensity in the bunch zone below the saturation point for anthocyanin accumulation would decrease grape colour. The effects of the light intensity on the grape colour obtained in this study are discussed in section 3.3.3. The increase in light intensity caused by L<sub>33</sub> was not significant (Table 3.4). Increases in light intensity due to defoliation in this study are in accordance with results previously obtained (Kliwer & Smart, 1989; Dry, 2000). The canopy microclimate due to leaf and lateral shoot removal in the bunch zone of increased the PPFR in the studies of Kliwer & Smart (1989) and Dry (2000).

**Table 3.6** Analysis of variance to test treatment and interaction effects of canopy management practices for different variables of Redglobe grapevines in the Hex River Valley (Grandview), South Africa, 2002/2003.

Source	Average light intensity ( $\mu\text{E.m}^{-2}.\text{s}^{-1}$ )			Bunch colour (visual)			Bunches with acceptable colour Far East (%)			Anthocyanin concentration in berry skins (mg/g)			Anthocyanin content/berry skin (mg)		
	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Block	3	22621.7	0.59	3	1.51	0.41	3	21.70	0.56	3	6.49	<0.01	3	0.165	0.05
Defoliation time (DT)	3	458165.2	<0.01	3	6.00	0.01	3	153.54	<0.01	3	2.73	0.10	3	0.448	<0.01
Lateral shoots = LS	2	1449279.1	<0.01	2	8.29	<0.01	2	162.07	<0.01	2	9.88	<0.01	2	0.642	<0.01
Leaves = L	1	105565.0	0.09	1	0.02	0.90	1	0.26	0.93	1	0.16	0.72	1	0.003	0.82
DT x LS	6	122720.7	<0.01	6	0.45	0.94	6	11.27	0.90	6	3.56	0.02	6	0.118	0.09
DT x L	3	12007.8	0.79	3	0.51	0.81	3	7.04	0.88	3	1.19	0.42	3	0.049	0.50
LS x L	2	15627.9	0.64	2	7.32	0.01	2	73.01	0.10	2	0.45	0.70	2	0.038	0.54
DT x LS x L	6	38867.9	0.36	6	0.63	0.87	6	12.04	0.89	6	2.55	0.07	6	0.061	0.44
<b>Error a</b>	<b>69</b>	<b>34767.8</b>													
Date (D)	7	36282.2	<0.01												
DT x D	15	2695.0	0.87												
LS x D	14	4010.4	0.55												
L x D	7	4733.5	0.38												
DT x LS x D	30	3255.4	0.84												
DT x L x D	15	2627.0	0.88												
LS x L x D	14	2369.1	0.91												
DT x LS x L x D	30	4918.7	0.31												
<b>Error b</b>	<b>394</b>	<b>4401.4</b>													
Corrected Total	621	10317899.7		69	1.55		69	31.33		67	1.25		68	0.062	

Non-Normality (P<W) <0.01 95 169.01 0.56 95 3318.74 <0.01 93 172.40 0.64 94 8.627 0.29

DF = Degrees of freedom. MS = Mean Square P = Probability of F-ratio test

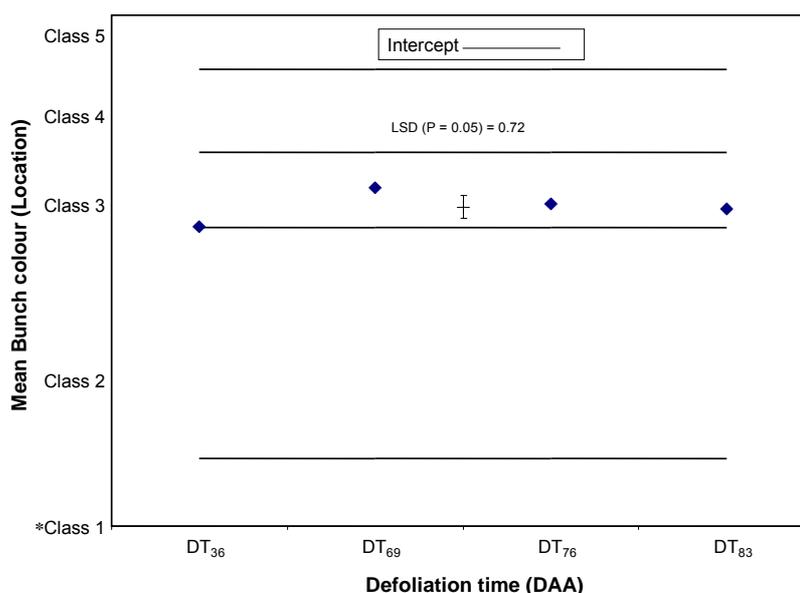
### 3.3.3 GRAPE COLOUR

Visual bunch evaluation showed that DT and LS x L significantly affected the mean grape colour (Table 3.6). Figure 3.10 shows that the mean bunch colour was in class three, but varied therein on account of the DT factor. Due to the nature of the statistical analysis, it is possible that significant differences can occur within classes, such as the differences within class three. It was found that DT<sub>69</sub> and DT<sub>76</sub> significantly decreased the bunch colour compared to DT<sub>36</sub> (Fig. 3.10). It therefore seems that bunch colour development is decreased by DT's later than DT<sub>36</sub>. The increased colour development that arises from DT<sub>36</sub> is ascribed to the possible increased photosynthetic capacity of the remaining leaves when leaves are removed at an early stage of bunch development, while removal at DT<sub>69</sub> impacted colour development the most. Similar results were obtained by Hunter *et al.* (1991). Fig. 3.11 shows that the mean bunch colour due to the LS x L interaction was in classes two and three. It was found that LS<sub>50</sub> x L<sub>0</sub>, LS<sub>100</sub> x L<sub>0</sub> and LS<sub>100</sub> x L<sub>33</sub> significantly decreased the bunch colour compared to LS<sub>0</sub> x L<sub>0</sub> while the colour development as a result of LS<sub>50</sub> x L<sub>33</sub> was also less (not significant) than LS<sub>0</sub> x L<sub>0</sub>. The darker colour that was found for the combinations involving LS<sub>0</sub> can be ascribed to the higher photosynthetic rates of the lateral shoots (Candolfi-Vasconcelos *et al.*, 1994). The mean bunch colour which resulted from LS<sub>0</sub> x L<sub>0</sub> is in class two, whereas the rest of the treatment combinations resulted in bunches located in class three. Although there were bunches in the other colour classes, the mean bunch colour resorted in classes two and three, thus still darker than optimal. However, considering the percentage of bunches with the acceptable colour, DT and LS had a significant effect on the percentage of bunches with the acceptable pink (the sum of the bunches in classes four and five expressed as a percentage of the total number of bunches evaluated for colour) colour (Table 3.4). The ideal colour (between classes four and five), as obtained in Chili, was not obtained in this study. DT<sub>69</sub> resulted in significantly more bunches with the acceptable colour than the effect of DT<sub>36</sub>, DT<sub>76</sub> and DT<sub>83</sub> (Table 3.4). Therefore, DT<sub>69</sub> seems to be the optimal DT that will potentially give the acceptable colour. The removal of leaf area for assimilates thus cause insufficient precursors to be available for colour development and therefore has the potential to decrease grape colour. Furthermore, compared to LS<sub>0</sub>, it was found that LS<sub>50</sub> and LS<sub>100</sub> also increased the percentage of bunches with the acceptable pink colour (Table 3.4). Although LS did result in lighter coloured grapes, too few bunches of which the colour acceptable for the Far Eastern market were obtained. LS can therefore not be recommended for application on a commercial scale for obtaining the required lighter colour for Redglobe. However, the impact on berry size, keeping quality and sugar must be kept in mind (Chapter 4).

For anthocyanin concentration there was a significant DT x LS interaction (Table 3.6). The effect of LS<sub>50</sub> and LS<sub>100</sub> at each defoliation time was compared to the average value for no lateral shoot removal at all DT's. Except for the effect of DT<sub>83</sub> x LS<sub>50</sub>, the removal of lateral shoots reduced the anthocyanin content of the

berries, although not always significantly. Anthocyanin concentration was significantly decreased by  $DT_{36} \times LS_{50}$ ,  $DT_{69} \times LS_{100}$  and  $DT_{76} \times LS_{100}$  and  $DT_{83} \times LS_{100}$  compared to  $LS_0$  (Fig. 3.12). For  $LS_{100}$ , there was a tendency that anthocyanin concentration decreased progressively for the later DT's.  $LS_{100}$  applied at  $DT_{76}$  and  $DT_{83}$  lowered the anthocyanin concentration significantly compared to  $LS_{100}$  applied at  $DT_{36}$ . Generally, anthocyanin concentration also decreased with increased levels of LS, except for  $DT_{36} \times LS_{100}$ . This is in accordance with results by Candolfi-Vasconcelos & Koblet (1990) and Vasconcelos & Castagnoli (2000) and was due to the removal of active leaf area in the period of colour accumulation (Candolfi-Vasconcelos & Koblet, 1990). Regardless of DT, the application of  $L_{33}$  did not affect the anthocyanin concentration in the berry skins significantly. Thus, the colour of Redglobe can not be manipulated by the removal of moderate levels of leaves ( $L_{33}$ ) on the main shoots.

