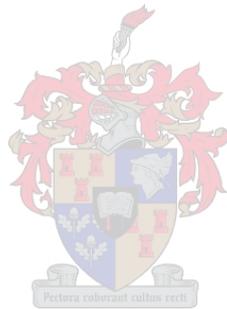


**Berry tannin structure and phenolics evolution in cv.
Cabernet Sauvignon (*Vitis vinifera* L.): effect of light and
temperature**

by

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated) that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 23 November 2015

Summary

This study investigated grape flavonoid (proanthocyanidins, flavonols and anthocyanins) accumulation and composition in grape seeds and skins from Cabernet Sauvignon (*Vitis vinifera* L.) under altered light intensities and temperatures, within the bunch zone in the Stellenbosch Wine of Origin District. Furthermore, the study examined the link between wine sensory properties and the harvest date.

This study was conducted in 2010/2011 and 2011/2012 and comprised of two main treatments with altered bunch microclimates in both seasons: no lateral shoot or leaf removal in the bunch zone (STD) and leaf removal in the bunch zone (LRW). The leaves were removed just after flowering on the western side of the canopy at the fruiting zone level (± 35 –40 cm above the cordon). Furthermore, to study the effect of change in light quality and quantity on fruit growth and composition, supplementary treatments were applied. In 2010/2011, a UV-B reducing sheet was added on the western side of the canopy to the STD (STD-UV-B) and LRW (LRW-UV-B) treatments. During the 2011/2012 season two types of UV-B reducing sheets were installed on both sides of the canopy to exclude the effect that the row direction can have on grape development. The latter resulted in the following treatments: LR (-UV-B, 2xOp50) and LR (-UV-B, 2xUHI).

The accumulated thermal time varied between the treatments and within a season. The 2010/2011 season had a higher accumulated thermal time than the 2011/2012 season. There was a significant difference in the photosynthetic active radiation (PAR) ($p \leq 0.001$) among the treatments indicating that the applied treatment were successful in creating variation in the amount of sunlight intercepted in the bunch zone. There were no significant differences in berry weights in 2010/2011, but a significant difference were observed in 2011/2012 ($p \leq 0.001$). Light and temperature had little effect on grape seed flavan-3-ol monomer and dimer concentration and content. Seed development after flowering potentially influenced light quality and quantity which impacted the seed number and affected flavan-3-ol concentration and content.

Grape skin flavan-3-ol concentration and content differed significantly among the treatments in 2010/2011, but not in 2011/2012. Generally, the seasonal impact was larger than those of the different treatments on flavonoid concentration and content during ripening resulting in significant differences among the treatments at harvest in the 2010/2011 season. However, treatment did not have a significant effect on either concentration or composition of a compound. Grape seed and skin (terminal and extension subunit) composition were influenced by the seasonal impact, rather than the treatment in both seasons. Moreover, the structural characteristics such as the percentage galloylation (%G), percentage prodelphinidins (%P), mean degree of polymerization (mDP) and average molecular mass (avMM) were influenced by seasonal variation.

The accumulation of flavonols was higher in the exposed treatments and low in treatments with UV-B reducing sheets. This indicates that flavonol synthesis is highly dependent on UV-B radiation. The accumulation of anthocyanins commenced at véraison and had two distinct patterns of accumulation in the respective seasons. The 2010/2011 season was characterised by a higher anthocyanin concentration and content compared to the cooler 2011/2012 season.

Grapes were harvested sequentially based on the sugar loading model at the fresh fruit stage (four treatments) and pre-mature (control treatment only) in 2010/2011. In the 2011/2012 season four treatments were harvested at the mature fruit stage. Identified aromas in the respective wines corresponded to the sugar loading model profile. Wine tannin, anthocyanin and flavonol concentrations were the highest in the LRW treatment in 2011/2012. Mouthfeel properties (adhesiveness, coarse, puckery – in and after expectoration) were rated higher in the treatments which were exposed to high light intensities in both seasons.

This research denotes the complex nature of flavonoid biosynthesis and composition. Therefore further research is needed to elucidate impact of the functioning of individual genes in the phenylpropanoid and flavonoid pathways which have an influence on the final concentration, content and composition of flavonoids at harvest.

Opsomming

Hierdie studie ondersoek druifflavonoïde (proantosianidiene, flavonole en antosianiene) se akkumulاسie en samestelling in druifsaad en -doppe van Cabernet Sauvignon (*Vitis vinifera* L.) onder veranderde ligintensiteite en temperature binne die trossone in die Stellenbosch Wyn van Oorsprong Distrik. Verder het die studie die verband tussen wysensoriese eienskappe en die oesdatum ondersoek.

Die studie is gedurende die 2010/2011- en 2011/2012-groeiseisoen uitgevoer en bestaan uit twee hoofbehandelings met veranderde mikroklimatiese toestande in beide seisoene: geen laterale loot- of blaarverwydering in die trossone (STD) nie en blaarverwydering in die trossone (LRW). Die blare is verwyder net tot ($\pm 35\text{--}40$ cm bo die kordon). Om die effek van ligkwaliteit en -kwantiteitverandering op die vrugontwikkeling en -samestelling verder te bestudeer, is aanvullende behandelings toegepas. In 2010/2011 is 'n UV-B-vermindingsplaat aan die westekant van die lower van die STD (STD-UV-B) en LRW (LRW-UV-B) -behandelings bygevoeg. Gedurende die 2011/2012- seisoen is twee tipes UV-B-vermindingsplate op beide kante van die lower geïnstalleer om die uitwerking wat die rygting op druifontwikkeling kan hê, uit te sluit. Laasgenoemde het tot die volgende behandelings, LR (-UV-B, 2xOp50) en LR (-UV-B, 2xUHI), gelei.

Die geakkumuleerde termiese tyd het tussen die behandelings en binne 'n seisoen gewissel. Die 2010/2011-seisoen het 'n hoër geakkumuleerd termiese tyd as die 2011/2012- seisoen. Daar was 'n beduidende verskil in die fotosintetiese aktiewe bestraling (PAR) ($p \leq 0.001$) onder die behandelings, wat aandui dat die toegepaste behandeling suksesvol was om variasie te skep in die hoeveelheid sonlig wat in die trossone onderskep is. Daar was geen betekenisvolle verskille in korrelgewigte in 2010/2011 nie, maar 'n beduidende verskil is waargeneem in 2011/2012 ($p \leq 0.001$). Lig en temperatuur het 'n geringe uitwerking op druifsaad se flavan-3-ol-monomeer en dimeerkonsentrasie en -inhoud. Saadontwikkeling na die blomperiode beïnvloed potensieel die ligkwaliteit en -kwantiteit, ingesluit die saad getal, en beïnvloed ook die flavan-3-ol-konsentrasie en -inhoud.

Die druifdop flavan-3-ol-konsentrasie en -inhoud het beduidend verskil tussen die behandelings in die 2010/2011-seisoen, maar nie in die 2011/2012-seisoen nie. Oor die algemeen was die seisoenale impak groter as dié van die verskillende behandelings op flavonoïd konsentrasie en -inhoud tydens rypwording, wat aanleiding gegee het tot aansienlike verskille tussen die behandelings by die oes in die 2010/2011-seisoen. Die behandeling het egter nie 'n beduidende uitwerking op die konsentrasie of samestelling van 'n verbinding gehad nie. Die druifsaad- en dop-(terminale en ekstensiesubeenheid) samestelling is beïnvloed deur die seisoen, eerder as die behandeling in beide seisoene. Verder is die strukturele eienskappe, soos die persentasie galloilasie (% G), persentasie prodelphinidiene (% P), gemiddelde graad van polimerisasie (mDP) en gemiddelde molekulêre massa (avMM) deur seisoenale variasie beïnvloed.

Die akkumulاسie van flavonole was hoër in die blootgestelde behandelings en laag in behandelings met UV-B-vermindingsplate. Dit dui daarop dat flavonolsintese hoogs afhanklik is van UV-B-bestraling. Die akkumulاسie van antosianiene begin by deurslaan en het twee afsonderlike patrone van die akkumulاسie in die onderskeie seisoene getoon. Die 2010/2011-seisoen is gekenmerk deur 'n hoër konsentrasie antosianien en inhoud in vergelyking met die koeler 2011/2012-seisoen.

Druive is opeenvolgende geoes, gebaseer op die suikerakkumulasiemodel by die varsvrugte- (vier behandelings) en voor-volwasse-stadia (kontrole behandeling alleen) in die 2010/2011-seisoen. In die 2011/2012-seisoen is vier behandelings op die volwassevrugtestadium geoes. Die wynaromas wat in die onderskeie wyne geïdentifiseer is, stem ooreen met die suikerakkumulasiemodelprofiel.

Wyntannien-, antosianien- en flavonolkonsentrasies was die hoogste in die LRW-behandeling in die 2011/2012-seisoen. Mondgevoeleienskappe (klewerigheid, grofheid, sametrekend - in en na ekspektorasië) is hoër in die behandelings wat blootgestel was aan hoë ligintensiteit in beide seisoene.

Hierdie navorsing dui op die komplekse aard van flavonoïdbiosintese en -samestelling. Verdere navorsing is dus nodig op die impak van die funksionering van individuele gene in die fenielpropanoïd- en flavonoïd paaie, wat 'n invloed op die finale konsentrasie, inhoud en samestelling van flavonoïede by oes het.

**This dissertation is dedicated to my husband Maarten Blancquaert
and son Josh Mathis Blancquaert**

Biographical sketch

Erna Blancquaert matriculated from Klein Nederburg Secondary in 2001. She studied BScAgric (Viticulture and Oenology) at Stellenbosch University from 2002, graduating in 2005. She completed her MScAgric (Viticulture) entitled "The ecophysiological characterisation of terroirs in Stellenbosch: the contribution of soil surface colour" graduating in March 2008. Erna has been employed as a junior lecturer in Viticulture at the Department of Viticulture and Oenology since May 2009.

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Preface

This dissertation is presented as a compilation of six chapters. Each chapter is introduced separately and is written according to the guide to authors of the South African Journal of Enology and Viticulture (SASEV).

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LIST OF ABBREVIATIONS

| | |
|--------------------|--|
| ANR | Anthocyanidin reductase |
| ANS | Anthocyanidin synthase |
| avMM | Average molecular mass |
| B1 | Dimer Ec-(4 β -8)-Cat |
| B2 | Dimer Ec-(4 β -8)-Ec |
| CHS | Chalcone synthase |
| CHI | Chalcone isomerase |
| DAA | Days after anthesis |
| DD | Degree days |
| DFR | Dihydroflavonol 4-reductase |
| DSA | Descriptive Sensory Analysis |
| F3'H | Flavanone 3 β -hydroxylase |
| F3'5'H | Flavanone 3,5 β -hydroxylase |
| HPLC-DAD | High performance Liquid Chromatography diode array |
| LAR | Leucoanthocyanidin reductase |
| LDOX | Leucoanthocyanidin dioxygenase |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| LRW | Leaf Removal West side of the bunch zone just after flowering |
| LRW-UV-B | Leaf Removal West and UV-sheet (Perspex'® Opal 050) on the western side of the bunch |
| LR (-UV-B, 2xOp50) | Leaf removal on both sides of the canopy (in the bunch zone) and ('Perspex'® Opal 050) on both sides of the bunch zone |
| LR (-UV-B, 2xUHI) | Leaf removal on both side of the canopy (in the bunch zone) and UV-sheet (UHI) extruded clear Acrylic was used on both sides of the bunch zone |
| mDP | Mean degree of polymerisation |
| PAR | Photosynthetic active radiation |
| RP-HPLC | Reverse phase high performance liquid chromatography |
| STD | Standard (control) treatment |
| STD-UV-B | Control treatment and UV-sheet (Perspex'® Opal 050) on the western side of the bunch |
| TSS | Total soluble solids |
| PAL | Phenylalanine ammonia-lyase |
| UDP-Glucose | Uridine diphosphate glucose |
| UFGT | Flavonoid-3-O-glucosyltransferase |
| UV | Ultraviolet radiation |

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Chapter 1

Introduction and project aims

INTRODUCTION AND PROJECT AIMS AND OBJECTIVES

1.1 INTRODUCTION

Grape berry phenolic compounds are widely described in literature (Ribéreau-Gayon, 1964; Cheynier *et al.*, 1998; Cheynier *et al.*, 2006). Phenolics can be divided in two main groups: flavonoids and non-flavonoids, of which the flavonoids are the most important. The two best known groups of flavonoids are the anthocyanins, which are responsible for the red colour in grapes, and the condensed tannins (also called proanthocyanidins), which are responsible for some major wine sensorial properties (astringency, browning and turbidity) and are involved in the wine ageing processes (Ricardo da Silva *et al.*, 1991a).

Differences in phenolics have been reported between grape seed and skins (Somers, 1971; Gawel, 1998; Santos-Buelga & Scalbert, 2000). Grape seed proanthocyanidins comprise of monomers constituting of (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-O-gallate (procyanidins) while grape skins comprise of both procyanidins and prodelfphinidins monomers ((-)-epigallocatechin and (+)-gallocatechin) (Prieur *et al.*, 1994; Souquet *et al.*, 1996). Oligomers are formed through interflavan linkages (C₄-C₈ or C₄-C₆) between the constituting monomers. The predominant interflavan bond is C₄-C₈ while a C₄-C₆ occurs less frequently. Proanthocyanidins can be found in the solid parts of the bunch (skins, seeds, stems and trace amounts in the pulp) and they are extracted during the winemaking process (Jordão *et al.*, 2001).

The composition and concentration of proanthocyanidins has been extensively studied in grapes and wine (Su & Singleton, 1969; Lea *et al.*, 1979; Romeyer *et al.*, 1986; Ricardo-da-Silva *et al.*, 1991b, Santos-Buelga *et al.*, 1995). The highest concentration of proanthocyanidins in grapes is found in the grape seeds, followed by (in decreasing order) stems, skins and pulp (Ricardo-da-Silva *et al.*, 1992a, b; Sun *et al.*, 2001; Downey *et al.*, 2003). Polymerisation levels are lower in seeds and stems compared to skin tannins (Prieur *et al.*, 1994; Souquet *et al.*, 1996; Souquet *et al.*, 2000). Total tannin content is reported to be significantly higher in seeds than skins, although the average degree of polymerisation (mDP) is generally much lower than (± 10 subunits) that of skins (± 30 subunits) (Prieur *et al.*, 1994; Escribano-Bailón *et al.*, 1995; Souquet *et al.*, 1996).

Furthermore, seed tannins are more galloylated (<35%) than skin tannins (<5%). However, Obreque-Slier (2010) reported galloylation percentages of 19% in skins and 16% in seeds at harvest for Cabernet Sauvignon grapes in Chile.

Harbertson *et al.* (2002) found that tannin levels in the skins (on a per berry basis) changed very little from véraison to harvest. Kennedy *et al.* (2001) found an increase in skin mDP throughout ripening in Shiraz under Australian conditions although the concentration of skin tannins (mg/g fresh weight) decreased. Variable results have been obtained in the literature for seed tannin evolution which can be ascribed to the analytical methods used during analysis. Obreque-Slier *et al.* (2010) determined a decrease in mDP for grape seed tannins with ripening. These qualitative differences are very important for wine quality and how this tannin will be perceived in the wine matrix (mouthfeel). It has been shown that both the mDP and galloylation influence tannin contribution to mouthfeel and their interaction in wine (Vidal *et al.*, 2003).

Numerous studies reported changes in grape flavonoid composition due to cultivar, season, location and viticultural practices (Oszmianski & Sapis, 1989; Jackson & Lombard, 1993; Katalinic & Males, 1997; de Freitas & Glories, 1999). Therefore, it is clear that the flavonoid content can be influenced by biotic and abiotic stimuli experienced in the particular season (Conde *et al.*, 2005; Obreque-Slier *et al.*, 2010). The response of flavonoids to light and temperature has been studied (Kliewer *et al.*, 1967; Kliewer & Antcliff, 1970; Price *et al.*, 1995; Haselgrove *et al.*, 2000; Tarara *et al.*, 2000; Bergqvist *et al.*, 2001; Spayd, 2002; Cortell & Kennedy, 2006; Ristic *et al.*, 2007; Berli *et al.* 2011; Gregan *et al.* 2012; Koyama *et al.*, 2012). Even if the microclimate was altered in many of these studies, there has been very little focus on the impact of UV-B radiation on flavonoid evolution and composition in grape seeds and skins. Understanding the consequences of UV-B radiation on phenolic compounds during berry growth is a major objective of this study as it has been shown that ultra-violet radiation has increased in the Southern Hemisphere over the last two decades (Gregan *et al.* 2012). It is therefore important to comprehend the potential scientific and economic impact for grape production in naturally high UV-light environments.

Plants have mechanisms for protection from herbivores, such as the accumulation of phenolic compounds in plant tissues. This causes an astringent (drying) sensation during consumption, as these phenolic compounds bind to the salivary proteins resulting in a decrease in lubrication (Bieza & Rodrigo, 2001; Mazid *et al.*, 2011). Additionally, carotenoids serve as light screens or internal traps protecting plants against high levels of solar radiation (Merzlyak & Solovchenko, 2002). The grapevine further responds to UV radiation by induction of flavonol biosynthesis in the leaves and berry skins (Kolb *et al.*, 2001 & 2003; Koyama *et al.* 2012). However, the regulation of proanthocyanidin accumulation and composition in seeds and skins in response to UV light in grapes is largely unknown. Furthermore, the sensory attributes of wines made under the former conditions are unfamiliar.

1.2 PROJECT AIMS

The study aims to make a contribution to the knowledge of flavonoid biosynthesis and chemical structures under altered microclimatic conditions in Cabernet Sauvignon grown in the Stellenbosch Wine of Origin District. The layout was designed to achieve these aims in an experimental vineyard to study the role of light quantity and quality at the fruit zone level (microclimate), while temperature was monitored in all treatments and water controlled to avoid constraints. Light quantity and thresholds were compared by having treatments with 100% of shaded bunches and treatments with 100% of exposed bunches. For shaded and exposed bunches, light quality was studied by comparing treatments where 99% of the UV-B were suppressed.

In order to achieve these aims, a field experiment was designed in a Cabernet Sauvignon vineyard during two consecutive seasons (2010/2011 and 2011/2012) in the Stellenbosch Wine of Origin District to investigate the following objectives:

- I: **Determination of the effect of light quantity, quality and temperature on seed and skin tannin biosynthesis and composition during berry development**
- II: **Determination of the effect of light quantity and quality and temperature on flavonol biosynthesis during berry development**

III: Determination of the effect of light quantity, quality and temperature on anthocyanin biosynthesis and composition during berry development

VI: Determination of the effect of light quantity and quality at the fruit zone level on wine sensory attributes

The following chapters describe the literature on this topic, experimental techniques which were applied and the investigations which were undertaken. Flavonoid biosynthesis during berry ripening is discussed in Chapter 3, while the potential impact of altered light and temperature conditions on tannin and anthocyanin composition during berry ripening in Cabernet Sauvignon (*Vitis vinifera* L.) are discussed in Chapter 4. The link between berry composition, wine composition and sensorial properties are discussed in the Chapter 5 followed by a general conclusion and future perspective in Chapter 6.

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Chapter 2

Literature review Phenolic compounds in the grape berry

LITERATURE REVIEW

2.1 INTRODUCTION

Globally, grapes are one of the most widely cultivated crops. In 2012 grapes covered 7.5 million hectares of arable land (OIV, 2013). Most of the grapes are fermented into wine, or used for table grapes and raisin production. Many researchers have shown that the growing site (climate and soil) and viticultural practices have a direct impact on grape maturity and the phenolic composition of the berry. Flavonoids play an important role in grape and wine quality. Therefore, an in depth understanding of flavonoid development and composition during berry development is needed under South African climatic conditions.

Grape berry development involves a complex series of physical and biochemical changes. These can be divided into three major phases: green growth, the lag phase and ripening phase. During these three phases, primary and secondary metabolites are synthesised under complex gene and enzymatic control. Primary metabolites, such as sugars, amino acids and organic acids are involved in normal growth, development and reproduction of plant species. Secondary metabolites, such as phenolics and stilbenoids have ecological functions, such as defence against predators, parasites and diseases (Conde *et al.*, 2007, Ali *et al.*, 2010). Phenolic compounds have a diversity of structures and can be divided into two main groups, namely flavonoids and non-flavonoids (Cheynier *et al.*, 2006). The phenolic compounds of interest in this study are the flavonoids and they will be discussed in depth in the following paragraphs.

2.2 FLAVONOID BIOSYNTHESIS

Flavonoid biosynthesis is the result of the shikimate and phenylpropanoid pathways (Dewick & Haslam, 1969; Heller & Forkmann, 1988). Flavonoids are characterised by two benzene rings (rings A and B), bonded by an oxygenated heterocyclic pyran ring (ring C); they therefore possess a C₆–C₃–C₆ skeleton (Fig. 2.1) (Somers & Vérette 1988; Ribéreau-Gayon, 2000). The heterocyclic ring is closed in most flavonoids, but remains open in chalcones and dihydrochalcones (Stafford,

1990). Variation in the oxidation state and substitution on ring C defines the different classes of flavonoids (Fig. 2.1).

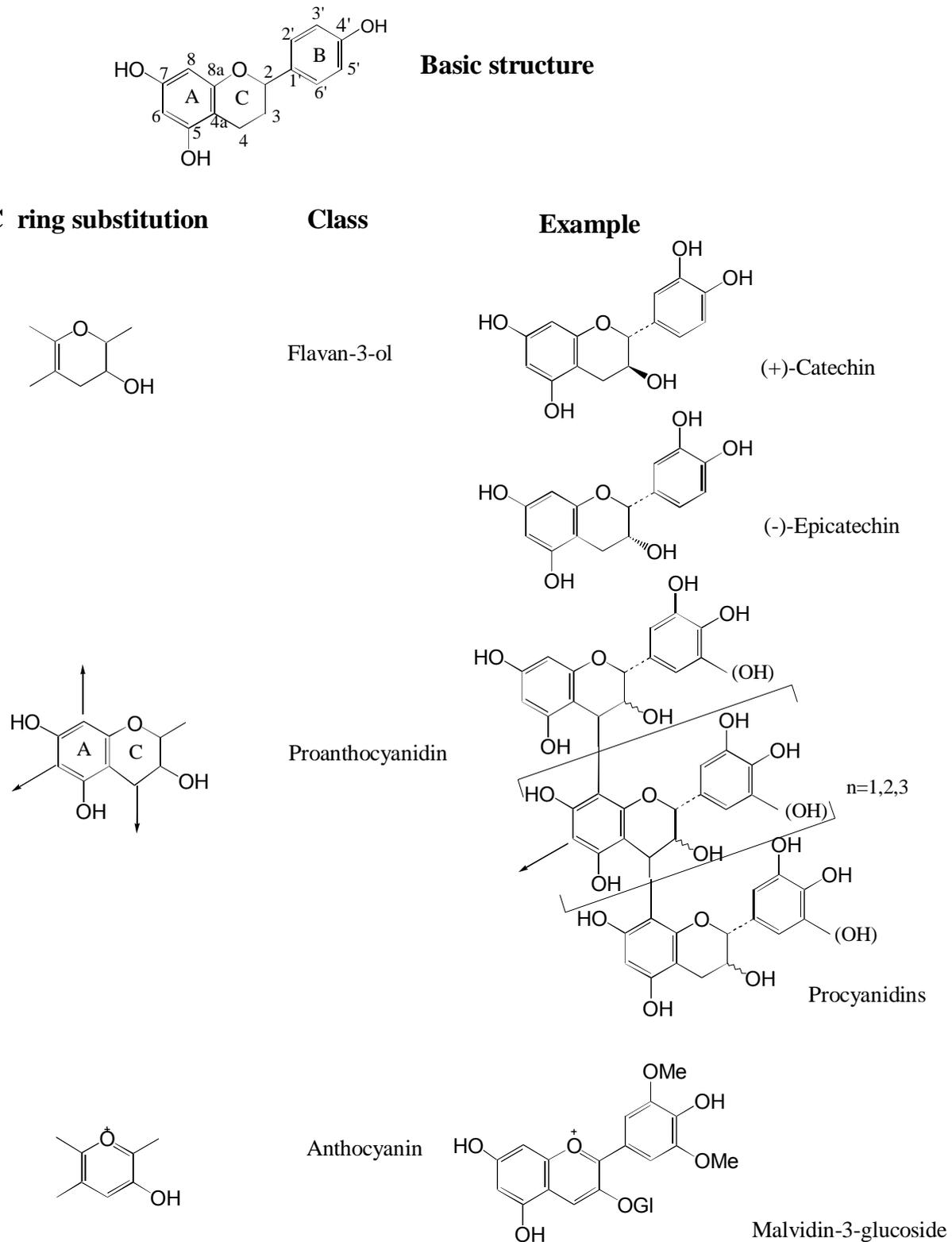


Figure 2.1. Flavonoid nomenclature (Somers & Vérette, 1988).

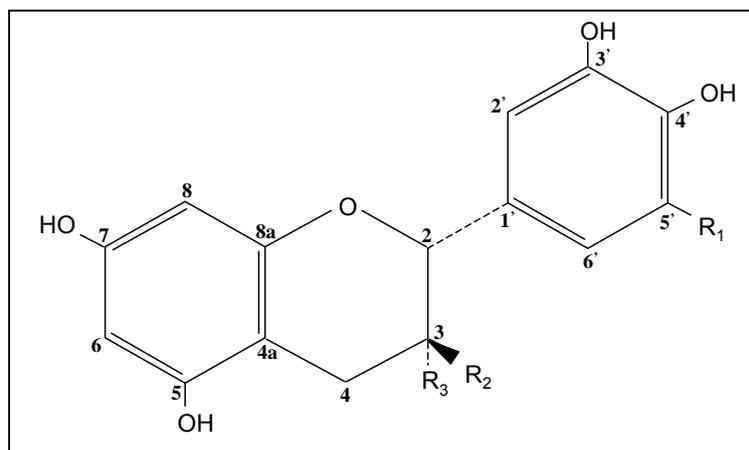
2.2.1 Flavan-3-ols

Flavan-3-ols are the most abundant class of flavonoid compounds in grape berries (Adams, 2006; Terrier *et al.*, 2009). Flavan-3-ols comprise of monomers (catechins), oligomers and polymers. They are also referred to as proanthocyanidins or condensed tannins (Cheynier & Rigaud, 1986; Ricardo-da-Silva *et al.*, 1991a, b). The major flavan-3-ol monomers in grape seeds are (+)-catechin, (-)-epicatechin and a galloylated form (-)-epicatechin-3-O-gallate (Fig. 2.2). (-)-Epigallocatechin and trace amounts of (+)-gallocatechin are also found in grapes. Flavan-3-ols in grape skins are represented by (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-O-gallate and (-)-epigallocatechin. The presence of (+)-gallocatechin in *Vitis vinifera* has been reported while (+)-catechin-2-gallate and (+)-gallocatechin-3-gallate have been detected in some non-*Vinifera* varieties (Piretti *et al.*, 1976; Czochanska *et al.*, 1979; Lee & Jaworski, 1987). Condensed tannins are formed during the polymerisation process and comprise of flavan-3-ol subunits connected by interflavan linkages (C₄-C₈ or C₄-C₆) (Fig. 2.3) (Haslam, 1998).

Proanthocyanidins, or condensed tannins, are mostly situated in the solid parts of the cluster (skins, seeds and stems) and to a lesser degree in the pulp (Sun *et al.*, 1999; Jordão *et al.*, 2001; Ó-Marques *et al.*, 2005). Seeds have the highest concentration of procyanidins (Ricardo-da-Silva *et al.*, 1992a). Within the grape berry, proanthocyanidins or condensed tannins are situated in the hypodermal layers of the skin and the soft parenchyma of the seed between the cuticle and the hard seed coat (Adams, 2006).

Skin tannins exhibit a higher degree of polymerisation than seed tannins expressed as the mean degree of polymerisation (mDP) (Adams, 2006). Kennedy *et al.* (2000a) and Downey *et al.* (2003a) found proanthocyanidin polymers with 25–40 subunits comprising of equal proportions (-)-epicatechin and (-)-epicatechin-3-O-gallate and (+)-catechin as terminal subunits. The polymer length remained constant until véraison and decreased to about 30 subunits four weeks after véraison and to approximately 20 subunits at harvest (Kennedy *et al.*, 2000; Downey, *et al.*, 2003a). The mDP in seeds varies between three to sixteen subunits, comprising of (+)-catechin,

(-)-epicatechin and (-) epicatechin-3-O-gallate (Cheynier *et al.*, 1998; Downey *et al.*, 2003a; Bogs *et al.*, 2005; Mané *et al.*, 2007). From fruit-set to one week pre-véraison, polymer length remained between five and six subunits. An increase in the terminal subunits at one week pre-véraison exceeded the accumulation of extension subunits, resulting in a decrease in polymer length to four subunits (Downey *et al.*, 2003a). Various average ranges of mDP's are reported for proanthocyanidins in grape berries. Prieur *et al.* (1994), Moutounet *et al.* (1996) and Labarbe *et al.* (1999) reported seed mDP ranging between 8 and 16 units for grape seeds, whilst Mané *et al.* (2007) reported values between 3 and 4 units. Skin mDP ranges between 27 and 45 units in average (Moutounet *et al.*, 1996; Souquet *et al.*, 1996; Mané *et al.*, 2007).



| | R ₁ | R ₂ | R ₃ |
|----------------------|----------------|----------------|----------------|
| (+)-catechin | H | OH | H |
| (+)-gallocatechin | OH | OH | H |
| (-)-epicatechin | H | H | OH |
| (-)-epigallocatechin | OH | H | OH |

Figure 2.2. Chemical structures of flavanols (Moutounet *et al.*, 1996)

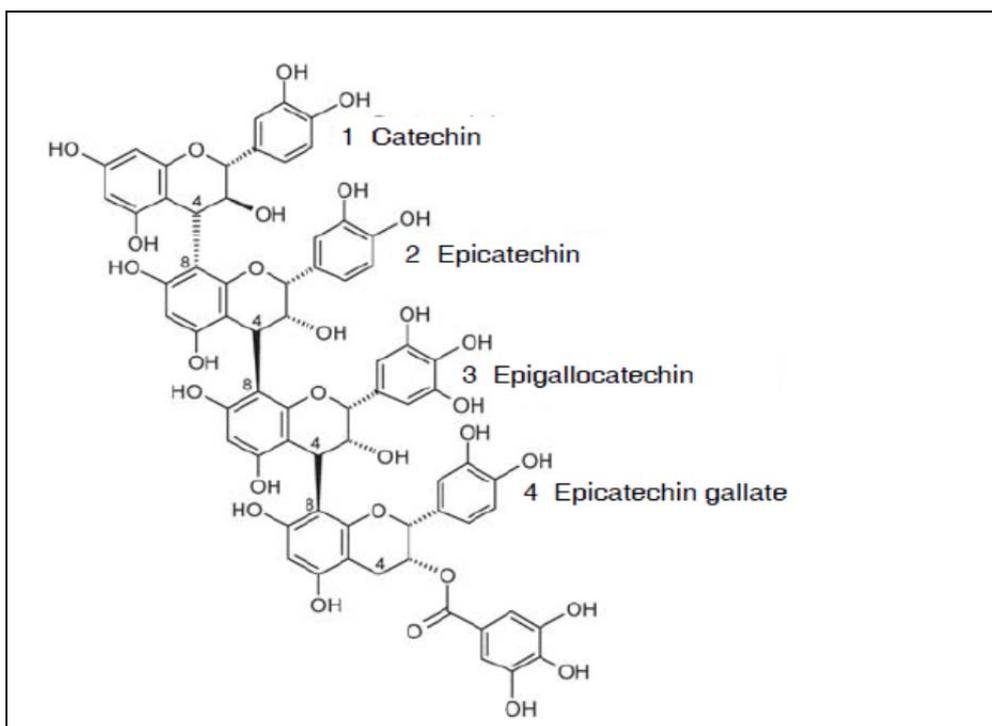


Figure 2.3. Condensed tannin and the four subunits: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and (-)-epicatechin gallate (Adams, 2006).

The flavan-3-ols are synthesised as part of the phenylpropanoid pathway (Fig. 2.4). Other secondary metabolites such as lignins, lignans, stilbenes and hydroxycinnamic acids are also produced in this pathway (Schwinn & Davies, 2004). Phenylalanine obtained via the shikimate pathway and malonyl-CoA derived from citrate produced by the tricarboxylic acid cycle (Davies & Schwinn, 2006) is the main flavonoid precursors. Phenylalanine is converted to the activated hydroxycinnamic acid p-coumaroyl-CoA by three enzymatic conversions, catalysed by phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase and 4-coumarate CoA ligase. Malonyl-CoA is required for flavonoid biosynthesis and acts as an “extender” molecule and acid moiety donor for acylation of flavonoid glycosides that form the first flavonoid. The point of entry into the flavonoid pathway is the formation of a chalcone (mostly naringenin), which is formed from p-coumaroyl-CoA and three acetate units from malonyl-CoA through the action of chalcone synthase (CHS). The chalcone then give rise to a flavonoid with a C15 backbone, which is directly or indirectly converted to a range of other flavonoids in a pathway of intersecting branches with intermediate compounds (Schwinn & Davies, 2004). A flavonoid with a heterocyclic C-ring is isomerised to a flavanone through the activity of chalcone isomerase (CHI) (Schwinn & Davies, 2004). Hydroxylation of flavanones is catalysed by flavanone 3 β -hydroxylase (F3H) which results

in dihydroflavonols. The latter is then subjected to catalysis by dihydroflavonol 4-reductase (DFR) resulting in leucocyanidins (which are colourless and unstable compounds). Proanthocyanidins are formed through interflavan linkages between the flavan-3-ol building blocks. Flavan-3-ols are formed via two biosynthetic routes: (i) 2,3-*trans*-flavan-3-ols are produced from leucocyanidins by leucoanthocyanidin reductase (LAR) and (ii) 2,3-*cis*-flavan-3-ols from cyanidin by anthocyanidin reductase (ANR). LAR removes the 4-hydroxyl from leucocyanidins to produce 2,3-*trans*-flavan-3-ols while the ANR converts cyanidin to the corresponding 2,3-*cis*-flavan-3-ols (Tanner *et al.*, 2003; Xie *et al.*, 2003) (Fig. 2.4). Flavan-3-ols are synthesised in the cytoplasm and transported to the vacuoles where polymerisation occurs and proanthocyanidins accumulate.

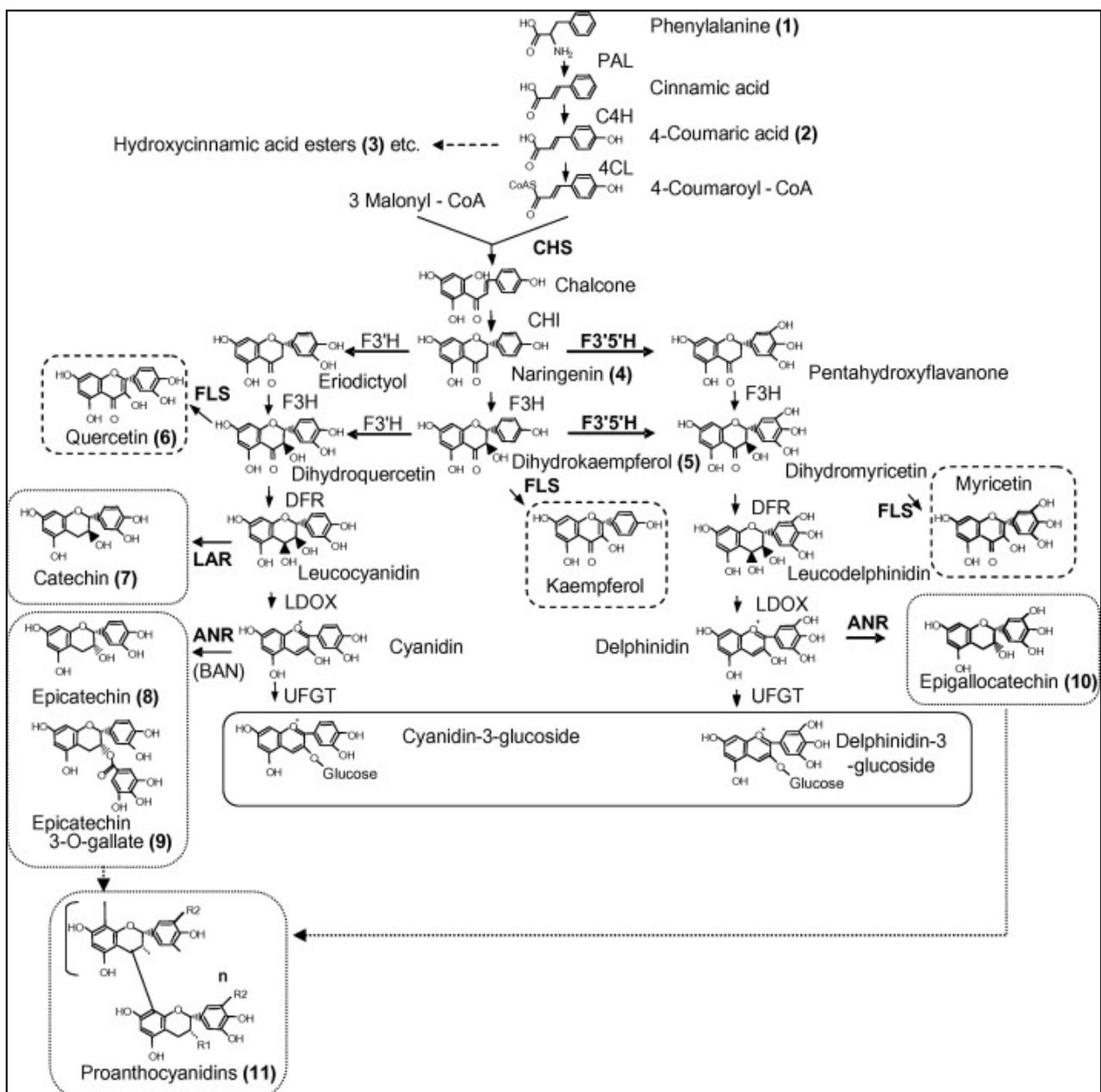


Figure 2.4. Phenylpropanoid pathway in the grape berry (Koyama *et al.*, 2012).

2.2.2 Anthocyanins

In the final step of anthocyanidin-3-O-glycoside biosynthesis pigments are formed through the activity of anthocyanidin synthase also referred to as leucoanthocyanidin dioxygenase (LDOX), and an anthocyanidin-3-glycosyltransferase to the corresponding anthocyanin (described in 2.2.1) (Fig. 2.5) (Davies & Schwinn, 2006). Schwinn & Davies (2004) suggested that hydroxylation has a key impact on anthocyanin colour. An increase in hydroxylation of the B-ring results in the shift in colour from red to blue, determining the type of anthocyanin produced.

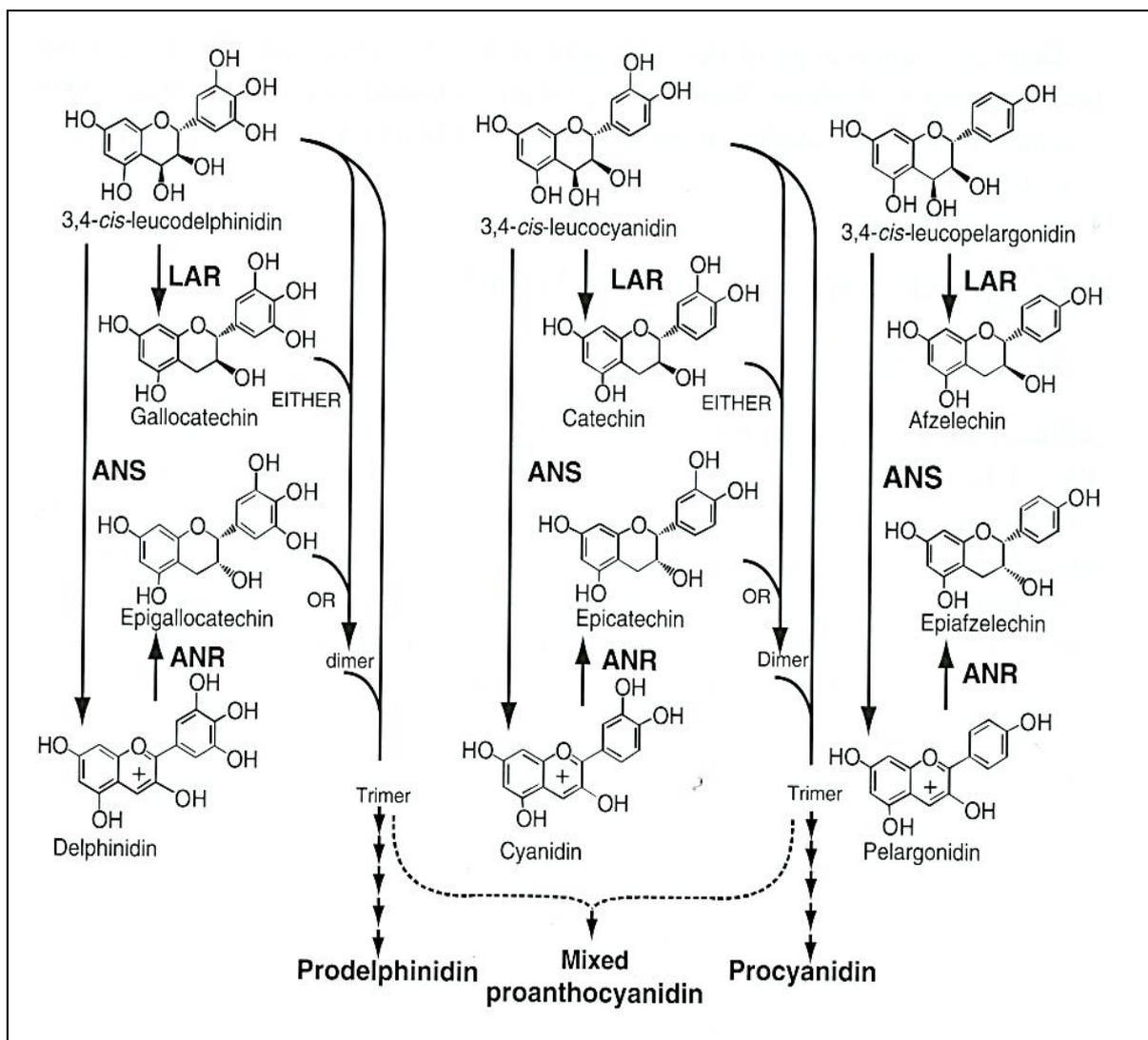


Figure 2.5. Biosynthesis of flavan-3-ols and proanthocyanidins (Tanner, 2006).

2.2.3 Flavonols

Flavonols are flavonoids that are found in higher plants in glycosidic forms formed during the flavonoid biosynthetic pathway (Mattivi *et al.*, 2006). Synthesis of flavonols predominately occurs in the grape skin (Price *et al.*, 1995). Quercetin-3-O-glucoside and quercetin-3-O-glucuronide have been identified as the main flavonols within the grape berries (Cheynier *et al.*, 1986; Price *et al.*, 1995; Downey *et al.*, 2003b). Various researchers have investigated the molecular structure and the expression of the main enzymes, and a general pathway for flavonol biosynthesis has been established (Downey *et al.* 2003b; Bogs *et al.* 2006; Castellarin *et al.* 2006; Mattivi *et al.* 2006) (Fig. 2.6).

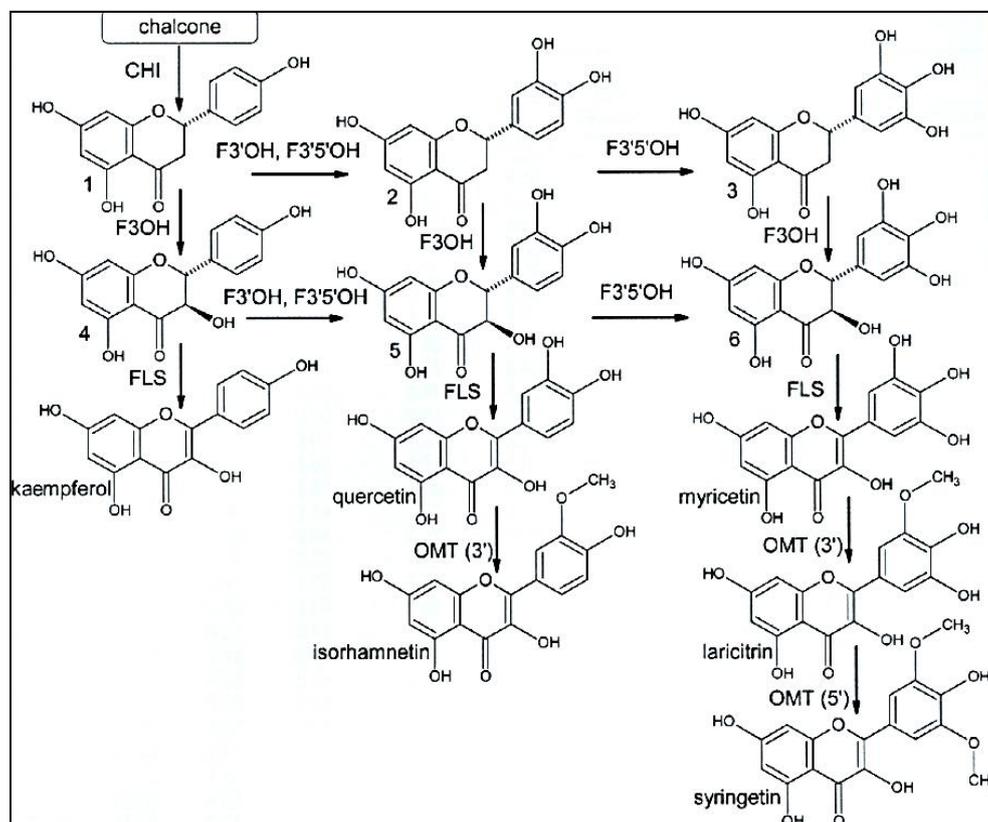


Figure 2.6. General pathway for flavonol biosynthesis (Mattivi *et al.*, 2006).

2.3 CHANGES IN FLAVONOID CONTENT WITH RIPENING

It is clear that the flavonoid biosynthesis is influenced by berry maturation. The overall tendency in grape seed tannin biosynthesis studied in grape cultivars such as Cabernet Sauvignon, Shiraz and Pinot noir indicated that the maximum concentration was reached at véraison, decreased thereafter, and remained constant during maturation (Kennedy *et al.*, 2000a; Kennedy *et al.*, 2000b; Jordão *et al.*, 2001; Downey *et al.*, 2003b; Downey *et al.*, 2006). Other studies reported that the concentration and composition were influenced by grape variety and the vintage (Ricardo-da-Silva *et al.*, 1991c & 1992b; Jordão *et al.*, 2001). Ribereau-Gayon *et al.* (2000) suggested that grape cultivars such as Cabernet franc, Pinot noir, Grenache and Tempranillo generally have higher levels of seed tannin compared to Cabernet Sauvignon and Merlot noir. As for grape skin tannin, investigators found a higher concentration at fruit set and noted a decrease and then an increase around véraison, followed by another decrease (Kennedy *et al.*, 2001; Kennedy *et al.*, 2002a, Downey *et al.*, 2003a).

Various researchers suggested that anthocyanin development and composition are influenced by cultivar, climatic conditions (abiotic factors such as light, temperature and water) and viticultural practices (Kliewer & Torres, 1972; Jackson & Lombard, 1993; Dokoozlian & Kliewer, 1996; Bergqvist *et al.*, 2001; Spayd *et al.*, 2002; Downey *et al.*, 2004). Downey *et al.* (2003b) reported that the total concentration of flavonols in berries was low pre-véraison and then increased post-véraison. Flavonoid composition clearly changes with ripening and research indicate potential influences of environmental parameters such as temperature and light.

2.4 ENVIRONMENTAL FACTORS AFFECTING DEVELOPMENT OF PHENOLIC COMPOUNDS IN GRAPE BERRIES

2.4.1 Cultivar and seasonal variability affecting flavonoid development

Phenolic development is affected by seasonal variation and cultivar characteristics (Cohen & Kennedy, 2010). Furthermore, the cultivation practices and resulting microclimate around the developing fruit affect the fruit composition e.g. total soluble solids, flavan-3-ol monomers, proanthocyanidins and pigmented polymers (Cortell *et al.*, 2005). Environmental factors such as sunlight, temperature, ultra-violet radiation (UV) and plant water status play a role in the accumulation of proanthocyanidins, flavonols and anthocyanins (Table 2) (Crippen & Morrison, 1986; Kennedy *et al.*, 2002b; Ojeda *et al.*, 2002; Downey *et al.*, 2004; Mori *et al.*, 2005 & 2007; Buchetti *et al.*, 2011; Gregan *et al.*, 2012; Koyoma *et al.*, 2012).

2.4.1.1 Light

Plant metabolism is greatly dependent on solar effects (Cohen & Kennedy, 2010). Zucker (1965) found that the functioning of PAL is affected by white light (visible light spectrum). Dokoozlian & Kliewer (1996) found that exposure of berries during growth stages I and II resulted in an increased PAL which resulted in a higher anthocyanin concentration. Investigations into the effect of light on grape composition resulted in varying outcomes, as described below.