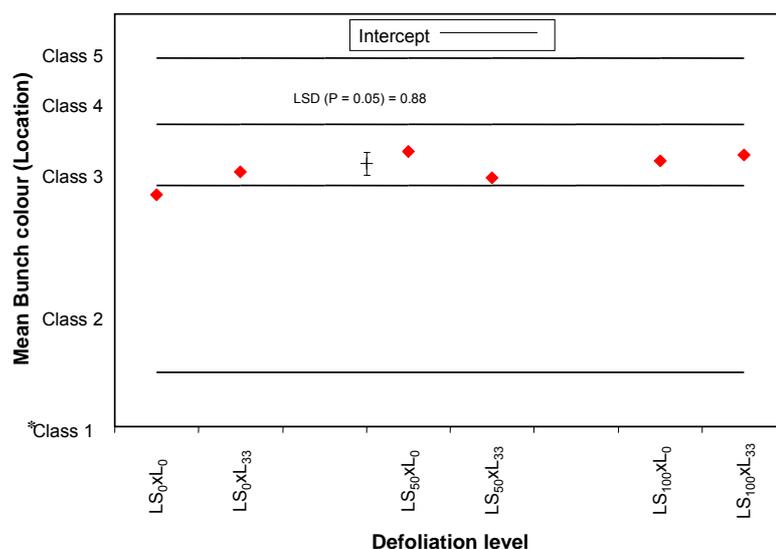
The anthocyanin content per berry skin was affected significantly by DT and LS. Their means can be seen in Table 3.4, where it is seen that defoliation at  $DT_{69}$ ,  $DT_{76}$  and  $DT_{83}$  decreased the anthocyanin content per grape berry skin. However, this decrease was not progressive with DT. This illustrates that the time of defoliation is important, pointing to the fact that the most active contribution made by leaves to anthocyanin production is at  $DT_{69}$  (véraison). Increased levels of lateral shoot removal also decreased the anthocyanin content per berry skin. This is in accordance with the pattern found for anthocyanin concentration in the berry skins and confirms the important contribution of lateral shoots to anthocyanin accumulation of Redglobe berries and their colour development. The implication is that increased colour development can be obtained by retaining the lateral shoots. Should a lighter colour be required, the removal of moderate levels of lateral shoots would decrease colour to more acceptable levels.



**Figure 3.10** The effect of DT on the mean bunch colour of Redglobe.

$DT_{36}$  = 36 DAA;  $DT_{69}$  = 69 DAA;  $DT_{76}$  = 76 DAA and  $DT_{83}$  = 83 DAA; DAA = Days after anthesis

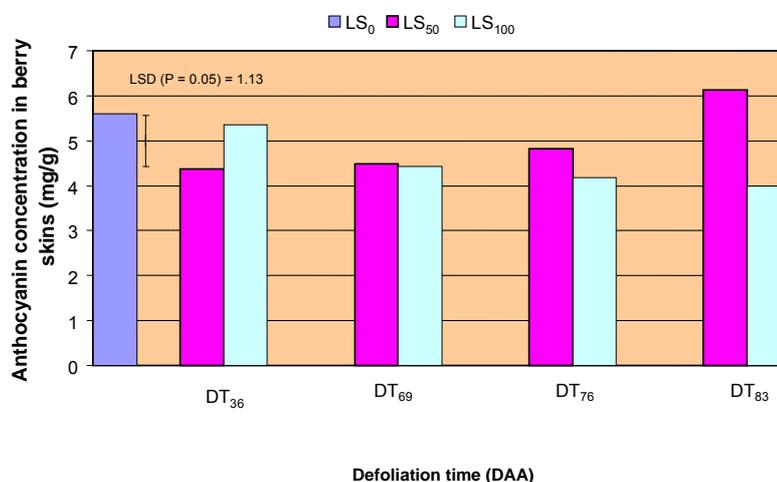
\*Class 1 is the darkest colour, while class 9 (not indicated) is the lightest colour – the ideal colour for Redglobe lies between classes 4 and 5



**Figure 3.11** The effect of LS x L on visual colour of Redglobe.

LS<sub>0</sub> x L<sub>0</sub> = 0% lateral shoot removal and 0% leaf removal; LS<sub>0</sub> x L<sub>33</sub> = 0% lateral shoot removal and 33% leaf removal; LS<sub>50</sub> x L<sub>0</sub> = 50% lateral shoot removal and 0% leaf removal; LS<sub>50</sub> x L<sub>33</sub> = 50% lateral shoot removal and 33% leaf removal; LS<sub>100</sub> x L<sub>0</sub> = 100% lateral shoot removal and 0% leaf removal; LS<sub>100</sub> x L<sub>33</sub> = 100% lateral shoot removal and 33% leaf removal

\*Class 1 is the darkest colour, while class 9 (not indicated) is the lightest colour – the ideal colour for Redglobe lies between classes 4 and 5.



**Figure 3.12** The effect of DT x LS on anthocyanin concentration of berry skins.

DT = defoliation time; DT<sub>36</sub> = 36 DAA; DT<sub>69</sub> = 69 DAA; DT<sub>76</sub> = 76 DAA; DT<sub>83</sub> = 83 DAA; DAA = Days after anthesis; LS<sub>0</sub> = 0% lateral shoot removal; LS<sub>50</sub> = 50% lateral shoot removal; LS<sub>100</sub> = 100% lateral shoot removal

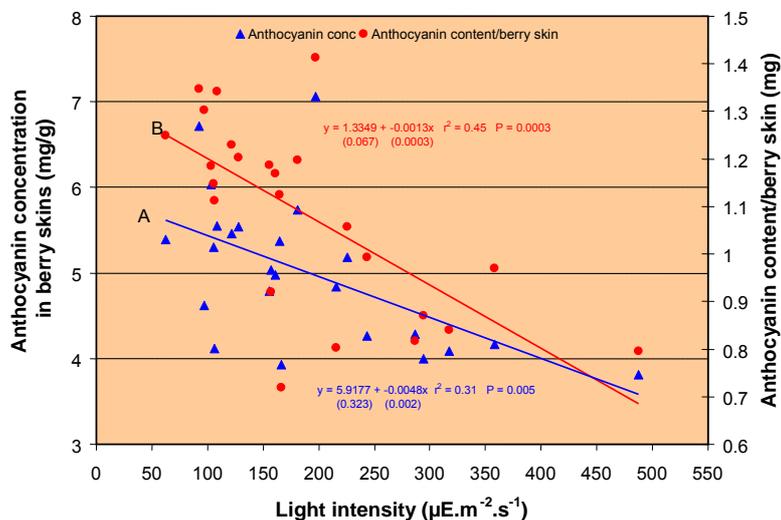
The reason why LS<sub>100</sub> applied at DT's later than DT<sub>69</sub> decreased anthocyanin content in the berry skins can be attributed to the fact that colour accumulation in berries is dependent on the available active leaf area between véraison and harvest, as found by Candolfi-Vasconcelos & Koblet (1990). These authors found that when

100% lateral shoots of Pinot Noir were retained, the skin colouration (expressed as percentage of the highest value obtained for optical density) was significantly increased compared when all the lateral shoots were removed. The fact that bunch colour was decreased (more acceptable for Redglobe) when lateral shoot removal was applied at DT<sub>69</sub> and DT<sub>76</sub>, accentuates the role of active leaf area during the period between véraison and harvest. Furthermore, the anthocyanin content decreased with increased levels of LS. This corresponds with results previously obtained (Reynolds & Wardle, 1989; Avenant, 1994; Candolfi-Vasconcelos & Koblet, 1990). Treatments that involved retaining all the lateral shoots resulted in more colour, as was the case in the study of Vasconcelos & Castagnoli (2000).

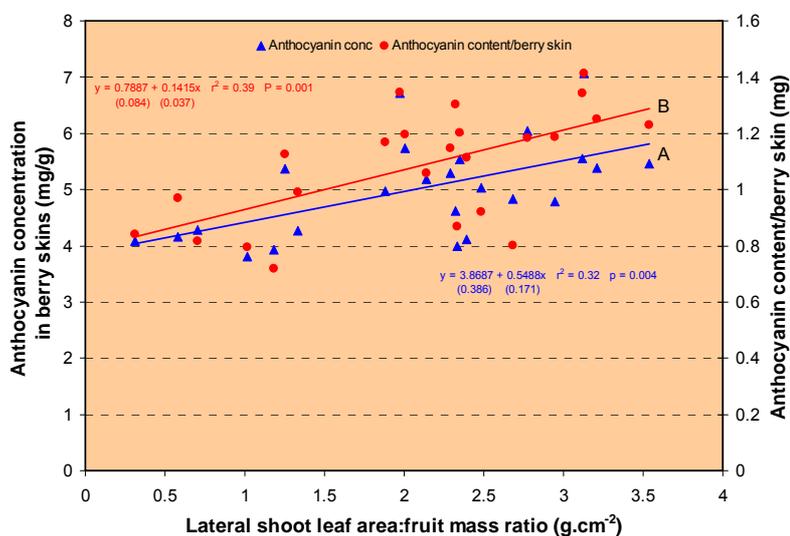
There was a significant negative correlation between light intensity in the bunch zone and colour, as measured by anthocyanin concentration and anthocyanin content per berry skin (Fig. 3.13). If the increased light levels were the reason for the decrease in colour, it is contrary to many studies that have shown that decreasing light levels decreased the colouration of berries (Le Roux, 1953; Weaver & McCune, 1960; Archer & Strauss, 1989; Rojas Lara & Morrison, 1989; Van Dyk & Saayman, 1989; Morrison & Noble, 1990; Iacono *et al.*, 1994; Smart & Robinson, 1991). A decrease in colour due to sub-optimal light conditions is ascribed to a reduction in PAL activity under shaded conditions (Roubelakis-Angelakis & Kliewer, 1986; Smart *et al.*, 1988). However, in this trial, light intensity was never below light compensation point (Tables 3.4 & 3.5) and if the findings of Kataoka *et al.* (2004) are applied in this study, light intensity was never below the critical saturation point for anthocyanin accumulation. Therefore insufficient light could not be the reason for a colour decrease. Effects of bunch exposure on anthocyanin content may be temperature related (Iland, 1989; Mabrouk & Sinoquet, 1998). Although temperature was not determined in this study, sun exposure increases the temperature of grape berries (Smart & Sinclair, 1976). Heating of berries has previously been correlated with a reduction in anthocyanin biosynthesis (Kliewer, 1977; Haselgrove *et al.*, 2000). The increase in bunch temperature may be directly proportional to the amount of leaf area removed (Kliewer *et al.*, 1988). Although temperature plays an important role in colour development (Kliewer & Torres, 1972; Kliewer, 1977; Pirie, 1979), the effect thereof was not investigated. Parallel to increased temperature, higher light intensities in the bunch zone is correlated with higher levels of defoliation, which has by now been shown to decrease colour development progressively. The lower anthocyanin contents with higher light intensity are therefore only an expression of the impact of higher defoliation levels.

The total leaf area:fruit mass ratio, main shoot leaf area per vine, lateral shoot leaf area per vine and total leaf area per vine did not correlate with the anthocyanin concentration (data not shown). This is conflicting to the results of Smart (1980) and Jackson (1986) whom found that the leaf area:fruit mass ratio correlated positively with wine colour and grape colour. A relatively fair positive relationship was found between lateral shoot leaf area:fruit mass ratio and colour (anthocyanin concentration and anthocyanin content per berry skin) (Fig. 3.14). This supports the findings of

Candolfi-Vasconcelos & Koblet (1990) and Vasconcelos & Castagnoli (2000) that young leaves are important for complete grape colouration. The role of the lateral shoot leaves is vital in the metabolic processes in the grape berry (Avenant, 1994; Hunter, 2000).



**Figure 3.13** The relationship between the average light intensity in the bunch zone and (A) anthocyanin concentration and (B) anthocyanin content per berry skin.  $\text{LS}_0 \times \text{L}_0 = 0\%$  lateral shoot removal and 0% leaf removal;  $\text{LS}_0 \times \text{L}_{33} = 0\%$  lateral shoot removal and 33% leaf removal;  $\text{LS}_{50} \times \text{L}_0 = 50\%$  lateral shoot removal and 0% leaf removal;  $\text{LS}_{50} \times \text{L}_{33} = 50\%$  lateral shoot removal and 33% leaf removal;  $\text{LS}_{100} \times \text{L}_0 = 100\%$  lateral shoot removal and 0% leaf removal;  $\text{LS}_{100} \times \text{L}_{33} = 100\%$  lateral shoot removal and 33% leaf removal



**Figure 3.14** The relationship between the lateral shoot leaf area:fruit mass ratio and (A) anthocyanin concentration and (B) anthocyanin content per berry skin.  $\text{LS}_0 \times \text{L}_0 = 0\%$  lateral shoot removal and 0% leaf removal;  $\text{LS}_0 \times \text{L}_{33} = 0\%$  lateral shoot removal and 33% leaf removal;  $\text{LS}_{50} \times \text{L}_0 = 50\%$  lateral shoot removal and 0% leaf removal;  $\text{LS}_{50} \times \text{L}_{33} = 50\%$  lateral shoot removal and 33% leaf removal;  $\text{LS}_{100} \times \text{L}_0 = 100\%$  lateral shoot removal and 0% leaf removal;  $\text{LS}_{100} \times \text{L}_{33} = 100\%$  lateral shoot removal and 33% leaf removal

### 3.4 CONCLUSIONS

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Generally, treatment combinations involving  $L_{33}$  lowered the main shoot leaf area, compared to  $L_0$  although this was not the case for  $DT_{36} \times LS_{50}$ ,  $DT_{69} \times LS_0$  and  $DT_{76} \times LS_{100}$  in combination with  $L_{33}$ . When LS and L was applied in combination with one another, the LS level did not seem to impact on the total vine leaf area. It is observed that, where  $L_{33}$  was applied in combination with any LS level, the total vine leaf area was always lower compared to where  $L_0$  was part of the treatment combination. These differences in vine leaf area probably affected the supply of photosynthates to the bunches. The impact of leaf area on the supply of assimilates was manifested in the effect of the lateral shoot leaf area on grape colour.

The application of  $LS_{100}$  at each DT, except  $DT_{69}$ , decreased the lateral shoot leaf area per vine in comparison to no lateral shoot removal ( $LS_0$ ). Lateral shoot removal implicates the removal of young photosynthetically active leaf area and thus the supply of assimilates in the grape berry.

Reducing these young leaves, through moderate levels of lateral shoot removal ( $LS_{50}$ ) at pea berry size ( $DT_{36}$ ), or through severe lateral shoot removal ( $LS_{100}$ ) at one week ( $DT_{76}$ ) or two weeks ( $DT_{83}$ ) after véraison, reduced anthocyanin concentration. The application of  $LS_{100}$  at  $DT_{76}$  and  $DT_{83}$  reduced the lateral shoot leaf area:fruit mass ratio thus emphasising the importance of photosynthetically active leaf area during the period after véraison. Furthermore, the positive relationship between the lateral shoot leaf area:fruit mass ratio and grape colour also indicates the importance of the lateral shoots for berry ripening. Due to the decreasing effect of lateral shoot removal on colour,  $LS_{50}$  and  $LS_{100}$  increased the percentage of bunches with the acceptable colour (pink).

Although a fair amount of bunches with the acceptable colour was obtained, the mean bunch colour brought about by DT and LS X L, was not in the acceptable class. On the other hand, DT and LS significantly impacted on the percentage of bunches with the acceptable colour. The results indicate that  $DT_{69}$  is the most appropriate DT and  $LS_{100}$  the most appropriate level of LS to obtain a certain percentage of bunches with the acceptable colour. This accentuates the role of active leaf area during véraison ( $DT_{69}$ ), when the grape berry act as a strong sink for assimilates. However, it is of the utmost importance that the effect thereof on yield and other grape quality parameters, such as total soluble solids (TSS) must be kept in mind since  $DT_{69}$  corresponds with véraison and thus the time of sugar accumulation in the grape berry.

Apart from leaf area, the different treatment combinations altered the age composition of the canopy. It was found that when the lateral shoot leaf area as percentage of the total leaf area decreased, the main shoot leaf area percentage increased and vice versa. Due to the fact that main and lateral shoot leaves contribute

differently to grape composition, assimilate supply to bunches was thus be affected. Regarding assimilate supply to bunches, this study made it clear that the lateral shoots play a vital role in grape colouration.