Haselgrove *et al.* (2000) and Spayd *et al.* (2002) studied the impact of light on Shiraz and Cabernet Sauvignon, respectively. According to Haselgrove *et al.* (2000), berries that received high levels of sunlight had high levels of quercetin-3-glucoside and low levels of malvidin-3-glucoside. The total anthocyanin levels were variable between treatments and dependent on the degree of bunch exposure. Spayd *et al.* (2002) found that berries that were exposed to sunlight had increased total skin monomeric anthocyanins regardless of the ambient temperature. Subsequent investigations showed that low light also reduced color in Emperor table grapes and in Pinot noir (Kliewer 1970,

1977). Similar results were later reported in Shiraz (Smart *et al.* 1985) and Cabernet Sauvignon (Morrison & Noble 1990, Hunter *et al.* 1991, Dokoozlian & Kliewer 1996). Together these results created a strong impression that light was necessary for colour formation in grapes, an impression reinforced by observations from other plant species, such as apple, where light is an absolute requirement for anthocyanin biosynthesis (Siegelman & Hendricks 1958, Chalmers & Faragher 1977, Lancaster 1992, Dong *et al.* 1998). Wicks & Kliewer (1983) and Dokoozlian & Kliewer (1996), suggested that low light intensities reduced anthocyanins and some other flavonoids, while increasing light intensity increased the flavonoid content of grapes. Results of these studies indicate that light is important in the colour formation in grapes. This theory is also supported for anthocyanin biosynthesis in other plant species such as apples (Siegelman & Hendricks, 1958; Lancaster, 1992; Dong *et al.*, 1998).

However, some investigations found contradictory results. Crippen & Morrison (1986) found that there were no significant differences at harvest between sun-exposed and shaded grapes although there were differences during berry development. Others reported that high light intensities ($>100 \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$) resulted in decreased anthocyanin levels (Bergqvist *et al.* 2001; Spayd *et al.*, 2002). Ristic *et al.* (2007) found that the anthocyanin content of grapes in a shade box treatment versus the non-shaded treatment did not differ significantly. Only a change in the deoxygenated anthocyanins was observed, and an increase in seed tannin and decrease in skin tannin between the shaded and non-shaded treatments. In a study on vine vigour, Cortell *et al.* (2005) found that seed proanthocyanidins composition in grapes were slightly different between vigour zones identified as high, medium and low, but the total amount was not affected. The total amount of epigallocatechin and mDP values of the skin proanthocyanidins were increased in the low vigour vines (vigour index of 0.09 and 0.44, respectively).

Many explanations have been suggested for the above mentioned differences in results ranging from differences in cultivar sites, vine vigour, vintage effects, sampling method and the analytical technique used (Downey *et al.*, 2006; Cortell *et al.*, 2005; Bucchetti *et al.*, 2011). Therefore, it can be deduced that:

- a) controlled conditions (e.g. greenhouse or growth chamber) are desirable to study the effect of abiotic factors (light intensity and quality, temperature and water) on berry phenolic composition and
- b) data should be presented on a per berry basis and in concentration to understand the dynamic of berry phenolic biosynthesis from berry set to maturation as well as the impact on overall concentration.

2.4.1.2 Temperature

An increase in plant temperature, either through direct heating by incident radiation or increased air temperatures will increase the rate of metabolic processes in the plant, with an associated increase in development and metabolite accumulation (Hawker, 1982; Ebadi *et al.*, 1995; Dokoozlian & Kliewer, 1996; Downey *et al.*, 2006). The accumulation/biosynthesis of total soluble solids and organic acids, the biosynthesis of aromatic precursors and colour components, and the process of photosynthesis are all enzyme-driven and therefore regulated by temperature, light and plant water status (Jackson & Lombard, 1993).

Gladstones (1992) suggests that pigment formation and the optimal physiological ripening of grapes for the synthesis of colour and aroma compounds takes place between 20 and 22°C. When the day temperature is high, low night temperatures are necessary to ensure a low pH and high natural acidity (Jackson & Lombard, 1993). Mori *et al.* (2005) found that that the metabolic pathways are altered when the ambient temperature reaches 30°C.

Chorti *et al.* (2010) and Mori *et al.* (2005) found that high night temperatures resulted in a decrease in the anthocyanin accumulation within the berry, but there was no change in the flavonol concentration. However, high temperatures inhibited the gene expression of CHS, F3H, dihydroflavanol 4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX) and UDP-glucose. Flavonoid 3-O-glucosyltransferase (UGT) activity decreased at véraison and was followed by an increase after véraison (Mori *et al.*, 2005). Buttrose *et al.* (1971) found that if the day temperatures were constant at 20°C for Cabernet Sauvignon it were favourable for colour

formation, but day temperatures $>30^{\circ}\text{C}$ resulted in less colour. Recently, Tarara *et al.* (2008) found that low light and high berry temperatures decreased the total skin anthocyanin (TSA). These findings are supported by the findings of Coombe (1987) which suggested that the primary metabolism of the berry is optimal at approximately $\sim 30^{\circ}\text{C}$ (Downey *et al.*, 2006).

Proanthocyanidin accumulation reaches a peak close to véraison and decreases towards harvest. This could be ascribed to its extractability rather than a degradation or turnover (Cheynier *et al.*, 1997; De Freitas & Glories, 1999; Kennedy *et al.*, 2001; Ó-Marques *et al.*, 2005). The amount of seed tannin in berries is related to the number of seeds per berry (Habertson & Adams, 2002). Cohen *et al.* (2008) studied the effect of temperature during the green berry stage and maturation. Proanthocyanidin accumulation was linearly related to the heat summation during the grape development period, but damping of the diurnal temperature by daytime cooling and night time heating resulted in a reduction in the proanthocyanidin mDP. Downey *et al.* (2004) suggested that shading had no significant effect on the levels of condensed tannins in the skins or seeds of ripe fruit. However, there were noticeable differences in the total condensed tannins over two vintages. These differences were ascribed to changes in the skin tannin content. Cohen *et al.* (2012) found that heating and cooling of berries from 20.5°C by $\pm 8^{\circ}\text{C}$, altered the initial rates of proanthocyanidin accumulation. However, the total proanthocyanidin accumulation was not related to the thermal time, but is more likely a function of berry development within a particular season.

2.4.1.3 Water constraint/stress

Irrigation of vineyards is a worldwide practice in arid and semi-arid regions, and it apparently affects the biosynthesis of phenolics (Cohen & Kennedy, 2010). Roby *et al.* (2004a; 2004b) found an increase in the skin tannins and anthocyanin amounts per berry and concentrations with an increased water deficit. Ojeda *et al.* (2001 & 2002) studied the impact of water deficits during different berry growth stages. Smaller berries and higher skin flavonol concentrations were correlated with water stress during berry green growth stages. Proanthocyanidin and anthocyanin concentrations were also impacted as the skin-to-pulp weight ratio increased due the induced

water stress applied before and/or after véraison. The latter findings correlate with other studies (Kennedy *et al.* 2002b; Petrie *et al.*, 2004; Salon *et al.*, 2005; Koundouras *et al.* 2006). Castellarin *et al.* (2007) found that water deficit before and after véraison resulted in a reduction in berry size and the flavonol concentrations were affected by the timing of the irrigation. An increase in proanthocyanidin concentration was noted after véraison, but was similar for all treatments (early and late deficit irrigation) at harvest. In general, the timing of water constraint/ waterstress (i.e. before or after véraison), the water constraint levels and the duration of the water constraints will affect the concentration of major phenols (Deloire *et al.*, 2004).

2.4.1.4 Ultra-violet radiation

Fruit composition is affected by photosynthetic, UV, thermal and phytochrome effects (Smart, 1987; Kolb *et al.*, 2001; Kolb *et al.*, 2003; Berli *et al.*, 2010). Light movement occurs through a passage of different tissue layers via light scattering therefore plants can be described as a complex optical system (Smith, 1975). After light passes through the plant surface, the spectral quality and quantity may be altered by wavelength dependent absorption (Smith, 1975).

Plant photosynthesis is sustained by the “visible” range of the spectrum on the earth surface (400–800 nm). However, when the visible spectrum radiation is gathered plants are also exposed to UV radiation in the wavelength range 290–400 nm. UV radiation can be divided in to UV-A (315–400 nm), UV-B (280–320 nm) and UV-C (<280 nm) ranges. Morphogenetic changes in plants have been caused by UV-B radiation (Rozema *et al.*, 1997). Furthermore, Jordan (1996), Rozema *et al.* (1997), Vass (1997) and Hollósy (2002) found damage to lipids, nucleic acids and proteins. Teramura & Sullivan (1994) reviewed the primary, secondary and indirect effects of UV-B radiation on photosynthesis. Plant morphogenetic parameters that are changed are plant height, leaf area, leaf thickness, branching and plant phenology (Tevini & Steinmuller, 1987; Barnes *et al.*, 1990; Ryel *et al.*, 1990; Bornman & Vogelmann, 1991; Teramura & Sullivan, 1994). UV-B radiation affects the secondary metabolism of plants. Secondary metabolite production can be stimulated by UV-B as well as the prevailing abiotic and biotic conditions (Rozema, *et al.*, 1997).

UV-B radiation affects some enzymes of the phenyl propanoid pathway. PAL and CHS activity are stimulated by UV-B radiation (Jansen *et al.* 1998; Pontin *et al.* 2010). PAL catalyses the deamination of phenylalanine to form trans-cinnamic acid. Hydroxycinnamic acids are particularly effective in screening out UV-B radiation as they absorb effectively in the 300 nm range of the UV-B spectral region whereas flavonoids absorb at 280 nm. Flavonoids absorb UV-B radiation and epidermal flavonoids in particular act as UV-B screens for interior tissues of leaves and stems. Elevated levels of UV-B radiation is known to cause a limited increase of tannins and lignin (Gehrke *et al.*, 1996).

The impact of UV radiation on grapevine functioning was at the centre of various studies. Kolb *et al.* (2001) found increased levels of hydroxycinnamic acids (coumaric and caffeic acid) in sun exposed grape leaves. In the berries, however, lower levels of hydroxycinnamic acids were obtained with increased radiation while, similar to grapevine leaves, quercetin and kaempferol increased (Kolb *et al.*, 2003). Spayd *et al.* (2002) studied the effect of UV barriers over the canopy and fruiting zone over a 2-year period. Flavonol biosynthesis was influenced by UV barriers as individual and total flavonol concentration was significantly reduced (Spayd *et al.*, 2002). Koyama *et al.* (2012) suggested that UV exclusion did not affect the concentration and composition of proanthocyanidins, but confirmed a decrease in flavonol concentration. Gregan *et al.* (2012) suggested that the composition of flavonols in the skins of Sauvignon blanc grapes is determined by UV-B radiation.

Table 2.1. Grapevine fruit responses to environmental factors (adapted from Cohen & Kennedy, 2010)

| Phenolic compounds | Environmental factors | Responses |
|--------------------|-----------------------|--|
| Anthocyanin | Light intensity | Increase per berry content in sun exposed versus canopy shaded fruit (Crippen & Morrison., 1986) Exposed berries (increase in per berry content) (Downey <i>et al.</i> , 2004) |
| | UV exposure | Total monomeric skin anthocyanin (TMSA) concentrations were not influenced by UV, but rather by the visible spectrum of light and temperature which played a crucial role (Spayd <i>et al.</i> , 2002) |
| | Temperature | Decrease at high temperatures (30 – 35°C) (Mori <i>et al.</i> , 2005 & 2007) Cooler temperatures increased TMSA (Spayd <i>et al.</i> , 2002) More anthocyanins at 20°C than 30°C (Yamane <i>et al.</i> , 2006) |
| | Irrigation | Water deficit increased concentration (Koundouras <i>et al.</i> , 2005) Early irrigation/ severe deficit: Lower concentration and amount per berry (Ojeda <i>et al.</i> , 2002) |
| Flavonols | Light intensity | Increase concentration with exposure (Price <i>et al.</i> , 1995 & Spayd <i>et al.</i> , 2002; Koyama <i>et al.</i> , 2012, Gregan <i>et al.</i> , 2012) |
| | UV exposure | Exclusion of solar UV remarkably decreased concentration (Koyama <i>et al.</i> , 2012) Increase with exposure (Spayd <i>et al.</i> , 2002) |
| | Temperature | No effect (Mori <i>et al.</i> , 2005) |
| | Irrigation | Deficit increased concentration (between anthesis and véraison / and véraison and harvest) (Ojeda <i>et al.</i> , 2002) |
| Proanthocyanidins | Light intensity | Exposure lead to an increase per berry whilst shade resulted in an increase in substitution positions within the molecule (Downey <i>et al.</i> , 2004) Exposure resulted in an increase in seeds and skins mDP (Downey <i>et al.</i> , 2004) |
| | UV exposure | UV exclusion did not affect the concentration and composition of PA's (Koyama <i>et al.</i> , 2012) |
| | Temperature | Heating and cooling berries altered the initial accumulation rate (<i>via</i> biosynthesis) pre-véraison (Cohen <i>et al.</i> , 2012) |

2.5 SENSORY PROPERTIES OF GRAPE AND WINE PHENOLICS

Phenolic compounds in wine contribute to the wine sensorial properties (wine colour, astringency, bitterness and mouthfeel) and antioxidative properties (Gawel, 1998). Phenolic levels in wine can be affected by several factors such as grape genotypes (Ricardo-da-Silva *et al.*, 1992b; Sun *et al.*, 2001) the winemaking practices and conditions of wine ageing and storage (Sun *et al.*, 1999; Sun *et al.*, 2001). The conversion of anthocyanins and proanthocyanidins to other polymeric species contributes to the change in colour and taste of a wine. The impact of polymerisation reactions on wine sensory properties is largely unknown. Some researchers suggest a contribution of newly formed polymeric pigments to astringency mouthfeel (Oberholster, 2009) and others suggest a decrease of wine astringency (Weber *et al.* 2013; Wollmann & Hofmann, 2013).

Monomeric and polymeric flavan-3-ols are the primary contributors to the astringency and bitterness character of red wine (Singleton & Trousdale, 1992). Astringency is a tactile sensation in

which drying, puckering and roughing is produced by the interactions of wine tannins with salivary proteins (Robichaud & Noble, 1990). Bitterness is a taste sensation perceived by each of the several thousand sensors on the tongue (Katsnelson, 2015). Astringency perception is not well understood, but can be caused by (i) an increase in friction, (ii) interaction between tannins and oral epithelial proteins/taste receptors and (iii) change in salivary viscosity (Gawel, 1998; McRae & Kennedy, 2011). Protein-polyphenol interactions can be divided into (i) hydrophobic interactions and (ii) hydrogen bonding which is influenced by the degree of polymerisation, galloylation and hydroxylation of tannins (Gawel, 1998; Peleg *et al.*, 1999). (+)-Catechin exhibits bitterness and astringency in white wine solutions (Arnold *et al.*, 1980; Robichaud & Noble, 1990). Five concentrations ranging between 0-1200 mg liter⁻¹ was evaluated by Robichaud & Noble (1990) while three concentrations between 160-300 mg liter⁻¹ was studied by Arnold *et al.* (1980) in base wine. Thorngate (1995) showed that the intensity of astringency and bitterness of two monomeric flavan-3-ols, (-)-epicatechin and (+)-catechin differs with (-)-epicatechin having a higher intensity than (+)-catechin. Three concentration levels (0.5, 0.9 and 1.2 g liter⁻¹ of (-)-epicatechin and (+)-catechin was assessed) in a model wine solution (Thorngate, 1995).

Chira *et al.* (2008) found a positive correlation between astringency intensity and mean degree of polymerisation (mDP) in grape skins (mDP 23.1 and 20.94 in 2006 and 2007 vintages, respectively).

Both astringent and bitterness perception thresholds are influenced by the concentrations, therefore a higher concentration results in an increase in the intensity of the sensation. Astringency and bitterness are influenced by the mDP of polymers. With an increase chain length both bitterness and astringency increase, however astringency increases faster than bitterness (Arnold *et al.*, 1980; Lea & Arnold, 1978; Gawel 1998). Peleg *et al.* (1999) found that bitterness is elicited by an interaction with a specific bitter membrane-bound receptor or through surface membrane interactions. Therefore, an increase in molecular size of procyanidins decreases bitterness by limiting the access to a membrane-bound receptor or by direct depolarisation of the taste receptor cell. Lea & Arnold (1978) suggested that the increase in the perceived astringency with the mDP is due to greater capacity of polyphenols to bind the proteins and stimulate astringency. Vidal *et al.*

(2003) suggested that the mDP in apple and grape extracts were the most discriminatory structural variable as astringency increased with an increase in polymerisation. An increase in the galloylation can result in an increase in coarseness while trihydroxylation of the B-ring decreased coarseness (Vidal *et al.*, 2004a). Vidal *et al.* (2004b) suggested that anthocyanins in their glucoside and coumaroylated forms did not influence astringency and bitterness of model wine solutions, but that polysaccharides play an important role in mouthfeel properties of wine. Acidic polysaccharides significantly decreased the astringency while neutral polysaccharides had less effect in a model wine solution (Vidal *et al.*, 2004b). Other parameters which affect the intensity and duration of astringent and bitter sensations by altering the salivary flow and composition are: (i) wine pH, (ii) ion concentration, (iii) temperature and (iv) ethanol concentration (Gawel, 1998).

2.6 CONCLUSION

The composition of wine grape berries is affected by genotype, clones, abiotic factors and cultural practices. Global climate change may affect the typicality of wines. Therefore, grape quality is a complex concept which is dependent on berry composition and size.

Plants are complex optical systems that are dependent on the light environment amongst other parameters. Light conditions are dependent on the source of light and the microclimatic conditions of the plant. The light environment is affected by both long-term (row direction, vine spacing, trellising system, etc.) and short-term practices (canopy manipulations, pruning and trellising which will affect the architecture of the canopy). Grape berry composition is affected by abiotic factors (light, temperature, soil water content, wind and air humidity), mainly at the meso- and microclimatic levels.

Berry temperature is important, as it is affected not only by sunlight exposure, but also by the availability of water to maintain transpiration. Increased exposure to sunlight from an early stage of berry development as well as the availability of water has an effect on the fruit growth and composition. Sunlight exposure ($>100 \mu\text{mol m}^{-2}/\text{s}^{-1}$) combined with simultaneous high temperatures lead to a decrease in phenolic compounds such as anthocyanins and total phenolics.

Clearly, vine metabolism (and overall performance) is affected by a complex interaction between natural and man-made factors. At the microclimatic level the management of light quantity and quality is a powerful tool to regulate the quantitative and qualitative performance of the vine. This study will contribute to the knowledge around the effect of sunlight and temperature on grapevine berry responses to bunch and canopy microclimatic changes. By determining under controlled conditions the possible effect of these two abiotic factors at the bunch microclimatic level, it should be possible to establish thresholds of light and temperature effect on berry growth and phenolic biosynthesis (stimulation or inhibition).

Working in a model vineyard, the impact of terroir concept will not be fully comprehended because of the number of variables linked to this notion (soil, meso climatic and vines differences). The prevailing conditions within a terroir unit therefore will impact the abiotic factors and the threshold values as it has been demonstrated within the literature study. Complicating our understanding of the effects of light, temperature and vine water status on berry phenolics composition.

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Chapter 3

Research results

Evolution of flavonoids under altered light and temperature conditions in Cabernet Sauvignon (*Vitis vinifera* L.) in two consecutive seasons (2010/2011 and 2011/2012)

CHAPTER 3

Evolution of flavonoids under altered light and temperature conditions in Cabernet Sauvignon (*Vitis vinifera* L.) in two consecutive seasons (2010/2011 and 2011/2012)

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3.2 ABSTRACT

The accumulation of flavan-3-ols, flavonols and anthocyanins were followed in Cabernet Sauvignon (*Vitis vinifera* L.) under altered temperature and light conditions. The study was conducted over two consecutive seasons 2010/2011 and 2011/2012 and comprised of two main treatments in which the light quantity was manipulated in the bunch zone: standard (STD) with no lateral shoot or leaf removal and treatment LRW with leaf removal on the western side of the bunch zone. Furthermore, the light quality was altered by installing ultra-violet-B suppressing sheets within the bunch zone in both seasons. The accumulated growing degree days (GDD) was higher in the 2011/2012 season. Temperature accumulation and threshold temperatures for grape functioning were influenced by the season rather than the treatment. Sugar accumulation followed a similar trend in the two seasons. Light exposure rather than temperature in a particular season significantly influenced tannin accumulation in grape skins but had little impact on grape seed

tannins. Additionally, flavonol accumulation was significantly influenced by the light quality which is known to be the main abiotic driver of flavonol biosynthesis regulation, whereas anthocyanin concentration and content were largely dependent on the temperature and light quality in a particular season.

3.3 INTRODUCTION

Flavonoids perform major roles in plants such as pollen fertilisation, auxin transport regulation, pigmentation, defence against pathogens and pests and provide protection from ultra-violet radiation. Additionally, flavonoids are important in wine because of their colour and astringency and the potential beneficial role in human health (Santos-Buelga & Scalbert, 2000; Winkel-Shirley, 2001). The three main groups of flavonoids identified in red grape berries are anthocyanins, flavonols and flavan-3-ols (tannin).

Anthocyanins are the pigmented compounds responsible for the colour in red grapes and wine (Ribereau-Gayon & Glories, 1986). Anthocyanins are synthesised and accumulate from véraison in the berry skins of most grape cultivars and contributes to the red and purple colour of the fruit (Adams, 2006). However, some *Vitis vinifera* cultivars i.e Alicante Bouschet and non *Vitis-vinifera* i.e hybrid cultivars show anthocyanins also in the pulp and are known as teinturier cultivars (Guan *et al.*, 2012). Flavonols are colourless compounds which are synthesised in the skins and accumulate after flowering and during ripening (Price *et al.*, 1995; Spayd *et al.*, 2002; Downey *et al.*, 2003a). They contribute to wine colour by forming co-pigments with anthocyanins (Asen *et al.*, 1972; Scheffeldt & Hrazdina *et al.*, 1978; Boulton, 2001). Moreover, flavonols are UV protectants and act as free radical scavengers (Flint *et al.*, 1985; Smith & Markham, 1998). Flavan-3-ols include a range of polyphenolic compounds which include flavan-3-ol monomers, dimers and various oligomers and polymers called condensed tannins (Adams, 2006). Proanthocyanidins are the most abundant class of grape phenols in the grape berry and are present in the seeds, skins, pulp and stems (Jordão *et al.*, 2001; Adams, 2006), which are connected by interflavan linkages (C₄–C₈ or C₄–C₆) (Ricardo-da-Silva *et al.*, 1991; Ricardo-da-Silva *et al.*, 1992 a,b).

A number of factors have been identified that can influence flavonoid accumulation in grapes. This include abiotic factors such as light, temperature and water status as well as others such as cultivar, crop level, nutritional status, soil type and plant growth regulators (Crippen & Morrison, 1986; Kennedy *et al.*, 2002; Ojeda *et al.*, 2002; Monagas *et al.*, 2003; Downey *et al.*, 2004; Downey *et al.*, 2006; Cortell *et al.*, 2005; Mori *et al.*, 2005 & 2007; Lorrain *et al.*, 2011; Cohen *et al.*, 2012; Gregan *et al.*, 2012; Koyoma *et al.*, 2012). The accumulation of flavonoids and their genes are up-regulated with exposure to light while shading down regulates the gene expression (Downey *et al.*, 2004; Jeong *et al.*, 2004; Cortell & Kennedy, 2006; Fujita *et al.* 2007). Varying results have been obtained on the effect of light exposure on anthocyanin accumulation. Wicks & Kliewer (1983), Gao & Cahoon (1994) and Dokoozlian & Kliewer (1996) reported a reduction in the anthocyanin content when fruit were shaded without altering the temperatures. Other authors reported no change in the anthocyanin content of shaded fruit compared to exposed fruit (Price *et al.*, 1995; Haselgrove *et al.*, 2000). Flavonol levels increased in grapes exposed to high levels of sunlight (Price *et al.*, 1995; Haselgrove *et al.*, 2000; Bergqvist *et al.*, 2001; Spayd *et al.*, 2002). Romeyer *et al.* (1986) and Downey *et al.* (2003b) reported that the pattern of tannin accumulation varied throughout berry development. However in general the seed and skin tannin content and concentration have been found to increase from flowering to véraison and reaching a maximum close to véraison followed by a decrease in concentration (Kennedy *et al.*, 2000 a & b; Downey *et al.*, 2003b; Downey *et al.*, 2004; Ristic *et al.*, 2007).

As flavonoids are important for red wine quality the purpose of this study was to investigate the evolution of the main flavonoids during berry development in an important international cultivar- Cabernet Sauvignon (*Vitis vinifera* L.). This work formed part of a larger study in which the flavonoid composition responses to light (quality and quantity) and temperature and an exploratory study in the resulting wines were studied under growing conditions in the Stellenbosch Wine of Origin District during two consecutive seasons (2010/2011 and 2011/2012).

3.4 MATERIALS AND METHODS

3.4.1 Vineyard characteristics

The study was conducted during the growing season of 2010/2011 and 2011/2012 seasons in a Stellenbosch University vineyard (GPS Coordinates: 33°56' 42" S 18°27' 43" E). The vineyard consists of *Vitis vinifera* L. cv. Cabernet Sauvignon clone CS 388C, grafted onto 101-14 *Mgt* (*Vitis riparia* X *Vitis rupestris*). The row orientation is north-west/south-east. The vines are trained on a six-wire vertical trellis system and the block was subjected to irrigation during critical phenological stages (e.g. fruit-set and véraison) and as required throughout the season to have a predawn leaf water potential between 0 and -0.3 MPa (Deloire & Heyns, 2011). The study comprised of two main treatments with altered bunch microclimates in both seasons: no lateral shoot or leaf removal in the bunch zone (STD) and leaf removal in the bunch zone (LRW) (Table 3.1). The leaves were removed just after flowering corresponding to growth stage 19 (Eichorn and Lorenz system) (Coombe, 1995) on the western side of the canopy at the fruiting zone level (\pm 35–40 cm above the cordon).

Furthermore, to study the effect of change in light quality on fruit growth and composition, supplementary treatments were applied. A UV sheet, reducing the UV-B radiation ('Perspex'® Opal 050), (Perspex South Africa (Pty) Ltd, Umbogintwini) (Table 3.2) was added to the Control/STD (STD-UV-B) and Leaf Removal West (LRW-UV-B) treatment in 2010/2011. During the 2011/2012 season the UV-B suppression sheets were installed on both sides of the canopy to exclude the effect that the row direction can have on grape development as in the 2010/2011 season. Additionally to the 'Perspex'® Opal 050 (Table 3.2) sheets a clear acrylic UV-sheet (UHI) was used during the 2011/2012 season. The latter resulted in the following treatments: LR (-UV-B, 2xOp50) and LR (-UV-B, 2xUHI) (Table 3.1). These sheets were installed just after flowering at \pm 35 cm above the cordon and suspended on 1.2 m custom made poles with hinges to open for sampling and for the spraying programme. The treatments were applied in a randomised block design. Each treatment was carried out in five replicates and each replicate comprised of three

panels (six vines between poles). Therefore, each of the four treatments in each season comprised of five replicates and each replicate consisted of 18 vines.

Table 3.1. Treatment description for 2010/2011 and 2011/2012 season.

| Treatments | |
|--|--|
| 2010/2011 | 2011/2012 |
| No lateral shoots or leaves were removed in the bunch zone and no water shoots were suckered Shaded (Control) (STD) | No lateral shoots or leaves were removed in the bunch zone and no water shoots were suckered Shaded (Control) (STD) |
| Leaf Removal West side of the bunch zone just after flowering Leaf Removal West (LRW) | Leaf Removal West side of the bunch zone just after flowering Leaf Removal West (LRW) |
| Control treatment and UV-sheet (Perspex' ® Opal 050) on the western side of the bunch STD with decreased UV-B radiation (STD-UV-B) | Leaf removal on both sides of the canopy (in the bunch zone) and ('Perspex' ® Opal 050) on both sides of the bunch zone Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone (LR-UV-B, 2xOp50) |
| Leaf Removal West and UV-sheet (Perspex' ® Opal 050) on the western side of the bunch LRW with decreased UV-B radiation (LRW-UV-B) | Leaf removal on both side of the canopy (in the bunch zone) and UV-sheet (UHI) extruded clear Acrylic was used on both sides of the bunch zone Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone (LR-UV-B, 2xUHI) |

Table 3.2. Optical properties of 'Perspex' ® and acrylic UV sheets.

| 2010/2011 | | | | | | | | | |
|------------------------------|----------------------|------------------|---------------------|-------------------|------------|---------------------|--------------------|---------------------|----------------|
| Perspex ® Opal 050 | Visible (380-780 nm) | | Solar (350-2100 nm) | | | | | Shading coefficient | UV elimination |
| | Light transmission | Light reflection | Total elimination | Direct reflection | Absorption | Direct transmission | Total transmission | | |
| | % | % | % | % | % | % | % | | |
| | 27 | 42 | 59 | 31 | 38 | 31 | 41 | 0.47 | 99 |
| 2011/2012 | | | | | | | | | |
| Extruded high impact acrylic | 89 | 78 | 12 | 8 | 6 | 86 | 88 | 1.0 | 99 |

3.4.2 Temperature measurements

Microclimate within the canopy and bunch zone was determined within each treatment with a tinytag (Tinytag Plus TGP-1500, West Sussex, United Kingdom). The tinytags were placed on the surface of the berry skins for the respective measurements. Berry temperatures were measured every 15 min from December until March (96 measurements daily) in both seasons. The average berry temperature was calculated per hour throughout the season for each treatment. Thermal time

in degree days (DD, °C) were calculated on each day and summed throughout each season. The latter was computed as follows.

$$DD = \frac{1}{n} \sum_{i=0}^n (T - T_b)$$

Where:

n: the number of averaged datalogger readings per day

T: Daily mean temperature (°C)

T_b: Base temperature (10°C) for grapevine growth

3.4.3 Light measurements

The incidence Photosynthetic Active Radiation (PAR) in $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was determined with a ceptometer (LP-80 AccuPAR, Decagon Devices Inc. Nebraska, USA). Five measurements were taken within the bunch zone on clear, sunny days at 11:00, 13:00 and 15:00 on three days during the growing season in both seasons.

3.4.4 Sampling procedure and preparation for analyses

Sampling occurred at regular intervals throughout the season (Table 3.3). Sampling was conducted between 06:00 and 08:00 at each sampling date from after fruit-set until harvest, 13-116 days after anthesis (DAA) during the 2010/2011 season and from 26-130 DAA in the 2011/2012 season (Table 3.3). Sampling corresponded with the Eichorn and Lorenz (E-L) system (Coombe, 1995) and started at stage 29 (peasize) until stage 38 (harvest) for phenolic analyses. Berry sampling for classical parameters (Total soluble solids (TSS), pH and titratable acidity) commenced on 41 DAA until 116 DAA in the 2010/2011 season and from 54 DAA until 130 DAA in the 2010/2012 season. The determination of the classical parameters entailed the sampling of thirty berries from each of the five treatment replicates (30x5=150) in the middle of the bunch. The hundred and fifty berries from each treatment were divided into three sub-samples of 50 berries each and processed immediately after sampling for TSS, pH and titratable acidity. The berries were crushed and the grape juice centrifuged. TSS were measured using an ATAGO PAL-1 pocket refractometer (Tokyo, Japan). The pH and TA were measured using an automatic titrator

(Metrohm, 702 SM Titrino, Herisau, Switzerland). Additional to the classical parameters, twenty berries from each of the five treatment replicates (20x5=100) were sampled in the middle of the bunch and kept separate for phenolic analyses.

Table 3.3. Sampling dates (for phenolic analysis) and days after anthesis for 2010/2011 and 2011/2012 seasons.

| Sampling and days after anthesis dates | | | |
|---|----------------------------|-------------------------|----------------------------|
| 2010-2011 season | | 2011-2012 season | |
| Sampling dates | Days after anthesis | Sampling dates | Days after anthesis |
| 7 December 2010 | 13 | 14 December 2011 | 26 |
| 11 December 2010 | 17 | 21 December 2011 | 33 |
| 16 December 2010 | 22 | 28 December 2011 | 40 |
| 11 January 2011 | 48 | 04 January 2012 | 47 |
| 25 January 2011 | 62 | 11 January 2012 | 54 |
| 08 February 2011 | 76 | 25 January 2012 | 68 |
| 22 February 2011 | 90 | 08 February 2012 | 82 |
| 20 March 2011 | 116 | 22 February 2012 | 96 |
| | | 06 March 2012 | 110 |
| | | 26 March 2012 | 130 |

3.4.5 Chemicals

All chromatographic solvents were HPLC grade. Ethanol, acetone, acetonitrile, methanol, L-ascorbic acid, gallic acid, (+)-catechin, (-)-epicatechin and quercetin were obtained from Sigma-Aldrich (Johannesburg, South Africa). Quercetin-3-glucoside was obtained from Fluka (Buchs, Switzerland), malvidin-3-glucoside from Polyphenols Laboratories AS (Norway), acetic acid and orthophosphoric acid obtained from Riedel-de Haën (Seelze, Germany). Water for the solvents was obtained from a Milli-Q filtration system (Millipore Filter Corp., Bedford, MA, USA).

3.4.6 Extraction of grape seeds and skins

The berries were processed immediately after sampling for the phenolic analysis. Each of the twenty berries from each treatment replicate was weighed. The berries were then frozen in liquid nitrogen. The berry samples were manually separated into skins and seeds, rinsed with water and dried with tissue paper. Isolated skins and seeds were weighed. During 2011/2012 the seed number from each treatment replicate was recorded. One milliliter of extraction solvent comprising of 70% acetone containing, 0.1% ascorbic acid was added for each 0.1 g of skins or seeds and

homogenised for 2 min using an IKA Ultra-Turrax T 18b (IKA Labortechnik, Staufen, Germany) homogeniser. The homogenate was extracted for 24 hours at 4°C stirring at 350 rpm with a Heidolph Unimax 1010 (Heidolph, Germany) followed by evaporation under reduced pressure at 35°C to remove the organic solvent. The aqueous solution was frozen overnight, lyophilised and stored at -20 degrees Celsius until further analysis were performed. The dried tannin extract representing one biological repetition of 20 berries was redissolved in 10 mL methanol for seeds and five milliliters methanol before reverse phase high performance liquid chromatography (RP-HPLC) analysis.

3.4.7 Analysis of individual flavan-3-ol monomers, dimers, flavonols, anthocyanins and tannins by RP-HPLC

Monomeric phenolic composition and polymeric procyanidins (seed) and proanthocyanidins (skin) (tannins) were quantified using RP-HPLC based on a method by Peng *et al.* (2001, 2002). Tannin elutes as an unresolved peak at the end of the run. This method gives an estimation of tannin content and can show trends among samples.

RP-HPLC was performed on a Hewlett Packard Agilent 1260 series HPLC system equipped with a diode array detector (Agilent Technologies, Palo Alto, CA, USA). Separations were carried out on a polystyrene/divinylbenzene reversed phase column (PLRP-S, 100Å, 250 × 4.6 mm, 5 µm) protected with a guard cartridge with the same packing material (PLRP-S, 10 × 4.6 mm). All purchased from Polymer Laboratories (Shropshire, UK). The following mobile phases were used: solvent containing MilliQ water with 1.5% v/v orthophosphoric acid and solvent B consisting of acetonitrile. A linear gradient was used: 0-78 min, from 6% to 31% B, staying constant for 8 min to 86 min, and then back to the starting conditions in 4 min until 90 min. The column was equilibrated for 15 min at the starting conditions before the next injection. A flow rate of 1 mL/min was used and the column was maintained at 35°C. The samples were filtered using a 0.45 µm Millipore filter (Millipore, Bedford, Mass., USA) before injection, and placed in a 1.5 mL amber HPLC vial.

Phenols were quantified using external standards: (+)-catechin hydrate, (-)-epicatechin, gallic acid, malvidin-3-glucoside and quercetin-3-glucoside. Monomeric and dimeric flavanols and polymeric phenols were quantified at 280 nm as mg/L catechin units with a quantification limit of 0.78 mg/L, and epicatechin with a LOQ of 0.89 mg/L. Gallic acid was also quantified at 280 nm in gallic acid units with a LOQ of 0.05 mg/L. Flavonol-glycosides were quantified at 360 nm as mg/L quercetin-3-glucoside with a LOQ of 0.20 mg/L. The following flavonols were identified, (i) quercetin-3-rutinoside, (ii) quercetin-3-galactoside, (iii) quercetin-3-glucoside and (iv) quercetin-3-glucuronide. Anthocyanins and polymeric pigments were quantified at 520 nm as mg/L malvidin-3-glucoside with a quantification limit of 0.19 mg/L. The corresponding LOD for the monomeric and dimeric flavanols and polymeric phenols was 0.23 mg/L for (+)-catechin units and 0.26 mg/L for (-)-epicatechin. Flavonol glycosides were quantified at 360 nm as mg/L quercetin-3-glucoside with a LOD of 0.06 mg/L. Anthocyanins and polymeric pigments were quantified at 520 nm as mg/L malvidin-3-glucoside with a LOD of 0.05 mg/L.

3.4.8 Statistical analysis

All analyses were carried out using Statistica 12 (Statsoft Inc., Tulsa, USA). Mixed model repeated measures ANOVAs were used and Fisher's least significant difference (LSD) corrections were used for *post-hoc* analyses. Significant differences were judged on a 5% significance level ($p \leq 0.05$).

3.5 RESULTS AND DISCUSSION

3.5.1 Growing degree days

The accumulation of growing degree days (GDD) was determined from December until March in both seasons. The amount of GDD for the 2010/2011 season was 1262 and in the 2011/2012 season was 1451 (base temperature of 10°C) (Fig. 3.1). The pattern of growing degree accumulation varied among the two seasons as the macroclimate in the 2010/2011 season was characterised by continuous drought and heat throughout the summer (VinPro, 2011). Whereas,

the 2011/2012 season was however, considered as an ideal growing season with a cool, and lengthened, harvesting period without rain or prolonged heat (VinPro, 2012).

The accumulation of fruit thermal degree days (DD) was affected by the season as well as by the treatments (Table 3.4). The DD among the treatments in the 2011/2012 season was lower compared to the 2010/2011 season. As anticipated, the STD had the lowest DD and LRW had the highest accumulated DD in the 2010/2011 season as shown in Table 3.4. The addition of the UV-B suppression sheets altered the fruit temperatures in the STD-UV-B treatment as it had a higher DD compared to the STD (Table 3.4). This suggests that the leaf layers in the STD-UV-B treatment and the combination of the UV-B sheet retained the heat and resulted in increased DD due to amplified solar radiation in the fruit zone.

In the 2011/2012 season the fruit from the LR (-UV-B, 2xOp50) and LR (-UV-B, 2xUHI) treatments had the lowest and highest DD, respectively (Table 3.4). This phenomenon can be ascribed to the spectral properties of the sheets that were used in the study. The Perspex Opal 050 sheet (Op50) has a shading coefficient of 0.47 which resulted in more shading of the fruit when compared to the extruded high impact acrylic sheet (UHI) with a shading coefficient of 1.0 (Table 3.2). This result coincides with that of Spayd *et al.* (2002) who observed that clusters exposed to UV-radiation reducing sheets had higher DD values than exposed fruit.

Smart & Sinclair (1976) reported that temperature is directly associated with the incident radiation. It is therefore difficult to separate the effects of light and temperature on fruit development. Berries in the LRW and to a lesser extent LRW-UV-B treatments received more direct sunlight as they were more exposed and therefore subjected to heating of the surface on the berry, while the STD treatments had indirect sunlight due to the higher leaf ratio and the STD-UV-B treatment due to the presence of the UV-B suppression sheet (Table 3.5). In the 2011/2012 season the PAR and percentage light intensity in the STD and LR (-UV-B, 2xOp50) were significantly lower ($p \leq 0.001$) than the LRW and LR (-UV-B, 2xUHI) treatments (Table 3.5). The latter had a significant impact on the berry temperatures at thresholds $>30^{\circ}\text{C}$ (Table 3.4). However, despite the significant

differences in temperature thresholds these differences were not large enough to have a real impact on the biosynthesis (Azuma *et al.*, 2012).

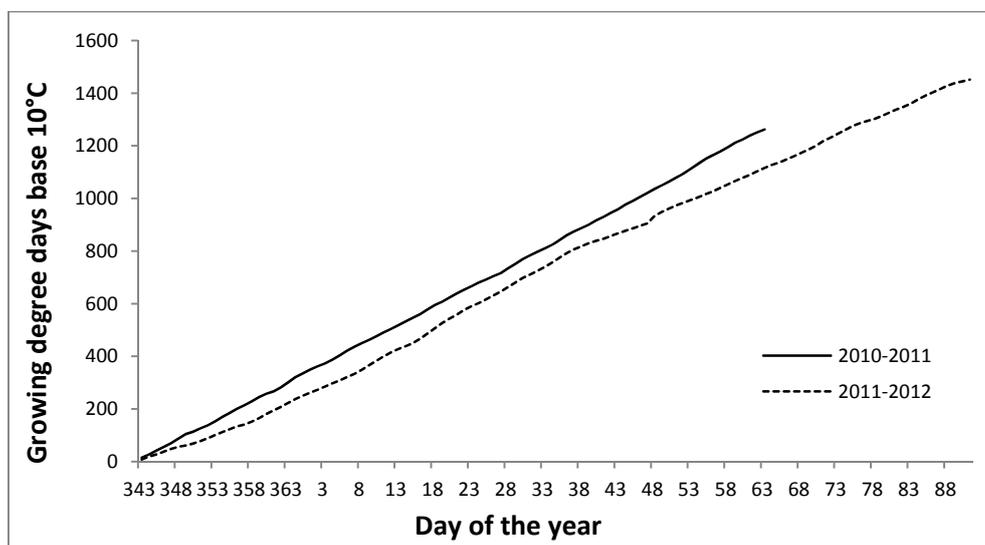


Figure 3.1. Growing degree accumulation from the 343th to 63th day of the year (2010/2011) and 343th to 88th day of the year (2011/2012).

Table 3.4. Accumulated thermal time and berry temperature and the average number of hours at thresholds in 2010/2011 and 2011/2012 seasons.

| Season | | Berry temp (°C) | | Number of hours berry temperature within the indicated temperature range | | | | |
|-------------------|--------------------------------------|-----------------|------------|--|--------------|--------------|--------------|---------------|
| 2010-2011 | | Mean | Max | <20 | 20-25 | 25-30 | 30-35 | >35 |
| Treatments | Thermal time (DD)^a | | | | | | | |
| STD | 731.3 | 23.4 | 32.5 b | 9.3 b | 5.5 a | 4.5 a | 3.8 b | 0.9 d |
| LRW | 757.8 | 23.9 | 35.4 a | 9.4 a | 5.2 b | 3.5 c | 4 b | 2.0 a |
| STD-UV-B | 756.1 | 23.8 | 33.8 b | 9.5 b | 4.8 c | 3.7 c | 4.5 a | 1.6 b |
| LRW-UV-B | 746.3 | 23.6 | 33.7 b | 9.3 b | 5.5 a | 4 b | 3.9 b | 1.4 c |
| p-value | | ns | *** | *** | *** | *** | ** | *** |
| 2011-2012 | | | | | | | | |
| Treatments | Thermal time (DD)^a | Mean | Max | <20 | 20-25 | 25-30 | 30-35 | >35 |
| STD | 684.6 | 23.8 ab | 40.4 a | 10.4 c | 4 b | 3.3 c | 3.2 b | 3.1 b |
| LRW | 686.7 | 23.2 ab | 37.1 b | 10.5 b | 4.1 a | 3.6 b | 3.3 b | 2.4 c |
| LR (-UVB, 2xOp50) | 680.9 | 22.8 b | 34.5 c | 10.7 a | 4 ab | 3.9 a | 3.5 ab | 1.8 d |
| LR (-UV-B, 2xUHI) | 729.7 | 24.2 a | 39.6 a | 10.5 bc | 3.5 c | 2.5 d | 3.7 a | 3.8 a |
| p-value | | * | *** | *** | *** | *** | * | *** |

^a Cumulated growing degree days over the season. STD (Shaded/Control); LRW (Leaf Removal West); STD-UV-B (STD with decreased UV-B radiation); LRW-UV-B (LRW with decreased UV-B radiation); LR (-UV-B, 2xOp50) (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR (-UV-B, 2xUHI) (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Table 3.5. Photosynthetic Active Radiation (PAR) and percentage light in the bunch zone in the 2010/2011 and 2011/2012 seasons.

| 2010-2011 | | | 2011-2012 | | |
|----------------|------------------|---------|-------------------|------------------|---------|
| Treatments | PAR ^a | % light | Treatments | PAR ^a | % light |
| STD | 175.3 bc | 0.10 c | STD | 72.0 b | 0.06 c |
| LRW | 517.7 a | 0.29 a | LRW | 278.9 a | 0.18 ab |
| STD-UV-B | 115.3 c | 0.06 c | LR (-UV-B,2xOp50) | 98.4 b | 0.07 cb |
| LRW-UV-B | 260.2 b | 0.16 b | LR (-UV-B,2xUHI) | 424.4 a | 0.19 a |
| <i>p-value</i> | *** | *** | <i>p-value</i> | *** | *** |

^aPhotosynthetic Active Radiation ($\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). STD (Shaded/Control); LRW (Leaf Removal West); STD-UV-B (STD with decreased UV-B radiation); LRW-UV-B (LRW with decreased UV-B radiation); LR (-UV-B, 2xOp50) (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR (-UV-B, 2xUHI) (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

3.5.2 Berry growth and development

The pattern of berry growth followed a typical double sigmoid pattern in both seasons. However, there was no significant difference in the average berry weight among the four treatments in the 2010/2011 season (Table 3.6). During 2010/2011 a rapid gain in berry weight was found just after fruit-set and the maximum weight were reached at 77 DAA (1.31, 1.23, 1.26 and 1.29 g for STD, LRW, STD-UV-B and LRW-UV-B, respectively) and thereafter it decreased to 1.26, 1.24, 1.22 and 1.21 g for STD, LRW, STD-UV-B and LRW-UV-B, respectively at 116 DAA. In the 2011/2012 season the initial gain in berry weight was very slow between 0-28 DAA. Thereafter a rapid increase in the berry weight was observed at 76 DAA for STD, LRW and LR (-UV-B, 2xOp50) (1.49, 1.35 and 1.31 g, respectively). The maximum berry weight of 1.20 g for LR (-UV-B, 2xUHI) was reached at 103 DAA. A significant difference ($p \leq 0.001$) in the average berry weight was observed among the four treatments in 2011/2012 (Table 3.6).

Mean berry weight of berries grown under lower light intensities (STD, LRW-UV-B and STD-UV-B) was slightly higher than the berries grown under higher light intensities (LRW) in 2010/2011 (Table 3.6). A similar pattern was observed in the 2011/2012 season as the STD had the highest average berry weight and the berries from the LR (-UV-B, 2xUHI) had the lowest mean berry weight and the highest light exposure (Table 3.5 & 6). This observed phenomenon could be due to the shaded berries having a lower transpiration rate which influenced the turgor pressure which will result in

enlargement of the berry as previously reported (Crippen & Morrison, 1986; Reynolds *et al.*, 1986; Blanke & Leyhe, 1987; Price *et al.*, 1995). The berries from the LR (-UV-B, 2xUHI) treatment had considerable lower berry weight compared to the other three treatments which can be ascribed to higher exposure to solar radiation and lower shading ability which resulted in higher transpiration rate (Table 3.6).

Table 3.6. Mean berry weights (n=3) determined during the 2010/2011 and 2011/2012 season.

| 2010-2011 | | 2011-2012 | |
|----------------|--------------|-------------------|------------------|
| Treatments | Berry weight | Treatments | Berry weight (g) |
| STD | 0.9 | STD | 1.0 a |
| LRW | 0.8 | LRW | 0.9 b |
| STD-UV-B | 0.9 | LR (-UV-B,2xOp50) | 0.9 b |
| LRW-UV-B | 0.9 | LR (-UV-B,2xUHI) | 0.8 c |
| p-value | ns | p-value | *** |

STD (Shaded/Control); LRW (Leaf Removal West); STD with decreased UV-B radiation (STD-UV-B); LRW with decreased UV-B radiation (LRW-UV-B); LR (-UV-B, 2xOp50) (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR (-UV-B, 2xUHI) (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Figure 3.2 shows the accumulation of total soluble solids and sugar (mg/berry) during the ripening period in the 2010/2011 and 2011/2012 season for each of the respective treatments. An active period of sugar accumulation was noted per berry from véraison at 41 DAA and 54 DAA in the respective seasons. Sugar accumulation for all treatments followed a similar trend although the 2010/2011 season was characterised by a rapid increase in sugar accumulation whilst the 2011/2012 season was characterised by a slow accumulation of sugars. The rapid increase in sugar accumulation in 2010/2011 could be due to water loss as a result of the increased temperatures and higher evaporation rate.