Apart from the role of leaf area, the altered light environment might also have affected grape colour. It was possible to alter the light environment in the bunch zone through moderate (LS<sub>50</sub>) and severe (LS<sub>100</sub>) LS at any DT. The significant negative relationship between light intensity and grape colour is an indication that the decreased colour due to defoliation might be ascribed to an inhibiting effect of light, or an accompanied increase in bunch temperature and the negative effects thereof on berry colour. However, in this study, it is the effect of the removal of active leaf area that decreased grape colour.

Generally, defoliation at DT<sub>69</sub> had the most significant impact on anthocyanin concentration of the berry skins, the anthocyanin content per berry skin and visual colour. This points to the fact that the most important period during which the leaves act as source of photosynthates for berry development (anthocyanin synthesis) is shortly after véraison.

It is evident that manipulating the grapevine canopy affected grape colour through changes in leaf area and/or light microclimatic conditions. However, recommendations based on the results obtained in this trial, cannot be made without considering the effect of these defoliation treatments on berry size and berry sugar concentration (See chapter 4). The latter plays a determining role in table grape quality and the time of ripening. Furthermore, since this is one season's results, one should beware of the temptation to make recommendations. The results of the 2001/2002 season were not included in this study due to a bacterial disease which affected the outcome of the same experiment done at a different location. Ideally the trial should have been conducted over three seasons.

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**RESEARCH RESULTS**  
**THE EFFECT OF DEFOLIATION TREATMENTS**  
**ON BERRY COMPOSITION AND YIELD**  
**COMPONENTS OF REDGLOBE**  
**(*VITIS VINIFERA* L.)**

# THE EFFECT OF DEFOLIATION TREATMENTS ON BERRY COMPOSITION AND YIELD COMPONENTS OF REDGLOBE (*VITIS VINIFERA* L.)

## ABSTRACT

The effect of the defoliation treatments on berry composition, i.e. total soluble solids (TSS), total titratable acidity (TTA), as well as pH and yield components (berry mass, and total yield) was investigated. Defoliation was conducted on six-year old Redglobe vines with moderate vigour. The experiment involved two leaf removal (L) levels ( $L_0$  = 0% leaf removal;  $L_{33}$  = 33% leaf removal) in combination with three lateral shoot removal (LS) levels ( $LS_0$  = 0% lateral shoot removal;  $LS_{50}$  = 50% lateral shoot removal;  $LS_{100}$  = 100% lateral shoot removal). The defoliation treatment combinations were done at four different times (DT): 36 (pea berry size), 69 (véraison), 76 (one week after véraison) and 83 (two weeks after véraison) days after anthesis (DAA), resulting in 24 treatments, replicated in four blocks.

Defoliation treatments affected TSS and berry mass. The TSS was subject to DT x LS and DT x L interactions. At  $DT_{69}$  (véraison), the application of  $LS_{50}$  and  $LS_{100}$  lowered TSS significantly. This illustrates the important role of lateral shoots in berry sugar accumulation. Likewise, main shoot leaf removal (L) at véraison decreased berry sugar accumulation. However, this decrease was not significant. These results suggest that the translocation of photosynthates, i.e. sugar, happens predominantly during the earlier part (first week after véraison) of ripening. The application of  $L_{33}$  at  $DT_{36}$  significantly increased the TSS as a result of the enhanced photosynthetic activity of the retained leaves, whereas  $L_{33}$  at  $DT_{83}$  significantly increased TSS due to enhanced sink strength.

For berry mass, DT x LS x L interactions were significant. Compared to the effect of  $LS_0$  x  $L_0$ , berry mass was affected significantly by defoliation at  $DT_{69}$  when  $LS_{100}$  x  $L_{33}$  and  $LS_{100}$  x  $L_0$  were applied. These results emphasise the role of active leaf area, especially lateral shoot leaf area, during véraison on berry development.

The negative effect of the treatment combinations involving LS on TSS must be kept in mind because the time of ripening is altered and thus the time of marketing.

## 4.1 INTRODUCTION

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Except for colour and shape, berry size and composition also determines berry quality. Berry size and composition are influenced by cultivation practices, such as canopy management in the form of leaf removal and lateral shoot removal. Leaf removal is a standard practice in table grape production and involves removal of the basal leaves at the beginning of véraison (Zeeman, 1983; Wagener *et al.*, 1985).

Berry sugar content is affected by leaf removal in various ways (Peterson & Smart, 1975; Kliewer *et al.*, 1988; Kingston & Van Epenhuijsen, 1989; Iacono *et al.*, 1994; Petrie *et al.*, 2000). Koblet (1987) and Koblet, (1988) found that basal leaf

removal increases berry sugar, while Vasconcelos & Castagnoli (2000), on the other hand, found that it decreased berry sugar. Titratable acidity (Kliwer *et al.*, 1988; Hunter *et al.*, 1991; Hunter *et al.*, 1995), pH (Kliwer *et al.*, 1988; Koblet *et al.*, 1994; Hunter *et al.*, 1995), berry mass (Candolfi-Vasconcelos & Koblet, 1990; Hunter & Visser, 1990b; Koblet *et al.*, 1994) and total yield (Koblet *et al.*, 1994; Hunter & Le Roux, 2000) are also influenced by the different effects of leaf removal on plant metabolism. The availability of assimilates needed for berry development are also decreased due to reduced leaf area (Peterson & Smart, 1975; Kingston & Van Epenhuijsen, 1989; Petrie *et al.*, 2000). Leaf removal furthermore enhances the light environment in the canopy (Hunter & Visser, 1988c; Hunter & Visser, 1990a; Hunter *et al.*, 1995). The photosynthetic activity of the remaining leaves (source) thus increase (Hunter & Visser, 1988b; Hunter & Visser, 1988c; Hunter & Visser, 1990a; Hunter *et al.*, 1991; Hunter *et al.*, 1995; Koblet *et al.*, 1996), which cause increases in the berry sugar (Hunter *et al.*, 1991). Apart from enhancing the source, leaf removal also leads to fruit exposure, thereby enhancing the sink strength. Mansfield & Howell (1981) found that bunches on completely defoliated Concord vines were powerful sinks that mobilised carbohydrates from parts of the vine other than the leaves. A lower source:sink ratio increase the export of photoassimilates (Hunter *et al.*, 1995; Hunter & Visser, 1988c) and thus contributes to an enhanced berry composition.

Since the physiological and biochemical functioning of the vine is affected by cultural practices, such as canopy manipulation techniques, the probability to influence the berry composition, berry mass and total yield of Redglobe through lateral shoot and main shoot leaf removal was investigated. The contribution of leaf area and the microclimatic light environment, on the mentioned variables in particular, was investigated.

## **4.2 MATERIALS AND METHODS**

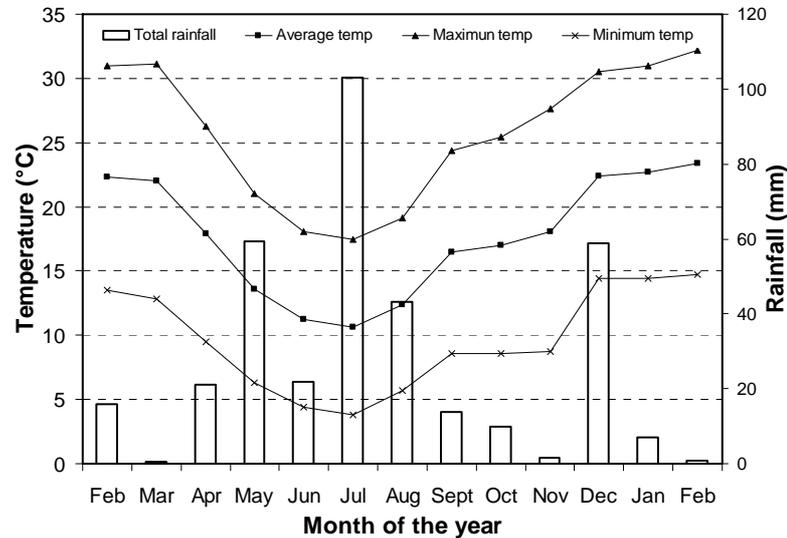
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### **4.2.1 EXPERIMENTAL VINEYARD**

The experiment was conducted on a virus-free six-year-old *Vitis vinifera* L. cv Redglobe grafted onto Ramsey (*Vitis Champinii*) vineyard with moderate vigour. It is situated on the farm Grandview (33° 30' 23" S; 19° 35' 43" E) in the Hex River Valley, De Doorns. The vines were spaced 2.74 m x 1.83 m on a sandy-loam soil and trained onto a gable trellis system as described by Zeeman (1981), with the rows orientated in an east-west direction. The vines were split into two double split cordons at the same height above ground level. Each vine was pruned to 20 spurs per vine, spaced evenly (15 cm apart) on four cordon arms.