The mean sugar content (mg/berry) differed significantly among treatments in both the 2010/2011 ($p \leq 0.001$) and 2011/2012 ($p \leq 0.05$) seasons. The mean sugar content in the 2010/2011 season was the highest in the LRW-UV-B treatment (231 mg/berry). The mean sugar content in the remaining treatments was 208.5, 204.8 and 216.8 mg/berry for STD, LRW and STD-UV-B, respectively. In the 2011/2012 season STD treatment had the highest sugar content (216.5 mg/berry) while LR (-UV-B, 2xUHI), LRW and LR (-UV-B, 2xOp50) exhibited sugar contents of

205.8, 199.6 and 198.3 mg/berry, respectively. The differences in the sugar content can be attributed to the variation in the berry size which were altered by the exposure at a given temperature and light intensity. Our findings support a study by Buttrose *et al.* (1971) that reported similar effects of air temperature on berry size and total soluble solids that varied with the duration of exposure on berry growth stage. Berry parameters at harvest are depicted in Addendum 1.

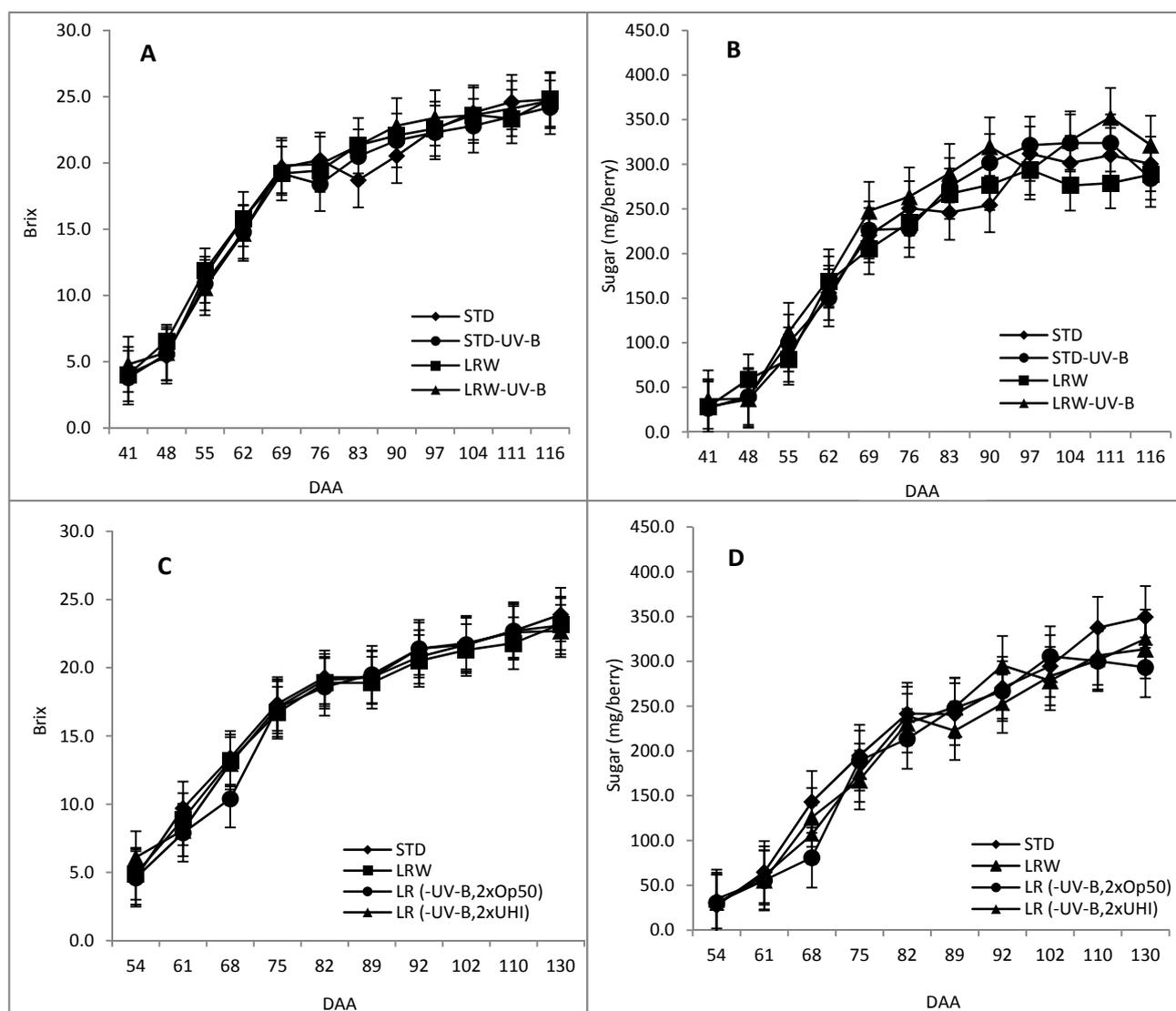


Figure 3.2. Total soluble solids and sugar accumulation determined in days after anthesis (DAA). (a) 2010/2011 TSS accumulation, (b) 2010/2011 sugar accumulation, (c) 2011/2012 TSS accumulation and (d) 2011/2012 sugar accumulation in 2011/2012. Each value represents the mean of 3 replicates \pm standard error.

3.5.3. The concentration and content of grape seed flavan-3-ol monomers, dimers and seed tannin

Evolution of seed flavan-3-ol monomer and dimer concentrations and content

The impact of light and temperature on the pattern of development of flavan-3-ol monomer, dimer and total tannin concentration (mg/g seed) and content (mg/berry) were determined in grape seeds during berry development. The main flavan-3-ol monomers present in the seeds were (+)-catechin, (–)-epicatechin and (–)-epicatechin-3-O-gallate with (+)-catechin having the highest concentration consistently throughout berry development in 2010/2011 and 2011/2012, respectively (Addendum 2 & 3). This is in agreement with the findings of Su & Singleton (1969), Prieur *et al.* (1994), Kennedy *et al.* (2000a) and Kennedy *et al.* (2000b) who found that (+)-catechin, (–)-epicatechin and (–)-epicatechin-3-O-gallate were the main seed flavan-3-ol monomers in different grape cultivars. Procyanidin B2 (EC-(4 β -8)-Ec) was the most abundant dimer in the seeds of the two measured (B1 (EC-(4 β -8)-Cat) and B2, Addendum 2 & 3). Our results are in accordance with that of Ricardo-da-Silva *et al.* (1992 a & b); Prieur *et al.* (1994), De Freitas *et al.* (2000) and Jordão *et al.* (2001) who reported that B2 was the predominant dimer in grape seeds irrespective of the grape cultivar.

The concentration and content of individual monomers and dimers followed a similar pattern increasing from fruit-set, reaching a maximum close to véraison followed by a decrease until harvest in both seasons (Fig. 3.3). In 2010/2011, a significant difference ($p \leq 0.05$) was observed in the seed monomeric and dimeric concentration and content amongst the treatments at 48 DAA. Similar concentrations and content were observed at 116 DAA (harvest) (Fig 3.3 a & b). In 2011/2012, the flavan-3-ol monomer and dimer concentration and content reached a maximum 14 days later in the LR (-UV-B, 2xOp50) and LRW treatments than the STD and LR (-UV-B, 2xUHI) treatments (Fig 3.3 a&b). Significant differences ($p \leq 0.001$) in flavan-3-ol monomer and dimer concentration and content were observed between 68–130 DAA. More specifically significant differences were also observed at 68 DAA ($p \leq 0.01$), 82 DAA ($p \leq 0.001$), 96 DAA and 110 DAA ($p \leq 0.001$) and 130 DAA ($p \leq 0.05$) (Fig. 3.3 c & d).

Our findings agrees with those of Kennedy *et al.* (2000a) who found that the flavan-3-ol monomers decreased from 1.34 mg/berry at véraison to 0.47 mg/berry at the last sampling date irrespective of the vine water status during ripening. Furthermore, Kennedy *et al.* (2000b) also reported an increase in the (+)-catechin, (–)-epicatechin and (–)-epicatechin-3-O-gallate flavan-3-ol monomers at 5 January (green berry stage) from 0.03, 0.04 and 0.02 to a maximum of 0.25, 0.22 and 0.07 mg/berry on 24 January (véraison). Downey *et al.* (2003b) observed a decline in the concentration (mg/g seed) of flavan-3-ol monomers during ripening when the concentration reached a maximum of ± 16 mg/g seed and a content of ± 1.8 mg/berry in Shiraz one week post-véraison. Thereafter, it was followed by a decrease reaching a concentration of ± 3 mg/g seed and 0.4 mg/berry at harvest. Cortell & Kennedy (2006) reported a decrease in the seed monomer concentration in shaded and compared to exposed treatments from véraison until harvest.

Lorrain *et al.* (2011) reported a decrease in the dimers B1 and B2 concentrations during maturation. However, due to the low concentrations of procyanidin B1 and B2 we discuss them in conjunction with the flavan-3-ol monomers.

In 2010/2011 the total monomeric and dimer concentration increased until a maximum of 23.1, 17.7, 18.4 and 15.3 mg/g seed was reached for STD, LRW, LRW-UV-B and STD-UV-B at véraison (48 DAA) respectively (Fig 3.3 a). Thereafter, the concentration sharply declined until 3.5, 2.6, 2.6 and 3.7 mg/g seed for STD, LRW, STD-UV-B and LRW-UV-B, respectively at 116 DAA (harvest). The content (mg/berry) of monomers and dimers followed the same pattern with a maximum observed at 48 DAA for all of the treatments (0.92, 0.57, 0.56 and 0.62 mg/berry for STD, LRW, LRW-UV-B and STD-UV-B, respectively). Thereafter it declined until 0.27, 0.16, 0.15 and 0.12 mg/berry for STD, LRW, LRW-UV-B and STD-UV-B, respectively at harvest (116 DAA) (Fig. 3.3 b).

In 2011/2012 the flavan-3-ol monomer and dimer concentration and content reached a maximum 14 days later in the LR (-UV-B, 2xOp50) and LRW treatments than the STD and LR (-UV-B, 2xUHI) treatments. A maximum concentration of 12.0 and 8.13 mg/g seed was reached at 54 DAA for STD and LR (-UV-B, 2xUHI) and 12.0 and 14.9 mg/g seed for LRW and LR (-UV-B, 2xOp50) was reached at véraison (68 DAA) followed by a decrease to 3.6, 2.7, 3.4 and 1.0 mg/g seed for

STD, LRW, LR (-UV-B,2xOp50) and LR (-UV-B, 2xUHI), respectively (Fig. 3.3 c). The monomeric and dimeric content reached a maximum of 0.67 and 0.62 mg/berry for STD and LR (-UV-B, 2xUHI) at 54 DAA while a maximum of 0.65 and 0.69 mg/berry was reached at 68 DAA for LR (-UV-B, 2xOp50) and LRW followed by a decrease until harvest 130 DAA (Fig. 3.3 d). The monomer and dimer concentration and content accumulated in an overall similar pattern in both seasons. The decrease in the monomer and dimer concentration and content confirms the findings of other studies that reported that the bulk of tannin synthesis occur before véraison followed by a decrease which can be ascribed to a reduction in the extractability of the seed tannin (Saint-Cricq de Gaulejac *et al.*, 1997; Kennedy *et al.*, 2000 a & b).

When comparing the STD and LRW treatments (the two treatments which were consistent among the two seasons) it is clear that the mean monomer and dimer concentrations and content of STD treatment were similar at harvest for the two seasons (Addendum 4). These results indicates a minimal light and temperature effect which are consistent with the findings of Dokoozlian & Kliewer (1996) and Downey *et al.* (2004) who suggested that shading showed minimal variation in seed chemistry. However, the LRW mean monomer and dimer concentration were significantly lower in 2011/2012 when compared to 2010/2011 (Addendum 4) indicating seasonal effects. In the treatments with the UV-B suppression sheets, we can see that the exclusion of UV-B radiation had an impact on the overall monomer and dimer concentration and content. However, we did not have similar UV-B suppression treatments in more than one season. Therefore, having the UV-B suppression sheets on one side of the canopy in the 2010/2011 season could possibly have minimized the effect of UV-B radiation exclusion on monomer and dimer concentration. At this stage the STD and LRW treatments indicate a larger seasonal impact of flavan-3-ol monomer and dimer concentration and content than the UV suppression sheet treatments. The more severe treatments in 2011/2012 exhibited clear differences among treatments. However, these findings require more study to determine the impact of UV-B suppression on seed monomer and dimer evolution, by using the same UV-B suppression material in multiple seasons.

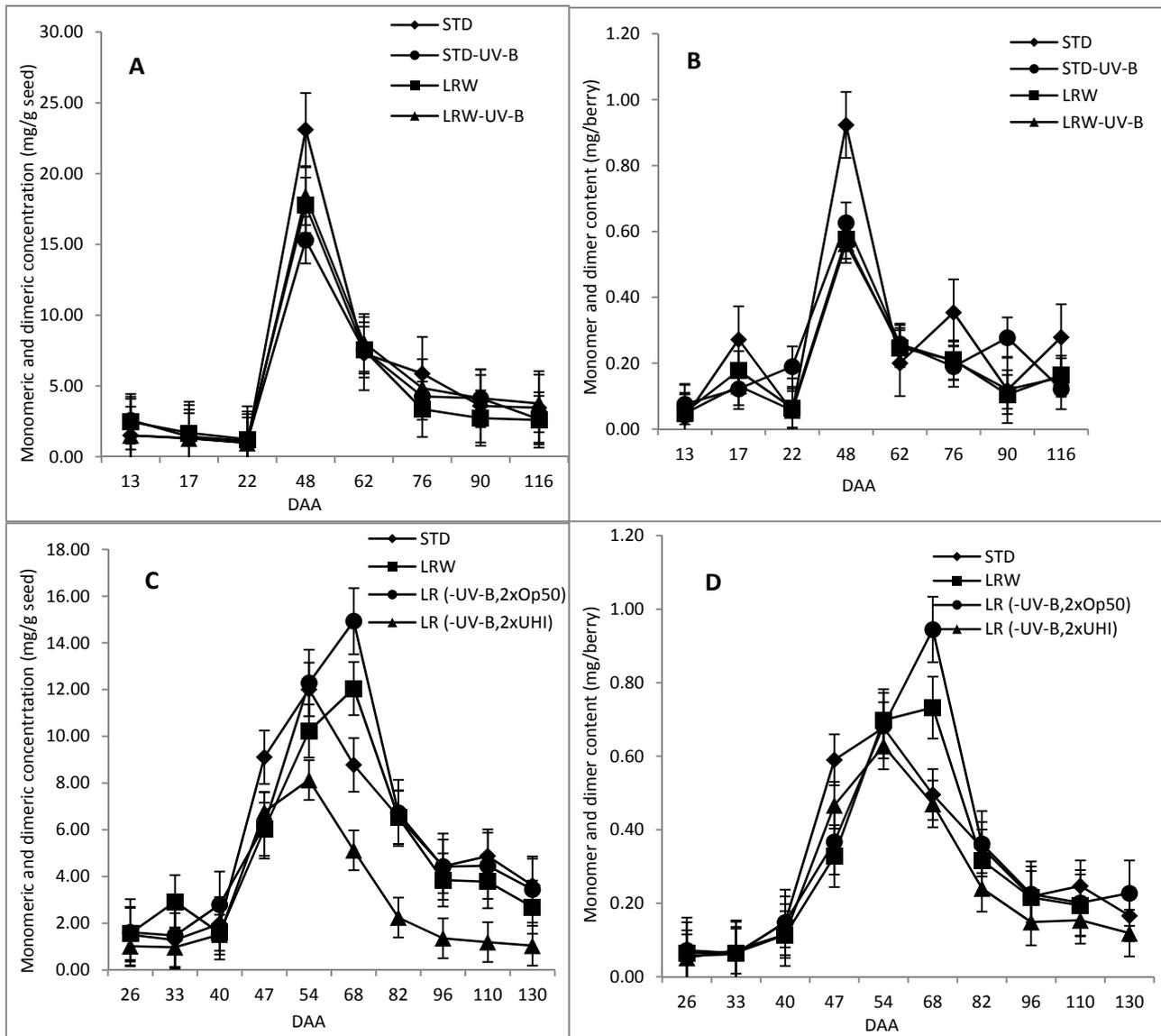


Figure 3.3. Developmental changes in the seed monomeric and dimeric concentration (mg/g seed weight) and content (mg/berry) during berry development under different light conditions. (a) 2010/2011 Grape seed flavan-3-ol monomer and dimer concentration (b) 2010/2011 grape seed flavan-3-ol monomer and dimer content (c) 2011/2012 grape seed flavan-3-ol monomer and dimer concentration and (d) 2011/2012 grape seed monomer flavan-3-ol content. Each value represents the mean of 5 replicates \pm standard error.

Evolution of seed tannin concentration and content

The pattern of seed tannin concentration (mg/g seed) and content (mg/berry) development differed among the seasons investigated according to RP-HPLC determination (Fig. 3.4). The 2010/2011 season was characterised by an increase in the seed tannin concentration and content until véraison, followed by a decrease and another increase from 76 DAA in all the treatments until harvest (Fig. 3.4 a & b). The 2011/2012 accumulation pattern fluctuated during berry ripening (Fig.

3.4 c & d). The LR (-UV-B, 2xUHI) treatment had significantly lower seed tannin concentration and content which can possibly be linked to the lower seed number in this treatment (Addendum 5).

The maximum seed tannin concentration was observed on 48 DAA at 70.9, 56.3, 53.9 and 52.8 mg/g seed for STD, LRW, STD-UV-B and LRW-UV-B, respectively in 2010/2011 (Fig.3.4 a). The content of total seed tannin followed the same pattern with a maximum observed at 48 DAA for all the treatments at 4.5, 3.3, 3.5 and 3.1 mg/berry for STD, LRW, STD-UV-B and LRW-UV-B, respectively (Fig. 3.4 b). Thereafter, a decrease was observed in the content and 2.6, 2.3, 1.9 and 1.8 mg/berry were obtained at 116 DAA in 2010/2011. In 2011/2012 the maximum concentration of 50.3 and 40.5 mg/g seed was observed at 47 DAA (12 weeks before harvest) STD and LR (-UV-B, 2xUHI) while a maximum concentration of 47.8 and 56.2 mg/g seed were reached at 54 DAA (11 weeks before harvest) for LRW and LR (-UV-B, 2xOp50) (Fig. 3.4 c). Thereafter, a sharp decline in the concentration of LR (-UV-B, 2xUHI) was observed in 2011/2012 until 130 DAA whereas the rest of the treatments the rest of the treatments decreased in concentration. In 2011/2012 the maximum content was reached at 47 DAA for STD and LR (-UV-B, 2xUHI) at 3.2 and 2.7 mg/berry while the maximum was reached at 54 DAA for LRW and LR (-UV-B, 2xOp50) at 3.3 and 3.1 mg/berry. Similar content were observed at 130 DAA (Fig. 3.4 d).

This data are in agreement with previous studies that reported an increase in seed tannin concentration with an increase in seed number per berry (Addendum 5) (Habertson *et al.* 2002; Roby *et al.*, 2004). Overall the 2010/2011 season had a higher concentration at harvest which could be potentially be due to a difference in seeds per berry and seed maturity compared to the 2011/2012 season. However, seed number per berry was only determined in 2011/2012 (Addendum 5) and not in 2010/2011. Ewart & Kliewer (1977), Habertson *et al.* (2002), Roby *et al.* (2004) and Pastor Del Rio & Kennedy (2006) observed that grapes with a higher number of seeds per berry showed an increase in proanthocyanidins per berry which are dependent on the accumulation of the flavan-3-ol monomers and dimers. Similar seed tannin concentration and content were achieved in the STD treatments in the two seasons (Addendum 4). However, variation in the mean seed tannin concentration was visible in the treatments where UV-B suppression sheets were present (Addendum 4). From our results we suggest that a high level of

exposure to solar radiation and the exclusion of UV-B (LR (-UV-B, 2xUHI)) could negatively impact seed tannin accumulation or extraction. Additional studies are needed to confirm this result. Both seasons showed a minimal impact of seed tannin concentration and content at harvest as demonstrated by treatments STD and LRW, which were constant for two seasons, although the seed tannin concentration obtained for LRW around harvest was significant. This indicates that seasonal factors did not have a large impact on the final seed tannin content.

Our results confirm the findings of Cortell & Kennedy (2006) and Bautista-Ortín *et al.* (2012) that reported a maximum seed concentration close to véraison in Monastrell, Cabernet Sauvignon and Shiraz with a decrease towards harvest. Bogs *et al.* (2005) suggested that the genes related to tannin synthesis were no longer detectable after véraison.

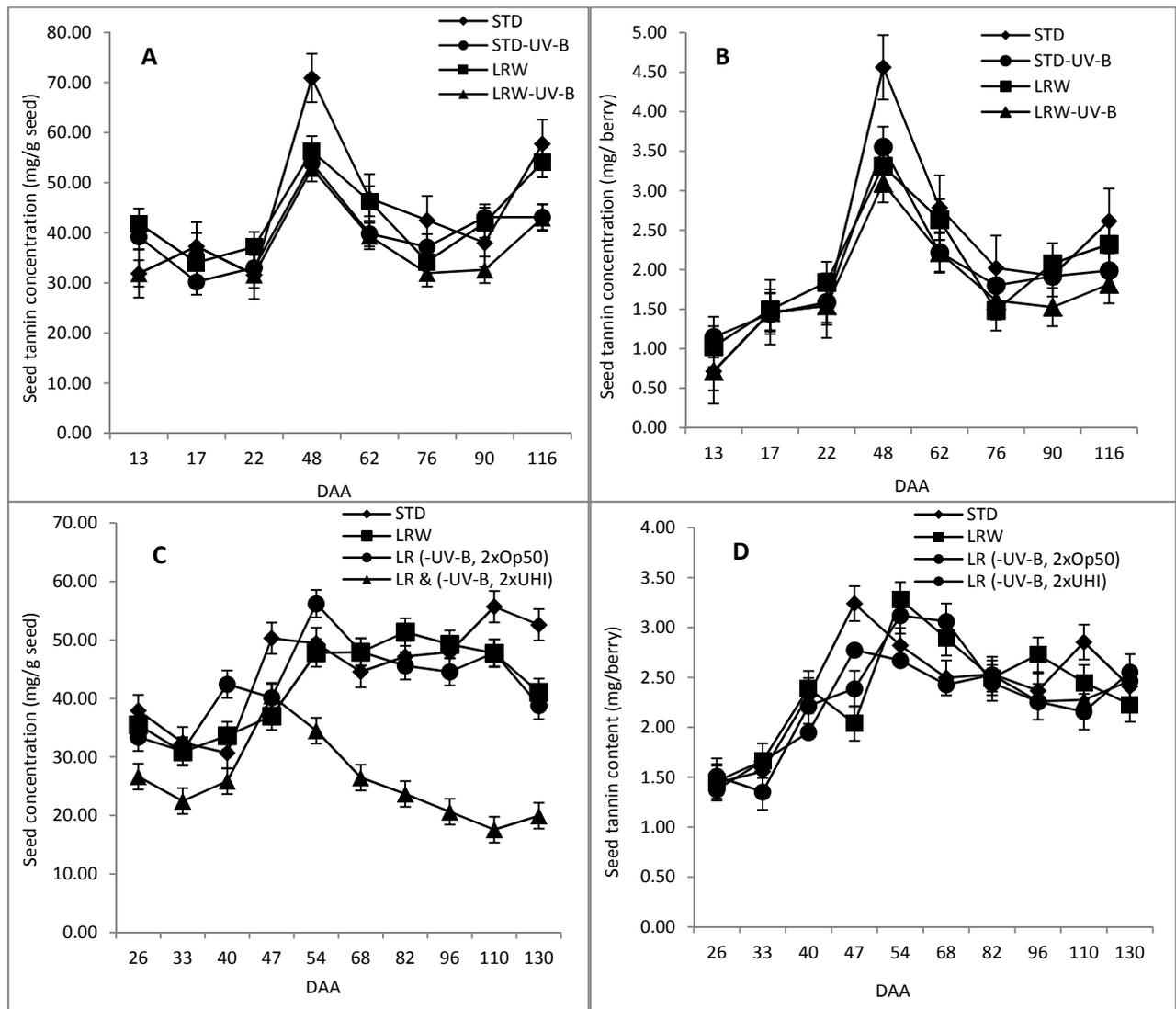


Figure 3.4. Pattern of seed tannin concentration expressed as mg/g fresh seed weight and content expressed as mg/berry during berry development under different light conditions. (a) 2010/2011 Grape seed tannin concentration (b) 2011/2012 grape seed tannin content (c) 2011/2012 grape seeds tannin concentration and (d) 2011/2012 grape seed tannin content. Each value represents the mean of 5 replicates \pm standard error.

Impact of temperature and PAR on seed flavan-3-ol monomers, dimers and total seed tannin evolution

Numerous studies have evaluated the evolution of seed flavan-3-ol monomers, dimers and total seed tannin and found that maximum concentration and content was reached around véraison followed by a decrease (Kennedy *et al.* 2000a; Kennedy *et al.* 2000b; Downey *et al.* 2003b; Pastor del Rio & Kennedy, 2006). Furthermore, Downey *et al.* (2004) and Cortell & Kennedy (2006) studied the effect of light and temperature on grape flavonoid synthesis during berry development.

From our results no real differences were observed in temperature in the respective treatments in both seasons (Table 3.4). It's been reported that temperature and light conditions play an essential role in berry development. Dokoozlian (2000) reported that germination and pollen growth are greatly reduced or even inhibited when temperatures fall below 15.6° or exceed 37.8°C. In the 2010/2011 season, December 2010 (berry set) was characterised by a warm and dry period which could have contributed to favourable conditions for seed development which resulted in a higher seed number and therefore influencing the evolution of the seed flavan-3-ol monomers, dimers and total tannin. In the 2011/2012 season, mid-October to November of 2011 (flowering until berry set) had temperatures considered as below normal with November being 2°C colder than average which resulted in a long, protracted flowering period. However, the treatments and the temperature loggers were only installed after flowering and it is difficult to assess the impact of temperature at flowering which will have a direct impact on the seed number and size per berry in a particular season. We hypothesize that the evolution of the flavan-3-ol monomers, dimers and total tannin were a function of the seed number per berry and were not influenced by the light and temperature conditions during the growing season. Although the concentration and content were consistent in the STD treatment indicating minimal seasonal impact other than delaying point of maximum concentration. This corresponds with the findings of other studies who suggested that seed proanthocyanidin accumulation is not highly responsive to environmental influences (Cortell *et al.* 2005). However, it is difficult to disseminate the impact of UV-B radiation on the monomer/dimer and seed concentration and content in our study as the UV-B exclusion treatments were not applied similarly over the two seasons.

Our study shows that the accumulation of seed tannins coincides with seed development and therefore commended at the early stages of berry development as seen before by many others. Researchers reported that the bulk of tannin synthesis occurred prior to véraison (Kennedy *et al.*, 2000a; Kennedy *et al.*, 2000b; Jordão *et al.*, 2001; Downey *et al.*, 2003b; Downey *et al.*, 2006). Thereafter the concentration decreased which can be ascribed to the decline in the extractability of the tannins, conjugation of proanthocyanidins with other cellular components and oxidative cross linking of polymers (Cheynier *et al.*, 1997; Saint-Cricq de Gaulejac *et al.*, 1997; Kennedy *et al.*,

2000b). Tannin accumulation has been studied extensively during ripening (Czochnska *et al.*, 1979; de Freitas & Glories, 1999; Kennedy *et al.* 2000a). From our study it appeared that the seed development after flowering could potentially be influenced by light quality and quantity, resulting in a variation in seed number (number of seeds per berry) and impacting flavan-3-ol concentration and content (Addendum 5). However, the number of seeds at each sampling date and treatment were only determined in 2011/2012 and should therefore be determined in additional seasons in order to confirm this. In conclusion, grape seed flavan-3-ol monomers, dimers and total tannin evolution are potentially dependent on the seed number per berry which may be influenced by the light and conditions during flowering.

3.5.4 The concentration and content of grape skin flavan-3-ol monomers, dimers and total skin tannins

Evolution of skin flavan-3-ol monomer and dimer concentrations and content

Flavan-3-ol monomer, dimer and total skin tannin concentration (mg/g skin) and content (mg/berry) evolution were studied under altered temperature and light conditions during berry development. The main flavan-3-ol monomers determined in the skins were (+)-catechin, (-)-epicatechin and (-)-epicatechin-gallate and the dimers EC-(4 β -8)-Cat (B1) and EC-(4 β -8)-Ec (B2) in both seasons (Addendum 6 & 7).

Numerous authors did not analyse the flavan-3-ol monomer concentrations due to the complex interactions between sugars and other phenolics resulting in low concentrations of flavan-3-ol monomers during quantification (Kennedy *et al.*, 2002, Monagas *et al.*, 2003; Cortell & Kennedy, 2006). The accumulation pattern of skin monomers and dimers differed among the two seasons. Overall, in 2011/2012 the skin monomer and dimer concentration and content accumulation pattern coincided with that of Kennedy *et al.* (2002), Downey *et al.* (2006) and Pastor del Rio & Kennedy (2006) who reported a peak in the skin proanthocyanidin concentration near véraison and then a decline with increasing maturity. However, 2010/2011 was characterised by an increase in concentration and content from 62–116 DAA in STD, LRW, LRW-UV-B, while STD-UV-B

decreased (Fig. 3.5 a & b). The observed increase in the 2010/2011 season are due to the increased (–)-epicatechin and (–)-epicatechin-3-O-gallate monomer concentration and content (Addendum 6). This suggests an up-regulation in the biosynthesis of (–)-epicatechin and (–)-epicatechin-3-O-gallate in the 2010/2011 season. STD-UV-B was the treatment with the lowest PAR and % light (Table 3.5) compared to the other three treatments. However, further studies are needed in the expression of the gene encoding biosynthetic enzyme (leucoanthocyanidin reductase) to confirm this phenomenon. In 2011/2012 a decrease in the monomer and dimer skin concentration and content was observed from 54–130 DAA (Addendum 7). Higher monomeric and dimeric concentration and content were obtained at harvest in the 2010/2011 season compared to the 2011/2012 season. However, the mean monomer and dimer concentration in the STD treatment were similar among the respective seasons (Addendum 8). However, the mean LRW skin flavan-3-ol monomer and dimer concentration were 50% less in 2010/2011 when compared to 2011/2012 (Addendum 8). The seasonal impact on skin flavan-3-ol monomers and dimers are unclear due to inconsistent results obtained from the STD and LRW treatments over the two seasons.

In 2010/2011 a significant difference ($p \leq 0.001$) in the monomeric and dimeric concentration and content among the treatments from 62–116 DAA (Fig. 3.5 a & b) was observed. The monomeric and dimeric skin concentration and content of STD-UV-B were significantly lower ($p \leq 0.01$) at 62 DAA than the other three treatments (Fig. 3.5 a & b). The significant differences in the monomeric and dimeric concentration and content among the STD and STD-UV-B treatments can be ascribed to importance of UV-B radiation for biosynthesis. In general the shaded treatment STD had the highest flavan-3-ol monomer and dimer concentration; however the other shaded treatment STD-UV-B had the lowest concentration and content. In the latter, UV-B radiation was additionally impacted indicating a potential role of UV-B radiation on flavan-3-ol synthesis. Downey *et al.* (2003b) also found an increase in the monomer concentration and content in shaded canopies and a decrease in exposed fruit. In 2011/2012 a similar accumulation pattern was observed among the different treatments with a maximum concentration at 54 DAA (véraison) for STD, LRW and LR (-UV-B, 2xUHI) followed by a decrease reaching similar levels at 130 DAA (harvest) (Fig. 5.5 c). The

LRW treatment had significantly higher ($p \leq 0.001$) flavan-3-ol monomer and dimer concentration and content from 68–110 DAA compared to the other treatments in the cooler season (Fig. 5.5 c & d). This indicate variable treatment effect on flavan-3-ol monomer and dimer evolution depending on the season, suggesting that season had a larger impact on flavan-3-ol concentration and content than the applied treatments. The treatments reducing UV-B radiation (LR (-UV-B, 2xOp50), LR (-UV-B, 2xUHI)) exposure in the bunch zone did result in decreased flavan-3-ol monomer and dimer concentration although there were no significant differences at harvest.

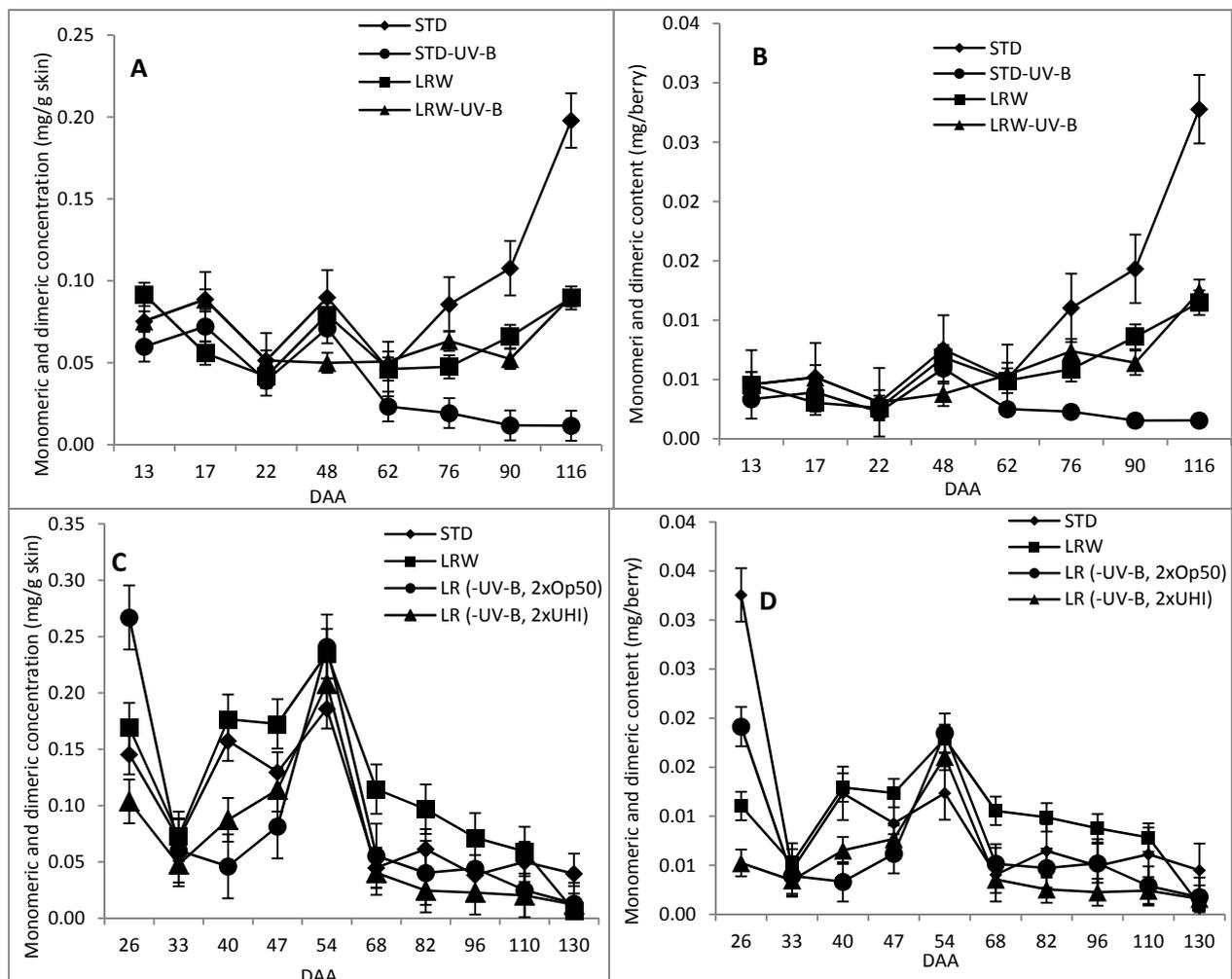


Figure 3.5. Developmental changes in the skin monomer and dimer concentration (mg/g skin) and content (mg/berry) during berry development under different light conditions: (a) 2010/2011 Grape skin monomer and dimer concentration (b) 2010/2011 grape skin monomer and dimer content (c) 2011/2012 grape skin monomer and dimer concentration and (d) 2011/2012 grape skin monomer and dimer content. Each value represents the mean of 5 replicates \pm standard error.

Evolution of total skin tannin concentration and content

The concentration and content of total skin tannins differed among the two seasons among all the treatments (Fig. 3.6). In 2010/2011 the total skin concentration (mg/g skin) reached a maximum at 48 DAA followed by a decrease in all the treatments and an increase from 62 DAA in LR-UV-B until harvest and an increase in the remainder of treatments from 76 DAA (Fig. 3.6 a). A similar pattern was observed in the skin total tannin content evolution in 2010/2011 with significant differences observed among different treatments from 62 DAA (post véraison) until 116 DAA (harvest) (Fig. 3.6 b). The 2011/2012 concentration evolution was characterised by a maximum at 54 DAA followed by a decrease in all the treatments and remained relatively constant until 130 DAA (harvest) (Fig. 3.6 c). Significant differences ($p \leq 0.05$) in the concentration were observed at 26 DAA ($p \leq 0.001$), 47 DAA ($p \leq 0.01$) and 82 DAA among the different treatments (Fig. 3.6 c). The skin total tannin content followed a similar pattern and similar tannin levels were obtained at harvest for all treatments. Significant differences were observed at 26 DAA ($p \leq 0.001$) and 47 DAA ($p \leq 0.01$) (Fig. 3.6 d). The significant differences observed among the treatments in a given season are potentially due to variation in the berry size and maturity as well as the interference of phenolic compounds and sugars.

Overall the skin concentration and content were higher in the 2010/2011 season when compared to the 2011/2012 season (Fig. 3.6; Addendum 8). In 2010/2011 skin tannin concentration and content from the LRW treatment were favoured by high light exposure (Table 3.5). These findings corresponds with that of Cortell *et al.* (2005) and Ristic *et al.* (2007) who found higher skin tannin concentration of tannins at harvest from low vigour vines (higher exposure) (Fig. 3.6 a & b). In the 2011/2012 season no clear pattern in the accumulation of the skin concentration and content were observed (Fig. 3.6 c & d). Numerous studies investigated the accumulation of skin tannins during fruit development in red grape cultivars (Kennedy *et al.*, 2001, 2002; Habertson *et al.*, 2002; Ojeda *et al.*, 2002; Downey *et al.*, 2003, 2004; Cortell *et al.*, 2005; Pastor del Rio & Kennedy, 2006; Castellarin *et al.*, 2007; Verries *et al.*, 2008; Hanlin & Downey, 2009). The variation in the skin concentration and content with season can be ascribed to the seasonal differences and the synergistic light exposure in a particular treatment. Our results show that skin tannin reaches a

maximum at véraison which corresponds with the findings of Cortell & Kennedy (2006) and Hanlin & Downey (2009) followed by a decrease. In our study the accumulation pattern increased again after véraison in both seasons (Fig. 3.6). Ojeda *et al.* (2002) also reported increasing tannin per berry throughout berry development in Shiraz. Similarly, Kennedy *et al.* (2002) showed an increase in tannin/berry throughout development in Cabernet Sauvignon. However, the analytical method (protein precipitation or HPLC after acid-catalysed depolymerisation techniques) used in the respective studies was identified as a reason for the variation in the evolution of the skin concentration and content. In our study the increase in skin tannin concentration after véraison could possibly be due to the RP-HPLC method used which accounts for all the phenolic material that do not separate into individual peaks. This value is an under estimation of the tannin as some is lost on the baseline (Peng *et al.*, 2001, 2002). Hanlin & Downey (2009) attributed the variability of skin tannin concentration and content to the growing region and the vineyard management practices. Numerous authors reported a variation in the harvest concentration of skin tannin due to fertilization, water potential and altitude (Mateus *et al.*, 2001; Kennedy *et al.*, 2002; Delgado *et al.*, 2004). From our data it is clear that the skin tannin evolution is influenced by seasonal variation. Downey *et al.* (2004) and Pastor del Rio & Kennedy (2006) also observed that the season has an impact on the skin tannin evolution. We can deduct from our study that light exposure in a given season can have a positive impact on skin tannin accumulation if the light quality is above a certain threshold which needs to be determined with further study. In 2010/2011 the PAR of $517.7 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for LRW resulted in a significant increase in tannin accumulation, but a PAR of $278.9 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in 2011/2012 did not. LR (-UV-B,2xUHI) also decreased UV-B light and had no impact on skin tannin accumulation. This may indicate a potential influence of UV-B light on tannin synthesis.

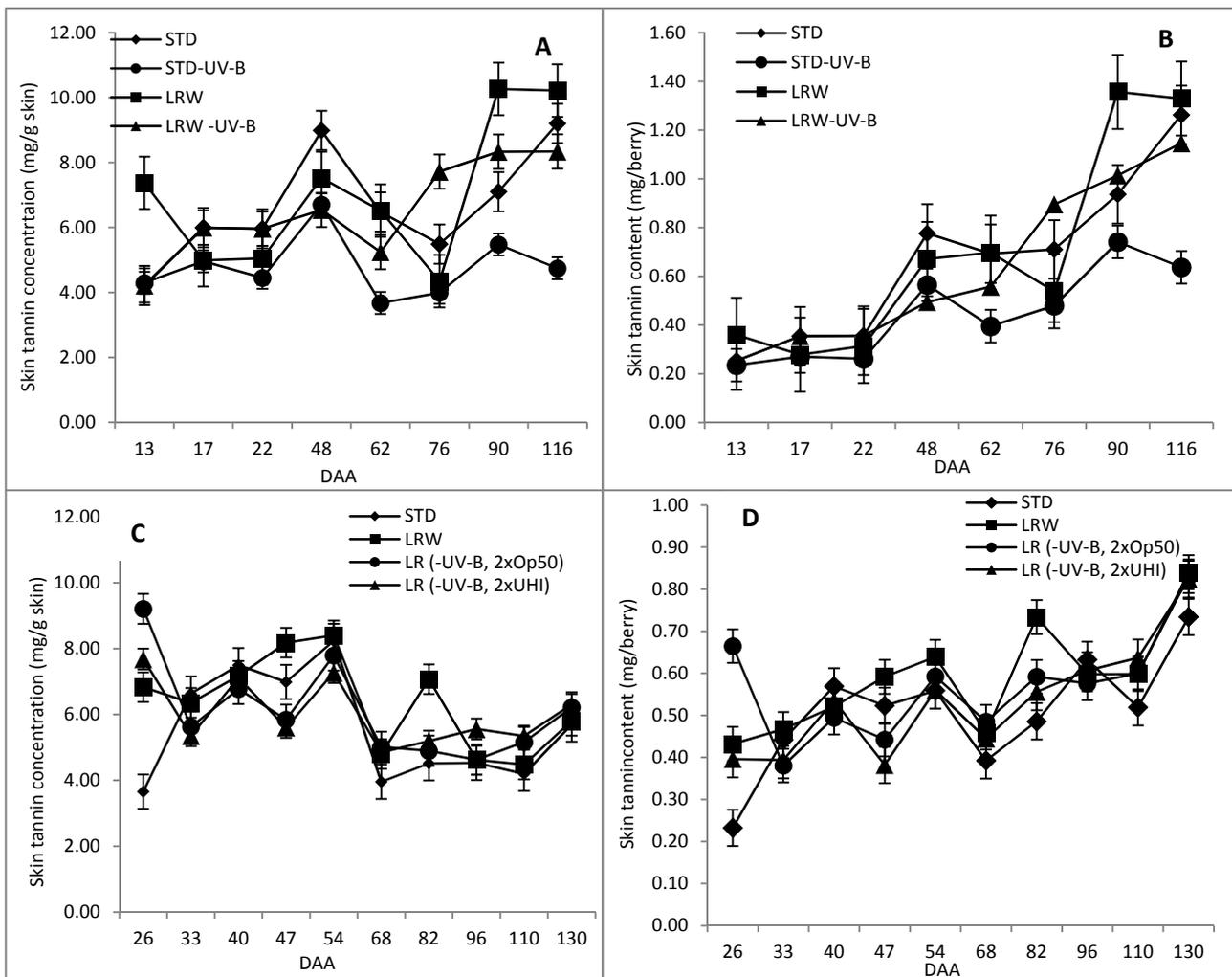


Figure 3.6. Developmental changes in the skin total tannin (mg/g skin) and content (mg/berry) during berry development under different light conditions: (a) 2010/2011 Grape skin total concentration, (b) 2010/2011 grape skin total content, (c) 2011/2012 grape skin total tannin concentration 2011/2012 and (d) grape skin total content in 2011/2012. Each value represents the mean of 5 replicates \pm standard error.

Impact of temperature and PAR on skin flavan-3-ol monomers, dimers and total tannin evolution

The accumulation pattern of flavan-3-ol monomers/dimers and total skin tannin was influenced by seasonal variation. Shading favoured the accumulation of (–)-epicatechin and (–)-epicatechin-3-O-gallate monomer and dimer concentration and content in 2010/2011 with a potential negative impact by UV-B restriction (Addendum 8). However, this could not be confirmed in the 2011/2012 season due to potential seasonal impact. The 2011/2012 season was cooler with lower DD amongst the treatments when compared to the 2010/2011 season. Furthermore, higher skin flavan-3-ol monomer/ dimer and total tannin concentration and content were observed in the

2010/2011 season compared to the 2011/2012 season. The 2011/2012 season favoured higher monomer and dimer concentrations from 68-110 DAA in the exposed treatments. It was difficult to distinguish whether seasonal differences and light or temperature influenced total skin concentration and content.

As mentioned earlier no real differences were observed in temperature of the respective treatments in both seasons (Table 3.4). From our results there is an indication that light quantity and quality can potentially have minor impact on flavan-3-ol and tannin accumulation in the skin. However, seasonal differences overshadowed treatment effects. These findings also highlight the photo-protective role tannin play in the berry skin. In 2010/2011 the total skin tannin concentration and content at 116 DAA in STD, LRW and LRW-UV-B differed significantly ($p \leq 0.05$) when compared to STD-UV-B. No significant differences were observed at 130 DAA in 2011/2012 amongst the treatments. In the 2010/2011 season the LRW treatment had a much higher PAR compared to other treatments, whereas the difference was less pronounced in the 2011/2012 season. Our results suggest that light exposure may promote skin tannin formation and further study is needed to confirm the potential impact of UV-B light.

3.6 The concentration and content of grape skin flavonols

Evolution of skin flavonol concentration and content

Flavonol concentration expressed as concentration of fresh tissue (mg/g skin) and content (mg/berry) were determined in the berry skins exposed to different temperatures and light conditions during ripening. Flavonol accumulation commenced after fruit-set until harvest in both seasons (Addendum 9 & 10). Throughout both seasons quercetin-glucoside and quercetin-glucuronide were the most abundant flavonol-glycosides with quercetin-rutinoside and quercetin-galactoside present in smaller quantities (Addendum 9 & 10). Mattivi *et al.* (2006) reported that myricetin is the major flavonol in Cabernet Sauvignon. The patterns of accumulation was characterised by an increase after fruit-set reaching maximum concentration and content at 76 DAA (4 weeks post-véraison) and remaining constant until 116 DAA in 2010/2011 (Fig. 3.7 a & b).

In 2011/2012 a maximum concentration and content was reached at 82 DAA followed by a decline until 130 DAA (Fig. 3.7 c & d).

A similar evolution pattern of skin flavonol concentration and content was observed in the two seasons. LRW treatments had significantly higher flavonol concentration and content throughout berry development (Fig.7; Addendum 11). STD had lower flavonol concentrations and content, while the treatments with the UV-B suppression sheets had the lowest concentrations and contents in both seasons (Fig. 3.7). Our results corresponds with the findings of Price *et al.* (1995), Haselgrove *et al.* (2000), Spayd *et al.* (2000), Downey *et al.* (2003a) and Downey *et al.* (2004) who reported that shaded fruit had lower flavonol-glucosides at harvest or during berry development in Cabernet Sauvignon, Shiraz and Merlot noir, respectively. In both seasons the patterns of accumulation were characterised by an increase after fruit-set reaching a maximum 4 weeks post-véraison and 5 weeks post-véraison in 2010/2011 and 2011/2012, respectively followed by small fluctuations in 2010/2011 or a decrease in 2011/2012 (Fig. 3.7 a-d). Downey *et al.* (2004) also found a decrease in flavonols per berry 2–4 weeks after véraison in both exposed and shaded fruit within one season, while the flavonol content fluctuated from véraison until harvest in the other seasons

Our results also indicate a clear seasonal impact on flavonol evolution during ripening and are due to the significant impact of the season on the the light quality and quantity (Table 3.5). The latter play a crucial role in the up-regulation of flavonol synthase (FLS) the gene that encodes the biosynthetic enzyme converting dihydroflavonols to flavonols (Spribille & Forkmann, 1984).