Figure 3.1 shows the monthly temperature and rainfall for the De Doorns experimental farm mechanical weather station for the 2002/2003 season, which is the period during which the trial was conducted. The mean February temperature (month during which the berries ripen) from 1963 to 2002 was 21.8 °C and the

Winkler index for the area is 1906. This area is thus classified into region III, which means that the climate of the Hex River valley is moderately warm and suitable for the production of red and black grapes (Winkler *et al.*, 1974). The small differences between day and night temperatures further contributes to sufficient grape colour (Kliewer & Torres, 1972).



**Figure 4.1** Monthly temperature and rainfall for the De Doorns Experimental Farm (2002/2003) Hex River Valley, South Africa (ARC-ISCW, 2005).

Suckering, crop control and bunch preparation took place as part of standard seasonal canopy management practices. Suckering involved the removal of infertile shoots and water shoots. Crop control involved the reduction of the potential yield to approximately 25 bunches per vine after berry set. During bunch preparation, the bunches were shortened to an approximate length of 12 cm. Berries that were smaller than the average berry size, as well as poorly coloured berries, were removed just before harvest. Gibberellic acid was applied at 10 ppm at 12 mm berry size for berry enlargement. No etrel was applied to enhance colour development. The vineyard was irrigated by means of scheduled micro-irrigation involving water application at 30 mm per week during the active shoot-growth period and 18 mm per week from véraison to harvest. For the rest of the year, the water requirements were supplemented by rainfall. To prevent heat damage during the ripening phase, an irrigation of 3 mm was applied every time the temperature was in excess of 30°C. Fertilisation was applied at three different growth stages: budbreak (N, P and K), berry set (K), 16 mm berry size (N and K) and after harvest (N, P and K). Fertilisation applications were done on the basis of information obtained from soil and leaf analyses to maintain optimal vegetative growth.

#### 4.2.2 EXPERIMENTAL DESIGN AND TREATMENTS

A randomised complete block design was used, with 24 treatment combinations replicated in four blocks with a single vine as experimental unit. The treatment

design was a 2 x 3 x 4 factorial. The factors were two leaf removal (L) levels ( $L_0$  = 0% leaf removal and  $L_{33}$  = 33% leaf removal, only on the main shoots), three lateral shoot removal (LS) levels ( $LS_0$  = 0% lateral shoot removal,  $LS_{50}$  = 50% lateral shoot removal and  $LS_{100}$  = 100% lateral shoot removal) and four defoliation times (DT): 36 (pea berry size), 69 (véraison), 76 (one week after véraison) and 83 (two weeks after véraison) days after anthesis (DAA). Treatment combinations were applied evenly, only on the main shoots and from side to side in the canopy. Only the results of the 2002/2003 season are presented due to the occurrence of Bacterial blight (*Xylophilus ampelinus*) at Clovelly in the 2001/2002 season.

#### **4.2.3 CANOPY MEASUREMENTS AND SAMPLING**

Determination of the photosynthetic photon fluence rate (PPFR) reaching the bunch zone and the leaf area is described in chapter three. At harvest, 50 berries per vine were sampled randomly. The berry samples were stored at -0.5°C for two days prior to the analyses and measurements being carried out.

#### **4.2.4 BERRY MEASUREMENTS, EVALUATION AND ANALYSES**

The berry samples were used for berry mass and standard juice analyses. Fresh berry samples consisting of 50 berries each were weighed to obtain the average berry mass. Usually, 100 berries are used to ensure a correct mass measurement. However, due to the limited number of bunches available for this study, 50 berries were used instead. The juice was analysed for total soluble solids (TSS in °Brix) by means of an Atago abx-30 refractometer. The total titratable acidity (TTA in g/L) and pH were determined with a Mettler DL21 titrator. At harvest (19 and 20 February 2003), each vine's bunches were counted and the total yield per vine was weighed (kg/vine).

#### **4.2.5 STATISTICAL ANALYSES**

Data obtained were subjected to statistical analyses by means of the SAS program, version 8.2 (SAS Institute Inc., 1999). An analysis of variance was performed using SAS version 8.2 (SAS Institute Inc., 1999). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). Student's t-Least Significant Differences (LSD) were calculated at a 5% significance level to compare the treatment means.

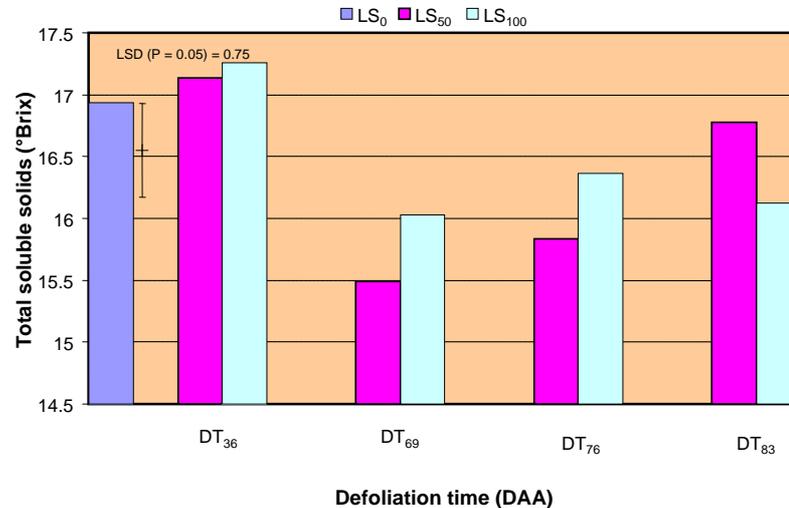
### **4.3 RESULTS AND DISCUSSION**

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#### **4.3.1 BERRY COMPOSITION**

Significant two-factor interactions (DT x LS, DT x L and LS x L) were evident for total soluble solids (TSS) (Table 4.1). The TSS at harvest was significantly affected by the

time of defoliation of both lateral shoot or main shoot leaf removal (DT x LS and DT x L). The effect of LS<sub>50</sub> and LS<sub>100</sub> on the TSS at each defoliation time was compared to the average value for no lateral shoot removal (LS<sub>0</sub>). In Fig. 4.2 it can be seen that the impact of both LS<sub>50</sub> and LS<sub>100</sub> is larger when removal is at DT<sub>69</sub> (véraison) compared to the later stages (DT<sub>76</sub> & DT<sub>83</sub>), which points to the importance of the lateral shoots for sugar accumulation during the early period of ripening (shortly after véraison). It suggests that this period appears to be the most important period of sugar accumulation, i.e. photosynthate translocation from the lateral shoots to the bunches. This is substantiated by the fact that at DT<sub>69</sub> and DT<sub>76</sub>, the impact of LS<sub>50</sub> was significant. Although not significant, the application of LS<sub>50</sub> applied at each DT, except at DT<sub>83</sub>, tended to lower the TSS compared to LS<sub>100</sub>. A reason for this anomaly at DT<sub>83</sub> might be the fact that the enhanced sink strength caused by the increased light intensity in the bunch zone (section 3.3.2) due to LS<sub>100</sub> was overcome by the loss in leaf area supplying assimilates to the bunches. The difference, however, was not significant. Furthermore, the early removal (DT<sub>36</sub>) of lateral shoots did not affect the TSS of the grapes at harvest negatively. This is in accordance with the observations made regarding colour development in the previous chapter and is explained by the observations of Hunter *et al.* (1991) and Koblet *et al.* (1996) where it was found that the photosynthetic capacity of the remaining leaves are enhanced by defoliation (when defoliation is done with caution).



**Figure 4.2** The effect of DT x LS on the juice total soluble solids.

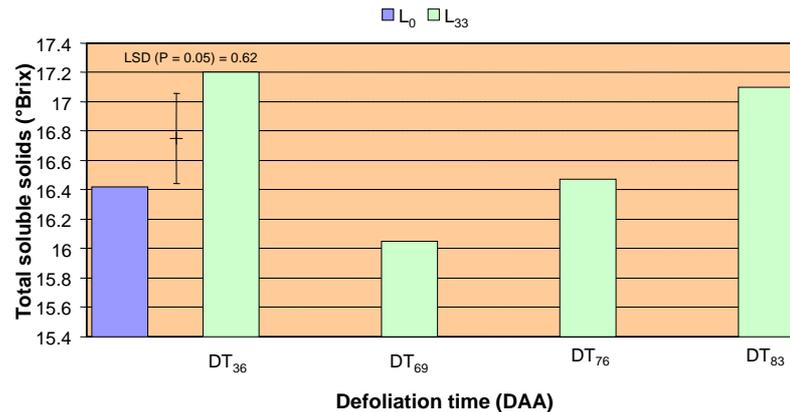
DT = defoliation time; DT<sub>36</sub> = 36 DAA; DT<sub>69</sub> = 69 DAA; DT<sub>76</sub> = 76 DAA; DT<sub>83</sub> = 83 DAA; DAA = Days after anthesis; LS<sub>0</sub> = 0% lateral shoot removal; LS<sub>50</sub> = 50% lateral shoot removal; LS<sub>100</sub> = 100% lateral shoot removal

A similar pattern is observed where main shoot leaves were removed. The average value for L<sub>0</sub> compared to L<sub>33</sub> applied at each DT is shown in Fig. 4.3. This tendency of the removal of main shoot leaves points to the importance of leaf area for sugar accumulation, especially at (DT<sub>69</sub>) véraison.