Impact of temperature and PAR on flavonol evolution

Despite higher temperatures in the LR (-UV-B, 2xUHI) treatment (Table 3.4) in 2011/2012, the flavonol concentrations obtained were lower when compared to the LR (-UV-B, 2xOp50) treatment. These findings suggest that temperature does not influence the flavonol accumulation. This is further supported by the findings of Spayd *et al.* (2002) who found that cluster temperature did not affect the flavonol concentration (quercetin-3-glucoside, myricetin-3-glucoside, kaempferol-3-glucoside) in exposed fruit. Azuma *et al.* (2012) observed flavonol synthase (FLS) was up-

regulated by light regardless of the temperature. In our study, temperature had little to no effect on flavonol concentration in grape skins.

Furthermore, it is clear that UV light stimulated flavonol biosynthesis as the concentration and content were significantly higher in the exposed treatment LRW in both seasons. Additionally, flavonol concentration and content decreased significantly when UV-B suppression sheets were present in both seasons. Therefore, we suggest that UV-B radiation play an important role in the photo-protection of the berry against light exposure. Increased sunlight radiation resulted in high levels of UV-exposure, resulting in increased flavonol levels. Shaded treatments exhibited lower concentrations compared to the LRW treatments. Our results indicate that flavonol concentration and content are dependent on the light quality. This in agreement with other studies who found that fruit exposed to different light qualities had higher flavonol-glucosides (Spayd *et al.*, 2002; Downey *et al.*, 2003a). The latter phenomenon confirms the findings of Flint *et al.* (1985) and Berli *et al.* (2011) who suggested that flavonols act as UV screening compounds, protecting the plant tissue from damage to light during berry ripening. This involves the accumulation of phenols in the epidermal cell vacuoles of leaf tissue and grape berries thereby protecting the photosynthetic mesophyll tissue (Olsen *et al.*, 1998; Kolb *et al.*, 2003; Berli *et al.*, 2011).

3.7 Concentration and content of anthocyanins in the berry skin

Evolution of anthocyanin concentration and content

Anthocyanin evolution during berry maturation was determined and the developmental changes expressed as concentration of fresh tissue (mg/g skin) and content (mg/berry). Mono-glucosides, acetyl-glucoside and coumaroyl-glucoside derivatives of delphinidin, petunidin, peonidin and malvidin were determined in both seasons (Addendum 12 & 13). The accumulation of the individual anthocyanins commenced at véraison, 48 DAA in 2010/2011 and 68 DAA in 2011/2012 (Fig. 3.8) as found by others Mori *et al.* (2005 & 2007) and Downey *et al.* (2004).

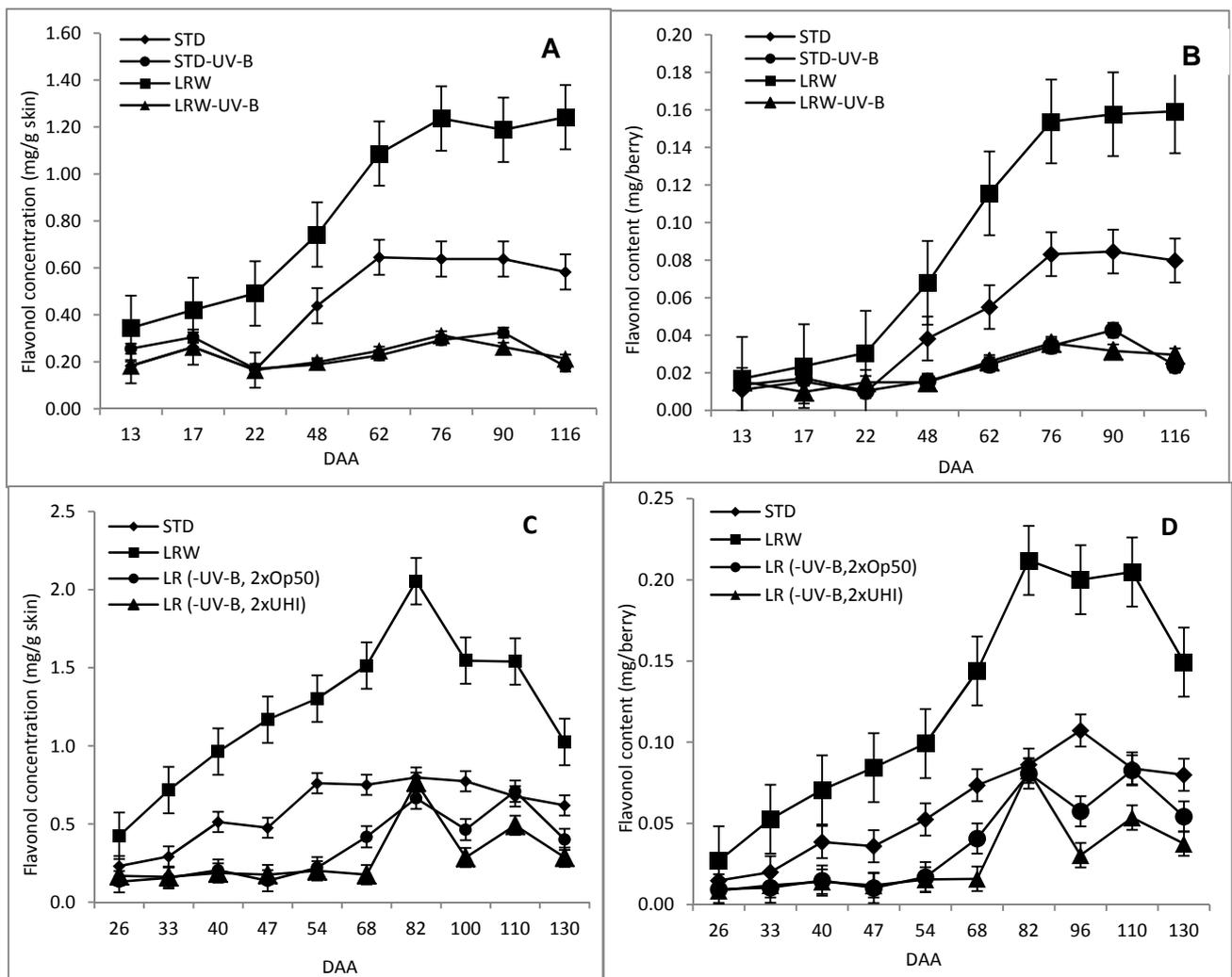


Figure 3.7. Developmental changes in the flavonol concentration expressed as mg/g fresh skin weight and content (mg/berry) during berry development under different light conditions: (a) 2010/2011 flavonol concentration (b) 2011/2012 flavonol concentration (c) 2010/2011 flavonol content in 2010/2011 and (d) 2011/2012 flavonol content. Each value represents the mean of 5 replicates \pm standard error.

The trend of anthocyanin accumulation differed between the two seasons as shown in Figure 3.8. The 2010/2011 season was characterised by an increase in concentration and content from véraison and a decrease from 90–116 DAA (Fig. 3.8 a & b). However, the 2011/2012 season was characterised by an increase after véraison between 68–82 DAA, a decrease between 83–96 DAA and another increase from 96–110 DAA, followed by a decrease from 110–130 DAA. In the 2011/2012 season, the anthocyanin concentration and content differed significantly ($p \leq 0.01$) among treatments for all of the sampling days (Fig. 8 c & d). The mean anthocyanin concentration and content were similar between the STD and LRW treatments in both seasons indicating no significant treatment impact as far as leaf removal goes (Addendum 14). However, in 2011/2012 season the shaded LR (-UV-B, 2xOp50) had the highest overall concentration and content when

compared to the other treatments. The LR (-UV-B, 2xUHI) treatment had the lowest concentration and content as shown in Addendum 14 while it was the treatment with the highest light exposure in addition to UV-B exclusion. Conflicting treatment results indicate that the season had a significant impact.

Overall there was no significant difference in the anthocyanin concentration and content in both the STD and LRW treatments for both seasons (Addendum 14). The mean anthocyanin concentration and content of the STD-UV-B and LRW-UV-B treatment in 2010/2011 were also similar (Addendum 14). Ryan & Revilla (2003) suggested that anthocyanin fingerprint of a grape cultivar grown in a given location changed slightly from year to year, probably as a consequence of anthocyanin biosynthesis modulation by weather conditions during ripening. Our results confirm that the treatments had little impact. In addition, temperatures exceeding 30°C preceding the sampling date at 96 DAA in 2011/2012 may have contributed to the observed decrease in anthocyanin concentration and content.

Furthermore there was a decrease in the individual anthocyanins under high temperatures (data not shown) (Addendum 13) in all the treatments. In general, treatments had less of an impact on anthocyanin evolution than the season. Other studies have also found significant differences between seasons (Brossaud *et al.* 1999; Spayd *et al.* 2002; Cortell *et al.* 2005) whereas Mazza *et al.* (1999) reported a minimal influence of the season in Cabernet franc, Merlot, and Pinot noir due to an atypical growing season over three seasons. Nevertheless, the 2010/2011 season was characterised by higher concentration and content at harvest when compared to the 2011/2012 season.

Impact of temperature and PAR on anthocyanin mono-glucosides and total anthocyanin evolution

In 2010/2011 the concentration of the total anthocyanins varied during ripening in all the treatments, but similar concentration were observed at harvest (116 DAA). As previously mentioned the 2010/2011 season was characterised by high light intensities (Table 3.4) which stimulated anthocyanin accumulation irrespective of the treatment resulting in higher anthocyanin

content at harvest compared to the 2011/2012 season. Several studies have found that light exposure has a positive effect on cluster anthocyanin concentration (Haselgrove *et al.*, 2000, Bergqvist *et al.*, 2001, Spayd *et al.*, 2002, Jeong *et al.*, 2004) while in contrast Downey *et al.* (2004) found that that anthocyanin biosynthesis is not readily affected by sunlight.

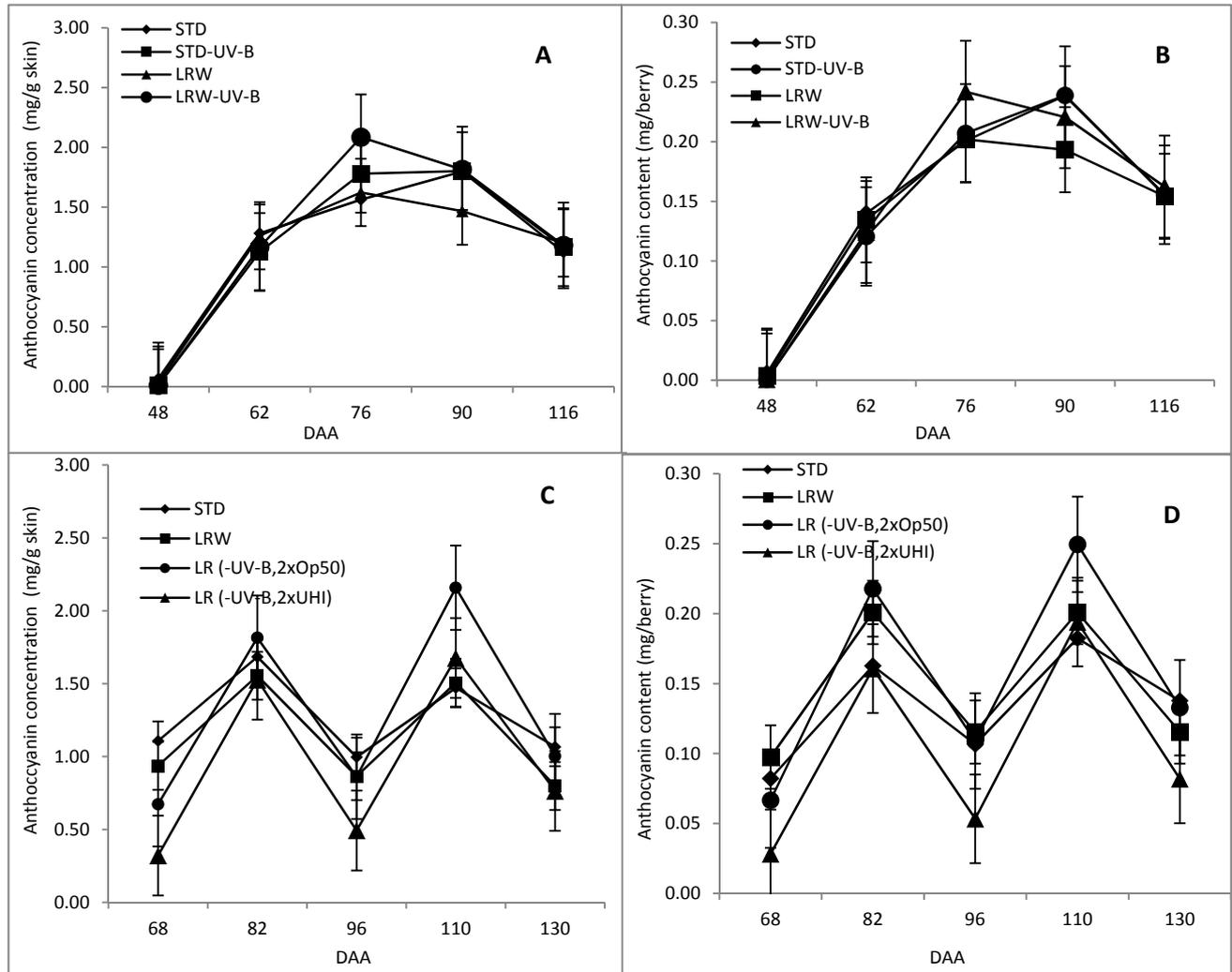


Figure 3.8. Developmental changes in the skin anthocyanin concentration expressed as mg/g fresh skin weight and content (mg/berry) during berry development under different light conditions: (a) 2010/2011 anthocyanin concentration, (b) 2011/2012 anthocyanin content, (c) 2011/2012 anthocyanin concentration and (d) 2011/2012 anthocyanin content. Each value represents the mean of 5 replicates \pm standard error.

3.8 CONCLUSION

From this study, we can conclude that flavonoid evolution is dependent on the prevailing light quality/quantity and temperatures during berry development in a particular season. The bulk of both seed and skins monomers, dimers and tannin were synthesised just after fruit-set and reached a maximum at véraison after which it decreased in both seasons. The post-véraison decrease of the seed and skins monomers, dimers and tannin concentration and content is ascribed to a reduction in the extractability of the tannin post-véraison. Seed tannin concentration and content were potentially influenced by the seed number per berry.

From our study we can deduce that light exposure in a particular season have the most significant impact on tannin accumulation in grape skins and little impact in grape seeds. We hypothesize that the light quality and quantity are a potential factor in the final skin total concentration and content as UV-B exclusion resulted in slightly lower concentration and content. Brown *et al.* (2005) found that low UV-B fluence rates, results in UV-B stimulation of some genes that are involved in a wide range of processes which are responsible for flavonoid and phenolic production (UV protection). Skin tannin may therefore play a photo-protective role within the berry. Flavonol accumulation was significantly influenced by the light quality which is known to be the main abiotic driver of skin flavonol biosynthesis regulation. Anthocyanin concentration and content were largely influenced by the season and not the treatments applied suggesting a synergistic influence of both light quantity and temperature.

Determination of the seed number data from both seasons is warranted for future studies and similarly the same UV-B exclusion treatments should be used in multiple seasons for conclusive results. However, with the data obtained from this study we established that: (i) light and to a lesser extent, temperatures had an impact at a microclimatic level on flavonoid biosynthesis, (ii) that a kinetic of flavonoid accumulation during berry development was established (iii) no real differences were obtained between temperatures of the respective treatments, but rather due to seasonal variations highlighting the effect of light quality and/or quantity in the accumulation of grape metabolites. These results contribute to a better understanding of the light and temperature effect

and possible interaction on flavonoid accumulation in the grape berry under climatic conditions of the Stellenbosch area. Further studies should also be conducted in a greenhouse or growth chamber where it is possible to control light and temperature although there are drawbacks using such techniques. Further research is also required at the transcriptomic and metabolomic levels to better understand the effect of combined abiotic factors on the fruit components and the flavonoid evolution as Dal Santo *et al.* (2013) identified the plastic transcriptome of the grapevine, allowing different developmental responses under diverse growing conditions.

3.9 LITERATURE CITED

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Chapter 4

Research results

Flavonoid composition under altered light and temperature conditions in Cabernet Sauvignon (*Vitis vinifera* L.) in two consecutive seasons (2010/2011 and 2011/2012)

CHAPTER 4

Flavonoid composition under altered light and temperature conditions in Cabernet Sauvignon (*Vitis vinifera* L.) in two consecutive seasons (2010/2011 and 2011/2012)

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4.2 ABSTRACT

Compositional changes in seed and skin proanthocyanidins and anthocyanins were determined during two consecutive seasons (2010/2011 and 2011/2012) in Cabernet Sauvignon (*Vitis vinifera* L.) under different light treatment. The study comprised of two main treatments in which the light quantity was manipulated in the bunch zone: standard (STD) with no lateral shoot or leaf removal and treatment LRW with leaf removal on the western side of the bunch zone. Furthermore, the light quality was altered by installing ultraviolet-B suppressing sheets within the bunch zone in both seasons. Grape seed and skin proanthocyanidin subunit composition skins changed during berry development. Seed extension subunit proportions were significantly different among the treatments, but not consistent over the two seasons. Seed proanthocyanidin concentration, content and galloylation were dependent on seasonal changes rather than the treatment. Skin proanthocyanidin terminal skin subunit concentration and content had varying results due to the

interference of phenolic compounds present in the skins. Similarly, anthocyanins composition was altered by the light and temperature conditions in the season rather than the individual treatments.

4.3 INTRODUCTION

Cabernet Sauvignon (*Vitis vinifera* L. cv.) is the most planted red grape cultivar globally (Mercer, 2014). Additionally, high number of plantings is found in South Africa and in the Stellenbosch Wine of Origin District. Despite the importance of this grape cultivar for winemaking there is still a lot not known about the flavonoid composition evolution of these compounds in grape berries during ripening under different light and temperature conditions. The effects of temperature and light on grape proanthocyanidin and anthocyanin composition have been studied extensively in Shiraz, Merlot and Pinot noir grape cultivars (Downey *et al.* 2004; Cortell *et al.* 2005; Cortell & Kennedy 2006; Ristic *et al.* 2007; Cohen *et al.* 2012; Koyoma *et al.* 2012). However, separating the effects of light and temperature on berry composition is difficult. According to literature phenolic content and composition are dependent on the grape variety and the phenological stage within a particular season.

Anthocyanin and proanthocyanidin synthesis shares common steps in the flavonoid pathway, which results in the synthesis of flavan-3,4-diols (such as leucocyanidin). The latter precursor in conjunction with the two enzymes leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR) can produce flavan-3-ol monomers required for the formation of proanthocyanidin polymers and anthocyanins (Stafford, 1990; Bogs *et al.*, 2005). Schijlen *et al.* (2004) suggested that the manipulation of ANR and LAR activity may have the potential to modify proanthocyanidin content and composition in plant tissue. Furthermore, Schijlen *et al.* (2004) identified structural and regulatory genes which encodes the enzymes that directly participate in the formation of flavonoids and that control the expression of the structural genes. Memelink *et al.* (2000), Martin *et al.* (2001) and Vom Endt *et al.* (2002) suggested that the regulation of these structural genes are dependent on the tissue type and the response to internal (i.e. hormones) and external signals (UV radiation). From the latter it is clear that the abiotic factors such as light and possibly temperature could have an impact on the flavonoid pathway.

Anthocyanins are responsible for the colour in grapes and young wines and accumulate in the berry skins from véraison onwards (Ribereau-Gayon & Glories, 1986). Several anthocyanins (cyanidin, petunidin, peonidin, delphinidin and malividin) are present in the grapes and vary between cultivars. Each of the anthocyanins is glycosylated at position 3 of ring C. A substitution of the glucoside with acetyl and coumaroyl moieties give rise to 15 different anthocyanins commonly found in grape berries (Mazza, 1995). Recent studies reported shifts in the anthocyanin composition with altering temperature and light conditions (Haselgrove *et al.*, 2000; Spayd *et al.*, 2002; Downey *et al.*, 2004). Proanthocyanidins are found in skins and seeds and their structure and composition have been studied: (i) during ripening (Czochanska *et al.*, 1979; Romeyer *et al.*, 1986; Katalinic & Males, 1997; Saint-Cricq de Gaulejac *et al.*, 1997; de Freitas & Glories, 1999; De Freitas *et al.*, 2000; Kennedy *et al.*, 2000 a & b) and (ii) at harvest (Kantz & Singleton, 1990; Prieur *et al.*, 1994; Escribano-Bailon *et al.*, 1995; Souquet *et al.*, 1996; Fuleki & Ricardo-da-Silva, 1997). The main flavan-3-ol subunits present in grape seeds are: (+)-catechin, (–)-epicatechin and (–)-epicatechin-3-O-gallate (Romeyer *et al.*, 1986; Prieur *et al.*, 1994; Souquet *et al.*, 1996; Downey *et al.*, 2003). In grape seeds the main terminal subunit was (+)-catechin (Prieur *et al.*, 1994; Escribano-Bailon *et al.*, 1996; Souquet *et al.*, 1996). Grape skins differ from seeds as (–)-epigallocatechin is present as well as a lower proportion of galloylated units. Furthermore, a higher degree of polymerisation occurs in grape skins (Somers, 1971; Gawel, 1998; Santos-Buelga, 2000). In grape skin, (+)-catechin is generally the predominant extension subunit (Prieur *et al.*, 1994; Escribano-Bailon *et al.*, 1995; Souquet *et al.*, 1996). (+)-Catechin, (–)-epicatechin, (–)epicatechin-3-O-gallate and (–)-epigallocatechin have all been identified as extension subunits in grape tannin, although the latter has only been identified in grape skins (Santos-Buelga *et al.*, 1995; Escribano-Bailon *et al.*, 1996; Souquet *et al.*, 1996; Cheynier *et al.*, 1997).

Flavan-3-ols (monomeric catechins and proanthocyanidins or condensed tannins) are responsible for the bitterness and astringency and structure of wines. Bitterness and astringency are determined by the molecular size of the condensed tannins (Peleg *et al.*, 1999). As flavonoids are important in red wine quality the purpose of this study was to investigate the potential impact of altered light and temperature conditions on tannin and anthocyanin composition during berry

ripening in Cabernet Sauvignon (*Vitis vinifera* L.). This work is part of a larger study in which the flavonoid evolution responses in Cabernet Sauvignon grapes to light and temperature and an exploratory study in the resulting wines were studied in the Stellenbosch Wine of Origin District during two consecutive seasons 2010/2011 and 2011/2012.

4.4 MATERIALS AND METHODS

4.4.1 Vineyard characteristics

Details of the vineyard characteristics are described in Chapter 3, paragraph 3.4.1.

4.4.2 Temperature measurements

Details of the temperature measurements are described in Chapter 3, paragraph 3.4.2.

4.4.3 Light measurements

Details of the light measurements are described in Chapter 3, paragraph 3.4.3.

4.4.4 Sampling procedure and preparation for analyses

Details of the sampling procedure and preparation for analysis are described in Chapter 3, paragraph 3.4.4.

4.4.5 Chemicals

All chromatographic solvents were HPLC grade. Methanol, acetone, acetonitrile, acetic acid, L-ascorbic acid, gallic acid, (+)-catechin, (-)-epicatechin and quercetin were obtained from Sigma-Aldrich (Johannesburg, South Africa). Acetic acid were obtained from Riedel-de Haën (Seelze, Germany). Phloroglucinol were obtained from Sigma-Aldrich (Johannesburg, South Africa) for the acid catalyses in the presence of excess phloroglucinol.

4.4.6 Extraction of grape skin and seed condensed tannin

Details of the extraction of grape seeds and skins are described in Chapter 3, paragraph 3.4.6.

4.4.7 Condensed tannin analysis by acid-catalyzed cleavage in the presence of phloroglucinol

Compositional analysis of proanthocyanidins was carried out following acid-catalysed cleavage in the presence of excess phloroglucinol (phloroglucinolysis) (Kennedy & Jones, 2001). The method provided information regarding the subunit composition, mean degree of polymerisation (mDP), percentage of galloylation (%G) and the percentage of prodelphinidin units (%P) in grape skins and seeds where applicable. The proanthocyanidin cleavage products were determined by RP-HPLC using a method adapted from Kennedy & Taylor (2003).

The chromatographic separation was carried out by two Chromolith Performance RP-18e columns in series (100 mm x 4.6 mm, 3 μ m) provided with a pre-column (Merck (Pty) Ltd, Johannesburg, South Africa) on a Waters Acquity Ultra Performance LC system (Waters, Waters Corp., Milford, MA, USA) equipped with a photo array detector (PAD e λ) (Milford, MA). The samples were filtered through a 0.22 μ m filter before the injection. Mobile phases were 1% (v/v) aqueous acetic acid (A) and acetonitrile containing 1% (v/v) acetic acid (B). Elution conditions were as follows: 3% B for 6 min, a linear gradient from 3 to 18% B in 15 min, and 80% B for 3 min. The column was washed with 3% B for 8 min and re-equilibrated for 3 min before the next injection. The column temperature was 30°C and the flow rate was 2 mL/min. The proanthocyanidin cleavage products were determined by means of their response factor relative to (+)-catechin, which was used as the quantitative standard. The molar absorptivity determined by Kennedy & Jones (2001a) was used. Each of the five biological replicates were analysed in duplicate. The integration of the chromatograms was carried out by Empower 2 software (Waters).

To calculate the mDP, the sum of all subunits (flavan-3-ol monomers (terminal units) and (extension units) phloroglucinol adducts, in moles) was divided by the sum of all flavan-3-ol monomers (in moles). The limit of detection (LOD) was defined as the lowest concentration of an analyte in a sample that results in a peak with a height three times as high as the baseline noise level and the limit of quantification (LOQ) as the minimum injected amount that gives a peak height

10 times higher than baseline noise. The LOD and LOQ determined for (+)-catechin were 0.0087 nmol and 0.0244 nmol, respectively.

4.4.8 Statistical analysis

Details of the statistical analysis are described in Chapter 3, paragraph 3.4.8.

4.5 RESULTS AND DISCUSSION

4.5.1 Seed analysis

From the RP-HPLC seed data obtained in Chapter 3 in the two seasons we hypothesized that the 2010/2011 season was characterised by a greater number of seeds per berry due to the high seed tannin concentration and content (Chapter 3, paragraph 3.5.3) when compared to 2011/2012 (Addendum 5).

4.5.2 Compositional changes in grape seeds

4.5.2.1 Evolution of terminal seed subunit concentration and content

The evolution of the terminal seed subunit concentration and content increased from fruit-set and reached a peak at 48 DAA followed by a decrease until 116 DAA in 2010/2011 (Fig. 4.1 a & b). In 2011/2012 the concentration and content reached a maximum near véraison (+/- 47-54 DAA) followed by a decrease until 96 DAA followed by a small fluctuations (Fig. 4.1 c & d). The concentration (mg/g seed) of the terminal seed subunits reached a maximum at 48 DAA (17.2, 11.4, 10.1 and 9.5 mg/g seed) for STD, LRW, STD-UV-B and LRW-UV-B, respectively in 2010/2011 (Fig. 4.1 a). The STD treatment had a significantly ($p \leq 0.01$) higher concentration and content than the other three treatments at 48 DAA. Similar levels were obtained among the treatments at 116 DAA (Fig. 4.1 a & b). The terminal subunit concentration (mg/g seed) (17.2, 11.4, 10.1 and 9.5) and content (mg/g berry) (1.1, 0.6, 0.6 and 0.5) reached a maximum at 48 DAA for STD, LRW, STD-UV-B and LRW-UV-B, respectively in 2010/2011 thereafter, a decrease was observed and similar concentration and content were observed at 116 DAA (harvest) (Fig. 4.1 b).

A significant ($p \leq 0.05$) difference in concentration and content were observed among treatments at 47 and 68 DAA (Fig. 4.1 c & d) in 2011/2012. A maximum concentration of 10.0 mg/g seed was reached at 47 DAA for STD, 9.7 mg/g seed for LR (-UV-B, 2xUHI) at 54 DAA and 10.7 and 7.8 mg/g seed for LR (-UV-B, 2xOp50) and LRW at 68 DAA, followed by a decrease thereafter until 130 DAA in 2011/2012 (Fig. 4.1 c). The seed proanthocyanidin content followed a similar accumulation pattern than the concentration. In our study the seed terminal unit concentration and content throughout fruit ripening mostly corresponded with that of Kennedy *et al.* (2000b) and Downey *et al.* (2003; 2004) which showed an increase in the terminal subunit concentration and content after fruit-set, reaching a peak at véraison followed by a decrease in Shiraz. From these results we can conclude that terminal subunit concentration and content increase from fruit-set and reach a maximum around véraison after which it decrease irrespective of the grape cultivar. The decrease can be ascribed to a decrease in the extractability of the tannin as result of the conjugation of proanthocyanidins with other cellular components (Cheynier *et al.* 1997, Saint-Cricq *et al.* 1997) while Kennedy (2000b) suggested that oxidative cross-linking of polymers would also decrease their extractability.

The terminal seed flavan-3-ol subunits that were identified are: (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-O-gallate (Fig. 4.2 & 4.3; Addendum 15 & 16). The proportional composition of terminal subunits changed throughout berry development in both seasons (Fig. 4.2 & 4.3; Addendum 15 & 16). The compositional changes in the terminal subunits during berry development were also observed by other authors (Kennedy *et al.* 2000 a & b; Downey 2003). (+)-Catechin was the predominant terminal monomer while (-)-epicatechin and (-)-epicatechin-3-O-gallate were present in lower proportions in both seasons (Fig. 4.2 & 4.3). This corresponds with the findings of Kennedy *et al.* (2000 a & b) and Downey (2003) who found similar proportions (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-O-gallate proportions to ours during berry development. There was a significant difference ($p \leq 0.001$) in the seasonal mean composition of (+)-catechin during ripening among the respective treatments in both seasons (Table 4.1). (+)-Catechin proportions were higher in the 2010/2011 season when compared to the 2011/2012 season. Similar proportions of (-)-epicatechin were found among the two seasons. However, a significant

difference ($p \leq 0.001$) was found in the (-)-epicatechin proportion amongst the treatments for each season (Table 4.1). In 2010/2011 the (-)-epicatechin contributions in the STD-UV-B and LRW-UV-B treatments were significantly higher compared to the STD and LRW treatments.

Furthermore, the (-)-epicatechin-3-O-gallate proportion was higher in 2011/2012 when compared to the 2010/2011 season (Table 4.1). A significant difference ($p \leq 0.001$) was observed in the (-)-epicatechin-3-O-gallate contributions among the treatments in 2010/2011 and no significant differences were observed among the treatments in 2011/2012 (Table 4.1). There was no clear trend in terminal unit composition changes with light quality and quantity changes due to treatment. The seasonal impact on the seed proanthocyanidin terminal subunit composition was larger than the treatment impact. This is primarily due to the higher light intensities observed in the 2010/2011 season.

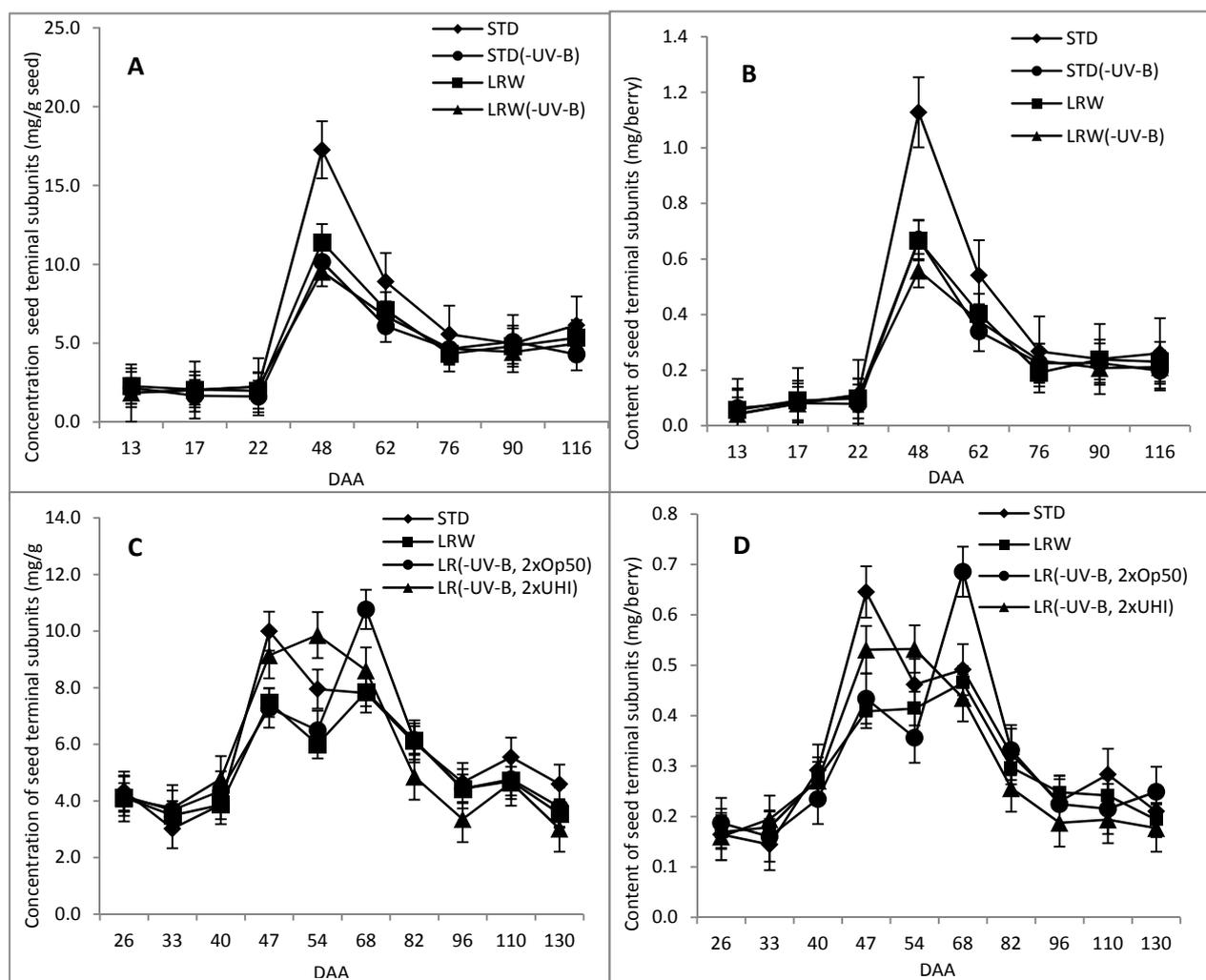


Figure 4.1. Developmental changes in the concentration (mg/g seed) and content (mg/berry) of terminal subunits of grape seeds during berry development in 2010/2011 and 2011/2012. (a) 2010/2011 terminal subunit concentration, (b) 2010/2011 terminal subunit content, (c) 2011/2012 terminal subunit concentration, (d) 2011/2012 terminal subunit content.

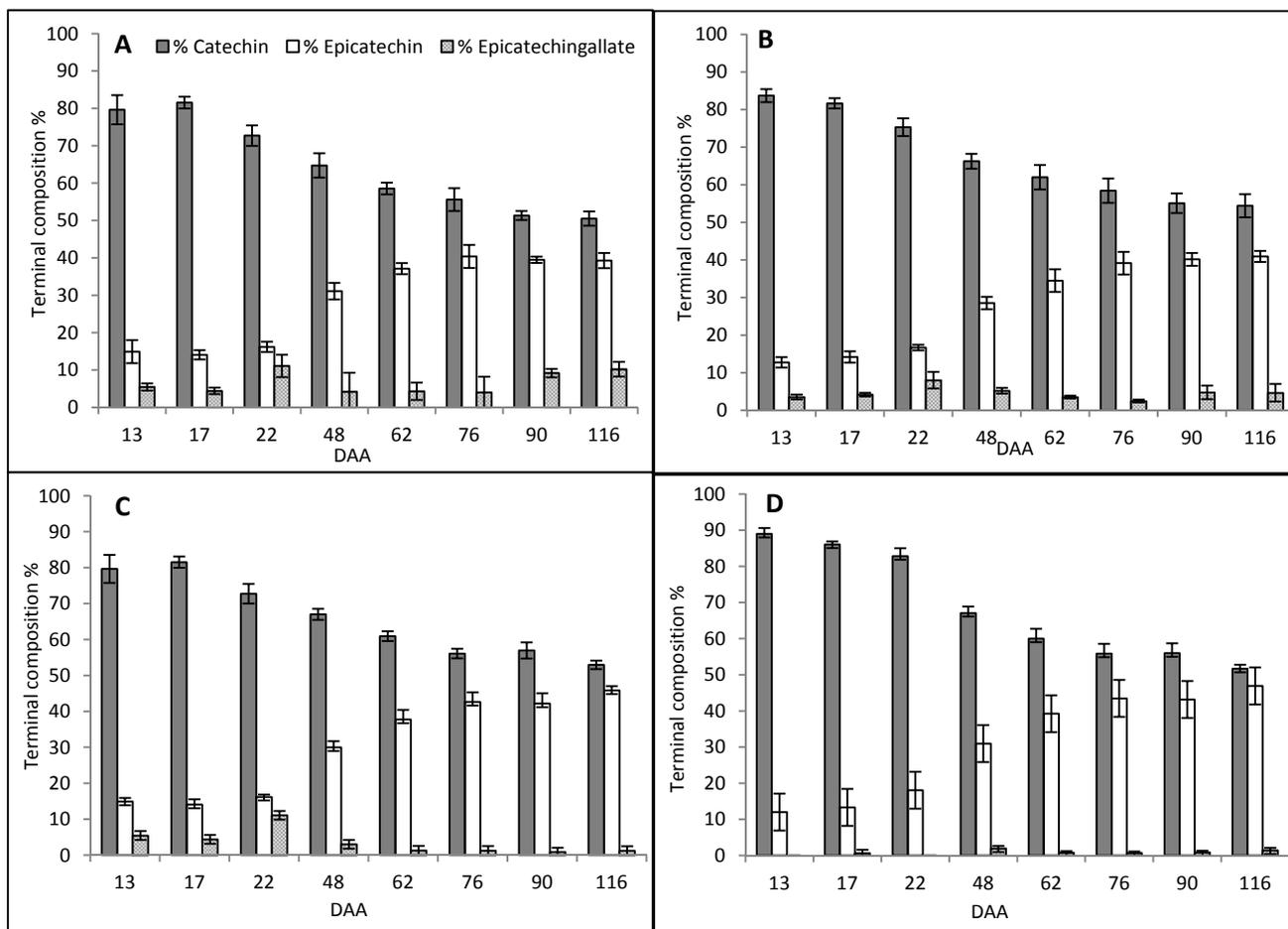


Figure 4.2. Developmental changes in the composition of terminal subunits in grapes seeds during berry development during 2010/2011 season. (a) Control (STD), (b) LRW, (c) LRW-UV-B and (d) STD-UV-B.

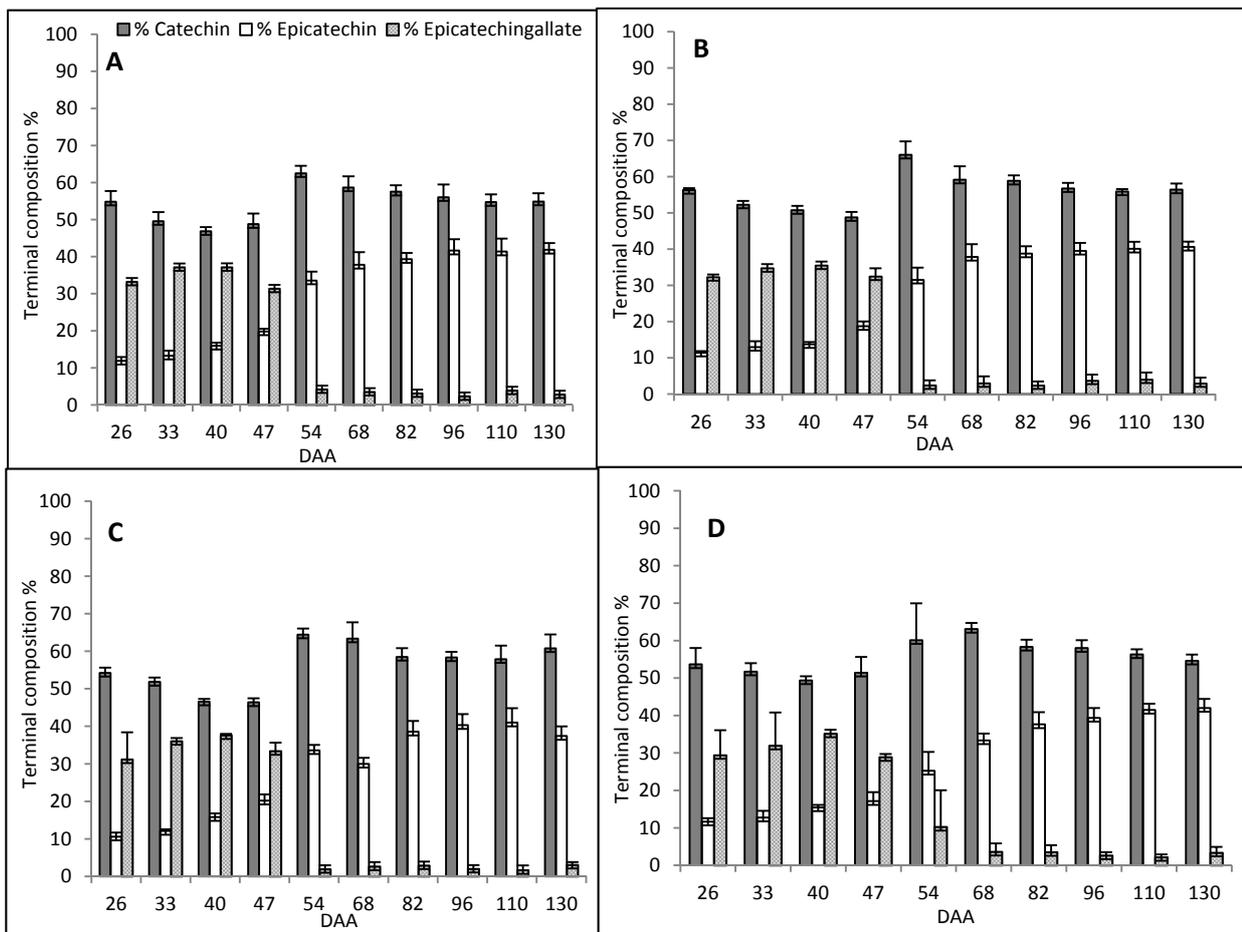


Figure 4.3. Developmental changes in the composition of terminal subunits in grapes seeds during berry development during 2011/2012 season. (a) Control (STD), (b) LRW, (c) LR (-UV-B, 2xOp50) and (d) LR (-UV-B, 2xUHI).

Table 4.1. Proportions of mean grape seed terminal subunits in 2010/2011 and 2011/2012 season.

| 2010/2011 | | | | 2011/2012 | | | |
|---------------------|--------|--------|-------|---------------------|---------|---------|------|
| Treatment | C | EC | ECG | Treatment | C | EC | ECG |
| Standard (Control) | 64.3 c | 29.1 b | 6.6 a | Standard (Control) | 54.4 b | 29.6 a | 15.9 |
| Leaf Removal West | 67.1 b | 28.4 b | 4.5 b | Leaf Removal West | 56.1 b | 28.5 ab | 15.4 |
| STD-UV-B | 68.6 a | 30.9 a | 0.8 c | LR (-UV-B, 2xOp50) | 56.2 a | 28.0 b | 15.3 |
| LRW-UV-B | 66.0 b | 30.4 a | 3.6 b | LR (-UV-B, 2xUHI) | 55.7 ab | 27.6 b | 15.1 |
| Significance | *** | *** | *** | Significance | *** | *** | ns |

Means in columns followed by a different letter are significantly different within one season.

Percent composition of proanthocyanidin terminal seed subunits C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate STD (Shaded/Control); LRW (Leaf Removal West); STD with decreased UV-B radiation (STD-UV-B); LRW with decreased UV-B radiation (LRW-UV-B); LR (-UV-B, 2xOp50)(Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR (-UV-B, 2xUHI) (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01 , 0.001 respectively; ns: not significant).

4.5.2.2 Evolution of seed extension subunit concentration and content

In 2010/2011 an increase in the seed proanthocyanidin extension concentration (mg/g seed) and content (mg/berry) were observed from fruit-set, reaching a maximum at 48 DAA followed by a decrease between 48–76 DAA. Thereafter changes were treatment depended (Fig.4.4 a & b). The 2011/2012 seed proanthocyanidin extension concentration (mg/g seed) and content (mg/berry) was characterised by a decrease from 26–33 DAA, followed by an increase and reaching a maximum 47 DAA followed by a decrease until 130 DAA with ripening. A decrease in the concentration was observed from 48 DAA until 76 DAA for all of the treatments followed by an increase for STD and LRW while STD-UV-B and LRW-UV-B remained constant until 116 DAA (Fig. 4.4 a). A higher maximum concentration of 43 mg/g seed was reached at 116 DAA in the STD treatment than at 48 DAA. A similar developmental pattern was observed in the content (mg/berry) in 2010/2011 (Fig. 4.4 b). Significant differences in concentration among treatments in 2010/2011 were observed at 17 DAA ($p \leq 0.01$), 22 DAA ($p \leq 0.001$), 62 DAA ($p \leq 0.001$), 90 DAA ($p \leq 0.001$) and 116 DAA ($p \leq 0.001$) (Fig. 4.4 a). Significant differences in the content ($p \leq 0.001$) were observed at 22, 62, 90 and 116 DAA (Fig. 4.4 b).

In 2011/2012 the seed proanthocyanidin subunit extension concentration reached a maximum at 47 DAA for all the treatments at 29.9, 30.7, 28.2 and 24.7 mg/g seed in LR (-UV-B, 2xUHI), STD, LRW and LR-UV-B, 2xOp50, respectively followed by a decrease until 116 DAA, reaching mostly similar levels at harvest (Fig.4.4 c). A similar accumulation pattern was observed in the content (mg/berry) (Fig. 4.4 d). In the 2011/2012 season significant differences in concentration were observed among the treatments at 26 DAA ($p \leq 0.05$), 47 DAA ($p \leq 0.05$), 54 DAA ($p \leq 0.05$), 96 DAA ($p \leq 0.05$), 110 DAA ($p \leq 0.01$) and 130 DAA ($p \leq 0.01$). Significant differences among the treatments were observed at 47 DAA ($p \leq 0.05$), 54 DAA ($p \leq 0.05$) and at 110 DAA ($p \leq 0.01$) for content. Results from the 2010/2011 season suggested a positive influence of shade and UV-B on the extension subunits due to the significantly higher concentration and content in the STD treatment. However, in the 2011/2012 season the impact of UV-B suppression was not visible despite the UV-B sheets being applied on both sides of the fruiting zone. Therefore, no clear trend among treatment and seed proanthocyanidin extension concentration and content could be found across both seasons

indicating that changes in the evolution of seed extension subunit concentration and content were mainly due to seasonal differences.

(-)-Epicatechin was the main constituent of the seed extension subunits with (+)-catechin and (-)-epicatechin-3-O-gallate being present in lower proportions in both seasons (Table 4.2). This observation coincides with that of other authors who found similar terminal seed proportions with (-)-epicatechin as the main extension subunit and lower proportions of (+)-catechin and (-)-epicatechin-3-O-gallate (Prieur *et al.*, 1994; Cortell *et al.*, 2005; Pastor del Rio & Kennedy, 2006; Moreno *et al.*, 2008; Obreque-Slier *et al.*, 2010). The proportional composition of extension subunits changed throughout berry development in both seasons (Fig. 4.5 & 4.6; Addendum 15 & 16). The proportional compositional changes during ripening correspond with that of other authors for different cultivars (Kennedy *et al.* 2000 a & b; Downey *et al.* 2003; Obreque-Slier *et al.* 2010). (+)-Catechin and (-)-epicatechin contributions remained constant from fruit-set until harvest while (-)-epicatechin-3-O-gallate proportions increased in the STD treatment during ripening (Fig. 4.5 a). (-)-Epicatechin-3-O-gallate proportions in the LRW treatment were similar throughout ripening (Fig. 4.5 b) while low proportions were present in the LRW-UV-B and STD-UV-B treatments (Fig. 4.5 c & d). (-)-Epicatechin proportions fluctuated in the 2011/2012 season while the (+)-catechin proportion remained relatively constant (Fig. 4.6; Addendum 16). (-)-Epicatechin-3-O-gallate contributions decreased after fruit-set until harvest in all the treatments (Fig. 4.6).

There was a significant difference ($p \leq 0.001$) in the average contribution of for both (+)-catechin and (-)-epicatechin to the seed proanthocyanidin extension composition amongst the treatments for both seasons (Table 4.2). There was only a significant difference ($p \leq 0.001$) between the average contribution of (-)-epicatechin-3-O-gallate in 2010/2011 (Table 4.2). There were significant differences among the extension subunit proportions among the different treatments this was not consistent over the two seasons studied and there are no clear trend with light exposure and UV-B radiation reduction. Our results agree with that of Fujita *et al.* (2007) and Cohen *et al.* (2008) who reported minimal variation in the seed proanthocyanidin composition with shading, heating and cooling of berries.