The juice TSS was enhanced by the application of  $L_{33}$  at  $DT_{36}$ . An increase in TSS as a result of leaf removal has been documented previously (Bledsoe *et al.*, 1988; Kliewer *et al.*, 1988). The lower  $L_0$  value compared to  $DT_{36} \times L_{33}$  is ascribed to the fact that the application of  $L_{33}$  at  $DT_{36}$  (pea berry size) probably enhanced the photosynthetic rate of the remaining leaves (Hunter & Visser, 1988b; Hunter & Visser, 1988c; Hunter & Visser, 1990a; Hunter & Visser, 1990b; Hunter *et al.*, 1991; Koblet *et al.*, 1996) for favourable sugar accumulation at the onset of véraison. The application of  $L_{33}$  at  $DT_{36}$  (pea berry size) therefore has an enhancing effect on sugar ripeness.

The application of  $L_{33}$  at  $DT_{83}$  also increased juice soluble solids compared to  $L_0$ . The reason for increased TSS due to  $L_{33}$  applied at  $DT_{83}$  can be ascribed probably to the enhanced sink strength of the bunches (Reynolds *et al.*, 1986; Hunter *et al.*, 1995). It is possible that an increase in fruit temperature increased the TSS (Reynolds *et al.*, 1986; Bledsoe *et al.*, 1988; Kliewer *et al.*, 1988) during this time, just after the onset of ripening, when the grape berry acts as a storage sink (Coombe, 1989). Thus, the application of  $L_{33}$  at  $DT_{83}$  (2 weeks after véraison) enhanced ripening in terms of TSS.

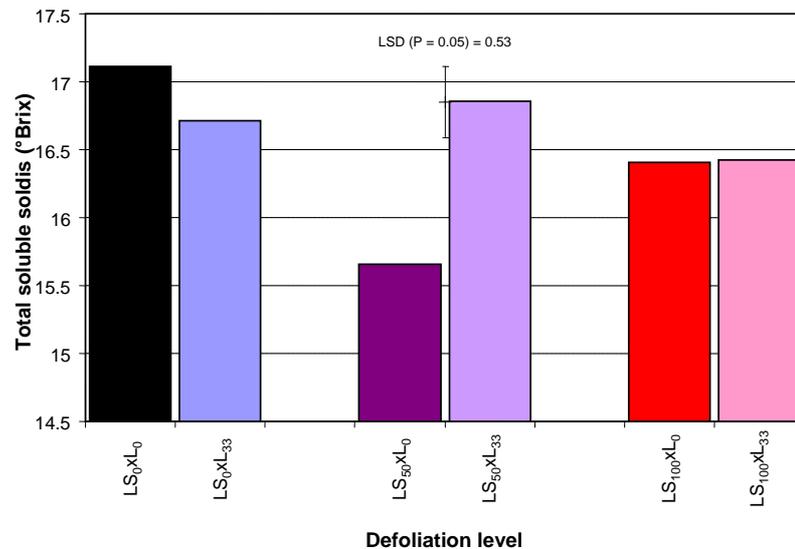
The increase in TSS due to  $L_{33}$  applied at  $DT_{36}$ , compared to  $L_{33}$  applied at  $DT_{69}$  and  $DT_{76}$  is in accordance with previous results which showed that TSS increases when vines are defoliated at set and four weeks after set (Bledsoe *et al.*, 1988; Kliewer *et al.*, 1988). It therefore seems that both LS and L, applied at  $DT_{69}$  (véraison) or shortly thereafter, delays ripening of Redglobe bunches, sometimes even significantly ( $DT_{69} \times LS_{50}$ ). This is explained by Candolfi-Vasconcelos & Koblet (1990) who states that the accumulation of sugar in grape berries depends on the active leaf surface area during the period between véraison and harvest. A loss in such functional leaf area reduce the availability of assimilates (May *et al.*, 1969; Kliewer & Antcliff, 1970; Peterson & Smart, 1975; Kriedeman, 1977; Kingston & Van Epenhuijsen, 1989; Petrie *et al.*, 2000). Severe defoliation, such as the treatment combinations that involved lateral shoot removal, imply the removal of highly functional leaf area because lateral shoot leaves have photosynthetic rates similar to those of the younger apical leaves (Candolfi-Vasconcelos *et al.*, 1994), which thus make them highly effective in terms of assimilate supply. Due to the fact that the photosynthetic capacity of a canopy increases with the presence of more lateral shoot leaves (Kriedeman *et al.*, 1970; Koblet, 1977; Candolfi-Vasconcelos *et al.*, 1994; Hunter *et al.*, 1994; Poni & Giachino, 2000), it seems that fruit maturation in this study was enhanced when more lateral shoots were present.



**Figure 4.3** The effect of DT x L on the juice total soluble solids.

DT = defoliation time; DT<sub>36</sub> = 36 DAA; DT<sub>69</sub> = 69 DAA; DT<sub>76</sub> = 76 DAA; DT<sub>83</sub> = 83 DAA; DAA = Days after anthesis; L<sub>0</sub> = 0% leaf removal; L<sub>33</sub> = 33% leaf removal.

The negative effect of main shoot leaf removal (L<sub>33</sub>) on TSS is also illustrated in Fig. 4.4 in that the berry TSS of LS<sub>0</sub> x L<sub>0</sub> was higher than that of LS<sub>0</sub> x L<sub>33</sub>. Figure 4.4 also shows that, with the defoliation time not taken into account, lateral shoot removal has a more pronounced effect on TSS than main shoot leaf removal. The lateral shoot leaves are therefore especially advantageous for TSS (Koblet, 1987; Candolfi-Vasconcelos & Koblet, 1990), which means that the removal thereof is expected to reduce the TSS. The reducing effects of LS<sub>100</sub> x L<sub>0</sub> and LS<sub>100</sub> x L<sub>33</sub> on TSS (Fig. 4.4) emphasised the importance of functional leaf area and especially lateral shoots. The lower TSS as a result of the treatment combinations that involved lateral shoot removal corresponds with previous results (Reynolds & Wardle, 1989; Avenant, 1994; Vasconcelos & Castagnoli, 2000). The berry sugar:acid ratio at harvest followed the same pattern than the TSS (Data not shown). Often the sugar:acid ratio is utilised as a maturity index. Obviously, this ratio is connected to the TSS content of the berries, which also explains why the sugar:acid ratio follows the same pattern than the TSS. Since the sugar:acid ratio is an indication of fruit maturity, it is commonly used by table grape producers. It can therefore be deduced that early (but cautious) defoliation benefits ripening. This can be brought into correlation with the results of Bledsoe *et al.* (1988) who found that early leaf removal advance sugar accumulation.