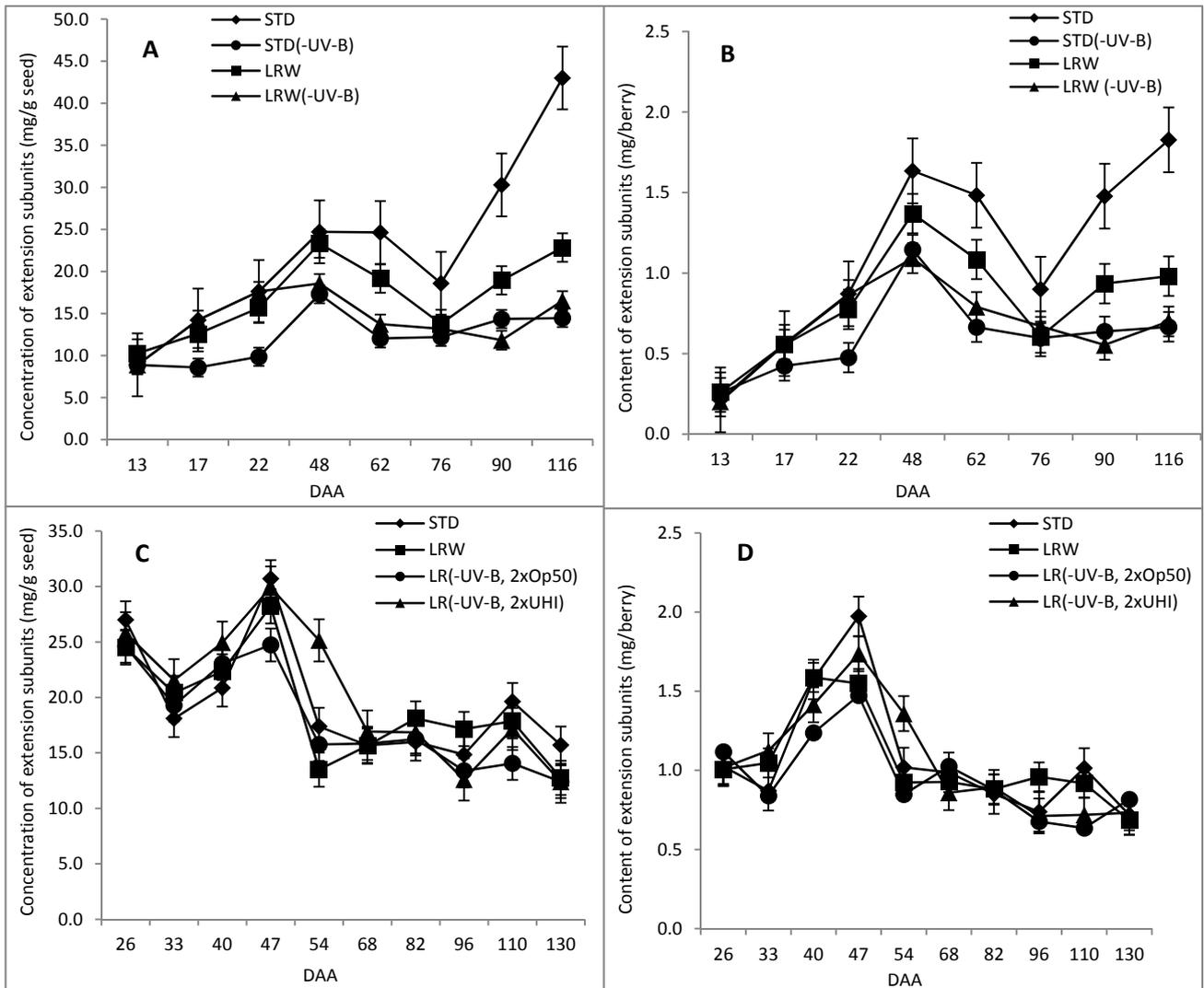


Figure 4.4. Developmental changes in the concentration (mg/g seed) and content (mg/berry) of extension subunits of grape seeds during berry development in 2010/2011 and 2011/2012. (a) 2010/2011 extension subunit concentration, (b) 2010/2011 extension subunit content, (c) 2011/2012 extension subunit concentration, (d) 2011/2012 extension subunit content.

Table 4.2. Proportions of mean grape seed extension subunits in 2010/2011 and 2011/2012 season.

| 2010/2011 | | | | 2011/2012 | | | |
|---------------------|---------|--------|-------|---------------------|---------|--------|-----|
| Treatment | C | EC | ECG | Treatment | C | EC | ECG |
| Standard (Control) | 10.7 c | 83.8 c | 5.5 a | Standard (Control) | 10.8 bc | 82.7 a | 6.2 |
| Leaf Removal West | 11.3 b | 86.4 b | 2.3 b | Leaf Removal West | 10.5 c | 81.7 a | 6.1 |
| STD-UV-B | 11.8 a | 88.2 a | 0.4 d | LR (-UV-B, 2xOp50) | 11.3 a | 75.0 b | 6.1 |
| LRW-UV-B | 11.6 ab | 87.1 b | 1.3 c | LR (-UV-B, 2xUHI) | 10.9 b | 74.8 b | 5.9 |
| Significance | *** | *** | *** | Significance | *** | *** | ns |

Means in columns followed by a different letter are significantly different within one season. Percent composition of proanthocyanidin extension seed subunits. C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate STD (Shaded/Control); LRW (Leaf Removal West); STD with decreased UV-B radiation (STD-UV-B); LRW with decreased UV-B radiation (LRW-UV-B); LR (-UV-B, 2xOp50) (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR (-UV-B, 2xUHI) (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01 , 0.001 respectively; ns: not significant).

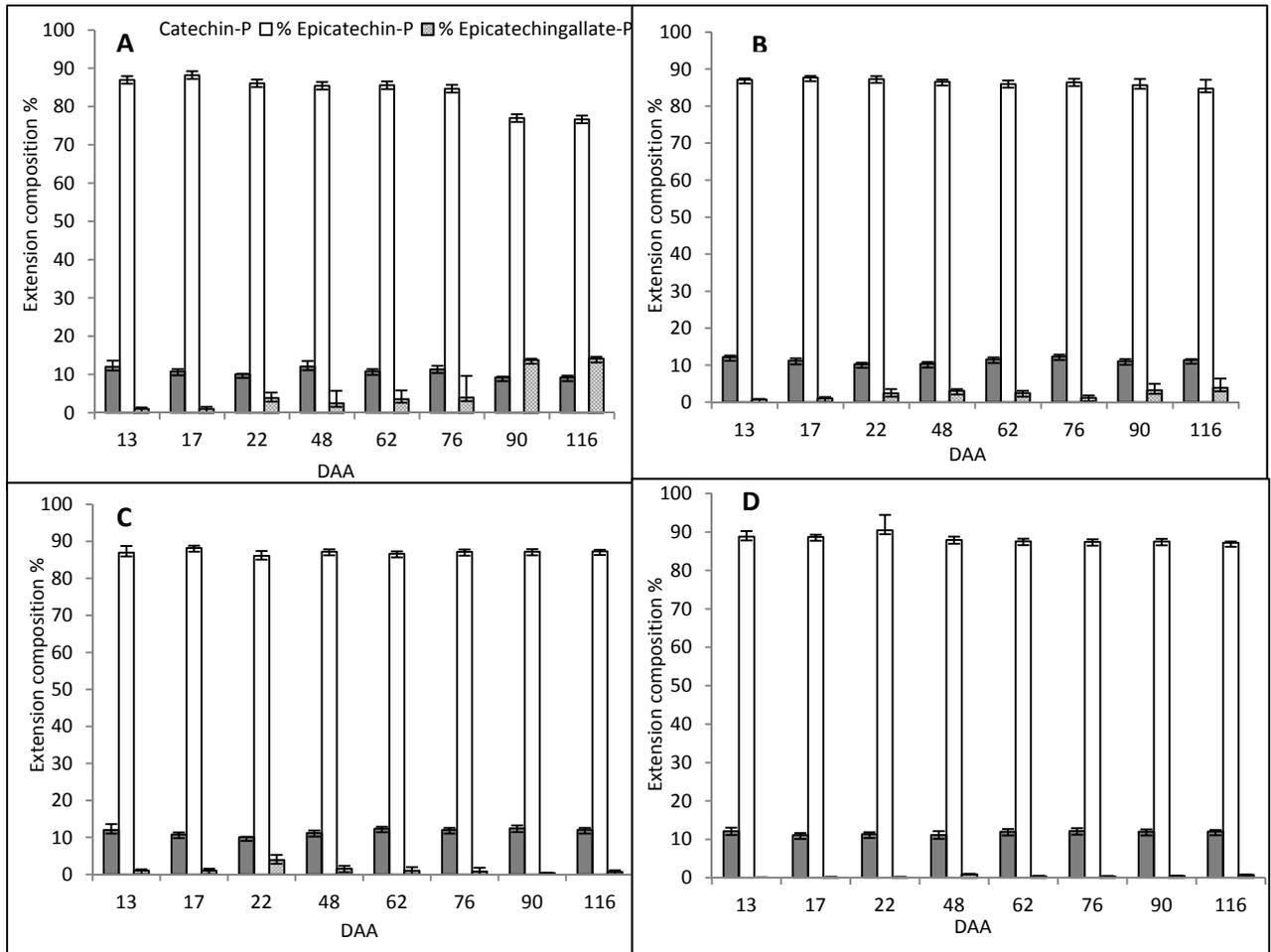


Figure 4.5. Developmental changes in the composition of extension subunits in grapes seeds during berry development during 2010/2011 season. (a) Control (STD), (b) LRW, (c) LRW-UV-B and (d) STD-UV-B.

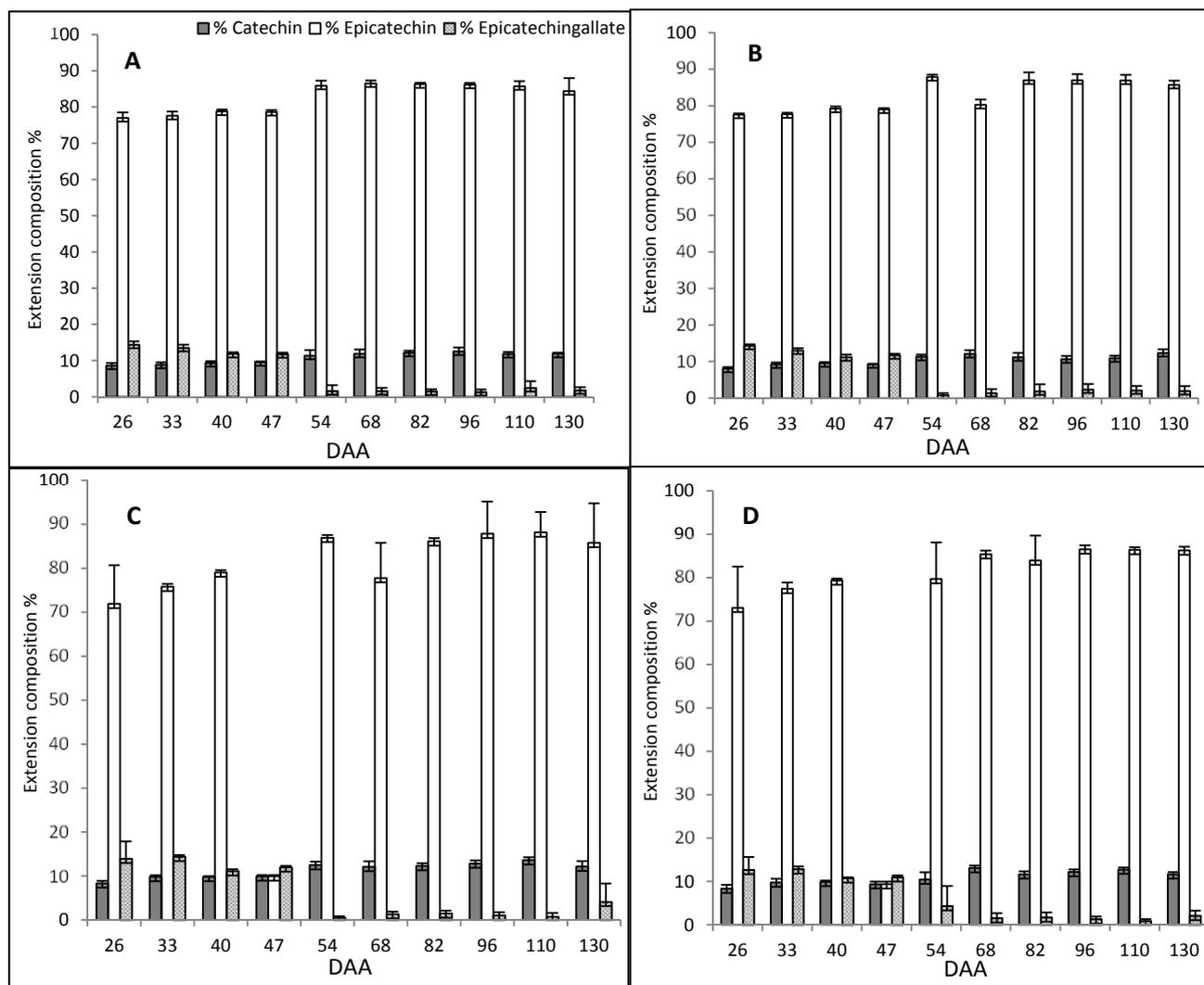


Figure 4.6. Developmental changes in the composition of extension subunits in grapes seeds during berry development during 2011/2012 season. (a) Control (STD), (b) LRW, (c) LR (-UV-B, 2xOp50) and (d) LR (-UV-B, 2xUHI).

4.5.3 Seed tannin concentration and content, mean degree of polymerisation, galloylation and average molecular weight

The seed tannin concentration evolution during ripening is shown in Addendum 15 & 16. In 2010/2011 the total seed tannin concentration (mg/g seed) increased from 13 DAA in STD, LRW and LRW-UV-B while STD-UV-B remained constant from 13–22 DAA (Fig. 4.7 a). STD-UV-B showed an increase from 22 DAA and reached a maximum concentration of 27.4 mg/g at 48 DAA followed by a decrease until 116 DAA (Fig. 4.7 a). A maximum concentration was also reached in the LRW (34.7 mg/g seed) and LRW-UV-B (28.1 mg/g seed) treatments at 48 DAA. Thereafter, it decreased until 116 DAA. STD reached a maximum concentration of 33.5 mg/g seed at 62 DAA

followed by a decrease until 76 DAA, with an increase until 116 DAA reaching a maximum concentration of 49.1 mg/g seed tannin (Fig. 4.7 a). The seed tannin content (mg/berry) followed a similar pattern than the concentration (Fig. 4.7 b). In 2011/2012 the concentration decreased from 26–33 DAA in all treatments, followed by an increase and reaching a maximum concentration in all the treatments at 47 DAA for STD, LR (-UV-B, 2xUHI), LRW and LR (-UV-B, 2xUHI) at 40.7, 39.0, 35.6, 32.0 mg/g tannin respectively (Fig. 4.7 c). After 47 DAA there was a fluctuation in the concentration until 130 DAA. The seed tannin content (mg/berry) followed a similar pattern than the concentration (Fig. 4.7 d). Kennedy *et al.* (2000a) also reported a decline in the seed procyanidin amount from 1.34 mg/berry at véraison (19 August) to 0.47 mg/berry on 18 October (harvest) in Cabernet Sauvignon subjected to standard irrigation and minimal and double irrigated vines. Significant differences in the concentration (mg/g seed) were observed amongst the treatments in 2010/2011 season at 17 DAA ($p \leq 0.001$), 22 DAA ($p \leq 0.001$), 48 DAA ($p \leq 0.001$), 62 DAA ($p \leq 0.001$), 90 DAA ($p \leq 0.001$) and 116 DAA ($p \leq 0.001$). Significant differences in the content (mg/berry) were observed amongst the treatments in 2010/2011 season at 22 DAA ($p \leq 0.001$), 48 DAA ($p \leq 0.05$), 62 DAA ($p \leq 0.001$), 90 DAA ($p \leq 0.001$) and 116 DAA ($p \leq 0.001$). Similarly to the proanthocyanidin terminal and extension unit changes, differences among the treatments were not consistent over the two seasons studied and there are no clear trends with light exposure and UV-B radiation. This corresponds with the findings of Downey *et al.* (2004) who found very little difference in the level of terminal and extension subunits in seeds of shaded and exposed fruit. Therefore, our results suggest that seed proanthocyanidin concentration and content are dependent on seasonal changes.

The mDP evolution during ripening and the average mDP for each treatment for both seasons were determined (Fig. 4.8 a & b; Addendum 15 & 16; Table 3.3). Seed mDP varied between 2.7–8.8 in the 2010/2011 and 2.9–7.7 in the 2011/2012 season during berry development amongst treatments (Addendum 15 & 16). Our findings are within the range found by other authors (Sun *et al.*, 1999; Monogas *et al.*, 2003; Vidal *et al.*, 2003; Chira *et al.*, 2009; Obreque-Slier *et al.*, (2010). STD had significantly higher ($p \leq 0.001$) mDP's compared to the other treatments at 90 and 116 DAA during the 2010/2011 season.

In the 2011/2012 season the respective treatments had higher amounts of extension subunits at the beginning of berry ripening than the 2010/2011 season resulting in a higher mDP (Addendum 15 & 16). As the terminal subunit concentration increased towards véraison, mean polymer size decreased to around 3 subunits and thereafter the extension subunits increased for all of the treatments from 68 DAA until 82 DAA and remained constant until 130 DAA (Fig. 4.8 b). These results agree with the findings of Obreque-Slier *et al.* (2010) who observed an increase in the mDP in both Carménère and Cabernet Sauvignon from harvest until the over-ripe stage. Significantly higher ($p \leq 0.001$) mDP's were observed from 54–110 DAA for the LR (-UV-B, 2xUHI) treatment compared to the other treatments in 2011/2012 (Addendum 15 & 16; Fig. 4.8b)

The percentage of gallyolated derivates was determined during both seasons (Addendum 15 & 16; Table 4.3). Our results coincides with the findings of Chira *et al.* (2009) who reported mean galloylation percentage of 3.6–5.5 and 2.4–3.0 % for respectively Cabernet Sauvignon and Merlot in grape seeds from five vineyards in Bordeaux. The percentage of galloylation varied throughout seed development (Addendum 15 & 16). A significantly higher ($p \leq 0.001$) percentage of galloylation was observed in the STD treatment when compared to the other three treatments in 2010/2011 (Table 4.3). During the 2011/2012 season no significant differences were observed between the galloylation percentages among the treatments. Our results suggest that galloylation was more influenced by the season than the treatments applied.

The average molecular weight (avMM) followed the same trend as mDP (Table 4.3). In 2010/2011 the avMM differed significantly ($p \leq 0.001$) amongst the treatments with the STD treatment having a significantly higher mDP and thus avMM. In 2011/2012 the LR (-UV-B, 2xUHI) treatment had a significantly higher avMM when compared to the STD and LR (-UV-B, 2xOp50) treatments (Table 4.3). The avMM in 2010/2011 decreased from after fruit-set and reached a minimum at 48 DAA followed by an increase until harvest. The decrease of mDP and avMM from fruit-set corresponds with the findings of Kennedy *et al.* (2000a), Kennedy *et al.* (2000b), Geny *et al.* (2003), Cortell *et al.* (2005), Pastor del Rio *et al.* (2006), Moreno *et al.* (2008), Obreque-Slier *et al.* (2010) which ascribes this phenomenon to the increase in terminal subunits and monomers which will have a direct impact on the structural and compositional characteristics of seed tannin.

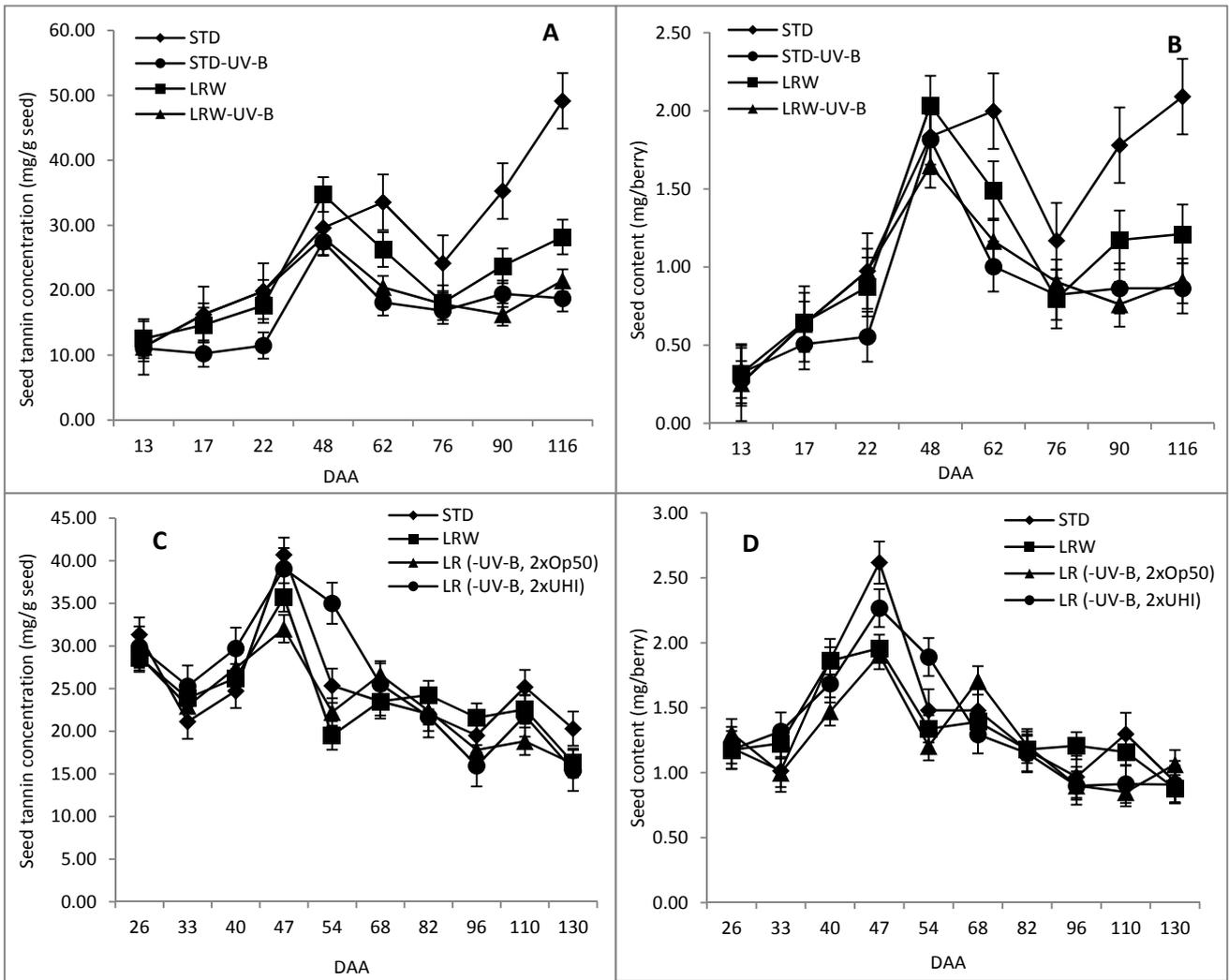


Figure 4.7. Developmental changes in the concentration (mg/g seed) and content (mg/g berry) composition of total grape seed tannin during berry development 2010/2011 and 2011/2012. (a) 2010/2011 total seed tannin concentration, (b) 2010/2011 total seed tannin content, (c) 2011/2012 total seed tannin concentration and (d) 2011/2012 total seed tannin content.

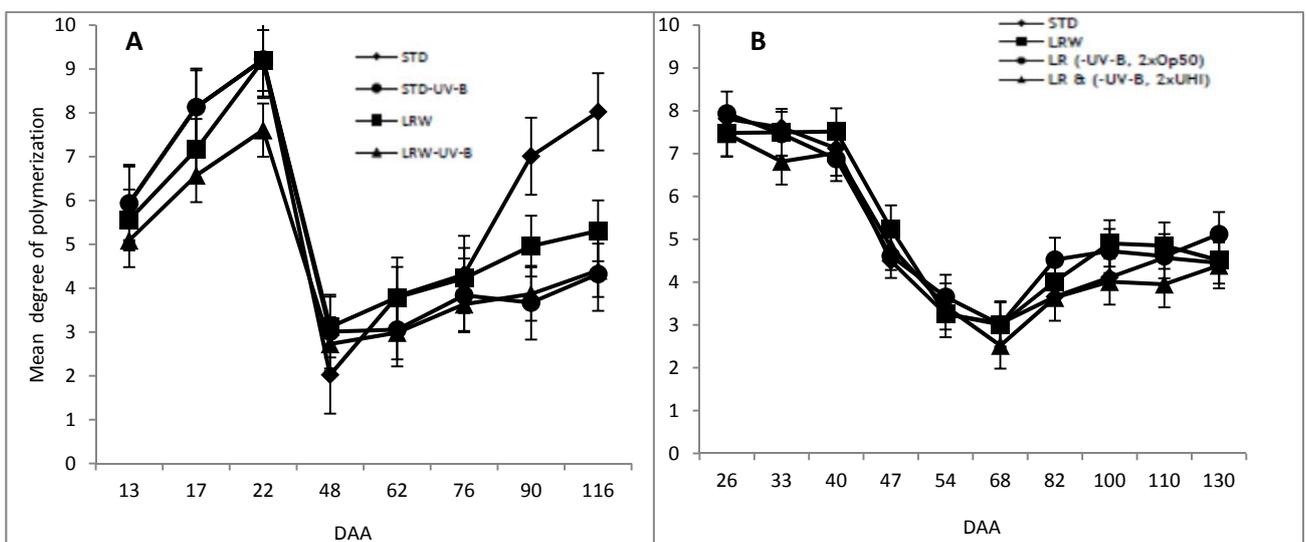


Figure 4.8. Average polymer length of Cabernet Sauvignon seeds during berry development. (a) 2010/2011 and (b) 2011/2012 season.

Table 4.3. Mean seed tannin structural characteristics in 2010/2011 and 2011/2012 season.

| 2010/2011 | | | | 2011/2012 | | | |
|---------------------|----------|-------|----------|---------------------|----------|-------|-----------|
| Treatment | Seed % G | mDP | avMM | Treatment | Seed % G | mDP | avMM |
| Standard (Control) | 5.5 a | 6.1 a | 1804.1 a | Standard (Control) | 7.8 | 5.0 b | 1518.6 cb |
| Leaf Removal West | 2.7 b | 5.4 b | 1582.5 b | Leaf Removal West | 7.5 | 5.2 a | 1581.6 ab |
| STD-UV-B | 0.6 d | 4.5 c | 1309.0 c | LR (-UV-B, 2xOp50) | 7.6 | 4.8 b | 1456.4 c |
| LRW-UV-B | 1.6 c | 5.1 b | 1499.2 b | LR (-UV-B, 2xUHI) | 7.9 | 5.3 a | 1587.9 a |
| <i>Significance</i> | *** | *** | *** | <i>Significance</i> | ns | *** | *** |

Means in columns followed by a different letter are significantly different within one season. Mass conversion based on percent recovery of proanthocyanidin by phloroglucinolysis; %G, percentage galloylation; mDP, mean degree of polymerization; avMM, average molecular mass. STD (Shaded/Control); LRW (Leaf Removal West); STD with decreased UV-B radiation (STD-UV-B); LRW with decreased UV-B radiation (LRW-UV-B); LR (-UV-B, 2xOp50) (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR (-UV-B, 2xUHI) (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

4.6 COMPOSITIONAL CHANGES IN GRAPE SKIN TANNIN

4.6.1 Evolution of proanthocyanidin terminal skin subunit concentration and content

The terminal subunit concentration (mg/g skin) and content (mg/berry) were studied under altered light and temperature conditions in two seasons. In 2011/2011 the terminal subunits fluctuated between 13 and 17 DAA (Fig. 4.9 a & b). A maximum concentration was reached for STD and LRW-UV-B at 17 DAA while LRW reached a minimum (Fig. 4.9 a). STD-UV-B increased from 13 DAA until 17 DAA. In general treatments experienced a decrease in terminal subunit concentration after 48 DAA. While the fluctuations in the terminal subunit content after 48 DAA can be ascribed to increases in berry size (Fig. 4.9. b). The evolution of the compositional and structural characteristics throughout the season is depicted in Addendum 17 & 18. We cannot explain the decrease in concentration and content for treatment LRW from 17–22 DAA, but it is possibly due to a sampling error as biological replicates of this treatment were similar. The 2011/2012 terminal skin subunit concentration and content followed a similar pattern with an early decrease from 26 to 33 DAA for all treatments staying relatively constant with small fluctuations among the treatments until 130 DAA (Fig. 4.9 c & d).

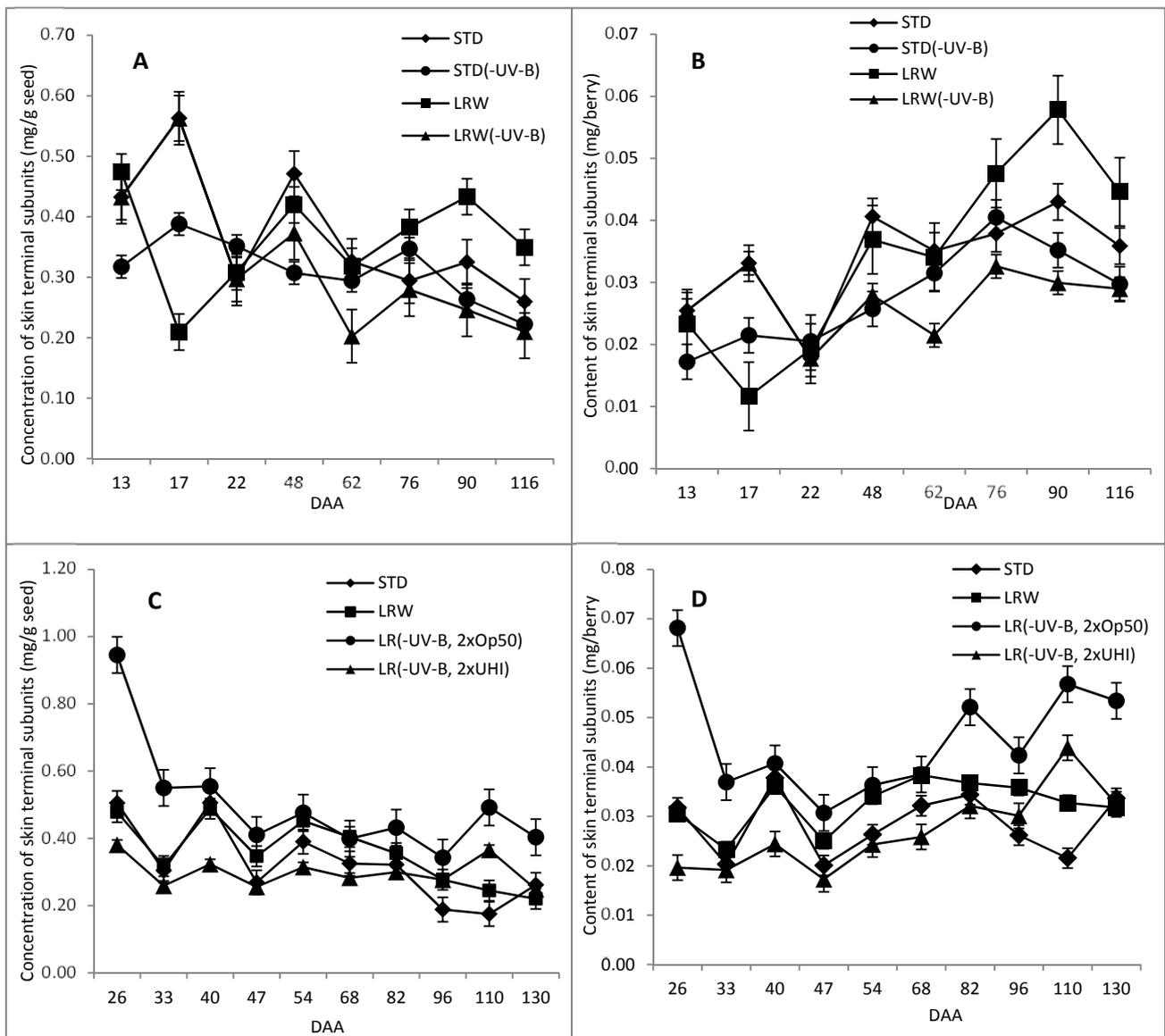


Figure 4.9. Developmental changes in the concentration (mg/g skin) and content (mg/berry) of terminal subunits of grape skins during berry development in 2010/2011 and 2011/2012. (a) 2010/2011 terminal subunit concentration, (b) 2010/2011 terminal subunit content, (c) 2011/2012 terminal subunit concentration, (d) 2011/2012 terminal subunit content.

There was a significant difference in the concentration (mg/g skin) and content (mg/berry) among the treatments at all the sampling dates except at 22 DAA in 2010/2011. LRW had a significantly higher concentration and content at 76 ($p \leq 0.05$), 90 ($p \leq 0.01$) and 116 DAA ($p \leq 0.01$) when compared to the other three treatments (Fig. 4.9 a & b). Significant differences were observed among the treatments throughout berry development in 2011/2012 as well (Fig. 4.9 c & d). In the 2010/2011 season, LRW had the highest terminal extension subunit amount whereas the LR (-UV-

B, 2xOp50) treatment had the highest concentration and content in the 2011/2012 season (Fig. 4.9 a & b).

(+)-Catechin, (-)-epicatechin and (-)-epicatechin-3-O-gallate were identified as the grape skin proanthocyanidin terminal subunits (Fig. 4.10 & 4.11). (+)-Catechin was the predominant compositional contributor with epicatechin and (-)-epicatechin-3-O-gallate present in lower proportions or not detected (Fig. 4.10 & 4.11). Our results are in alignment with the findings of Souquet *et al.* (1996), Kennedy *et al.* (2001b), Downey *et al.* (2003), Monagas *et al.* (2003), Cortell & Kennedy (2006) and Hanlin & Downey (2009) who reported the same compositional and proportional contributions. There was a significant difference in the mean (+)-catechin terminal subunit contribution between the two seasons ($p \leq 0.001$) (Table 4.4).

In 2010/2011 the (+)-catechin proportions were significantly higher in the treatments with UV-B suppression sheets (STD-UV-B and LRW-UV-B) while that was not the scenario in 2011/2012. (-)-Epicatechin proportions differed significantly ($p \leq 0.001$) among treatments in 2010/2011, but not in 2011/2012. (-)-Epicatechin-3-O-gallate was only detected from 62 DAA in 2010/2011, but was present from the beginning of the 2011/2012 season (Fig. 4.10 & 4.11). The mean (-)-epicatechin-3-O-gallate proportions were significantly higher ($p \leq 0.001$) in the LR (-UV-B, 2xOp50) treatment (Table 4.4). Similar to what was seen in the grape seed tannin, the seasonal impact was greater than those of the treatments applied as the treatment impact was not consistent across the two seasons. The results suggest that the variation between the treatments could potentially be an artefact of quantification difficulties due to the inconsistent interference of anthocyanins and flavonols present in the grape skins. Furthermore, covalent and non-covalent bonds can be formed between anthocyanins.

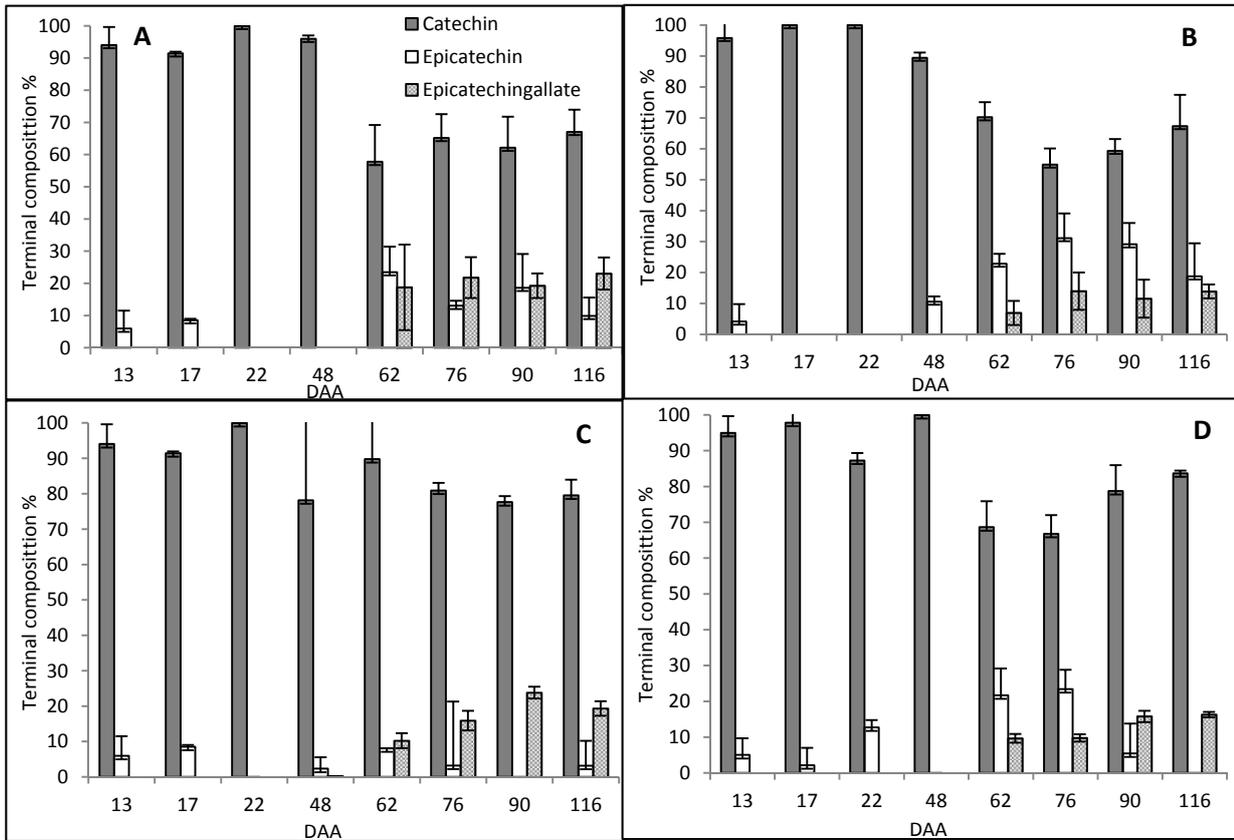


Figure 4.10. Developmental changes in the composition of terminal subunits in grapes skins during berry development during 2010/2011 season. (a) Control (STD), (b) LRW, (c) LRW-UV-B and (d) STD-UV-B.

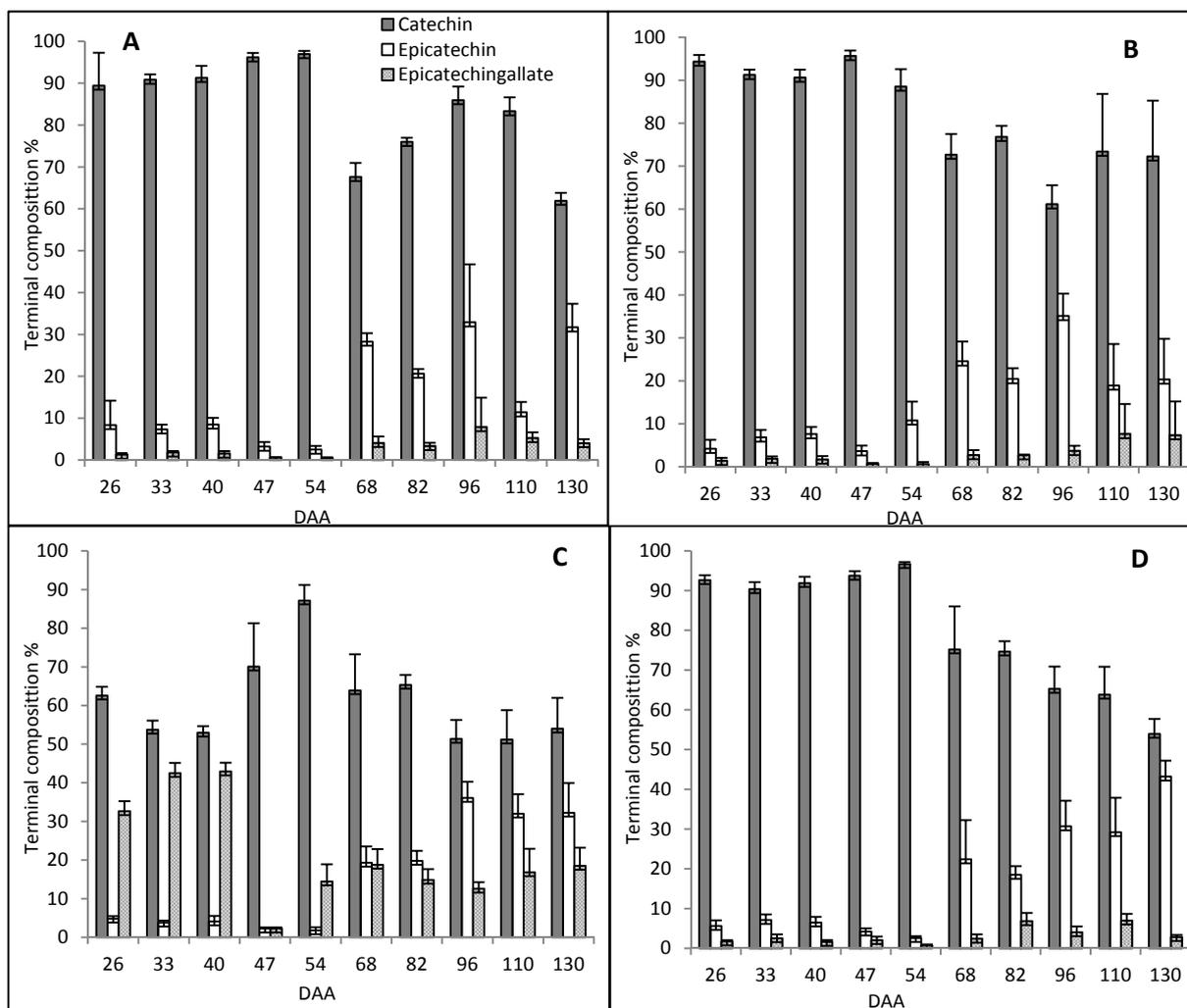


Figure 4.11. Developmental changes in the composition of terminal subunits in grapes skins during berry development during 2011/2012 season. (a) Control (STD), (b) LRW, (c) LR (-UV-B, 2xOp50) and LR (-UV-B, 2xUHI).

Table 4.4. Proportions of mean grape skin terminal subunits in 2010/2011 and 2011/2012 season. Means in columns followed by a different letter are significantly different within one season.

| 2010/2011 | | | | 2011/2012 | | | |
|---------------------|--------|--------|--------|---------------------|---------|------|--------|
| Treatment | C | EC | ECG | Treatment | C | EC | ECG |
| Standard (Control) | 79.2 b | 9.9 b | 10.4 a | Standard (Control) | 83.9 a | 15.5 | 3.1 b |
| Leaf Removal West | 79.6 b | 14.6 a | 5.8 b | Leaf Removal West | 81.7 ab | 15.3 | 3.1 b |
| STD-UV-B | 84.8 a | 8.8 b | 6.4 b | LR (-UV-B, 2xOp50) | 61.2 c | 15.6 | 21.6 a |
| LRW-UV-B | 86.4 a | 3.9 c | 8.7 a | LR (-UV-B, 2xUHI) | 79.8 b | 17.0 | 3.2 b |
| Significance | *** | *** | *** | Significance | *** | ns | *** |

Percent composition of proanthocyanidin terminal skin subunits C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate. STD (Shaded/Control); LRW (Leaf Removal West); STD with decreased UV-B radiation (STD-UV-B); LRW with decreased UV-B radiation (LRW-UV-B); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

4.6.2 Evolution of skin proanthocyanidin extension subunit concentration and content

In 2010/2011 the accumulation pattern of skin extension subunits varied between 13 and 22 DAA and reached a maximum concentration of 17.9, 16.1, 13.1 and 16.7 mg/g skin for STD, LRW, STD-UV-B and LRW-UV-B, respectively at 48 DAA (véraison) thereafter it was followed by a decrease in all the treatments until harvest to 10.6, 7.5, 4.6 and 7.8 mg/g skin for LRW, STD-UV-B, STD and LRW-UV-B at 116 DAA (Fig.4.12 a & b). The content followed a similar pattern than the concentration. In 2011/2012 a similar concentration and content pattern were observed with a maximum concentration (22.5 and 19.6 mg/g) was observed for LR (-UV-B, 2xUHI) and LR (-UV-B, 2xUHI) at 26 DAA while a STD and LRW reached a maximum at 33 DAA (Fig. 4.12 c & d) followed by a decrease until 130 DAA.

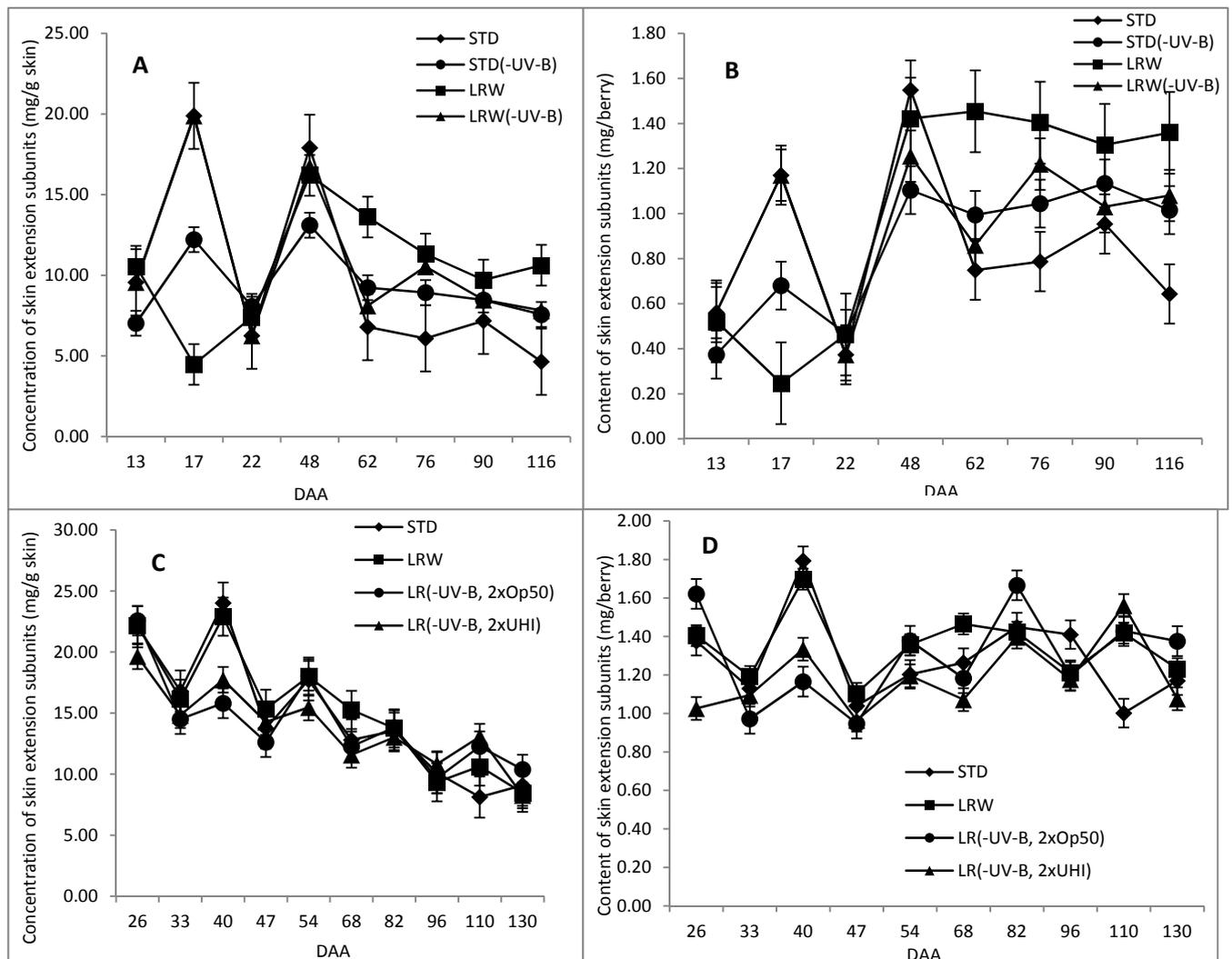


Figure 4.12. Developmental changes in the concentration (mg/g skin) and content (mg/berry) of extension subunits of grape skins during berry development in 2010/2011 and 2011/2012. (a) 2010/2011 extension subunit concentration, (b) 2010/2011 extension subunit content, (c) 2011/2012 extension subunit concentration and (d) 2011/2012 extension subunit content.