**Figure 4.4** The effect of LS x L on the juice total soluble solids.

LS<sub>0</sub> x L<sub>0</sub> = 0% lateral shoot removal and 0% leaf removal; LS<sub>0</sub> x L<sub>33</sub> = 0% lateral shoot removal and 33% leaf removal; LS<sub>50</sub> x L<sub>0</sub> = 50% lateral shoot removal and 0% leaf removal; LS<sub>50</sub> x L<sub>33</sub> = 50% lateral shoot removal and 33% leaf removal; LS<sub>100</sub> x L<sub>0</sub> = 100% lateral shoot removal and 0% leaf removal; LS<sub>100</sub> x L<sub>33</sub> = 100% lateral shoot removal and 33% leaf removal

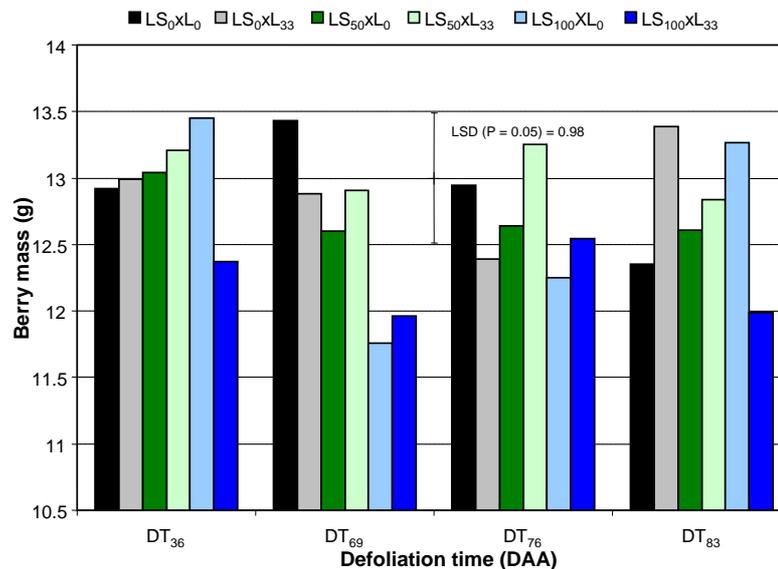
László & Loubser (1995) found that the palatability rating of Redglobe with a sugar:acid ratio of 45.4 and higher, together with a TSS of 16.8 °Brix and higher was acceptable. In this regard, the sugar:acid ratio caused by the different treatment combinations did not impair eating quality (data not shown). It is, however, the severe reduction in TSS that is a point of concern because, according to the standards for export (Anon., 2004), the minimum TSS of Redglobe should be 15.5 °Brix, whereas it was lowered below this by DT<sub>69</sub> x LS<sub>50</sub> (Fig. 4.2). The implication is that because of the potential that lateral shoot removal has to delay ripening and reduce sugar accumulation, this practice must be prevented or applied very conservatively. No treatment factor or treatment combination affected TTA significantly (data not shown). In accordance with the normal pattern of ripening, the TTA decreased during ripening (Winkler *et al.*, 1974; Alleweltdt, 1977) for all the treatment combinations (data not shown). Although DT had a significant effect on pH (data not shown), the difference was a mere 0.05 units and is thus for all practical reasons considered to be of no value for the purposes of this experiment. The TTA did not reflect the same pattern and therefore deductions regarding the effect thereof on pH cannot be made.

#### 4.3.2 YIELD COMPONENTS

There was a lack of correlation between the leaf area:fruit mass ratio and berry mass (data not shown). This is in contrast with the results of Jackson (1986), as well as Kliever & Weaver (1971) who found a higher average berry mass with a higher leaf area:fruit mass ratio. Berry mass was also not correlated with the average light intensity in the bunch zone (data not shown). The lack of the abovementioned

correlation can be ascribed to the effect of bunch preparation, and in particular bunch thinning, on the source:sink dynamics.

However, a significant three-factor interaction for berry mass occurred (Table 4.1). The means of this interaction can be seen in Fig. 4.5. The application of the most drastic level of defoliation ( $LS_{100} \times L_{33}$ ) was the only treatment that consistently reduced, berry mass. The important role of lateral shoots in berry development and berry size, especially at véraison, is emphasised by the decreasing effect of  $LS_{100} \times L_{33}$  and  $LS_{100} \times L_0$  at  $DT_{69}$  compared to  $LS_0 \times L_0$  at the same DT. In accordance with results obtained in this study, Avenant (1994) found that lateral shoot removal at just after pea berry size significantly decreased berry mass. Defoliation at  $DT_{36}$ ,  $DT_{76}$  and  $DT_{83}$  did not affect berry mass negatively. The effects of LS in any treatment combination lead to similar results of bunch colour and anthocyanin concentration (See Chapter 3). In this study, berry mass therefore decreased as a result of the mentioned treatment combinations that involved only LS. This is in accordance with the results of Kliewer & Antcliff (1970), as well as Sidahmed & Kliewer (1980).



**Figure 4.5** The effect of DT x LS x L on the berry mass of Redglobe.

DT = defoliation time;  $DT_{36}$  = 36 DAA;  $DT_{69}$  = 69 DAA;  $DT_{76}$  = 76 DAA;  $DT_{83}$  = 83 DAA; DAA = Days after anthesis;  $LS_0 \times L_0$  = 0% lateral shoot removal and 0% leaf removal;  $LS_0 \times L_{33}$  = 0% lateral shoot removal and 33% leaf removal;  $LS_{50} \times L_0$  = 50% lateral shoot removal and 0% leaf removal;  $LS_{50} \times L_{33}$  = 50% lateral shoot removal and 33% leaf removal;  $LS_{100} \times L_0$  = 100% lateral shoot removal and 0% leaf removal;  $LS_{100} \times L_{33}$  = 100% lateral shoot removal and 33% leaf removal

The reason for this contradiction might be attributed to one, or possibly more, of the reasons discussed below, together with physiological reactions within the plant.

The decreased berry mass can be ascribed to the restriction of the leaf area providing photosynthates (Kliewer, 1970; Kingston & Van Epenhuijsen, 1989; Hunter & Visser, 1990b). Lateral shoot removal lowered berry mass, as was the case in a study of Reynolds & Wardle (1989). The lateral shoots probably acted as full blown production sites (Hale & Weaver, 1962) during the time of their removal. Removal thereof thus deprived the grape berries of assimilates such as sugars. Since glucose

and fructose account for approximately 12 to 27% of berry mass (Winkler *et al.*, 1974), berry mass might have decreased as a result of the removal of the source of sugars which is, according to Kriedeman (1977), the leaves. Similarly, Kliewer & Antcliff (1970) found the greatest reduction in berry mass when the apical leaves of the shoots were removed. Apical leaves are the younger leaves in the canopy and the photosynthetic activity of these recently unfolded leaves is higher (Kriedeman *et al.*, 1970; Koblet, 1977; Hunter & Visser, 1988a; Hunter & Visser, 1988b; Hunter & Visser, 1988c; Hunter *et al.*, 1994).

The reduction in berry mass can also be explained by the changes in microclimate due to defoliation. Due to the fact that shade was not a factor in this study, the reduction in berry weight associated with LS<sub>100</sub> at DT<sub>69</sub> and DT<sub>76</sub> (Fig. 4.5) can probably be attributed to transpiration. Thus, water loss accounts for the loss in berry mass, as was found by McCarthy & Coombe (1999). On the other hand, if the grapes in this study were shaded, then the berries might have been larger and heavier due to reduced transpiration as a result of lower temperatures (Crippen & Morrison, 1986; Reynolds *et al.*, 1986).

Yield per vine was subject to DT x L interaction (Table 4.1). Although it is possible that removal of leaf area supplying assimilates contributing to berry mass lowered total yield to a certain extent, the yield pattern can mainly be attributed to the number of bunches per vine which followed exactly the same trend (data not shown). The number of bunches per vine was allocated when berries were pea size. Furthermore, manual bunch manipulations also could have contributed to the eventual yield obtained.

**Table 4.1** Analysis of variance to test treatment and interaction effects of canopy management practices for different variables of Redglobe grapevines in the Hex River Valley (Grandview), South Africa, 2002/2003.

Source	Total soluble solids (°Brix)			Berry mass (g)			Total yield (kg)		
	DF	MS	P	DF	MS	P	DF	MS	P
Blok	3	2.0764	0.01	3	2.3673	<0.01	3	31.08	0.36
Defoliation time (DT)	3	3.7804	<0.01	3	0.7387	0.21	3	146.52	<0.01
Lateral shoot removal (LS)	2	3.6978	<0.01	2	2.1897	0.01	2	2.64	0.91
Leaf removal (L)	1	1.8635	0.07	1	0.0598	0.72	1	22.13	0.38
DTxLS	6	1.3699	0.03	6	0.7359	0.17	6	22.61	0.58
DTxL	3	1.4610	0.05	3	0.1536	0.81	3	96.45	0.02
LSxL	2	4.7978	<0.01	2	1.2397	0.08	2	13.48	0.63
DTxLSxL	6	1.0631	0.08	6	1.2033	0.03	6	33.03	0.35
<b>Error</b>	64	0.5345		68	0.4734		68	28.81	
<b>Corrected Total</b>	90	89.6138		94	60.5261		94	3169.74	

Non-normality (P<W)

0.87

0.57

0.71

DF = Degrees of freedom.

MS = Mean Square.

P = Probability of F-ratio test.