Significant differences in the extension subunit concentration and content were observed among the treatments at 17 DAA ($p \leq 0.001$), 62 DAA ($p \leq 0.001$) and 116 DAA ($p \leq 0.001$) in the 2010/2011 season (Fig. 4.12 a & b). LRW were significantly higher than the other three treatments from 62 DAA until 116 DAA. In the 2011/2012 season significant differences in concentration and content were observed at 68 DAA ($p \leq 0.05$), 82 DAA ($p \leq 0.05$), 110 DAA ($p \leq 0.001$) and 130 DAA ($p \leq 0.001$) among the treatments (Fig. 4.12 c & d). From our results the extension subunits concentration and content was significantly higher in the LRW treatment in 2010/2011 however this was not seen in 2011/2012 (Fig. 4.12; Addendum 17 & 18). The varied results for the STD and LRW treatment across two seasons indicate that differences seen are due to seasonal difference rather than treatment effects. Our results from 2010/2011 are in agreement with that of Downey *et al.* (2004) who found high skin extension subunits in exposed fruit in 2000/2001 season, but similar levels at harvest.

Four extension subunits were detected in the skins during both seasons (Fig. 4.13 & 4.14). (-)-Epigallocatechin was the predominant extension subunits followed by epicatechin and similar lower levels of (+)-catechin and (-)-epicatechin-3-O-gallate in were found both seasons (Table 4.5). These results are in agreement with that reported by Kennedy *et al.* (2001b); Downey *et al.* (2003) and Hanlin & Downey (2009) who found similar extension subunit proportions in skins. The composition of (-)-epigallocatechin in the respective treatments ranged between 55–57 % in 2010/2011 and 58–59% in 2011/2012 (Table 4.5). Our results contradict that of Fernandez *et al.* (2007) who identified epicatechin as the main contributor to skin extension subunits with present lower (-)-epigallocatechin proportions present when studying Carménère skins. However, the (+)-catechin and (-)-epicatechin-3-O-gallate proportions obtained between the seasons were similar to that of Fernandez *et al.* (2007). The (-)-epigallocatechin extension composition did not differ significantly (Table 4.5; Addendum 17 & 18) in either of the two seasons, but varied throughout the season (Fig. 4.12 & 4.13). In our study light exposure did not have a significant impact on the extension unit composition in either of the seasons investigated.

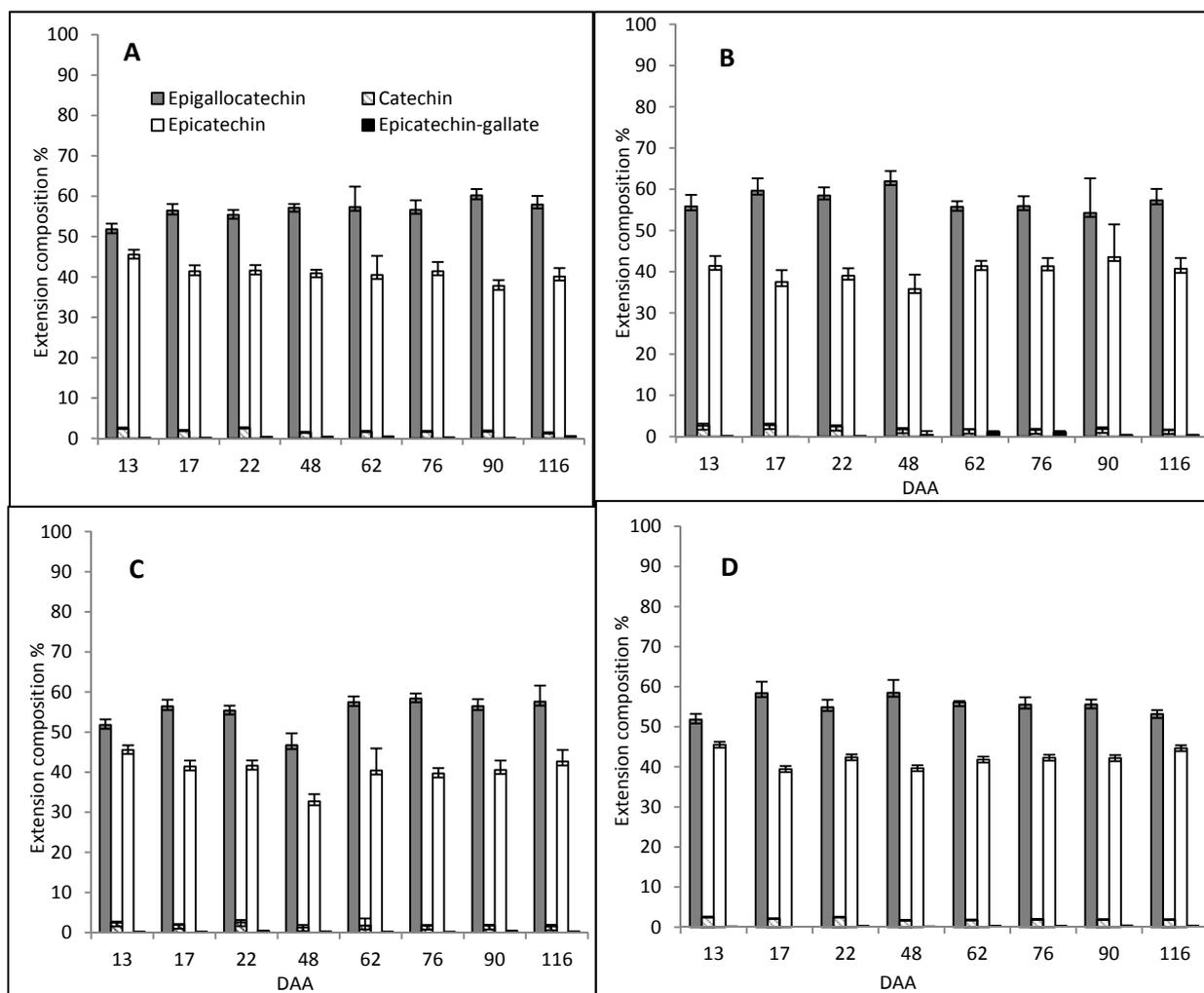


Figure 4.13. Developmental changes in the composition of extension subunits in grapes skins during berry development during 2010/2011 season. (a) Control (STD), (b) LRW, (c) LRW-UV-B and (d) STD-UV-B.

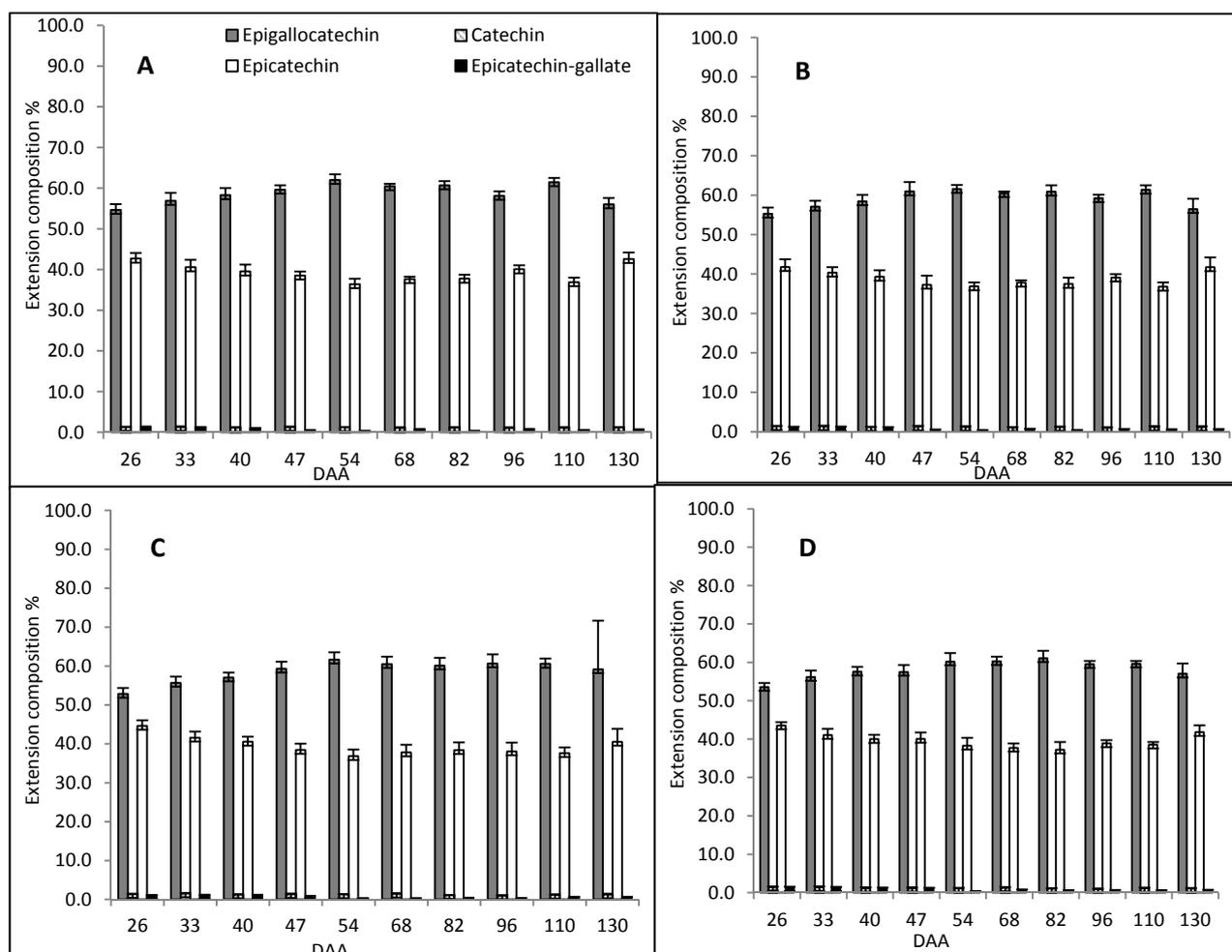


Figure 4.14. Developmental changes in the composition of extension subunits in grapes skins during berry development during 2011/2012 season. (a) Control (STD), (b) LRW, (c) LR (-UV-B, 2xOp50) and (d) LR (-UV-B, 2xUHI).

Table 4.5. Proportions of mean grape skin proanthocyanidin extension subunit in 2010/2011 and 2011/2012 season. Means in columns followed by a different letter are significantly different within one season.

| 2010/2011 | | | | | 2011/2012 | | | | |
|---------------------|--------|------|--------|------|---------------------|-------|---------|-------|------|
| Treatment | C | EC | ECG | EGC | Treatment | C | EC | ECG | EGC |
| Standard (Control) | 1.9 b | 41.2 | 0.3 ab | 56.6 | Standard (Control) | 1.2 b | 39.3 ab | 0.7 b | 58.8 |
| Leaf Removal West | 2.1 a | 40.1 | 0.4 a | 57.4 | Leaf Removal West | 1.3 b | 38.8 b | 0.7 b | 59.2 |
| STD-UV-B | 1.9 ab | 40.6 | 0.2 b | 55.0 | LR (-UV-B, 2xOp50) | 1.3 a | 39.4 ab | 0.6 c | 58.8 |
| LRW-UV-B | 2.0 b | 42.2 | 0.2 b | 55.5 | LR (-UV-B, 2xUHI) | 1.2 b | 39.7 a | 0.8 a | 58.3 |
| Significance | ** | ns | *** | ns | Significance | *** | * | *** | ns |

Percent composition of proanthocyanidin extension skin subunits C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate; EGC, (-)-epigallocatechin. STD (Shaded/Control); LRW (Leaf Removal West); STD with decreased UV-B radiation (STD-UV-B); LRW with decreased UV-B radiation (LRW-UV-B); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05, 0.01, 0.001$ respectively; ns: not significant).

4.6.3 Total skin tannin, mean degree of polymerisation, galloylation and prodelphinidins percentages in grape skins

In 2010/2011 the skin total tannin concentration (mg/g skin) and content (mg/berry) increased after initial fluctuations between 13–22 DAA due to potential sampling error as discussed previously (section 4.6.1) (Fig. 4.15 a & b). Thereafter, a maximum concentration was observed at 48 DAA followed by a decrease until 116 DAA. Content (mg/berry) followed a similar pattern of skin tannin development throughout the season (Fig. 4.15 b). The skin tannin concentration was significantly different in the LRW treatment from the other treatments at 62 DAA ($p \leq 0.001$), 76 DAA ($p \leq 0.001$), 90 DAA ($p \leq 0.05$) and 116 DAA ($p \leq 0.001$) (Fig. 4.15 a). The content followed a similar pattern with significant differences observed among the treatments from 62–116 DAA ($p \leq 0.001$). In 2011/2012 only small differences among the treatments for concentration (mg/g skin) and content (mg/berry) throughout berry development was observed (Fig. 4.15 c & d). Significant differences among the treatments were observed at 110 DAA ($p \leq 0.001$) and 130 DAA ($p \leq 0.05$). Grapes in the LRW treatment in 2010/2011 was exposed to the highest % light and PAR suggesting that light quantity had a positive effect on skin tannin concentration and content. However, in 2011/2012, the highest PAR was in the LR (-UV-B, 2xUHI) treatment which was similar to the other treatments (Table 3.4; Chapter 3). Therefore the results on total skin tannin accumulation are inconclusive with no clear influence due to light quality and quantity.

Grape skin mDP varied between 20–45 in 2010/2011 and 27–55 in 2011/2012 (Fig. 4.16 a & b; Addendum 17 & 18) which were in range of the mDP's determined by other authors (Moutounet *et al.* 1995; Souquet *et al.* 1996; Kennedy *et al.*, 2001; Downey *et al.*, 2003; Cortell *et al.*, 2005; Mane *et al.*, 2007). During 2010/2011 the STD treatment had the lowest mDP at 116 DAA when compared to the other three treatments (Fig. 4.16 a). In the 2011/2012 season the highest average polymer length was observed in the LR (-UV-B, 2xUHI) treatment at the beginning of the season while the LR (-UV-B, 2xOp50) treatment showed constantly the lowest mDP's. The high mDP observed in the LR (-UV-B, 2xUHI) treatment can be a result of the high PAR and temperature (Table 3.4; Chapter 3). Chorti *et al.* (2010) reported that excessive sunlight exposure could result in

excessive sunburn which could influence skin proanthocyanidins in the grape berry. Additionally, Lacampagne *et al.* (2010) suggested that skin mDP is correlated with the state of skin cell walls.

Grape skin tannin differs from grape seed tannin as they have a lower percentage of galloylation (Table 4.3 & 4.6). Skin tannins exhibit a higher degree of polymerisation than seed tannins expressed as the mean degree of polymerisation (mDP) (Adams, 2006). Similar mDP values were obtained between the seeds in the respective seasons (Table 4.3). Skin mDP were higher in 2011/2012 when compared to 2010/2011 (Table 4.6). Kazuya & Goto-Yamamoto (2008) reported that shading favoured galloylation in grape skins. In our result not all the shaded treatments had consistently higher galloylation percentages compare to the more exposed treatments. The percentage prodelphinidins varied between 53.9%–55.3% in 2010/2011 and 56.7%–57.5% in 2011/2012 (Table 4.6). Although, high these prodelphinidins percentage are consistent with what has been reported by others (Souquet *et al.*, 1996, Monagas *et al.*, 2003 and Cosme *et al.*, 2009).

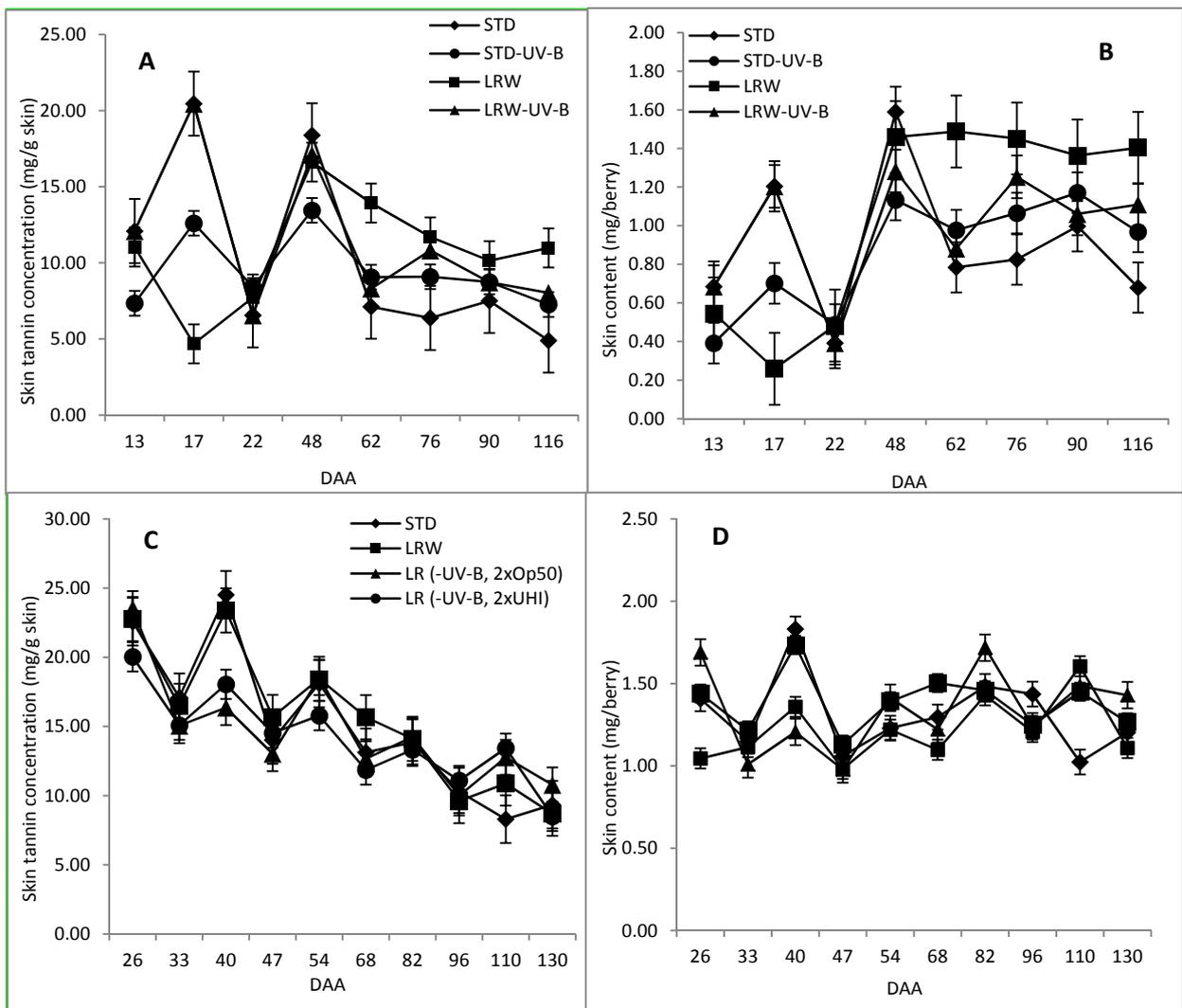


Figure 4.15. Developmental changes in the concentration (mg/g skin) and content (mg/berry) of total grape skin tannin during berry development in 2010/2011 and 2011/2012. (a) 2010/2011 total skin tannin concentration, (b) 2010/2011 total skin tannin content, (c) 2011/2012 total skin tannin concentration, (d) 2011/2012 total skin content.

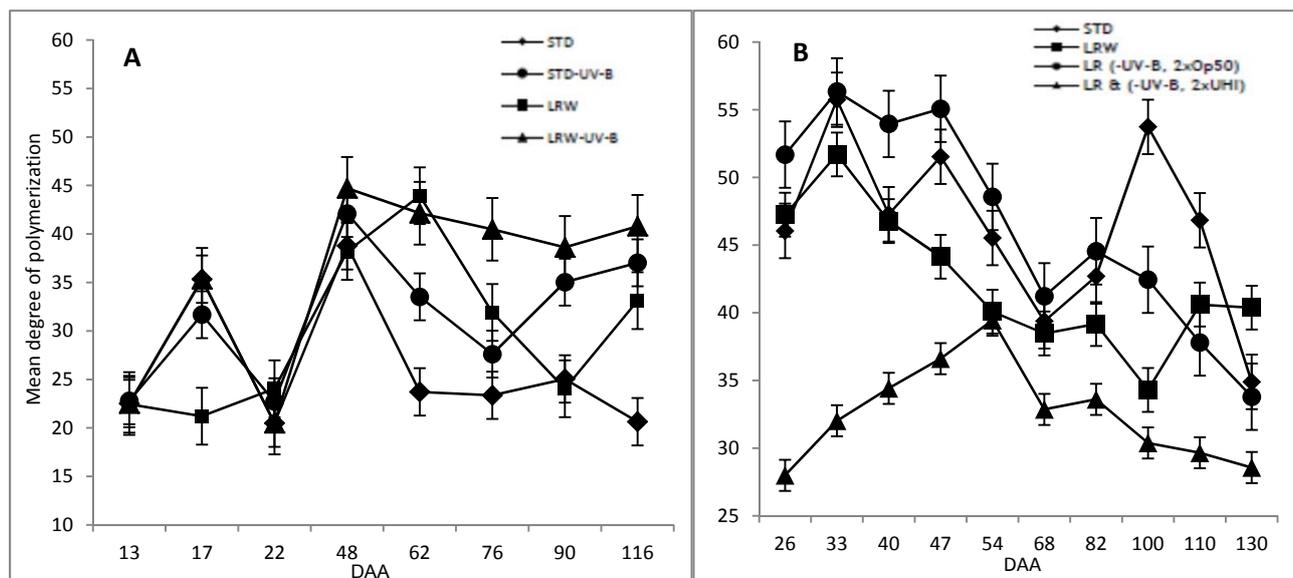


Figure 4.16. Average polymer length of Cabernet Sauvignon skin during berry development. (a) 2010/2011 and (b) 2011/2012 season.

Table 4.6. Mean skin tannin structural characteristics in 2010/2011 and 2011/2012 season.

| 2010/2011 | | | | | 2011/2012 | | | | |
|---------------------|----------|----------|--------|-----------|---------------------|----------|----------|--------|-----------|
| Treatment | Skin % G | Skin % P | mDP | avMM | Treatment | Skin % G | Skin % P | mDP | avMM |
| Standard (Control) | 0.8 a | 54.3 | 26.2 c | 7824.1 c | Standard (Control) | 0.8 c | 57.5 ab | 46.4 a | 13837.0 a |
| Leaf Removal West | 0.6 b | 55.3 | 29.9 b | 8899.4 b | Leaf Removal West | 0.7 c | 57.8 a | 42.3 b | 12620.5 b |
| STD-UV-B | 0.4 c | 53.9 | 31.4 b | 9330.5 b | LR (-UV-B, 2xOp50) | 1.4 a | 56.7 c | 32.6 c | 9740.8 c |
| LRW-UV-B | 0.5 bc | 54.7 | 35.6 a | 10614.2 a | LR (-UV-B, 2xUHI) | 0.9 b | 56.9 cb | 46.5 a | 13895.6 a |
| <i>Significance</i> | *** | ns | *** | *** | <i>Significance</i> | *** | *** | *** | *** |

Means in columns followed by a different letter are significantly different within one season. Mass conversion based on percent recovery of proanthocyanidin by phloroglucinolysis; mDP, mean degree of polymerization; %G, percentage galloylation; %P, percentage prodelphinidins; avMM, average molecular mass. STD (Shaded/Control); LRW (Leaf Removal West); STD with decreased UV-B radiation (STD-UV-B); LRW with decreased UV-B radiation (LRW-UV-B); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

4.7 COMPOSITIONAL CHARACTERISTICS OF ANTHOCYANINS

Anthocyanin mono-glucoside, acetyl-glucoside and coumaroyl proportions were determined in the grape skins (Table 4.7). In the total anthocyanin pool mono-glucoside was the predominant form, while acetyl-glucoside and coumaroyl-glucoside forms were present in lower proportions (Table 4.7). No significant differences among the treatments for the mean anthocyanin glucoside derivatives were observed in 2010/2011 (Table 4.7). However, significant differences were observed in the acetyl-glucoside ($p \leq 0.01$) and coumaroyl-glucoside ($p \leq 0.001$) contributions in 2011/2012 (Table 4.7).

Table 4.7. Mean proportions of anthocyanins in mono-glucoside, acetyl-glucoside and coumaroyl-glucoside form in the skins in 2010/2011 and 2011/2012 vintages.

| Anthocyanin proportions (%) | | | | | | | |
|-----------------------------|----------------|------------------|---------------------|---------------------|----------------|------------------|---------------------|
| 2010/2011 | | | | 2011/2012 | | | |
| Treatment | Mono-glucoside | Acetyl-glucoside | Coumaroyl-glucoside | Treatment | Mono-glucoside | Acetyl-glucoside | Coumaroyl-glucoside |
| Standard (Control) | 57.4 | 30.4 | 12.2 | Standard (Control) | 57.5 | 30.3 a | 12.2 a |
| Leaf Removal West | 56.0 | 28.3 | 11.7 | Leaf Removal West | 58.0 | 29.4 ab | 12.7 a |
| STD-UV-B | 55.1 | 32.3 | 12.6 | LR (-UV-B, 2xOp50) | 59.2 | 26.5 b | 10.3 b |
| LRW-UV-B | 54.6 | 30.0 | 15.4 | LR (-UV-B, 2xUHI) | 58.0 | 29.6 a | 12.4 a |
| <i>Significance</i> | ns | ns | ns | <i>Significance</i> | ns | ** | *** |

Each value represents the mean of 5 replicates. Means followed by different letters are significantly different within one season. STD (Shaded/Control); LRW (Leaf Removal West); STD with decreased UV-B radiation (STD-UV-B); LRW with decreased UV-B radiation (LRW-UV-B); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01 , 0.001 respectively; ns: not significant).

Malvidin-3-glucoside was the dominant anthocyanin and malvidin-3-acetyl glucoside was the major acylated anthocyanin in all treatments and in both seasons (Table 4.8). Malvidin-3-glucoside and its derivatives represented between 66–77 % of the total anthocyanin-mono-glucosides for the respective treatments during 2010/2011 and 83–89% during the 2011/2012 season (Table 4.8). The proportion of petunidin-3-glucosides and its derivatives varied between 6.5–13.5 % while delphinidin and peonidin were present at similar proportions during the respective seasons (Table 4.8). Furthermore, the delphinidin mono-glucosides were significantly ($p \leq 0.05$) higher in the STD treatment (Table 4.8). STD-UV-B had significantly higher malvidin-acetyl glucoside proportions when compared to the other three treatments.

In 2011/2012 significant differences ($p \leq 0.001$) was present in all the derivatives of mono-glucoside (Table 4.8). LR-(UV-B, 2xOp50) had significantly higher mono-glucoside derivatives which can be ascribed to the absence of UV-B radiation and lower PAR values (Table 3.5, Chapter 3) during the growing season. Acetyl-glucoside proportions were altered by the light conditions but no clear trend was visible. Significant differences were observed in the derivatives of all the coumaroyl glucoside proportions except petunidin coumaroyl-glucoside (Table 4.8). From our results it is clear that anthocyanin composition was not consistently influenced by treatment, however the composition was altered by light and temperature conditions within a particular season. As previously mentioned (Chapter 3, paragraph 3.5.1) 2010/2011 was characterised by extensive drought and heat throughout the summer (VinPro, 2011), whereas, the 2011/2012 season was considered as an ideal growing season with a cool, and lengthened, harvesting period without rain or extensive heat (VinPro, 2012). This corresponds with the findings of Ryan & Revilla (2003) and Downey *et al.* (2004) who found vintage effects to play an important role in anthocyanin composition.

Table 4.8. Mean proportions of anthocyanin mono-glucoside, acetyl-glucoside and coumaroyl-glucoside in the grape skins in 2010/2011 and 2011/2012.

| Mono-glucoside proportions (%) | | | | | | | | | |
|-------------------------------------|-----------------------------------|---------------------------------|--------------------------------|--------------------------------|---------------------|-----------------------------------|---------------------------------|--------------------------------|--------------------------------|
| 2010/2011 | | | | | 2011/2012 | | | | |
| Treatment | Delphinidin mono-glucoside % | Petunidin mono-glucoside % | Peonidin mono-glucoside % | Malvidin mono-glucoside % | Treatment | Delphinidin mono-glucoside % | Petunidin mono-glucoside % | Peonidin mono-glucoside % | Malvidin mono-glucoside % |
| Standard (Control) | 8.5 a | 6.1 | 6.1 a | 36.7 | Standard (Control) | 1.6 b | 5.0 b | 3.7 b | 39.6 a |
| Leaf Removal West | 7.1 ab | 6.2 | 6.6 a | 38.5 | Leaf Removal West | 1.6 b | 5.5 b | 4.1 b | 31.7 b |
| STD-UV-B | 5.6 ab | 5.2 | 4.1 b | 40.1 | LR (-UV-B, 2xOp50) | 2.4 a | 8.3 a | 5.0 a | 40.5 a |
| LRW-UV-B | 6.4 b | 5.7 | 4.3 b | 38.2 | LR (-UV-B, 2xUHI) | 1.5 b | 5.2 b | 3.5 b | 43.3 a |
| <i>Significance</i> | * | ns | *** | ns | <i>Significance</i> | *** | *** | *** | *** |
| Acetyl-glucoside proportions (%) | | | | | | | | | |
| 2010/2011 | | | | | 2011/2012 | | | | |
| Treatment | Delphinidin acetyl-glucoside % | Petunidin acetyl-glucoside % | Peonidin acetyl-glucoside % | Malvidin acetyl-glucoside % | Treatment | Delphinidin acetyl-glucoside % | Petunidin acetyl-glucoside % | Peonidin acetyl-glucoside % | Malvidin acetyl-glucoside % |
| Standard (Control) | 2.4 | 2.7 | 2.6 | 22.7 b | Standard (Control) | 1.6 b | 2.1 c | 1.7 b | 22.9 a |
| Leaf Removal West | 2.3 | 2.5 | 2.9 | 21.8 b | Leaf Removal West | 1.6 b | 3.4 a | 1.7 ab | 16.6 c |
| STD-UV-B | 1.9 | 2.2 | 2.1 | 26.1 a | LR (-UV-B, 2xOp50) | 2.4 a | 2.9 b | 1.9 a | 20.4 b |
| LRW-UV-B | 2.2 | 2.7 | 2.1 | 23.0 b | LR (-UV-B, 2xUHI) | 1.5 a | 2.2 c | 1.7 ab | 24.3 a |
| <i>Significance</i> | ns | ns | ns | *** | <i>Significance</i> | *** | *** | * | *** |
| Coumaroyl-glucoside proportions (%) | | | | | | | | | |
| 2010/2011 | | | | | 2011/2012 | | | | |
| Treatment | Delphinidin coumaroyl-glucoside % | Petunidin coumaroyl-glucoside % | Peonidin coumaroyl-glucoside % | Malvidin coumaroyl-glucoside % | Treatment | Delphinidin coumaroyl-glucoside % | Petunidin coumaroyl-glucoside % | Peonidin coumaroyl-glucoside % | Malvidin coumaroyl-glucoside % |
| Standard (Control) | 0.7 | 0.9 | 1.7 | 9.0 | Standard (Control) | 0.5 c | 1.0 | 1.0 b | 9.0 b |
| Leaf Removal West | 0.7 | 1.0 | 1.8 | 8.5 | Leaf Removal West | 1.0 a | 0.9 | 1.2 a | 7.1 c |
| STD-UV-B | 0.8 | 0.8 | 1.8 | 12.4 | LR (-UV-B, 2xOp50) | 0.7 b | 1.0 | 1.0 ab | 7.9 c |
| LRW-UV-B | 0.5 | 0.9 | 1.6 | 9.2 | LR (-UV-B, 2xUHI) | 0.6 c | 0.9 | 1.0 b | 9.9 a |
| <i>Significance</i> | ns | ns | ns | ns | <i>Significance</i> | *** | ns | * | *** |

Means followed by different letters are significantly different within one season. STD (Shaded/Control); LRW (Leaf Removal West); STD with decreased UV-B radiation (STD-UV-B); LRW with decreased UV-B radiation (LRW-UV-B); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01 , 0.001 respectively; ns: not significant).

4.8 CONCLUSIONS

From this study we can conclude that tannin and anthocyanin composition is influenced by complex interactions within a particular season. These interactions include seasonal climatic patterns and particularly the light quality and quantity from flowering until harvest which has a direct impact on the berry size and seed number as well as the accumulation of flavonoids. There was no clear impact of treatment and thus light quality and quantity on either seed tannin composition or concentration. In the case of skin tannin there was an indication of increased skin tannin with light exposure, but this was only visible in the 2010/2011 seasons indicating that seasonal variability had a larger impact than the individual treatments applied to alter the light quantity and quality. Therefore, seasonal differences should be taken into account. Differences between the anthocyanin compositional data varied between the two seasons with no clear treatment or seasonal effect.

These results contribute to a better understanding of the light and seasonal interaction on flavonoid accumulation in the grape berry under growing conditions in Stellenbosch. However, the experimental conditions of 2011/2012 should be repeated for at least 2 years to have a clearer understanding of the seasonal impact on flavonoid composition. Furthermore, additional studies performed in a greenhouse or growth chamber, to control the temperature and light conditions to have a clearer understanding of the abiotic factors influencing flavonoid composition in the berry, could be beneficial.

4.9 LITERATURE CITED

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Chapter 5

Research results

Sensory profiles of wines made from sequentially harvested Cabernet Sauvignon (*Vitis vinifera* L.) grapes

CHAPTER 5

Sensory profiles of wines made from sequentially harvested Cabernet Sauvignon (*Vitis vinifera* L.) grapes

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5.2 ABSTRACT

Harvest time has an influence on the aromatic and phenolic composition of the grapes and the resulting wines. The aim of this study was to evaluate wines harvested sequentially using the berry sugar accumulation model as a physiological indicator. Two seasons and treatments in which the light quality and quantity were altered at the fruit zone were compared. The grapes were harvested at two ripening stages at 20-25 days and approximately 35 days after the sugar loading plateau was reached named the fresh fruit and pre-mature stage in 2010/2011. In the 2011/2012 season grapes were harvested 45 days after the sugar loading plateau was reached and was named the mature fruit stage. Vegetative aromas were synonymous with the fresh fruit stage in 2010/2011, while the 2011/2012 wines were characterised by raisin, prune and spicy aromas which are associated with the mature fruit harvest date. In both seasons the STD treatments was rated high in satin in the mouth in and after expectoration. The mouthfeel attribute coarseness were rated the highest in wines in which the UV-B radiation was excluded - STD-UV-B and LR (-UV-B, 2xUHI)

during berry growth in the respective two seasons. Higher acidity content in the LR (-UV-B, 2xUHI) treatment enhanced the astringency perception in the wine. Flavonol concentration in 2011/2012 wine was higher in the exposed LRW treatment compared to the other treatments. Anthocyanin concentration in the wine was favoured by high light intensities in 2011/2012 season.

5.3 INTRODUCTION

Grape ripening is multi-faceted as it includes numerous physical and biochemical modifications (Jackson & Lombard, 1993; Le Moigne *et al.*, 2008; Dai *et al.*, 2010; Deloire, 2013). Numerous classes of primary (sugars and organic acids) and secondary metabolites (phenolics) as well as hormones and aromatic precursors are synthesised prior and post-véraison while others are provided by the roots and leaves (Ollat & Gaudillère, 1996; Deloire, 2013). The concentration and content of the primary and secondary metabolites change during grape berry ripening stages which are controlled by independent regulated synthesis pathways that are affected by genotype, environmental factors as well as viticultural practices (Jackson & Lombard, 1993; Le Moigne *et al.*, 2008; Dai *et al.*, 2010).

Optimal berry ripeness depends on the wine style goal. The sensory characteristics of the finished wine and thus the quality is strongly dependant on the perception of the primary and secondary metabolites and the alcohol level. Numerous studies have been conducted on the relationship between berry composition and wine phenolic composition. Garcia-Beneytez *et al.* (2002), Harbertson *et al.* (2002), Hazak *et al.* (2005) and Koundouras *et al.* (2006) found no clear relationship between the amount of phenolic compounds found in grapes at harvest and the amount found in the finished wines. The latter are ascribed to factors that influence the extraction of phenolics such as skin thickness, fermentation temperature and alcohol content. Preys *et al.* (2006) suggested that there are some relationship between sensory properties and polyphenolic composition in the final wine. Somers & Evans (1974), Ough & Nagaoka (1984), Bravdo *et al.* (1985) and Hunter *et al.* (1991 & 1995) reported that there is a relationship between berry composition and sensory attributes which is attributed to the applied treatment, vineyard attributes and seasonal variation. More recently, Bindon *et al.* (2013) and Bindon *et al.* (2014) ascribed

significant changes in wine matrix chemistry to grape maturity and yeast metabolism which had a direct impact on the sensory attributes of Cabernet Sauvignon.

Consequently, it will be valuable to be able to predict the future wine style in relation with harvest time (Deloire, 2013). Various ripening tools have been developed to determine berry maturity objectively and accurately at harvest. Berry maturity indices include (i) total soluble solids (TSS), (ii) titratable acidity (TA), (iii) pH and (iv) combinations thereof (maturity indexes) (Amerine & Winkler, 1941; Du Plessis & Van Rooyen, 1982; Van Rooyen, 1984; Boulton *et al.*, 1996; Iland *et al.*, 2000; Ribéreau-Gayon *et al.*, 2006; Botes, 2009). Kourakou (1974), Carbonneau *et al.* (1998) and Schneider *et al.* (2002) identified three types of grape maturity levels: (i) technological maturity, which corresponds to maximum sugar accumulation/concentration and low acidity, (ii) phenolic maturity, defined as the level and concentrations of phenolics in the skins and seeds and (iii) aromatic maturity, associated with the decrease in vegetal notes and the evolution of wine volatile profile.

Cabernet Sauvignon grapes often have a characteristic aroma described as vegetative, herbaceous, grassy or green (Lacey *et al.*, 1991). Deloire (2011) suggested a sugar loading concept which defines sugar loading as the evolution of the sugar quantity (mg/berry) from véraison onward. The evolution of sugar accumulation per berry gives an indication of the ripening time and could be used as a physiological indicator in direct relation with the potential wine styles. Three sugar loading profiles are distinguished: continual and rapid loading, slow sugar loading (inhibition of ripening) and sugar loading presenting a plateau phase. Depending on whether the grapes are picked in the early, mid or late stages of the plateau, the wine will be characterised as 'fresh fruit', 'neutral-spicy' or 'pre-mature' and 'mature fruit' (Deloire, 2011). No peer-reviewed publication at this stage for the berry aromatic sequence concept. The aroma attributes present in the grapes are attributed to the evolution of volatile precursors during berry development which are dependent on enzyme activity and specificity. An in depth understanding of the secondary metabolites during berry development may provide predictive information between the grape and wine aroma (Kalua & Boss, 2009). These aromatic stages require sensory analysis to verify which sensory attributes associate with the respective stages.

The two best known groups of phenols are the condensed tannins (also called proanthocyanidins) and anthocyanins, which are responsible for the red colour in red grapes and wine. Condensed tannins are mainly responsible for bitterness and astringency as well as colour development due to the role it plays in wine ageing processes such as polymerisation reactions with anthocyanins to form polymeric pigments (Ricardo-da-Silva *et al.*, 1991). Wine colour is affected by the level and composition of anthocyanins, tannins and flavonols extracted during vinification (Baranowski & Nagel 1983; Bakker *et al.* 1993; Picinelli *et al.* 1994; Dallas *et al.*, 1996; Cheynier *et al.*, 2000; Romero & Bakker 2000; Eglinton *et al.*, 2004; Ristic *et al.*, 2007). Flavonols form co-pigments with anthocyanins and protect the flavylium cation against the nucleophilic attack of water, peroxide, and sulfur dioxide bleaching and pH changes (Gordillo *et al.*, 2015).

As said before, flavan-3-ols or their oligomers referred to as proanthocyanidins, contribute to bitterness and astringency. The low molecular weight flavan-3-ols exhibit more bitterness than astringency, however as the flavan-3-ols increase in size, astringency increases faster than bitterness (Joslyn & Goldstein, 1964; Rossi & Singleton, 1966; Lea & Arnold, 1978; Delcour, 1984; Noble, 1990; Robichaud & Noble, 1990; Kennedy *et al.*, 2006). Thus, the low molecular weight flavan-3-ols which are associated more with grape seeds have a lower astringency to bitterness ratio than the high molecular weight flavan-3-ols of grape skins. Astringency is a tactile sensation in which drying, puckering and roughing are the result of increased friction between the tongue and the surfaces inside the mouth (Lea & Arnold, 1978; Robichaud & Noble, 1990). Recently, Ferrer-Gallego *et al.* (2015) reported that astringent perception are modulated by an increase in the volatile compounds. Bitterness is a taste sensation perceived by each of the several thousand sensors on the tongue (Katsnelson, 2015). Gonzalo-Diago *et al.* (2014) found that bitterness was highly correlated with in-mouth persistence. Wine phenol composition and thus astringency and bitterness are altered by grape maturity at harvest, winemaking techniques and wine ageing. The rate of phenolic extraction into the wine is dependent on: (i) ripeness of the fruit, (ii) berry size, (iii) the concentration in the grapes, (iv) temperature, (v) sulphur dioxide, (vi) extraction or winemaking techniques, (vii) final ethanol content (viii) as well as the ageing conditions (Berg & Akiyoshi, 1958;

Singleton & Draper, 1964; Ribereau-Gayon, 1974; Ozmianski *et al.*, 1986; Ricardo-da-Silva *et al.*, 1992 a & b; Gomez *et al.*, 1995; Canals *et al.*, 2005; Walker *et al.*, 2005).

In view of the above, the aim of this study was to evaluate wines produced by grapes that were harvested at different ripeness levels using berry sugar accumulation as a physiological indicator. Sequential harvest dates for the STD treatment in 2010/2011 were used to understand the possible effect of the evolution of fruit ripening on the wine matrix and sensory properties. The potential effect of the phenolic composition and volatile compounds on the wine sensory attributes was studied in the 2011/2012 season. The results presented are preliminary results and several more seasons and more detailed chemical analyses are needed to link fruit and wine chemical composition and wine sensory profile of grapes harvested sequentially.

5.4 MATERIALS AND METHODS

5.4.1 Vineyard characteristics

Details of the vineyard characteristics are described in Chapter 3, paragraph 3.4.1.

5.4.2 Sampling procedure

Details of the sampling procedure and preparation for analysis are described in Chapter 3, paragraph 3.4.4.

5.4.3 Harvesting

Sequential harvest dates were predicted using the berry sugar loading model (Deloire 2011 & 2013). Grapes of the 2010/2011 season were harvested at the following times (i) fresh fruit period (for all four treatments) (20–25 days after the sugar loading plateau was reached) on the 28th of February 2011 and (ii) pre-mature period (\pm 35 days after the sugar loading plateau was reached) on the 20th of March 2011 (only the STD treatment was harvested). The reason for only studying STD treatment at the pre-mature period was to confirm the aroma attributes of wines made at this stage according to the sequential harvest using a berry physiological indicator as described by

Deloire (2011). The study aimed to assess the potential aromatic profile of the wine made from grapes harvested at the pre-mature stage which is thought to deliver a neutral or pre-mature wine style. The grapes of all the treatments in 2011/2012 season were harvested at the mature fruit period (45 days after the sugar loading plateau was reached) on the 26th of March 2012.

5.4.4 Small scale winemaking

Standard winemaking procedures of the experimental cellar of the Department of Viticulture and Oenology, Stellenbosch University were followed. The grapes were crushed and destemmed into 20L plastic drums and 30 mg/L SO₂ was added. Juice samples for pH, titratable acidity and °B was taken before the SO₂ addition. Four wines were made from the fresh fruit stage and additionally the control was vinified at the pre-mature stage in 2010/2011. In 2011/2012, four wines were from the grapes harvested at mature fruit stage. The crushed grapes were inoculated with 30 g/hL *Saccharomyces cerevisiae* (WE 372 ®, Anchor) and Lalvin (ICV-D21®, Lallemand) and 30 g/hL Go Ferm Protect (Lallemand) in the rehydration water in 2010/2011 and 2011/2012, respectively. Co-inoculation with 0.01 g/L *Oenococcus oeni* (Enoferm ® Alpha, Lallemand) was carried out 24 hours after the yeast inoculation in order to start the malolactic fermentation. Fermentation took place at 25 °C and punch downs were done three times a day. The rate of fermentation was measured daily with a hydrometer. After 5 °B sugar was fermented 0.25 g/L Fermaid K (Lallemand) was added. The fermentation took about 5 days after which the skins were pressed at -1 °B (press to 1 bar) and moved to 20 °C in order to finish the malolactic fermentation. Once the malolactic fermentation was completed (malic and lactic acids determined enzymatically by the Central Analytical Facility, Stellenbosch University, South Africa), the wines were racked off the lees and an addition of 50 mg/L SO₂ was made. The wines underwent cold stabilization for 3 weeks at -4 °C before adjusting the free SO₂ to 40 mg/L and bottled in 750 mL dark green glass bottles, sealed with screw caps and stored at 15°C, 1 day after bottling. The wines were subjected to sensorial analysis six months after bottling.

5.4.5 Chemicals

Compounds were quantified using external calibration curves for malvidin-3-glucoside (from Extrasynthese, Genay Cedex, France), as well as caffeic acid, *p*-coumaric acid, (+)-catechin, (–)-epicatechin, (–)-epicatechin-3-*O*-gallate, gallic acid and 2,6-dimethyl-hepten-2-ol (all from Sigma-Aldrich St. Louis, MO, U.S.A.). All anthocyanins and other pigments were quantified at 520 nm as malvidin-3-glucoside units, whereas proanthocyanidins and polymeric phenols were quantified at 280 nm as (+)-catechin equivalents. (+)-Catechin, (–)-epicatechin, (–)-epicatechin-3-*O*-gallate were quantified as itself at 280 nm. Phloroglucinol and sodium acetate was obtained from Sigma-Aldrich (Johannesburg, South Africa) for the acid catalyses in the presence of excess phloroglucinol.

Ethyl acetate and isoamyl acetate was purchased at Riedel de Haën (Seelze, Germany). Methanol, hexanol, acetic acid and 2-phenylethanol standard as well diethyl ether, ethanol and NaSO₄ were purchased from Merck (Darmstadt, Germany). Ethyl butyrate, propanol, isobutanol, butanol, hexyl acetate, ethyl lactate, propionic acid, iso-butyric and butyric acid, iso-valeric acid, diethyl succinate, valeric acid, 2-phenylethyl acetate, 4-methyl-2-pentanol and hexane were from Fluka (Buchs, Switzerland). Hexanoic acid, octanoic acid, isoamyl alcohol, ethyl caprylate, ethyl caprate were from Aldrich (Steinheim, Germany). Decanoic acid and ethyl hexanoate were purchased from Sigma (St. Louis, USA).

5.4.6 Method for analysis and quantification of aroma compounds

Aroma compounds were quantified in the 2011/2012 wines. Gas Chromatography–Flame Ionization Detector (GC-FID) analyses were performed to determine the volatile components and monoterpenes in the 2011/2012 wines. Volatile component analysis was carried out using 5 mL of wine with an internal standard (4-methyl-2-pentanol). The extraction was carried out with 1 mL diethyl ether by placing the ether/wine mixture in an ultrasonic bath for 5 min. The wine/ether mixture was then centrifuged at 4000 rpm for 3 min and the ether layer was removed and the extract dried on anhydrous sodium sulphate. This extract was injected into the GC-FID (Coetzee *et al.*, 2005a). Monoterpene analysis was carried out using 50 mL of wine with an internal standard (150 mg/L solution of 2,6-dimethyl-hepten-2-ol dissolved in ethanol). Monoterpenes were extracted

with 2 mL of dichloromethane. The dichloromethane was removed and dried on anhydrous sodium sulphate. The monoterpenes extract was then injected into the GC-FID for analyses (Coetzee *et al.*, 2005b).

5.4.7 Isolation, purification and characterization of proanthocyanidins/tannins

The proanthocyanidins/tannins were characterised and quantified in the 2011/2012 wines. Proanthocyanidins/tannins were isolated in triplicate from different wine treatments using Toyopearl® HW-40 (Tosoh Bioscience, Stuttgart, Germany) size exclusion columns (60 mm x 14.5 mm) as described previously Oberholster *et al.* (2013). In short, dimers and smaller phenolics were washed off the column after loading of the wine (2 mL) with ethanol/water (55/45) containing 0.05% trifluoroacetic acid (TFA). Larger proanthocyanidins/tannin were eluted with 30 mL of acetone/water (60/40) containing 0.05% TFA which was collected and concentrated under reduced pressure at 35°C to remove excess solvent.

The phloroglucinolysis protocol described in Oberholster *et al.* (2013) was implemented and the proanthocyanidin cleavage products were analyzed by RP-HPLC using an Agilent® Poroshell 120 SB-C18 column (4.6 x 150mm, 2.8 µm particle) on an Agilent® Infinity series 1260 HPLC system (Agilent Technologies, Inc., Deerfield, IL, USA) equipped with a DAD detector. Mobile phase A was 0.1 % (v/v) formic acid (Sigma-Aldrich, St. Louis, MO, USA) and mobile phase B acetonitrile containing 0.1% (v/v) formic acid (Sigma-Aldrich, St. Louis, MO, USA). Linear elution conditions were as follows: column temp 35°C; 2 ml/min; 2.96 min at 3% B; 3 to 16% B in 10.30 min, 16 to 20% B in 0.1 min, 1.7 min at 20% B, 20 to 80% B in 0.90 min, column clean-up at 80% B for 1.34 min, and back to 3% B in 1.00 min. The column was equilibrated for 8 min at 3% B before the next injection. Chromatograph integration was performed using Agilent® CDS ChemStation software.

The proanthocyanidin cleavage products were quantified by means of their response factor relative to catechin, which was used as the quantitative standard (Kennedy & Jones, 2001). All samples were analyzed in duplicate. The LOQ and LOD determined for (+)-catechin (Sigma Chemicals, St. Louis, MO) were, respectively 0.0244 nmol and 0.0087 nmol where LOQ was defined as the minimum injected amount that gives a peak height seven times higher than baseline noise and

LOD as the lowest concentration of an analyte in a sample that results in a peak with a height three times as high as the baseline noise level.

5.4.8 Descriptive sensory analysis (DSA)

The wines were evaluated 6 months after bottling by a panel of ten female judges (28–65 years old) for the 2010/2011 season during four replicate sessions. The 2011/2012 wines were evaluated by a panel of nine female judges (29–65 years old) during six replicate sessions. Prior to testing the panel members underwent training and assessment of panel performance in six two hour session in both seasons. The first training session involved standardisation of the panellist on the aroma standards provided in 2011 and 2012 as well as touch standards using different materials (Table 5.1). The mouthfeel properties of the wines were evaluated with touch standards using the mouthfeel wheel (Gawel *et al.*, 2000). The samples were evaluated for an array of aroma attributes, as well as taste and mouthfeel attributes before and after expectoration using 100-point unstructured line scales. Wine samples were served in standard ISO wine tasting glasses, with each glass containing 30 mL of wine. Each sample was coded with a 3-digit random code and served in a complete randomised order (Lawless & Heymann, 2010). Panellists performed the analysis in individual booths, with each booth being fitted with a data collecting system (Compusense® five, Version 5.2, Compusense Inc., Guelph, Ontario, Canada). The testing area was light- and temperature-controlled (21°C).

Table 5.1. Aroma and touch reference standards for mouthfeel evaluations used in the 2010/2011 and 2011/2012 vintages.

| Aroma attributes | Reference standard composition |
|--------------------------------|---|
| Jammy ^a | 30 g red berry jam |
| Strawberry ^a | Sliced fresh strawberries, (ca. 10mm x 10mm) and steeped in wine for ca. 45 minutes |
| Blackberry ^{ab} | 20 g blackberries |
| Blackcurrant ^a | 30 g blackcurrant crushed and steeped in wine |
| Raspberry ^a | 30g raspberries steeped in wine |
| Dark berries ^b | 15 g dark berries blackcurrants and 15 g raspberries steeped in wine |
| Strawberry ^b | 30 g strawberry steeped in wine |
| Prune ^b | 10 mL prune extract |
| Earthy/Dusty ^b | 10 g vacuum dust and 10 g saw dust steeped in wine |
| Vegetative green ^{ab} | Sliced fresh green pepper, (ca 12mm x 10 mm) steeped in wine for 60 minutes |
| Green plum ^a | 1 fresh green plum, (ca 5mm x10mm) without the stone on a petri dish |
| Cooked green ^a | 2 tinned green beans and 10 mL brine |
| Raisin ^a | 50 g raisins |
| Spicy ^a | 5 g Robertson® cinnamon and cloves spice |
| Touch attributes | Reference standard |
| Satin | Satin material |
| Silk | Silk material |
| Course emery | Emery paper |

All standard were made up 150 mL unwooded Cabernet Sauvignon

^a (Attributes used for the 2010/2011 wines).

^b (Attributes used for the 2011/2012 wines).

5.4.9 Statistical analysis

Univariate analysis of variance (ANOVA) was performed on the sensory data using the GLM (General Linear Model) Procedure of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). Sensory data were pre-processed and subjected to a test–retest analysis of variance (ANOVA), using SAS. The latter was performed to test for panel reliability. The Shapiro–Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t test least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998). A probability level of $p \leq 0.05$ was considered significant for all the significance tests. Data were also subjected to multivariate methods of analysis such as principal component analysis (PCA) (XLStat, Version 2011, Addinsoft, New York, USA) to visualise and then interpret the relationships between the samples and their attributes. Multifactor analysis (MFA) was performed on a combination of the grape chemical data and the descriptive analysis data in 2010/2011. Furthermore, MFA analysis was performed on the grape chemical data, descriptive analysis and the wine chemical data in 2011/2012. The MFA function of Statistica 13 (Statsoft Inc., Tulsa, USA) was used.

5.5 RESULTS AND DISCUSSION

5.5.1 Berry composition

At harvest total soluble solids (TSS), pH and titratable acidity (TA) were determined for grapes from each of the treatments in both seasons (Table 5.2). In the 2010/2011 season, the TSS varied significantly ($p \leq 0.01$) at harvest among the treatments (Table 5.2). STD treatment had significant lower TSS ($p \leq 0.01$) compared to the other three treatments in 2010/2011 (Table 5.2). We did not observe an increase in a similar low TSS in the STD-UV-B, treatments despite the similar, low measured light intensities when compared with the STD treatment (Table 3.5; Chapter 3). Spayd *et al.* (2002), Joscelyne *et al.* (2007) and Ristic *et al.* (2007) reported a delay in ripening due to shading which was caused by a greater proportion of leaves. However, Haselgrove *et al.* (2000) found no difference in TSS of shaded or exposed treatments. The thermal time (DD) (Table 3.4; Chapter 3) was the lowest in the STD treatment, but STD-UV-B had similar DD than the other treatments showing an interactive effect of temperature and light. When comparing the premature harvest data with the fresh fruit harvest data for the STD treatment there was an increase in TSS and a simultaneous decrease in TA as expected, but the pH remained the same between the two harvest dates.

In the 2011/2012 season the TSS at harvest was significantly higher ($p \leq 0.001$) in the STD treatment compared to the other treatments although all values were within 1.3 Brix of each other. pH were significantly lower ($p \leq 0.001$) in the STD, LRW and LR (-UV-B, 2xOp50) treatments compared to LR (-UV-B, 2xUHI) in 2011/2012 (Table 5.2). Additionally, a significant lower TA ($p \leq 0.001$) was observed in the LR (-UV-B, 2xUHI) treatment when compared to the other three treatments (Table 5.2). This can be ascribed to the higher exposure level and the absence of leaves which degrade the acid in the berry (Chapter 3; Table 3.5). Rojas-Lara & Morrison (1989), Morrison & Noble (1990) and Downey *et al.* (2006) reported differences in pH and TA in response to light and temperature as shaded fruit had higher pH and potassium levels. From our results there was no clear relation between the grape classical parameters and the impact of treatment on

light and temperature parameters indicating that differences were rather driven by seasonal influences.

Table 5.2. Berry parameters at harvest for the 2010/2011 and 2011/2012 season.

| Treatment | TSS | pH | TA | Fresh mass (g) | Sugar per berry (mg) |
|--------------------------------|---------|--------|--------|----------------|----------------------|
| 2010/2011 | | | | | |
| Fresh fruit harvest | | | | | |
| STD | 20.5 b | 3.6 a | 5.9 ab | 60.3 b | 290.9 b |
| LRW | 22.4 a | 3.7 a | 6.0 a | 58.3 b | 282.9 b |
| STD-UV-B | 22.4 a | 3.6 ab | 6.2 a | 52.1 c | 285.2 b |
| LRW-UV-B | 22.9 a | 3.4 b | 5.5 b | 63.1 a | 316.7 a |
| <i>Significance</i> | ** | *** | * | *** | *** |
| 2010/2011 | | | | | |
| Premature fruit harvest | | | | | |
| STD | 24.8 | 3.6 | 5.5 | 60.1 | 299.1 |
| 2011/2012 | | | | | |
| Mature fruit harvest | | | | | |
| STD | 23.9 a | 3.4 b | 5.4 b | 72.7 a | 348.0 a |
| LRW | 23.1 bc | 3.4 b | 5.3 b | 68.4 b | 327.3 b |
| LR (-UV-B, 2xOp50) | 23.1 b | 3.4 b | 6.1 a | 68.4 b | 289.7 d |
| LR (-UV-B, 2xUHI) | 22.6 c | 3.6 a | 4.8 c | 63.4 c | 305.1 c |
| <i>Significance</i> | *** | *** | *** | *** | *** |

Each value represents the mean of 3 replicates. Means in columns followed by different letters are significantly different within one season. Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001, respectively).

5.5.2 Wine composition 2011/2012

The wine chemical composition of the 2011/2012 wines differed significantly between the treatments (Table 5.3). Wines made from LRW and LR (-UV-B, 2xUHI) treatments had the highest % alcohol while the LR (-UV-B, 2xOp50) contained significantly less, alcohol. Wine pH from the STD and LRW treatments were significantly higher compared to the LR (-UV-B, 2xOp50) and LR (-UV-B, 2xUHI) treatments (Table 5.3). TA values differed significantly among the wines with LR (-UV-B, 2xUHI) treatment having the highest value (Table 5.3). There was no clear relationship between the grape and wine chemical parameters indicating the influence of oenological factors.

The proanthocyanidin composition of the wine tannins was determined by phloroglucinolysis. (+)-Catechin was the predominant terminal unit in the wine in each of the treatments (Table 5.4). This corresponds with the findings of Fernández *et al.* (2007) who reported similar (+)-catechin proportions in different Carménère and Cabernet Sauvignon wines. There were small although

significant differences in the tannin composition of the different wine treatments (Table 5.4). (–)–Epicatechin was the predominant extension subunit as found by other authors (Fernández *et al.* 2007). Most notably the higher %P in LR (-UV-B, 2xOp50) indicates larger contribution from skin tannin. However results were not consistent with light exposure as it is known to increase skin tannin concentration although we only found a small impact of light in our study (Chapter 4, paragraph 4.6.3) (Price *et al.*, 1995; Cortell & Kennedy, 2006; Ristic *et al.* 2007). The treatments with the highest % light intensity, LR (-UV-B, 2xUHI) and LRW (Chapter 3, Table 3.5) did not both have higher %P compared to the other more shaded treatments. Although the tannin concentration was significantly higher in the LRW treatment the STD was not different from LR (-UV-B, 2xOp50). The high tannin concentration observed in our study can be ascribed to the time elapsed between the end of fermentation/maceration and the tannin analysis date (+/- 10 months) which resulted in structural changes. This corresponds to the concentrations obtained by Cosme *et al.* (2009) after six months of storage.

Wine flavonol concentration was higher in the LRW treatment (9.1 mg/L) compared to STD, LR (-UV-B, 2xOp50) and LR (-UV-B, 2xUHI) treatments (7.0, 3.56 and 3.99 mg/L), respectively. This corresponds to our findings of flavonol concentration and content in the fruit as discussed in Chapter 3, paragraph 3.5.4, where higher flavonol concentration were observed in the LRW treatment throughout berry development. The anthocyanin concentration was the highest in the most exposed treatments - LRW and LR (-UV-B, 2xUHI) (173.9 and 139.9 mg/L, respectively) while wines made from the shaded treatments LR (-UV-B, 2xOp50) and STD wines were lower at 92.5 and 124.4 mg/L, respectively. Our results corresponds with the findings of Cortell & Kennedy (2006) and Song *et al.* (2015) who found high anthocyanin concentrations, wine colour density, total pigments and total phenolic and tannin in bunches exposed to sunlight in model solutions and wine.

Table 5.3. Wine parameters of 2012 wines 6 months after bottling.

| Treatment | Alcohol (% vol) | pH | TA |
|--------------------|-----------------|-------|-------|
| STD | 12.9 c | 3.1 a | 8.0 b |
| LRW | 13.6 b | 3.1 a | 7.2 d |
| LR (-UV-B, 2xOp50) | 12.7 d | 3.0 b | 7.6 c |
| LR (-UV-B, 2xUHI) | 14.5 a | 3.0 b | 8.7 a |
| Significance | *** | * | *** |

Each value represents the mean of 3 replicates. Means in columns followed by different letters are significantly different. Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001, respectively)

Table 5.4. Wine compositional and structural characterisation of 2011-2012 wines.

| Terminal units ^a | | | | Extension units ^a | | | | mDP | %G | %P | avMM | Tannin mg/L |
|-----------------------------|--------|--------|------|------------------------------|---------|-------|--------|------|-------|---------|--------|-------------|
| Treatment | C | EC | ECG | C | EC | ECG | EGC | | | | | |
| STD | 74.2 d | 25.5 a | 0.25 | 4.8a | 70.9 a | 2.9 a | 21.3 b | 8.5 | 2.6 a | 18.9 b | 2534.0 | 162.5 b |
| LRW | 82.3 a | 17.6 d | nd | 4.2 ab | 74.3 a | 2.3 b | 19.1 b | 10.1 | 2.0 b | 17.2 b | 2987.8 | 249.3 a |
| LR (-UV-B, 2xOp50) | 79.8 b | 19.8 c | 0.25 | 3.8 b | 57.2 b | 2.2b | 36.6 a | 10.8 | 2.0 b | 33.3 a | 3222.1 | 219.5 a |
| LR (-UV-B, 2xUHI) | 77.6 c | 21.8 b | 0.56 | 3.9 ab | 60.9 ab | 2.3 b | 32.7ab | 10.3 | 2.1 b | 29.5 ab | 3068.2 | 233.3 a |
| Significance | *** | *** | ns | ns | ns | * | ns | ns | * | ns | ns | ** |

Each value represents the mean of 3 replicates. ^aPercent composition of proanthocyanidin subunits (in moles) C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate. mDP, mean degree of polymerization; %G, percentage galloylation; %P, percentage gallo unit; avMM, average molecular mass; nd, not detected;. Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001, respectively, ns: not significant).

5.5.3 Aroma composition of 2011/2012 wines

Volatile compounds were quantified in the 2011/2012 wines using GC-FID. Volatile analysis indicated that limonene, linalool_oxide 1, linalool_oxide 2, linalool, linalyl acetate, α -terpeneol, citronellol, nerol, geraniol, α -ionone, β -ionone and β -farnesol 1 concentrations were higher in the LR (-UV-B, 2xUHI) treatment when compared to the other three treatments (Addendum 19). This corresponds with the findings of Song *et al.* (2015) who found significantly higher concentrations of citronellol, nerol and geraniol in sunlight exposed and UV-radiation blocked treatments. Furthermore, Fang & Qian (2006) suggested that high level of sun exposure results in a higher level of terpene alcohols due to grape maturity. From our results we can confirm that wine aromas are altered by the light conditions of a treatment.

5.5.4 Sensory profile of the wines

The sensory profile of a wine is greatly influenced by the primary and secondary metabolites of the berries at harvest as well as the techniques used during vinification (Boss *et al.* 2010). In our study we investigated the accumulation of grape flavan-3-ol monomers, dimers, total tannin, flavonols and anthocyanins in Chapter 3, paragraphs 3.5.3, 3.5.4, 3.5.5, 3.5.5 as well as the compositional changes of the seed and skin tannin and anthocyanins in Chapter 4, paragraphs 4.5.1, 4.5.2, 4.5.3, 4.4, 4.7 and 4.8 during ripening. Overall the accumulation and compositional data were altered by the light quality/quantity within a particular season. Wines were made from different grape treatments harvested at different maturity levels and evaluated by using descriptive sensorial analyses. Descriptive sensory analysis was used to characterise differences in the perceived aroma and mouthfeel attributes of the wines made with grapes at the different maturity stages of sequential harvesting.

Table 5.5 lists the wine attributes evaluated in the wines made in the 2010/2011 season. The wines made from the different treatments differed significantly for 11 of the 22 sensory attributes, these include the aromas vegetative green ($p \leq 0.001$) and green plum ($p \leq 0.001$) and the in mouth palate attributes: acidity ($p \leq 0.001$), fullness ($p \leq 0.001$), drying ($p \leq 0.05$), satin ($p \leq 0.05$) and coarse emery ($p \leq 0.05$) as well as the attributes experienced after expectoration, drying ($p \leq 0.001$), adhesive ($p \leq 0.001$), hotness (≤ 0.001) and fruit flavour persistence ($p \leq 0.001$) (Table 5.5). Wines made from STD and STD-UV-B treatment grapes scored significantly higher for green plum (Table 5.5). High levels of green plum can be ascribed to the low light intensities through natural shading (STD) and the addition of the UV-B sheets (STD-UV-B) (Chapter 3; Table 3.5). This corresponds to the findings of Heymann & Noble (1987) and Morrison & Noble (1990) that reported an increase in the 3-isobutyl-2-methoxypyrazine (IBMP) (vegetative, herbaceous and grassy) concentration as a result of increased canopy density and bunch shading. The LRW treatment was rated high in vegetative green character. This is the opposite of what we expected, however the score value was low showing that it was not a major aroma attribute. The descriptors used for wines made from the fresh fruit stage of the sequential harvest model were not influenced by the applied treatment, but resulted in wines with fresh fruit, green plant like aromas and unripe plum (Table 5.5). This

corresponds to the findings of Nell (2015) in Merlot noir and Cabernet Sauvignon harvested at the fresh fruit stage. Treatments mostly influenced the intensity of the attributes as discussed above. When the STD wine from the fresh fruit stage and that of the pre-mature stage is compared it is evident that the latter wine had significantly less green plum aromas and more blackberry aromas (Table 5.5).

Wines made from the STD treatment grapes were rated significantly higher in satin in the mouth compared to the other treatment wines (Table 5.5). This finding coincides with that of Ristic *et al.* (2007) who found shaded berries to be less coarse and grainy. After expectoration, “drying” and “adhesive” was rated the highest for the STD-UV-B treatment, indicating a higher perception of astringency. Numerous authors attribute the increase in astringency perception to greater concentration of tannins, polymerised phenols and the variation in tannin structures (Vidal *et al.*, 2003; Kennedy *et al.*, 2006, Mercurio & Smith 2008; Oberholster *et al.*, 2009). From the grape composition results (discussed in Chapter 3 & Chapter 4) the STD-UV-B treatment did not have significantly higher concentration or content of tannins at harvest. This can be due to a result of to increased tannin interactions with berry cell wall material during winemaking which results in the berries and the resulting wine having different phenolic compositions (Adams & Scholz, 2007; Holt *et al.*, 2008). Furthermore, wine made from the STD treatment grapes harvested at the pre-mature stage were rated as being less ‘adhesive’ after expectoration compare to the STD treatment from the fresh fruit stage which indicate a decrease in astringency. Thus the STD wine made from the pre-mature fruit had less green character and decreased astringency compared to the STD wine from the fresh fruit stage. As we did not analyse the wines chemically in 2010/2011, it is not possible to confirm and/or relate the sensory differences to changes in the wine composition.

Table 5.5. Mean score on a 100-point scale of different treatment wines from the 2010/2011 season.

| Treatments | | | | | | |
|----------------------------|---------|---------|----------|----------|----------------|---------|
| Attribute | STD | LRW | STD-UV-B | LRW-UV-B | STD PRE-MATURE | p-value |
| Aroma | | | | | | |
| Blackberry | 41.6 b | 46.1 a | 43.8 ab | 45.0 a | 44.6 a | 0.13 |
| Blackcurrant | 21.9 a | 17.5 ab | 16.2 b | 21.3 ab | 19.8 ab | 0.25 |
| Raspberry | 19.3 ab | 21.7 a | 14.8 b | 1.9 ab | 24.1 a | 0.09 |
| Vegetative green | 1.9 ab | 4.4 a | 1.9 ab | 0 b | 1.7 ab | *** |
| Cooked green | 2.8 ab | 3.2 ab | 1.2 b | 4.4 a | 3.5 ab | 0.34 |
| Green plum | 21.5 b | 10.2 c | 37.2 a | 12.2 c | 8.8 c | *** |
| In the mouth | | | | | | |
| Acidity (in) | 42.2 bc | 48.2 a | 48.0 a | 41.0 c | 45.7 ab | *** |
| Fullness (Viscosity) | 36.6 b | 35.3 b | 42.7 a | 37.5 b | 35.6 b | *** |
| Hotness (% alc. burn) | 38.0 a | 37.5 a | 37.8 a | 42.1 a | 38.5 b | 0.14 |
| Drying | 38.6 ab | 40.7 ab | 44.6 a | 39.6 ab | 38.2 b | * |
| Satin | 11.2 a | 5.7 c | 6.6 c | 7.2 bc | 9.5 b | * |
| Silk | 34.4 a | 35.1 a | 36.2 a | 35.4 a | 35.0 ab | 0.46 |
| Coarse/Emery | 3.9 c | 8.2 a | 7.7 ab | 5.3 bc | 4.9 c | * |
| After expectoration | | | | | | |
| Acidity (out) | 43.6 a | 46.1 a | 45.9 a | 43.2 a | 45.8 a | 0.17 |
| Satin (out) | 2.8 a | 1.3 b | 1.1 b | 2.5 ab | 1.4 ab | 0.16 |
| Silk (out) | 31.1 a | 30.8 a | 31.4 a | 31.2 a | 30.9 a | 0.99 |
| Coarse/Emery (out) | 9.8 b | 11.5 ab | 12.6 a | 11.6 ab | 10.2 b | 0.37 |
| Drying | 41.1 b | 45.7 ab | 49.8 a | 40.9 b | 40.6 b | *** |
| Puckery | 12.7 a | 13.6 a | 15.6 a | 12.9 a | 11.5 a | 0.19 |
| Adhesive | 20.5 b | 22.2 ab | 24.4 a | 20.0 bc | 16.6 c | *** |
| Hotness (% alc. burn) | 38.2 b | 37.7 b | 39.6 ab | 43.8 a | 36.9 b | *** |
| Fruit flavour persistence | 34.4 b | 34.8 b | 40.7 a | 35.0 b | 34.5 b | *** |

Each value represents the mean of 4 replicates. Means in columns followed by different letters are significantly different amongst treatments. STD, LRW, STD-UV-B and LRW-UV-B harvested at the fresh fruit stage of the sequential harvesting model. Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001, respectively)

Wines made from the 2011/2012 season differed significantly among treatments in both aroma and mouthfeel attributes for 20 of the 27 attributes investigated (Table 5.6), these include the aromas prune ($p \leq 0.001$), raisin ($p \leq 0.001$), spice ($p \leq 0.001$), earthy ($p \leq 0.05$) and cooked vegetable ($p \leq 0.001$). In the palate acidity ($p \leq 0.001$), satin ($p \leq 0.05$), silk ($p \leq 0.05$), coarse emery ($p \leq 0.001$), drying ($p \leq 0.001$), hotness ($p \leq 0.001$) and puckery ($p \leq 0.001$) and after expectoration acidity ($p \leq 0.05$), satin ($p \leq 0.05$), silk ($p \leq 0.05$), coarse/emery ($p \leq 0.001$), drying ($p \leq 0.001$), hotness (% alc. burn) ($p \leq 0.001$), puckery ($p \leq 0.05$), adhesive ($p \leq 0.001$) and astringent persistence ($p \leq 0.001$) were significantly different among wine treatments (Table 5.6). The aroma attributes that were perceived by the panel can be associated with over-matured fruit indicating a longer hanging time and corresponds with the sequential harvest model of Deloire (2011). The over-matured fruit and spicy aroma attributes found in our study corresponds with the findings of Nell (2015) in Merlot noir and Cabernet Sauvignon. The LR (-UV-B, 2xUHI) wine scored higher for prune ($p \leq 0.001$), raisin

($p \leq 0.001$), spice ($p \leq 0.001$) and cooked vegetative ($p \leq 0.05$) attributes when compared to the other treatments (Table 5.6). The latter result can be ascribed to the grapes from this treatment being exposed to higher % light in the visible spectrum (380–780nm). The latter resulted in the LR (-UV-B, 2xUHI) treatment having a shading coefficient of 1.0, thermal time of 729.7 and a maximum mean temperature of 39.6°C (Table 3.4; Chapter 3).

In general the wine from treatment LR (-UV-B, 2xUHI) was rated significantly higher than the other three treatments in most of the mouth and after expectoration attributes (Table 5.6). Gawel *et al.* (2007) suggested that an increase in ‘puckery’ sensation were characterised by low anthocyanin levels, high acidity and high pigmented polymer and tannin concentrations. Although wine treatment LR (-UV-B, 2xUHI) rated higher than the other treatments in all of the astringency related attributes except for satin, the wine analyses does not really support this. Tannin analysis (Table 5.4) indicates that there were no significant differences between treatment LR (-UV-B, 2xUHI) and treatments LRW and LR (-UV-B, 2xOp50) in tannin concentration and mDP values. Phenolic profile results from RP-HPLC analysis support this. There were differences in anthocyanin (7.0, 9.0, 3.9 and 3.5 mg/L) and flavonol content (124.4, 173.9, 139.9 and 92.5 mg/L) for STD, LRW, LR (-UV-B, 2xOp50) and LR (-UV-B, 2xUHI), respectively.

The perception of astringency in wines is however also influenced by other parameters such as pH, acidity, ethanol concentration and polysaccharides (Cheynier *et al.*, 2006; Bajec & Pickering, 2008; Ma *et al.*, 2014). From our results the LR (-UV-B, 2xUHI) treatment had significantly higher ($p \leq 0.001$) (Table 5.3) acidity which could enhance the astringency perception of the phenolic compounds.

Table 5.6. Mean score on a 100-point scale of different treatment wines from the 2011/2012 season

| Treatment | | | | | |
|----------------------------|--------|---------|--------------------|-------------------|---------|
| Attribute | STD | LRW | LR (-UV-B, 2xOp50) | LR (-UV-B, 2xUHI) | p-value |
| Aroma | | | | | |
| Dark berries | 35.9 a | 38.4 a | 36.7 a | 37.7 a | 0.39 |
| Strawberry | 20.1 a | 17.3 a | 18.9 a | 18.2 a | 0.79 |
| Prune | 10.4 c | 12.9 b | 11.7 bc | 15.9 a | *** |
| Raisin | 7.4 bc | 8.7 b | 5.3 c | 12.3 a | *** |
| Spice | 4.5 b | 4.7 b | 5.6 b | 9.6 a | *** |
| Earthy | 2.7 bc | 2.5 c | 3.6 ab | 4.5 a | * |
| Fresh vegetative green | 12.8 a | 12.5 a | 13.5 a | 10.8 a | 0.28 |
| Cooked vegetative | 1.8 b | 1.1 b | 0.8 b | 6.8 a | *** |
| Buttery | 6.2 a | 5.6 a | 4.3 a | 5.9 a | 0.16 |
| In the mouth | | | | | |
| Acidity | 22.9 b | 22.7 b | 24.0 b | 26.5 a | *** |
| Satin | 12.8 a | 11.5 a | 11.7 a | 7.4 b | *** |
| Silk | 25.3 a | 25.7 a | 25.3 a | 27.0 a | 0.09 |
| Coarse/Emery | 1.3 b | 3.4 a | 1.7 b | 4.2 a | *** |
| Drying | 18.8 b | 18.6 b | 18.6 b | 21.8 a | * |
| Hotness | 23.8 b | 23.7 a | 25.2 b | 29.5 a | *** |
| Fullness | 24.5 a | 24.1 a | 27.7 a | 25.4 a | 0.40 |
| After expectoration | | | | | |
| Acidity | 29.4 b | 30.0 b | 30.2 b | 32.5 a | * |
| Satin | 5.7 a | 5.5 ab | 5.1 ab | 3.7 b | * |
| Silk | 28.0 b | 28.1 b | 29.1 ab | 30.7 a | * |
| Coarse/Emery | 3.6 b | 4.4 b | 4.2 b | 7.8 a | *** |
| Drying | 24.5 b | 25.3 b | 26.1 b | 29.3 a | *** |
| Hotness | 29.8 b | 30.4 b | 29.2 b | 35.9 a | *** |
| Fullness | 25.5 a | 25.5 a | 26.0 a | 28.0 a | 0.06 |
| Puckery | 12.5 b | 14.0 ab | 12.9 b | 15.3 a | * |
| Adhesive | 14.2 b | 15.5 b | 15.5 b | 18.6 a | *** |
| Fruit flavour persistence | 22.7 a | 22.2 a | 22.2 a | 23.7 a | 0.24 |
| Astringent persistence | 15.4 b | 16.8 b | 16.8 b | 20.1 a | *** |

Each value represents the mean of 6 replicates. Means in rows by different letters are significantly different amongst the treatments. STD, LRW, LR (-UV-B, 2xOp50) and LR(-UV-B, 2xUHI) harvested at the fresh fruit stage of the sequential harvesting model. Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001, respectively).

5.5.5 Multivariate associations of sensory attributes and treatments

Principle component analysis (PCA) was performed for all the aroma and mouthfeel properties for wines from both seasons in an attempt to discriminate among the treatments and the perceived attributes. Cumulatively, PC1 and PC2 explained 80.08% in 2010/2011 and 92.23% in 2011/2012 season (Fig. 5.1 & 5.2) of the variance.

In the 2010/2011 season the LRW and STD-UV-B treatments associate with most of the mouthfeel attributes, whereas STD, LRW-UV-B and STD_Pre-mature associated with three of the aroma attributes i.e raspberry, cooked green and black currant as well as the mouthfeel attributes satin after expectoration and hotness (% alcohol burn) (Fig.1). Differences were driven by higher scores in blackcurrant aroma, alcohol hotness and satin mouthfeel for wines from treatments STD and LRW-UV-B in addition to lower scores in mouthfeel terms drying, puckery and adhesive. STD pre-

mature separated from STD fresh due to mainly an increase in raspberry aroma and a decrease in green plum. Our results corresponds with the findings of Archer & Strauss (1990), Morrison & Noble (1990) and Price *et al.* (1995) who reported that grapes grown in shaded conditions were characterised as green or grassy with limited differences in the wine composition, but the wines from exposed treatments were rated higher in overall quality due to the intensity of the aromas and darker colour. The treatments did not follow any specific trend except for descriptives corresponding with the sequential harvest model (Deloire, 2011).

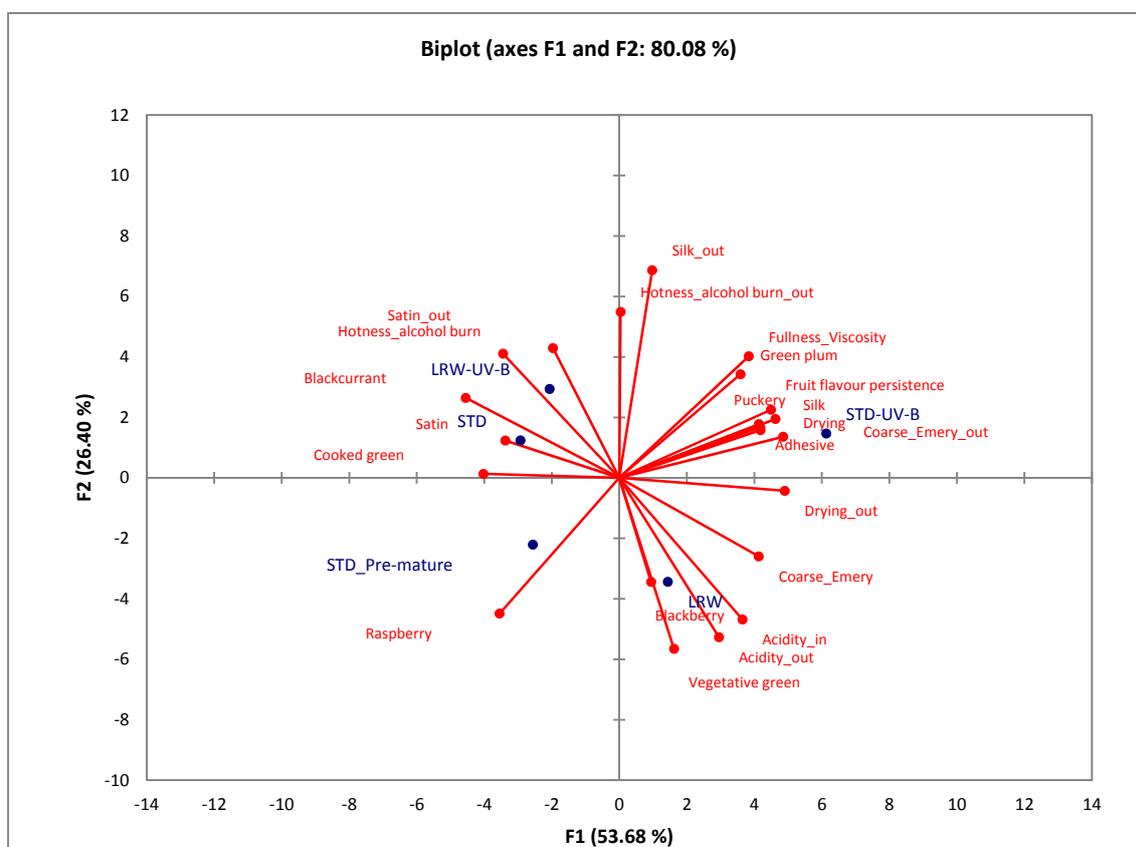


Figure 5.1. The bi-plot for the 2010/2011 Cabernet Sauvignon harvested at the fresh fruit and pre-mature stages based on the perceived sensory attributes. STD (Shaded/Control); LRW (Leaf Removal West); STD-UV-B (STD with decreased UV-B radiation); LRW-UV-B (LRW with decreased UV-B radiation). Harvesting stages: STD, LRW, STD-UV-B and LRW-UV-B (fresh fruit stage) and STD_Premature (Pre-mature stage).

In the 2011/2012 season separation of the wine treatments was due to much higher scores for most aroma and palate attributes for the LR (-UV-B, 2xUHI) treatment compared with the other treatments except for the fresh vegetative and satin attributes. There was thus a clear separation of wines in the 2011/2012 according to light exposure (72, 278.9, 98.4 and 424.4 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for STD, LRW, LR (-UV-B, 2xOp50) and LR (-UV-B, 2xUHI) respectively) based on sensory attributes.

According to the 2011/2012 results (Fig. 5.2) it is clear that a limited number of sensory attributes on the left side of the PCA bi-plot, i.e strawberry and fresh vegetative aromas and satin (in and after expectoration) can be ascribed to the light quantity and not quality as the LR (-UV-B, 2xOp50) was closely related to the LRW and STD treatment. The LR (-UV-B, 2xUHI) treatment was associated with the majority of the sensory attributes, especially the mouthfeel attributes (Fig. 5.2). It is clear that the development of aroma and mouthfeel properties is dependent on light exposure as the LR (-UV-B, 2xUHI) were characterised by high visible light exposure. However, in the 2010/2011 season similar differences in light intensity (175.3, 517.7, 115.3, 260.2 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for STD, LRW, STD-UV-B and LRW-UV-B, respectively) did not result in clear separation of the treatments. The impact of the climate can however be seen if the light intensities for the STD treatment in both seasons are compared. In this study it appears that seasonal variation had a larger impact than treatments on wine sensory attributes. However the grapes were not harvested at the same stages in the different seasons, making final conclusions more difficult. When comparing the two seasons (Fig. 5.1 & 5.2), the aroma attributes perceived in both seasons were found to be significantly different in the assessed wines. However, it corresponded to the sugar loading model of Deloire (2011).

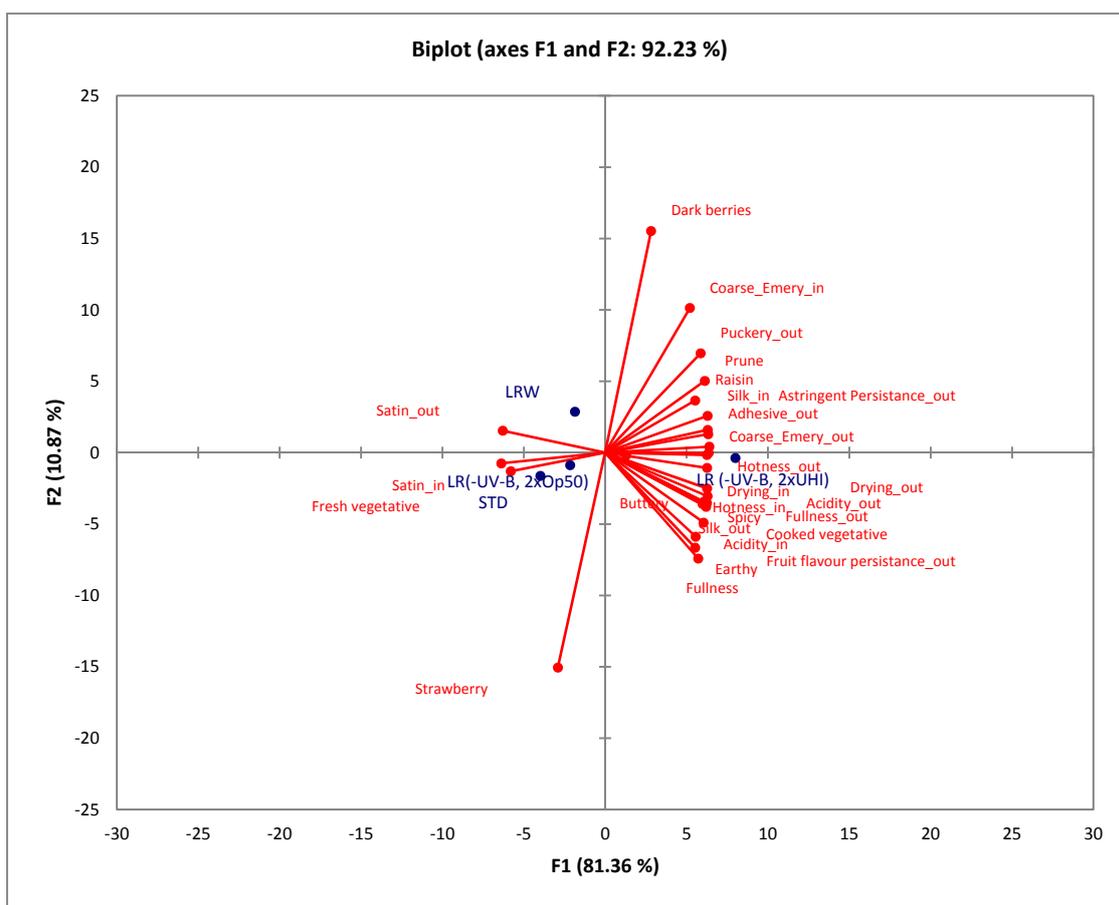


Figure 5.2. The PCA loadings plot and bi-plot for the 2011/2012 Cabernet Sauvignon wines harvested at the mature fruit stage based on the perceived sensory attributes. STD (Shaded/Control); LRW (Leaf Removal West); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone harvest at the mature fruit stage).

5.5.6 Multivariate associations of grape and wine chemical data with sensory attributes

The grape and wine chemical data and sensory analysis data were evaluated by multi-factorial analysis in both seasons (Addendum 20 & 21). Cooked green and raspberry aromas were negatively correlated with the fruit flavour persistence attribute in 2010/2011 (Addendum 20). Most of the mouthfeel attributes of the 2010/2011 wines i.e. coarse emery, drying, adhesiveness, puckery, silk and percentage alcohol burn (hotness), fullness (viscosity and fruit flavour persistence) was closely associated with the grape skin attributes i.e. grape skin mDP, grape skin extension EC %, grape skin terminal C% as well as grape seed terminal EC % and grape skin extension ECG % (Addendum 20). Fullness and viscosity were associated with the anthocyanin content, while the grape flavonols were grouped together. Acidity (in and after expectoration) correlated with coarse emery (in the mouth) and negatively associated with satin (in and after

expectoration). Satin (in and after expectoration) were closely associated with grape skin percentage galloylation and grape seed mDP (Addendum 20).

The 2011/2012 season wine aromas could not be associated with particular grape or wine chemical attributes (Addendum 21). In 2011/2012 satin (in and after expectoration) were negatively correlated with all the other mouthfeel properties (Addendum 21). The remainder of the mouthfeel attributes were strongly correlated with wine mDP, average MW and EGC-P (Addendum 21). This corresponds with the findings of Cerpa-Calderón & Kennedy (2008) who reported a plateau in the skin proanthocyanidin concentration with crushing and an increase in seed proanthocyanidin throughout maceration.

5.6 CONCLUSION

In both seasons berry composition was influenced more by seasonal effects rather than the applied treatment. For most compounds analysed, there were not a linear relation between grape chemical composition and the composition of the resulting wines. Wines in the respective seasons corresponded with sequential harvest using a berry physiological indicator as wines were classified as fresh, green characters in 2010/2011 and prune and raisin characters in 2011/2012. Wines from the STD treatment were consistently rated as having higher satin properties in and after expectoration. Interesting correlations between positive mouthfeel attributes and grape skin and seed tannin parameters as well as grape anthocyanin content were found and needs further investigation.

Sequential harvest is an interesting way to determine the evolution of ripening and the aromas in the associated wines. The study should be conducted over more seasons with the same treatments to investigate the impact of seasonal variability. This will be of interest as we are still trying to relate wine composition with specific mouthfeel attributes and determine how the matrix influences it. Additionally, grapes from the respective treatments should be harvested at different ripeness levels as it would be interesting to determine whether ripeness (i.e harvest time) has more

of a sensory impact than light quantity and quality due to either seasonal or treatment effect as we could not discern it from this study.

5.7 LITERATURE CITED

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Chapter 6

General discussion and conclusions

CHAPTER 6

6.1 INTRODUCTION

Grape berry development involves a complex series of physical and biochemical changes which occurs during the three phases of grape development. Primary and secondary metabolites are synthesised under complex genes and enzymatic control which are altered by the environmental conditions. Primary metabolites such as sugars, amino acids and organic acids are involved in normal growth, development and reproduction of plant species. Secondary metabolites such as phenolics and stilbenoids have ecological functions such as defenses against herbivores, parasites and diseases (Conde *et al.*, 2007, Ali *et al.*, 2010).

Phenolic compounds play an important role in the grape and wine quality. Numerous authors reported on the changes in phenolic development and accumulation which can be attributed to seasonal and cultivar variation in the respective studies (Esteban *et al.* 2001; Spayd *et al.* 2002; Downey *et al.* 2004; Giorgi *et al.* 2005). Furthermore, the cultivation practices and resulting microclimate around the developing fruit affect the fruit composition (flavan-3-ol monomers, proanthocyanidins and pigmented polymers) (Cortell *et al.*, 2005). Environmental factors such as sunlight, temperature, ultra-violet radiation (UV) and plant water status play a role in the accumulation of anthocyanins and proanthocyanidins (Crippen *et al.*, 1986; Kennedy *et al.*, 2002; Ojeda *et al.*, 2002; Downey *et al.*, 2004; Mori *et al.*, 2005 & 2007; Berli *et al.* 2011; Gregan *et al.*, 2012; Koyoma *et al.*, 2012). However, these studies did not investigate the response of seed and skin tannins throughout berry development to light quality, light quantity and temperature variations in the bunch zone. Numerous studies investigated the relationship between berry composition, wine (phenolic) composition and sensory. Garcia-Beneytez *et al.* (2002), Habertson *et al.* (2002), Hazak *et al.* (2005) and Koundouras *et al.* (2006) found no clear relationship between the amount of phenolic compounds found in grapes at harvest and the amount found in the finished wines. The latter are ascribed to factors which influence the extraction of phenolic such as skin thickness, fermentation temperature and alcohol content. Preys *et al.* (2006) suggested that there are some relationship between sensory properties and polyphenolic composition in the final wine. Somers &

Evans (1974), Ough & Nagaoka (1984), Bravdo *et al.* (1985) and Hunter *et al.* (1991 & 1995) reported that there is a relationship between berry composition and sensory attributes which is attributed to the applied treatment, vineyard attributes and seasonal changes.

Very little is known about the response of seed and skin flavonoids accumulation, composition and the resulting wine sensory properties to an altered microclimate under the growing conditions in the Stellenbosch Wine of Origin district. Therefore, this study was proposed to improve our understanding of the impact of light quality (UV-B exclusion), light quantity and temperature on flavonoid (proanthocyanidins, anthocyanin and flavonol) biosynthesis and concentration during berry growth and maturation in skins and seeds and the resulting impact on wine phenolic composition and sensory properties.

6.2 EXPERIMENTAL TREATMENTS

The study was conducted during two consecutive seasons 2010/2011 and 2011/2012 in a Stellenbosch University vineyard (GPS Coordinates: 33°56' 42" S 18°27' 43" E). The vineyard consists of *Vitis vinifera* L. cv. Cabernet Sauvignon clone CS 388C, grafted onto 101-14 *Mgt* (*Vitis riparia* X *Vitis rupestris*). The study comprised of two main treatments with altered bunch microclimates in both seasons: no lateral shoot or leaf removal in the bunch zone (STD) and leaf removal in the bunch zone (LRW) (Chapter 3; Table 3.1). The leaves were removed just after flowering corresponding to growth stage 19 (Eichorn and Lorenz system) (Coombe, 1995) on the western side of the canopy at the fruiting zone level (\pm 35–40 cm above the cordon). Furthermore, to study the effect of change in light quality on fruit growth and composition, supplementary treatments were applied. A UV sheet, reducing the UV-B radiation ('Perspex'® Opal 050), (Perspex South Africa (Pty) Ltd, Umbogintwini) (Chapter 3; Table 3.2) was added to the Control/STD (STD-UV-B) and Leaf Removal West (LRW-UV-B) treatment in 2010/2011. During the 2011/2012 season the UV-B suppression sheets were installed on both sides of the canopy to exclude the effect which row direction can have on grape development. Additionally to the 'Perspex'® Opal 050 (Chapter 3; Table 3.2) sheets a clear acrylic UV-sheet (UHI) was used

during the 2011/2012 season. The latter resulted in the following treatments: LR (-UV-B, 2xOp50) and LR (-UV-B, 2xUHI) (Chapter 3; Table 3.1).

Overall, the 2010/2011 season was characterised by high light intensities when compared to the 2011/2012 season (Chapter 3; Table 3.5). The addition of the UV-B reduction sheets altered the PAR and percentage light in the fruiting zone. The LR (-UV-B, 2xUHI) had a higher PAR due to the shading coefficient which was 1.0 and resulted in a high percentage of visible light in the fruit zone (Chapter 3; Table 3.2).

6.3 GENERAL DISCUSSION

Objective I: Determination of the effect of light quantity, quality and temperature on seed and skin tannin biosynthesis and composition during berry development

Berry development followed a double sigmoid curve (Chapter 3, paragraph 3.5.2). The study showed that seed development coincided with berry development and that changes in berry size altered the tannin content and concentration. An increase in the monomer and dimer concentration and content was observed post-flowering and a maximum was reached at véraison, followed by a decline until harvest in both seasons. Grape seed tannin content and concentration was higher in the 2010/2011 season. This can be ascribed to seasonal variation in the light intensity. Furthermore, seed tannin concentration and content in 2010/2011 was characterised by a second phase of tannin accumulation later in the ripening stage. This could be ascribed to the higher light intensity in the 2010/2011 season which potentially up-regulated the PAL and CHI enzymes in flavonoid synthesis. Logeman & Hahlbrock (2002) suggested that UV-B radiation modifies the metabolism and promotes the synthesis of UV-protective flavonoids as the key enzymes in the phenylpropanoid (phenylalanine-lyase, PAL) and flavonoid (chalcone synthase, CHS and chalcone isomerase, CHI) biosynthetic pathways are up-regulated by UV-B radiation. In our study seasonal variability had a larger impact on seed phenol composition than the applied treatments.

Skin monomers, dimers and tannins accumulated in a similar pattern than those in the seeds and reached a maximum at véraison followed by a decrease post-véraison. The decline after véraison confirms the findings of other authors (Kennedy *et al.*, 2000a; Kennedy *et al.*, 2000b; Downey *et*

al., 2003a; Pastor del Rio & Kennedy, 2006; Cortell & Kennedy, 2006 and Hanlin & Downey, 2009) who found that maximum seed and skin flavan-3-ol concentration and content was reached around véraison followed by a decrease. The decrease in both seed and skin flavan-3-ols from post-véraison is attributed to the decline in the extractability of tannins and the conjugation of proanthocyanidins with other cellular components and the formation of oxidative cross linking polymers (Cheynier *et al.*, 1997; Saint-Cricq de Gaulejac *et al.*, 1997; Kennedy *et al.*, 2000b). Grape skin flavan-3-ol content was significantly influenced in the 2010/2011 by light intensity resulting in significantly higher skin tannin concentrations in shaded STD treatment. However, this trend was not observed in the 2011/2012 season and need further investigation. The two seasons had different patterns of accumulation with 2010/2011 being characterised by a second increase prior to harvest (between 62–116 DAA).