#### 4.4 CONCLUSIONS

The different treatment combinations applied in this study affected grape quality and berry size. The decreasing effect of lateral shoot removal on TSS was very pronounced at DT<sub>69</sub> and afterwards. TSS was also decreased by LS<sub>100</sub> applied at each DT from véraison and afterwards. When LS x L interactions involved LS<sub>100</sub>, the TSS was also lowered. The impact of a reduction in leaf area at véraison (DT<sub>69</sub>) also manifested in the tendency for a lowered TSS due to L<sub>33</sub> applied at DT<sub>69</sub>. The fact that both leaf and lateral shoot removal at DT<sub>69</sub> (véraison) had a negative (significant with lateral shoot removal) impact on TSS points to the importance of an active leaf area during the time of sugar accumulation and in particular, the week following véraison. Lateral shoot removal had a more pronounced effect on TSS than leaf removal meaning that the lateral shoot leaves are extremely advantageous for TSS accumulation in bunches. The same pattern that was observed for TSS, occurred for the sugar:acid ratio. These two variables is determinant for grape quality and in this case, the lowered values might impact negatively on export quality.

At each DT, LS<sub>100</sub> x L<sub>33</sub> decreased berry mass compared to treatments involving no foliage removal. This, together with the fact that LS<sub>100</sub> x L<sub>0</sub> applied at DT<sub>69</sub> also lowered the berry mass compared to treatments with no foliage removal at that DT stresses the contribution that the lateral shoots make at véraison and, shortly thereafter.

The TSS caused by LS<sub>100</sub> x L<sub>0</sub> and LS<sub>100</sub> x L<sub>33</sub> applied at DT<sub>69</sub> is a point of concern since it affects grape quality and ripening. The reduced berry mass is associated with smaller berries and defoliation treatments, such as those applied in this study, might thus reduce berry size below the standards set by exporters and consumers. Furthermore, these results must be considered together with the effects of defoliation on berry colour, because canopy manipulations hold no merit if one variable is positively influenced, i.e. if the desired colour is obtained, but the TSS is reduced to such an extent that the export quality is impaired.

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# **GENERAL DISCUSSION AND CONCLUSIONS**

## GENERAL DISCUSSION AND CONCLUSIONS

The optimisation of quality in red table grapes has been restricted by the development of excessive colour or insufficient colour in certain cultivars. In South Africa, Flame Seedless, Crimson Seedless, Ralli Seedless, and Redglobe have problems developing sufficient colour in the hot regions. Redglobe, on the other hand, tends to develop excessive colour under certain conditions, making it unacceptable for the Far Eastern markets. Therefore, strategies are needed to either increase or decrease grape colour. Such strategies include canopy management in the form of leaf removal. However, only basic guidelines for these practices are currently available. Canopy management strategies for table and wine grapes have been investigated several times to manipulate grape colour. These are either enhanced or decreased by defoliation. Decreases in colour are evident if the source of assimilates (leaves) is decreased. On the other hand, discreet leaf removal does not necessarily counteract the effect of the enhanced light environment, it rather increases grape colour.

The aims of this study were to test the impact and usefulness of lateral shoot removal (LS) and leaf removal (L) at different defoliation times (DT), after anthesis, on the colour of Redglobe grapes. The focus was in particular to investigate the possibility to obtain the ideal pink berry colour suitable for the Far Eastern markets. The effect of these treatment combinations on grape quality and berry size was determined.

The role of the leaf area and especially the lateral shoot leaf area were emphasised by the effect of the different treatment combinations on grape colour, total soluble solids (TSS) and berry mass. The lighter mean bunch colour obtained, as well as the significant increase in the percentage of bunches with the acceptable colour when defoliation was applied at DT<sub>69</sub> (véraison) points to the importance of an adequate leaf area (for ripening) at the onset of ripening. The importance of an adequate assimilate supply (for colouration) during DT<sub>69</sub> (véraison) and shortly afterwards (1 week after véraison and 2 weeks after véraison) was emphasised by the decreasing effect on anthocyanin concentration when LS<sub>50</sub> and LS<sub>100</sub> was applied at DT<sub>69</sub> (véraison), as well as the significant decrease caused by LS<sub>50</sub> at DT<sub>76</sub> (one week after véraison) and LS<sub>100</sub> applied at DT<sub>83</sub> (two weeks after véraison). This, together with the fact that the mean bunch colour reduced and the percentage of bunches with the acceptable colour increased when LS<sub>50</sub> and LS<sub>100</sub> was applied, points to the role of the lateral shoots as vital suppliers of assimilates for maximum colour development.

Similar observations were made for the TSS. Moderate (LS<sub>50</sub>) to severe (LS<sub>100</sub>) levels of lateral shoot removal at véraison (DT<sub>69</sub>) decreased the TSS. Lateral shoot removal at DT<sub>69</sub> (véraison) had the most negative impact on TSS. Despite the lack of significance, L<sub>33</sub> at DT<sub>69</sub> (véraison) also had a decreasing effect on TSS compared to L<sub>0</sub>. Thus, lateral shoot removal had a more pronounced effect on TSS than leaf

removal. The role of an adequate leaf area, especially lateral shoot leaf area, on TSS during véraison is therefore emphasised. The lateral shoots, in particular, seem to contribute immensely to grape colour and TSS and by retaining them a darker colour and a higher TSS are ensured. It is therefore clear that assimilate supply has to be sustained throughout the ripening period.

At DT<sub>69</sub> (véraison), lateral shoot removal had the most significant impact on berry mass. The importance of leaf area at véraison on berry mass was illustrated by the decreasing effect of LS<sub>100</sub> x L<sub>33</sub> on berry mass when applied at DT<sub>69</sub>. Berry mass was thus reduced as a result of a loss in leaf area. Removal of leaf area thus implies removal of assimilates that contributes to berry mass.

Apart from the role of leaf area, the altered light environment might also have contributed to grape colour because it was possible to alter the light environment in the bunch zone through moderate (LS<sub>50</sub>) and severe (LS<sub>100</sub>) lateral shoot removal at any DT. The mean bunch colour was increased (darker) and the TSS was also enhanced significantly by DT<sub>36</sub> because the photosynthetic capacity of the remaining leaves possibly increased, which ensured sustainable assimilate supply to the bunches during véraison. The increase due to L<sub>33</sub> at DT<sub>83</sub> is ascribed to an increase in the metabolic activity of bunches which enhanced the sink strength and thus increased the demand for assimilates from the leaves. The significant negative relationship between light intensity and grape colour might be due to an inhibiting effect of a high light intensity, or due to an increase in bunch temperature and the negative effects thereof on berry colour.

Although the larger percentage of bunches still had a colour that was darker than the acceptable colour, it was possible to reduce the colour of Redglobe through the application of leaf and lateral shoot removal at different defoliation times. However, the question arises as to whether the treatment combinations used in this study are worthwhile to pursue. Although the percentage of bunches that had an acceptable colour was increased, the extent thereof must be weighed up against the following factors:

1. The mean bunch colour was still not ideal for the targeted markets.
2. TSS and berry mass was also decreased. These treatment combinations therefore have the potential to negatively affect export quality.
3. Whether it can financially be justified in terms of the labour cost, needs to be explored.
4. The impact of terroir must be kept in mind, since differences between regions and vineyards will also affect grape quality.

If the abovementioned is considered, and these treatment combinations are to be applied to reduce grape colour, it must be approached cautiously to ensure that overall grape quality is not lowered below the standards for export.

The role of active leaf area (lateral shoot leaf area) during véraison and shortly afterwards (1 week after véraison and 2 weeks after véraison) was emphasised by the decrease in mean bunch colour and the increase in the percentage of bunches

with the desired colour when defoliation was done during this period. It is thus clear that lateral shoot removal during this stage has the potential to decrease grape colour. However, the negative effect of defoliation (lateral shoot removal and leaf removal) during véraison on TSS rules out such a recommendation. The negative impact of the most severe levels of defoliation ( $LS_{100} \times L_0$  and  $LS_{100} \times L_{33}$ ) on berry mass also does not motivate recommending such a practice. Furthermore, in cases where insufficient colour is already experienced, the practice of defoliation will only aggravate this problem.

Considering the positive effect of  $L_{33}$  at  $DT_{36}$  (pea berry size) on TSS, as well as the fact that  $L_{33}$  applied at  $DT_{36}$  did not have a significant negative effect on berry mass, makes it clear that leaf removal, for the purposes of better canopy aeration, and easier application of cultivation practices is, can be conducted at pea berry size.

In the table grape industry, the commercial tendency is to remove leaves at véraison. This study has shown that colour and TSS are negatively affected (decreased) when leaves and especially lateral shoots are removed at véraison. However, this study has shown that leaf removal two weeks after véraison increased TSS significantly compared to the application of no leaf removal, without affecting colour or berry mass negatively. Therefore, instead of leaf removal at véraison, leaf removal should rather be conducted at least three weeks after véraison, in order to prevent the negative effects of the removal of active leaf area during the ripening period.

Although it was possible to make meaningful conclusions from the results obtained in this study, it has to be stressed that these findings have to be confirmed by the results of one more season in order to make a final recommendation to the industry.