In terms of grape skin and seed flavan-3-ol compositional data, there was no clear trend with light or UV-B exposure across both seasons indicating that seasonal impact was larger than that of the applied treatments. Our results agree with that of Downey *et al.* (2006), Fujita *et al.* (2007) and Cohen *et al.* (2008) who reported minimal variation in the seed compositional proanthocyanidins exposed to different light and temperature regimes. There were clear trends with light exposure and UV-B radiation reduction in the grape skin terminal proportions while grape skin extension subunit proportions showed no clear trends with light exposure and UV-B radiation.

The temperature differences among treatments were too small to significantly influence the flavonoid biosynthesis and composition (Chapter 3; Table 3.4). The temperature was strongly associated with the seasonal influence. Therefore, it is advisable that the study be performed at the macroclimatic, meso and nano-climate scale for at least two years to elucidate the impact of temperature on the flavonoid evolution. The scientific contribution made in this objective is that an accurate characterisation of the seed and skin tannin biosynthesis and composition under altered berry microclimatic conditions were made.

Objective II: Determination of the effect of light quality and temperature on flavonol biosynthesis during berry development

Flavonol concentration and content were the highest in the most exposed treatment (LRW) in both seasons. The shaded treatments (STD) exhibited the second highest concentrations whereas the treatments with the UV suppression sheets exhibited the lowest concentrations. These findings are in agreement with that of other authors (Price *et al.*, 1995; Haselgrove *et al.*, 2000; Spayd *et al.*, 2002; Downey *et al.*, 2003b & 2004; Cortell *et al.*, 2005) who found that exposed fruit had higher flavonol-glycoside content compared to shaded fruit. In 2011/2012 the LR (-UV-B, 2xUHI) treatment had the highest percentage light exposure (Table 3.5; Chapter 3), but still the lowest flavonol concentration and content which indicate that UV-B light is essential for flavonol biosynthesis. This indicates that flavonol biosynthesis is dependent on the light quality rather than the prevailing temperatures in a particular season.

Objective III: Determination of the effect of light quantity and temperature on anthocyanin biosynthesis and composition during berry development

Several studies have found that light exposure have a positive effect on cluster anthocyanin concentration (Haselgrove *et al.*, 2000, Bergqvist *et al.*, 2001, Spayd *et al.*, 2002, Jeong *et al.*, 2004) while in contrast, Downey *et al.* (2004) found that anthocyanin biosynthesis is not readily affected by sunlight. The 2010/2011 season was characterised by high light intensities (Chapter 3; Table 3.4) which stimulated anthocyanin accumulation irrespective of the treatment. Anthocyanin concentration and content were largely influenced by the season and not the treatments applied suggesting a synergistic influence of both light and temperature. Our results are supported by numerous authors (Smart *et al.*, 1985; Crippen & Morrison, 1986; Smart *et al.*, 1988) who found similar results in Cabernet Sauvignon and Shiraz.

Grape anthocyanin monomers are cyanidin, peonidin, delphinidin, petunidin, and malvidin which are glucosylated at position 3 of ring C. The glucoside can be substituted with acetyl and coumaroyl moieties which results in the 15 main anthocyanins found in *Vitis vinifera* grapes (Mazza, 1995). No significant effect of treatment on the anthocyanin proportions were observed in 2010/2011 (Chapter 4; Table 4.7). However, significant differences were observed in the acetyl-

glucoside ($p \leq 0.01$) and coumaroyl-glucoside ($p \leq 0.001$) proportions in 2011/2012 (Chapter 4; Table 4.7). Our results indicate that anthocyanin composition is altered by light and temperature conditions within a particular season. Furthermore, relevant knowledge on the impact of sunlight and UV radiation effect on anthocyanin biosynthesis and composition are provided.

Objective VI: Determination of light quantity and quality at the fruit zone level on wine sensory attributes

The classical parameters (TSS, pH and TA) of the grapes at harvest were a function of the season rather than the applied treatment. Grapes were harvested according to the sugar loading model (Deloire, 2011) at the fresh fruit period and at the pre-mature fresh period for the control (STD) in 2010/2011. In 2011/2012 grapes from all treatments were harvested at the mature fruit stage.

Aromas attributes of the 2010/2011 wines corresponded with the fresh fruit stage (vegetative, herbaceous and grassy) of the sugar loading model (Deloire, 2011). Wine made from grapes of the shaded (STD and STD-UV-B) treatments had significantly higher ratings for green plum. The 2011/2012 wines corresponded with the sugar loading model as mature fruit characters such as raisin, spicy and prune characters were prevalent. The variation in the mouthfeel characteristics can be attributed to the concentration and compositional differences. STD was rated higher in satin in the mouth and after expectoration in both seasons. This finding coincides with that of Ristic *et al.* (2007) who found shaded berries (from shading boxes) to be less coarse and grainy.

Wine chemical composition was only determined in the 2011/2012 wines. Mouthfeel properties (adhesiveness, coarse, pucker – in and after expectoration) were rated higher in the treatments which were exposed to high light intensities in both seasons. The scientific contribution made in this objective is that the application of field treatments provide a realistic view on the microclimatic impact on the oenological aspects.

6.4 PERSPECTIVES AND DIRECTIONS FOR FUTURE RESEARCH

The results obtained in this study indicate that the concentration, content and composition of flavonoids in the grape seeds and skins are dependent on complex interactions between light quality and light quantity. Further work should be done due to the complexity of the study. One way is to conduct the study in a glass-house or growth chamber to control light and temperature to understand the physiological response to UV radiation. However, conducting experiments under glass-house and growth chamber conditions provides unrealistic PAR and UV-radiation which lacks ecological relevance (Krizek, 2004). Therefore, conducting outdoor studies (i.e UV supplementation and exclusion studies) like ours are essential to realistically evaluate the biological effects of solar UV-B radiation. The study can further be enhanced by studying flavonoid responses at the respective UV, Red and Far-red wavelengths in Cabernet Sauvignon. It will be beneficial to be able to repeat the 2011/2012 treatments for two years to be able to study the light quality and quantity versus seasonal impact further. This will enable us to better predict the impact of altered light conditions on grape composition and thus wine quality.

Furthermore, transcriptomic and metabolomics analysis are essential to understand the gene expression under different light and temperature conditions. Essentially, the study should investigate plant responses under different doses of UV-B radiation and the photosynthetic acclimation of UV RESISTANCE LOCUS8 (UVR8) photoreceptor. Few studies focused on the molecular mechanism of UV-B sensitivity of photosynthesis in maize (Caldwell *et al.*, 2007; Casati & Walbot 2003) and recently in *Vitis vinifera* cv. Sauvignon blanc (Liu *et al.*, 2015). To our knowledge, transcriptomic and metabolic work has never been conducted in South Africa on Cabernet Sauvignon under altered light and temperature conditions. This will result in a better understanding of the potential scientific and economic consequences under increasing global warming conditions for the most planted red grape cultivar in South Africa. This could off course also be extended to other cultivars to aid in increasing the quality of these cultivars.

6.5 LITERATURE CITED

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ADDENDUMS

Addendum 1. Berry parameters at harvest of 2010/2011 and 2011/2012.

| Treatment | Total soluble solids | Fresh mass (g) | Sugar per berry |
|---------------------|----------------------|----------------|-----------------|
| 2010/2011 | | | |
| STD | 20.5 b | 60.3 b | 290.9 b |
| LRW | 22.4 a | 58.3 b | 282.9 b |
| STD-UV-B | 22.4 a | 52.1 c | 285.2 b |
| LRW-UV-B | 22.9 a | 63.1 a | 316.7 a |
| Significance | ** | *** | *** |
| 2011/2012 | | | |
| STD | 23.9 a | 72.7 a | 348.0 a |
| LRW | 23.1 bc | 68.4 b | 327.3 b |
| LR (-UV-B, 2xOp50) | 23.1 b | 68.4 b | 289.7 d |
| LR(-UV-B, 2xUHI) | 22.6 c | 63.4 c | 305.1 c |
| Significance | *** | *** | *** |

Each value represents the mean of 5 replicates (\pm) standard deviation. Treatments: STD (Shaded/Control); LRW (Leaf Removal West); STD-UV-B (STD with decreased UV-B radiation); LRW-UV-B (LRW with decreased UV-B radiation); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Addendum 2. Concentration and content of seed flavan-3-ol monomers and dimers in 2010/2011.

| Concentration (mg/g seed) | | | | | | | Content (mg/berry) | | | | | | |
|---------------------------|---------------------|--------------|----------------|----------------|----------------|---------------|--------------------|---------------------|---------------|---------------|---------------|-------------|---------------|
| DAA | Treatment | C | EC | ECG | B1 | B2 | DAA | Treatment | C | EC | ECG | B1 | B2 |
| 13 | Standard (Control) | 0.67 ± 0.33 | 0.14 ± 0.15 b | 0.60 ± 0.31 | 0.08 ± 0.04 | 0.01 ± 0.01 b | 13 | Standard (Control) | 0.02 ± 0.01 b | 0.00 ± 0 b | 0.01 ± 0.01 b | 0.00 ± 0 b | 0.00 ± 0 c |
| | Leaf Removal West | 0.95 ± 0.20 | 0.29 ± 0.06 ab | 0.92 ± 0.23 | 0.10 ± 0.02 | 0.20 ± 0.04 a | | Leaf Removal West | 0.02 ± 0.01 a | 0.01 ± 0 a | 0.02 ± 0 a | 0.00 ± 0 b | 0.00 ± 0 b |
| | STD-UV-B | 1.04 ± 0.13 | 0.32 ± 0.06 a | 0.85 ± 0.12 | 0.13 ± 0.02 | 0.23 ± 0.03 a | | STD-UV-B | 0.03 ± 0 a | 0.01 ± 0 a | 0.02 ± 0.01 a | 0.00 ± 0 a | 0.00 ± 0 a |
| | LRW-UV-B | 0.68 ± 0.33 | 0.14 ± 0.15 b | 0.60 ± 0.31 | 0.08 ± 0.04 | 0.01 ± 0.01 b | | LRW-UV-B | 0.02 ± 0.01 b | 0.00 ± 0 b | 0.01 ± 0 b | 0.00 ± 0 b | 0.01 ± 0 c |
| | Significance | <i>ns</i> | * | <i>ns</i> | <i>ns</i> | *** | | Significance | * | *** | *** | *** | *** |
| 17 | Standard (Control) | 0.60 ± 0.07 | 0.10 ± 0.03 c | 0.48 ± 0.06 | 0.09 ± 0.02 | 0.03 ± 0.02 b | 17 | Standard (Control) | 0.02 ± 0.01 | 0.00 ± 0 b | 0.02 ± 0 | 0.00 ± 0 | 0.00 ± 0 a |
| | Leaf Removal West | 0.69 ± 0.12 | 0.23 ± 0.04 a | 0.51 ± 0.02 | 0.10 ± 0.02 | 0.17 ± 0.03 a | | Leaf Removal West | 0.03 ± 0.01 | 0.01 ± 0 a | 0.02 ± 0 | 0.00 ± 0 | 0.01 ± 0 b |
| | STD-UV-B | 0.55 ± 0.14 | 0.18 ± 0.04 b | 0.48 ± 0.09 | 0.08 ± 0.03 | 0.14 ± 0.04 a | | STD-UV-B | 0.03 ± 0.01 | 0.01 ± 0 a | 0.02 ± 0 | 0.00 ± 0 | 0.01 ± 0 a |
| | LRW-UV-B | 0.60 ± 0.07 | 0.10 ± 0.03 c | 0.48 ± 0.06 | 0.09 ± 0.02 | 0.03 ± 0.02 b | | LRW-UV-B | 0.02 ± 0.01 | 0.00 ± 0 b | 0.02 ± 0 | 0.00 ± 0 | 0.00 ± 0 b |
| | Significance | <i>ns</i> | *** | <i>ns</i> | <i>ns</i> | *** | | Significance | <i>ns</i> | *** | <i>ns</i> | <i>ns</i> | *** |
| 22 | Standard (Control) | 0.32 ± 0.05 | 0.15 ± 0.02 | 0.41 ± 0.02 | 0.07 ± 0.01 | 0.02 ± 0 b | 22 | Standard (Control) | 0.02 ± 0 | 0.01 ± 0 | 0.02 ± 0 | 0.00 ± 0 | 0.00 ± 0 b |
| | Leaf Removal West | 0.40 ± 0.04 | 0.16 ± 0.02 | 0.45 ± 0.02 | 0.08 ± 0.01 | 0.14 ± 0.03 a | | Leaf Removal West | 0.02 ± 0 | 0.01 ± 0 | 0.02 ± 0.01 | 0.00 ± 0 | 0.01 ± 0 a |
| | STD-UV-B | 0.34 ± 0.07 | 0.15 ± 0.04 | 0.43 ± 0.10 | 0.08 ± 0.03 | 0.12 ± 0.03 a | | STD-UV-B | 0.02 ± 0 | 0.01 ± 0 | 0.02 ± 0 | 0.00 ± 0 | 0.01 ± 0 a |
| | LRW-UV-B | 0.32 ± 0.05 | 0.15 ± 0.02 | 0.41 ± 0.02 | 0.07 ± 0.01 | 0.02 ± 0 b | | LRW-UV-B | 0.02 ± 0 | 0.01 ± 0 | 0.02 ± 0.01 | 0.00 ± 0 | 0.00 ± 0 b |
| | Significance | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | | Significance | <i>ns</i> | *** | <i>ns</i> | <i>ns</i> | *** |
| 48 | Standard (Control) | 11.13 ± 3.87 | 4.73 ± 1.85 a | 6.48 ± 2.45 | 0.43 ± 0.24 | 0.33 ± 0.19 | 48 | Standard (Control) | 0.71 ± 0.36 | 0.30 ± 0.17 a | 0.42 ± 0.22 | 0.03 ± 0.02 | 0.02 ± 0.02 |
| | Leaf Removal West | 8.53 ± 1.17 | 3.36 ± 0.64 ab | 5.30 ± 1.05 | 0.35 ± 0.04 | 0.23 ± 0.02 | | Leaf Removal West | 0.50 ± 0.05 | 0.20 ± 0.03 a | 0.31 ± 0.06 | 0.02 ± 0 | 0.01 ± 0 |
| | STD-UV-B | 8.68 ± 1.76 | 0.29 ± 0.04 c | 5.81 ± 0.71 | 0.31 ± 0.06 | 0.22 ± 0.01 | | STD-UV-B | 0.57 ± 0.07 | 0.02 ± 0 b | 0.38 ± 0.03 | 0.02 ± 0 | 0.01 ± 0 |
| | LRW-UV-B | 8.4 ± 1.73 | 3.34 ± 0.63 b | 5.27 ± 0.67 | 0.29 ± 0.04 | 1.12 ± 1.91 | | LRW-UV-B | 0.49 ± 0.08 | 0.20 ± 0.03 a | 0.31 ± 0.01 | 0.02 ± 0 | 0.06 ± 0.10 |
| | Significance | <i>ns</i> | *** | <i>ns</i> | <i>ns</i> | <i>ns</i> | | Significance | <i>ns</i> | *** | <i>ns</i> | <i>ns</i> | <i>ns</i> |
| 62 | Standard (Control) | 3.1 ± 2.04 | 2.30 ± 0.79 | 1.30 ± 0.51 | 0.27 ± 0.06 | 0.33 ± 0.06 b | 62 | Standard (Control) | 0.18 ± 0.12 | 0.14 ± 0.04 | 0.08 ± 0.03 | 0.02 ± 0 | 0.02 ± 0 b |
| | Leaf Removal West | 3.9 ± 1.27 | 2.10 ± 0.39 | 1.14 ± 0.23 | 0.25 ± 0.03 | 0.18 ± 0.03 c | | Leaf Removal West | 0.22 ± 0.07 | 0.12 ± 0.02 | 0.06 ± 0.01 | 0.01 ± 0 | 0.01 ± 0 c |
| | STD-UV-B | 3.7 ± 0.88 | 2.20 ± 0.14 | 1.11 ± 0.29 | 0.24 ± 0.04 | 0.25 ± 0.04 c | | STD-UV-B | 0.21 ± 0.07 | 0.12 ± 0.02 | 0.06 ± 0.02 | 0.01 ± 0 | 0.01 ± 0 b |
| | LRW-UV-B | 4.05 ± 0.33 | 2.28 ± 0.12 | 1.01 ± 0.21 | 0.26 ± 0.02 | 0.45 ± 0.07 a | | LRW-UV-B | 0.21 ± 0.03 | 0.13 ± 0.02 | 0.06 ± 0.02 | 0.01 ± 0 | 0.02 ± 0 a |
| | Significance | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | | Significance | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** |
| 76 | Standard (Control) | 2.75 ± 1.90 | 1.85 ± 0.96 | 0.70 ± 0.55 | 0.24 ± 0.05 ab | 0.34 ± 0.06 b | 76 | Standard (Control) | 0.13 ± 0.09 | 0.09 ± 0.05 | 0.03 ± 0.03 | 0.01 ± 0 ab | 0.02 ± 0 b |
| | Leaf Removal West | 1.60 ± 0.46 | 1.06 ± 0.25 | 0.40 ± 0.10 | 0.18 ± 0.03 c | 0.12 ± 0.02 c | | Leaf Removal West | 0.07 ± 0.03 | 0.05 ± 0.02 | 0.02 ± 0.01 | 0.01 ± 0 c | 0.01 ± 0 c |
| | STD-UV-B | 1.85 ± 0.37 | 1.42 ± 0.10 | 0.42 ± 0.09 | 0.22 ± 0.02cb | 0.36 ± 0.03 b | | STD-UV-B | 0.09 ± 0.02 | 0.07 ± 0.01 | 0.02 ± 0 | 0.01 ± 0 a | 0.02 ± 0 b |
| | LRW-UV-B | 1.92 ± 0.43 | 1.44 ± 0.20 | 0.61 ± 0.06 | 0.27 ± 0.02 a | 0.61 ± 0.06 a | | LRW-UV-B | 0.10 ± 0.03 | 0.07 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0 ab | 0.03 ± 0.01 a |
| | Significance | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | *** | | Significance | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | *** |
| 90 | Standard (Control) | 1.46 ± 0.30 | 1.26 ± 0.24 a | 0.28 ± 0.07 ab | 0.22 ± 0.03 ab | 0.36 ± 0.05 b | 90 | Standard (Control) | 0.07 ± 0.01 | 0.06 ± 0.01 | 0.01 ± 0 | 0.01 ± 0 | 0.02 ± 0 |
| | Leaf Removal West | 1.25 ± 0.32 | 0.93 ± 0.11 b | 0.21 ± 0.10 b | 0.20 ± 0.02 b | 0.14 ± 0.04c | | Leaf Removal West | 0.06 ± 0.02 | 0.05 ± 0.01 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 |
| | STD-UV-B | 1.90 ± 0.76 | 1.40 ± 0.31 a | 0.39 ± 0.12 a | 0.26 ± 0.05 a | 0.16 ± 0.02 c | | STD-UV-B | 0.08 ± 0.03 | 0.06 ± 0 | 0.02 ± 0 | 0.01 ± 0 | 0.01 ± 0 |
| | LRW-UV-B | 1.77 ± 0.39 | 1.22 ± 0.12 ab | 0.29 ± 0.08 ab | 0.26 ± 0.05 a | 0.59 ± 0.06 a | | LRW-UV-B | 0.08 ± 0.01 | 0.06 ± 0.01 | 0.01 ± 0 | 0.01 ± 0 | 0.03 ± 0 |
| | Significance | <i>ns</i> | * | * | * | *** | | Significance | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** |
| 116 | Standard (Control) | 1.40 ± 0.51 | 1.16 ± 0.37 | 0.18 ± 0.03 a | 0.28 ± 0.05 ab | 0.46 ± 0.08 b | 116 | Standard (Control) | 0.07 ± 0.03 | 0.05 ± 0.02 | 0.01 ± 0 | 0.01 ± 0 | 0.02 ± 0.01 |
| | Leaf Removal West | 1.10 ± 0.23 | 1.02 ± 0.40 | 0.12 ± 0.03 b | 0.23 ± 0.03 b | 0.14 ± 0.02 c | | Leaf Removal West | 0.05 ± 0.01 | 0.04 ± 0.01 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 |
| | STD-UV-B | 1.10 ± 0.28 | 1.13 ± 0.11 | 0.13 ± 0.02 b | 0.25 ± 0.03 b | 0.13 ± 0.11 c | | STD-UV-B | 0.05 ± 0.01 | 0.05 ± 0.01 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 |
| | LRW-UV-B | 1.40 ± 0.23 | 1.07 ± 0.23 | 0.16 ± 0.03 ab | 0.32 ± 0.04 a | 0.77 ± 0.11 a | | LRW-UV-B | 0.06 ± 0.01 | 0.05 ± 0.01 | 0.01 ± 0 | 0.01 ± 0 | 0.03 ± 0 |
| | Significance | <i>ns</i> | <i>ns</i> | * | * | *** | | Significance | <i>ns</i> | <i>ns</i> | * | <i>ns</i> | *** |

Each value represents the mean of 5 replicates ± standard deviation of the concentration (mg/g seed) and content (mg/berry). Monomers: C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate. Dimers: B1, Ec-(4β-8-Cat); B2, Ec-(4β-8-Ec). Treatments: STD (Shaded/Control); LRW (Leaf Removal West); STD-UV-B (STD with decreased UV-B radiation); LRW-UV-B (LRW with decreased UV-B radiation); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at p ≤ 0.05, 0.01, 0.001 respectively; ns: not significant).

Addendum 3. Concentration and content of monomeric and dimeric seed flavan-3-ols in 2011/2012.

| Concentration (mg/g seed) | | | | | | | Content (mg/berry) | | | | | | |
|---------------------------|--------------------|----------------|----------------|----------------|----------------|----------------|---------------------|--------------------|----------------|----------------|----------------|----------------|-------------|
| DAA | Treatment | C | EC | ECG | B1 | B2 | DAA | Treatment | C | EC | ECG | B1 | B2 |
| 26 | Standard (Control) | 0.60 ± 0.14 a | 0.16 ± 0.03 a | 0.56 ± 0.05 ab | 0.08 ± 0.01 a | 0.12 ± 0.02 a | 26 | Standard (Control) | 0.06 ± 0.01 b | 0.02 ± 0 b | 0.05 ± 0bc | 0.01 ± 0 | 0.01 ± 0 |
| | Leaf Removal West | 0.67 ± 0.02 a | 0.17 ± 0.01 a | 0.52 ± 0.02 b | 0.08 ± 0.01 a | 0.12 ± 0.01 a | | Leaf Removal West | 0.07 ± 0.01 a | 0.02 ± 0 ab | 0.06 ± 0 b | 0.01 ± 0 | 0.01 ± 0 |
| | LR (-UV-B, 2xOp50) | 0.64 ± 0.09 a | 0.76 ± 0.02 a | 0.60 ± 0.06 a | 0.08 ± 0.01 a | 0.12 ± 0.02 a | | LR (-UV-B, 2xOp50) | 0.07 ± 0.01 a | 0.02 ± 0 a | 0.06 ± 0.01 a | 0.01 ± 0 | 0.01 ± 0 |
| | LR(-UV-B, 2xUHI) | 0.39 ± 0.11 b | 0.12 ± 0.03 b | 0.36 ± 0.08 c | 0.06 ± 0.02 b | 0.09 ± 0.02 b | | LR(-UV-B, 2xUHI) | 0.05 ± 0.01 b | 0.01 ± 0 b | 0.04 ± 0.01 c | 0.01 ± 0 | 0.01 ± 0 |
| Significance | | *** | *** | *** | * | * | Significance | | *** | ** | *** | ns | ns |
| 33 | Standard (Control) | 0.37 ± 0.09 | 0.16 ± 0.01 | 0.61 ± 0.04 | 0.06 ± 0.01 | 0.09 ± 0.02 | 33 | Standard (Control) | 0.03 ± 0.01 | 0.01 ± 0 | 0.05 ± 0.01 | 0.01 ± 0 b | 0.01 ± 0 |
| | Leaf Removal West | 0.87 ± 1.01 | 0.32 ± 0.40 | 1.33 ± 1.79 | 0.17 ± 0.22 | 0.22 ± 0.27 | | Leaf Removal West | 0.04 ± 0 | 0.01 ± 0 | 0.06 ± 0.01 | 0.01 ± 0 ab | 0.01 ± 0 |
| | LR (-UV-B, 2xOp50) | 0.47 ± 0.06 | 0.17 ± 0.02 | 0.65 ± 0.10 | 0.07 ± 0.01 | 0.11 ± 0.02 | | LR (-UV-B, 2xOp50) | 0.05 ± 0.01 | 0.02 ± 0 | 0.07 ± 0.01 | 0.01 ± 0 b | 0.01 ± 0 |
| | LR(-UV-B, 2xUHI) | 0.29 ± 0.08 | 0.11 ± 0.03 | 0.45 ± 0.08 | 0.06 ± 0.01 | 0.08 ± 0.02 | | LR(-UV-B, 2xUHI) | 0.05 ± 0.02 | 0.02 ± 0.01 | 0.07 ± 0.01 | 0.01 ± 0 a | 0.01 ± 0 |
| Significance | | ns | ns | ns | ns | ns | Significance | | ns | ns | ns | * | ns |
| 40 | Standard (Control) | 0.43 ± 0.12 b | 0.33 ± 0.05 ab | 1.10 ± 0.28 b | 0.08 ± 0.02bc | 0.04 ± 0.01 | 40 | Standard (Control) | 0.05 ± 0.01 | 0.04 ± 0 | 0.13 ± 0.03 | 0.01 ± 0 | 0.00 ± 0 |
| | Leaf Removal West | 0.40 ± 0.04 b | 0.25 ± 0.04 b | 0.79 ± 0.18 b | 0.09 ± 0.01 b | 0.06 ± 0.03 | | Leaf Removal West | 0.05 ± 0.01 | 0.03 ± 0.01 | 0.09 ± 0.02 | 0.01 ± 0 | 0.01 ± 0 |
| | LR (-UV-B, 2xOp50) | 0.70 ± 0.09 a | 0.40 ± 0.09 a | 1.51 ± 0.24 a | 0.12 ± 0.03 a | 0.06 ± 0.02 | | LR (-UV-B, 2xOp50) | 0.06 ± 0.01 | 0.04 ± 0.01 | 0.14 ± 0.02 | 0.01 ± 0 | 0.01 ± 0 |
| | LR(-UV-B, 2xUHI) | 0.32 ± 0.08 b | 0.24 ± 0.07 b | 0.86 ± 0.22 b | 0.07 ± 0.01 c | 0.03 ± 0.01 | | LR(-UV-B, 2xUHI) | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.11 ± 0.03 | 0.01 ± 0 | 0.00 ± 0 |
| Significance | | *** | *** | *** | ** | ** | Significance | | ns | ns | ns | ns | ns |
| 47 | Standard (Control) | 3.03 ± 1.34 | 1.43 ± 0.38 a | 4.36 ± 1.28 | 0.15 ± 0.03 | 0.13 ± 0.03 | 47 | Standard (Control) | 0.20 ± 0.09 a | 0.09 ± 0.03 a | 0.28 ± 0.09 a | 0.01 ± 0 | 0.01 ± 0 a |
| | Leaf Removal West | 1.87 ± 0.72 | 0.95 ± 0.27 b | 2.96 ± 0.66 | 0.14 ± 0.02 | 0.10 ± 0.03 | | Leaf Removal West | 0.10 ± 0.04 b | 0.05 ± 0.01 b | 0.16 ± 0.03 b | 0.01 ± 0 | 0.01 ± 0 b |
| | LR (-UV-B, 2xOp50) | 1.77 ± 0.62 | 1.04 ± 0.27 b | 3.16 ± 0.66 | 0.13 ± 0.02 | 0.10 ± 0.02 | | LR (-UV-B, 2xOp50) | 0.10 ± 0.04 b | 0.06 ± 0.02 b | 0.19 ± 0.04 b | 0.01 ± 0 | 0.01 ± 0 b |
| | LR(-UV-B, 2xUHI) | 2.46 ± 0.44 | 0.89 ± 0.18 b | 3.15 ± 0.34 | 0.14 ± 0.02 | 0.12 ± 0.03 | | LR(-UV-B, 2xUHI) | 0.17 ± 0.04 ab | 0.06 ± 0.01 b | 0.22 ± 0.04 ab | 0.01 ± 0 | 0.01 ± 0 a |
| Significance | | ns | * | ns | ns | ns | Significance | | * | * | * | ns | * |
| 54 | Standard (Control) | 4.51 ± 1.57 | 2.43 ± 0.75 | 4.62 ± 1.03 | 0.23 ± 0.05 ab | 0.22 ± 0.04 ab | 54 | Standard (Control) | 0.38 ± 0.15 | 0.20 ± 0.07 | 0.39 ± 0.11 | 0.02 ± 0.01 ab | 0.02 ± 0 |
| | Leaf Removal West | 3.75 ± 1.37 | 2.03 ± 0.73 | 4.01 ± 1.06 | 0.23 ± 0.05 b | 0.20 ± 0.04cb | | Leaf Removal West | 0.36 ± 0.12 | 0.19 ± 0.06 | 0.38 ± 0.08 | 0.02 ± 0 a | 0.02 ± 0 |
| | LR (-UV-B, 2xOp50) | 4.98 ± 0.96 | 2.10 ± 0.51 | 4.67 ± 0.94 | 0.29 ± 0.04 a | 0.25 ± 0.04 a | | LR (-UV-B, 2xOp50) | 0.43 ± 0.06 | 0.18 ± 0.03 | 0.40 ± 0.05 | 0.03 ± 0 a | 0.02 ± 0 |
| | LR(-UV-B, 2xUHI) | 2.91 ± 0.76 | 1.60 ± 0.34 | 3.33 ± 0.60 | 0.14 ± 0.02 c | 0.15 ± 0.03 c | | LR(-UV-B, 2xUHI) | 0.37 ± 0.10 | 0.20 ± 0.05 | 0.43 ± 0.10 | 0.02 ± 0 b | 0.02 ± 0 |
| Significance | | ns | ns | ns | *** | ** | Significance | | ns | ns | ns | * | ns |
| 68 | Standard (Control) | 3.74 ± 2.16cb | 2.39 ± 1.21 a | 2.12 ± 0.86 b | 0.21 ± 0.08 ab | 0.32 ± 0.16 a | 68 | Standard (Control) | 0.21 ± 0.10 b | 0.12 ± 0.06 cb | 0.11 ± 0.05 c | 0.01 ± 0 c | 0.02 ± 0.01 |
| | Leaf Removal West | 5.64 ± 1.14 ab | 2.70 ± 0.21 a | 3.09 ± 0.65 a | 0.27 ± 0.03 a | 0.34 ± 0.04 a | | Leaf Removal West | 0.35 ± 0.13 ab | 0.16 ± 0.03 ab | 0.19 ± 0.07 ab | 0.02 ± 0 ab | 0.02 ± 0 |
| | LR (-UV-B, 2xOp50) | 7.53 ± 2.09 a | 3.07 ± 0.65 a | 3.68 ± 0.74 a | 0.28 ± 0.07 a | 0.37 ± 0.07 a | | LR (-UV-B, 2xOp50) | 0.48 ± 0.12 a | 0.20 ± 0.03 a | 0.24 ± 0.05 a | 0.02 ± 0 a | 0.02 ± 0 |
| | LR(-UV-B, 2xUHI) | 2.33 ± 1.17 c | 1.12 ± 0.51 b | 1.39 ± 0.35 b | 0.13 ± 0.04 b | 0.16 ± 0.07 b | | LR(-UV-B, 2xUHI) | 0.25 ± 0.13 b | 0.12 ± 0.06 c | 0.15 ± 0.05 cb | 0.01 ± 0 b | 0.02 ± 0.01 |
| Significance | | *** | *** | *** | *** | * | Significance | | *** | * | ** | * | ns |
| 82 | Standard (Control) | 2.81 ± 0.45 a | 1.87 ± 0.26 a | 0.81 ± 0.16 b | 0.31 ± 0.03 a | 0.73 ± 0.09 a | 82 | Standard (Control) | 0.12 ± 0.02 a | 0.1 ± 0.02 a | 0.03 ± 0.01 | 0.01 ± 0 | 0.03 ± 0.01 |
| | Leaf Removal West | 2.83 ± 0.86 a | 1.72 ± 0.34 a | 1.07 ± 0.30 a | 0.32 ± 0.03 a | 0.58 ± 0.06 b | | Leaf Removal West | 0.12 ± 0.03 ab | 0.08 ± 0.01 a | 0.05 ± 0.01 | 0.01 ± 0 | 0.02 ± 0 |
| | LR (-UV-B, 2xOp50) | 2.98 ± 0.51 a | 1.87 ± 0.15 a | 0.89 ± 0.10 ab | 0.30 ± 0.02 a | 0.69 ± 0.06 ab | | LR (-UV-B, 2xOp50) | 0.13 ± 0.03 a | 0.09 ± 0.01 a | 0.04 ± 0.01 | 0.01 ± 0 | 0.03 ± 0 |
| | LR(-UV-B, 2xUHI) | 0.87 ± 0.27 b | 0.57 ± 0.11 b | 0.34 ± 0.07 c | 0.14 ± 0.03 b | 0.33 ± 0.15 c | | LR(-UV-B, 2xUHI) | 0.08 ± 0.03 b | 0.06 ± 0.01 b | 0.03 ± 0.01 | 0.01 ± 0 | 0.03 ± 0.01 |
| Significance | | *** | *** | *** | *** | *** | Significance | | * | *** | ns | ns | ns |
| 96 | Standard (Control) | 1.73 ± 0.30 ab | 1.31 ± 0.15 a | 0.53 ± 0.04 a | 0.23 ± 0.02 a | 0.63 ± 0.13 a | 96 | Standard (Control) | 0.06 ± 0.01 ab | 0.04 ± 0.01 a | 0.02 ± 0 a | 0.01 ± 0 | 0.02 ± 0.01 |
| | Leaf Removal West | 1.43 ± 0.33 b | 1.13 ± 0.21 a | 0.51 ± 0.06 a | 0.24 ± 0.04 a | 0.54 ± 0.08 a | | Leaf Removal West | 0.06 ± 0.02 ab | 0.05 ± 0.01 a | 0.02 ± 0 a | 0.01 ± 0 | 0.02 ± 0 |
| | LR (-UV-B, 2xOp50) | 1.85 ± 0.12 a | 1.25 ± 0.14 a | 0.50 ± 0.08 a | 0.24 ± 0.02 a | 0.57 ± 0.08 a | | LR (-UV-B, 2xOp50) | 0.07 ± 0.01 a | 0.05 ± 0.01 a | 0.02 ± 0 ab | 0.01 ± 0 | 0.02 ± 0 |
| | LR(-UV-B, 2xUHI) | 0.48 ± 0.14 c | 0.35 ± 0.06 b | 0.18 ± 0.04 b | 0.10 ± 0.02 b | 0.25 ± 0.03 b | | LR(-UV-B, 2xUHI) | 0.04 ± 0.01 b | 0.03 ± 0 b | 0.02 ± 0 b | 0.01 ± 0 | 0.02 ± 0 |
| Significance | | *** | *** | *** | *** | *** | Significance | | * | ** | * | ns | ns |
| 110 | Standard (Control) | 1.86 ± 0.57 a | 1.43 ± 0.32 a | 0.52 ± 0.15 a | 0.32 ± 0.04 a | 0.75 ± 0.09 a | 110 | Standard (Control) | 0.06 ± 0.02 a | 0.07 ± 0.01 a | 0.02 ± 0 | 0.01 ± 0 | 0.03 ± 0 a |
| | Leaf Removal West | 1.43 ± 0.14 a | 1.10 ± 0.11 ab | 0.43 ± 0.10 a | 0.27 ± 0.03 a | 0.54 ± 0.05 c | | Leaf Removal West | 0.05 ± 0.01 a | 0.04 ± 0 b | 0.02 ± 0 | 0.01 ± 0 | 0.02 ± 0 b |
| | LR (-UV-B, 2xOp50) | 1.80 ± 0.47 a | 1.26 ± 0.13 ab | 0.44 ± 0.10 a | 0.31 ± 0.06 a | 0.65 ± 0.03 b | | LR (-UV-B, 2xOp50) | 0.06 ± 0.02 a | 0.05 ± 0 b | 0.02 ± 0 | 0.01 ± 0 | 0.02 ± 0 b |
| | LR(-UV-B, 2xUHI) | 0.40 ± 0.07 b | 0.33 ± 0.05 c | 0.15 ± 0.02 b | 0.11 ± 0.02b | 0.21 ± 0.03 d | | LR(-UV-B, 2xUHI) | 0.04 ± 0.01 b | 0.03 ± 0 c | 0.02 ± 0 | 0.01 ± 0 | 0.02 ± 0 b |
| Significance | | *** | *** | *** | *** | *** | Significance | | ** | *** | ns | ns | *** |

Addendum 3 (cont.)

| DAA | Treatment | C | EC | ECG | B1 | B2 | DAA | Treatment | C | EC | ECG | B1 | B2 |
|---------------------|--------------------|----------------|---------------|---------------|---------------|----------------|---------------------|--------------------|---------------|---------------|-------------|-----------|-------------|
| 130 | Standard (Control) | 1.34 ± 0.30 ab | 1.05 ± 0.17 a | 0.27 ± 0.04 a | 0.32 ± 0.02 a | 0.64 ± 0.04 a | 130 | Standard (Control) | 0.05 ± 0.01 b | 0.04 ± 0 ab | 0.01 ± 0 | 0.01 ± 0 | 0.02 ± 0 |
| | Leaf Removal West | 0.96 ± 0.12 b | 0.76 ± 0.11 b | 0.28 ± 0.08 a | 0.24 ± 0.03 b | 0.45 ± 0.09 b | | Leaf Removal West | 0.04 ± 0.01 b | 0.03 ± 0 cb | 0.01 ± 0 | 0.01 ± 0 | 0.02 ± 0 |
| | LR (-UV-B, 2xOp50) | 1.53 ± 0.57 a | 0.84 ± 0.18 b | 0.27 ± 0.06 a | 0.27 ± 0.06 b | 0.53 ± 0.19 ab | | LR (-UV-B, 2xOp50) | 0.09 ± 0.03 a | 0.05 ± 0.01 a | 0.02 ± 0 | 0.01 ± 0 | 0.03 ± 0.01 |
| | LR(-UV-B, 2xUHI) | 0.33 ± 0.12 c | 0.29 ± 0.08 c | 0.11 ± 0.08 b | 0.11 ± 0.33 c | 0.20 ± 0.05 c | | LR(-UV-B, 2xUHI) | 0.03 ± 0.01 b | 0.03 ± 0 c | 0.01 ± 0.01 | 0.01 ± 0 | 0.02 ± 0 |
| <i>Significance</i> | | *** | *** | *** | *** | *** | <i>Significance</i> | | *** | * | <i>ns</i> | <i>ns</i> | <i>ns</i> |

Each value represents the mean of 5 replicates ± standard deviation of the concentration (mg/g seed) content (mg/berry). Monomers: C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate. Dimers: B1, Ec-(4β-8-)Cat; B2, Ec-(4β-8-)Ec. Treatments: STD (Shaded/Control); LRW (Leaf Removal West); STD-UV-B (STD with decreased UV-B radiation); LRW-UV-B (LRW with decreased UV-B radiation); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Addendum 4. The mean monomer/dimer and seed tannin concentration and content in 2010/2011 and 2011/2012.

| Treatment | Monomer and dimer concentration (mg/g seed) | Monomer and dimer content (mg/berry) | Total seed tannin concentration (mg/g seed) | Total seed tannin content (mg/berry) |
|--------------------|---|--------------------------------------|---|--------------------------------------|
| 2010/2011 | | | | |
| Standard (Control) | 5.89 | 0.33 | 44.61 a | 2.20 |
| Leaf Removal West | 4.93 | 0.26 | 43.26 a | 2.02 |
| STD-UV-B | 4.88 | 0.27 | 39.96 ab | 1.96 |
| LRW-UV-B | 5.37 | 0.28 | 37.58 b | 1.75 |
| Significance | ns | ns | * | ns |
| 2011/2012 | | | | |
| Standard (Control) | 5.41 a | 0.30 a | 44.88 a | 2.40 |
| Leaf Removal West | 0.34 c | 0.29 a | 42.22 a | 2.36 |
| LR (-UV-B, 2xOp50) | 0.09 c | 0.01 b | 42.80 a | 2.31 |
| LR(-UV-B, 2xUHI) | 2.94 b | 0.25 a | 25.88 b | 2.24 |
| Significance | *** | *** | *** | ns |

Each value represent the mean of 5 replicates at 8 sampling dates in 2010/2011 and 10 sampling dates in 2011/2012. Means in columns followed by a different letter are significantly different within one season. Monomers: C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate. Dimers: B1, Ec-(4 β -8-)Cat; B2, Ec-(4 β -8-)Ec. Treatments: STD (Shaded/Control); LRW (Leaf Removal West); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Addendum 5. Seed number in the 2011/2012 season. Each value represents the mean of 5 replicates (\pm) standard deviation.

| DAA | Treatment | Seed number |
|---------------------|--------------------|---------------------|
| 26 | STD | 25.8 \pm 3.70 |
| 26 | LRW | 23.6 \pm 1.8 2 |
| 26 | LR (-UV-B, 2xOp50) | 26.00 \pm 2.24 |
| 26 | LR(-UV-B, 2xUHI) | 26.00 \pm 2.74 |
| Significance | | ns |
| 33 | STD | 26.20 \pm 1.30 ab |
| 33 | LRW | 28.60 \pm 5.41 a |
| 33 | LR (-UV-B, 2xOp50) | 23.60 \pm 2.07 b |
| 33 | LR(-UV-B, 2xUHI) | 28.20 \pm 1.30 a |
| Significance | | ns |
| 40 | STD | 31.40 \pm 5.94 ab |
| 40 | LRW | 34.60 \pm 4.22 a |
| 40 | LR (-UV-B, 2xOp50) | 27.80 \pm 4.71 b |
| 40 | LR(-UV-B, 2xUHI) | 30.60 \pm 3.91 ab |
| Significance | | ns |
| 47 | STD | 29.60 \pm 4.10 |
| 47 | LRW | 27.20 \pm 3.63 |
| 47 | LR (-UV-B, 2xOp50) | 27.00 \pm 2.12 |
| 47 | LR(-UV-B, 2xUHI) | 29.00 \pm 2.83 |
| Significance | | ns |
| 54 | STD | 28.40 \pm 4.83 ab |
| 54 | LRW | 31.20 \pm 4.38 a |
| 54 | LR (-UV-B, 2xOp50) | 25.40 \pm 2.51 b |
| 54 | LR(-UV-B, 2xUHI) | 26.00 \pm 2.74 b |
| Significance | | ns |
| 68 | STD | 26.40 \pm 2.30 ab |
| 68 | LRW | 27.60 \pm 3.85 a |
| 68 | LR (-UV-B, 2xOp50) | 24.00 \pm 2.83 ab |
| 68 | LR(-UV-B, 2xUHI) | 23.40 \pm 2.41 b |
| Significance | | ns |
| 82 | STD | 25.00 \pm 1.22 |
| 82 | LRW | 23.20 \pm 1.30 |
| 82 | LR (-UV-B, 2xOp50) | 23.80 \pm 4.09 |
| 82 | LR(-UV-B, 2xUHI) | 25.00 \pm 3.54 |
| Significance | | ns |
| 96 | STD | 27.80 \pm 2.17 |
| 96 | LRW | 29.80 \pm 2.59 |
| 96 | LR (-UV-B, 2xOp50) | 28.40 \pm 2.88 |
| 96 | LR(-UV-B, 2xUHI) | 27.40 \pm 0.55 |
| Significance | | ns |
| 110 | STD | 28.60 \pm 3.44 ab |
| 110 | LRW | 29.80 \pm 1.92 a |
| 110 | LR (-UV-B, 2xOp50) | 25.00 \pm 1.58 c |
| 110 | LR(-UV-B, 2xUHI) | 24.50 \pm 1.29 cb |
| Significance | | * |
| 130 | STD | 26.00 \pm 2.74 ab |
| 130 | LRW | 23.20 \pm 1.92 b |
| 130 | LR (-UV-B, 2xOp50) | 28.60 \pm 2.51 a |
| 130 | LR(-UV-B, 2xUHI) | 25.83 \pm 2.79 b |
| Significance | | * |

Each value represents the mean of 5 replicates \pm standard deviation. Treatments: STD (Control); LRW (Leaf Removal West); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Addendum 6. Concentration and content of skin flavan-3-ol monomers and dimers in 2010/2011.

| Concentration (mg/g skin) | | | | | | | Content (mg/berry) | | |
|---------------------------|--------------------|------------------|-----------------|------------------|------------------|-----------------|---------------------|--------------------|--|
| DAA | Treatment | C | EC | ECG | B1 | B2 | DAA | Treatment | Skin monomeric and dimeric flavan-3-ols (mg/berry) |
| 13 | Standard (Control) | 0.05 ± 0.61 | 0.005 ± 0.001 | 0.01 ± 0.007 | 0.007 ± 0.002 | 0 b | 13 | Standard (Control) | 0.005 ± 0.002 |
| | Leaf Removal West | 0.05 ± 0.02 | 0.006 ± 0.001 | 0.01 ± 0.009 | 0.006 ± 0.002 | 0.61 ± 0.003 a | | Leaf Removal West | 0.005 ± 0.002 |
| | STD-UV-B | 0.03 ± 0.02 | 0.005 ± 0.001 | 0.005 ± 0.002 | 0.004 ± 0.002 | 0.01 ± 0.005 a | | STD-UV-B | 0.003 ± 0.002 |
| | LRW-UV-B | 0.05 ± 0.01 | 0.005 ± 0.001 | 0.01 ± 0.007 | 0.007 ± 0.002 | 0 b | | LRW-UV-B | 0.005 ± 0.002 |
| Significance | | ns | ns | ns | ns | *** | Significance | | ns |
| 17 | Standard (Control) | 0.06 ± 0.01 | 0.007 ± 0.003 | 0.003 ± 0.001 | 0.01 ± 0.005 | 0 c | 17 | Standard (Control) | 0.005 ± 0.001 |
| | Leaf Removal West | 0.03 ± 0.03 | 0.006 ± 0.002 | 0.006 ± 0.008 | 0.006 ± 0.007 | 0.004 ± 0.002 b | | Leaf Removal West | 0.003 ± 0.003 |
| | STD-UV-B | 0.04 ± 0.02 | 0.007 ± 0.004 | 0.006 ± 0.005 | 0.004 ± 0.001 | 0.008 ± 0.004 a | | STD-UV-B | 0.004 ± 0.002 |
| | LRW-UV-B | 0.06 ± 0.01 | 0.007 ± 0.003 | 0.003 ± 0.001 | 0.01 ± 0.005 | 0 c | | LRW-UV-B | 0.005 ± 0.001 |
| Significance | | ns | ns | ns | ns | *** | Significance | | ns |
| 22 | Standard (Control) | 0.03 ± 0.01 a | 0.006 ± 0.003 | 0.003 ± 0.002 | 0.007 ± 0.002 | 0 b | 22 | Standard (Control) | 0.003 ± 0.001 |
| | Leaf Removal West | 0.03 ± 0.01 b | 0.007 ± 0.001 | 0.002 ± 0.003 | 0.008 ± 0.003 | 0.004 ± 0.001 a | | Leaf Removal West | 0.003 ± 0.001 |
| | STD-UV-B | 0.01 ± 0.01 b | 0.006 ± 0.002 | 0.006 ± 0.002 | 0.005 ± 0.002 | 0.004 ± 0.002 a | | STD-UV-B | 0.002 ± 0.001 |
| | LRW-UV-B | 0.03 ± 0.01 a | 0.006 ± 0.003 | 0.003 ± 0.002 | 0.007 ± 0.002 | 0 b | | LRW-UV-B | 0.003 ± 0.001 |
| Significance | | * | ns | ns | ns | *** | Significance | | ns |
| 48 | Standard (Control) | 0.05 ± 0.03 | 0.007 ± 0.003 | 0.008 ± 0.008 | 0.01 ± 0.01 | 0 b | 48 | Standard (Control) | 0.008 ± 0.004 |
| | Leaf Removal West | 0.04 ± 0.01 | 0.004 ± 0.002 | 0.009 ± 0.007 | 0.01 ± 0.004 | 0.008 ± 0.001 a | | Leaf Removal West | 0.007 ± 0.002 |
| | STD-UV-B | 0.03 ± 0.01 | 0.008 ± 0.004 | 0.008 ± 0.004 | 0.01 ± 0.003 a | 0.007 ± 0.003 a | | STD-UV-B | 0.006 ± 0.002 |
| | LRW-UV-B | 0.02 ± 0.01 | 0.006 ± 0.002 | 0.007 ± 0.005 | 0.007 ± 0.003 | 0.006 ± 0.001 a | | LRW-UV-B | 0.004 ± 0.002 |
| Significance | | ns | ns | ns | ns | *** | Significance | | ns |
| 62 | Standard (Control) | 0.009 ± 0.004 b | 0.01 ± 0.005 | 0.01 ± 0.001 ab | 0.01 ± 0.004 a | 0 b | 62 | Standard (Control) | 0.005 ± 0.001 a |
| | Leaf Removal West | 0.006 ± 0.002 b | 0.01 ± 0.003 | 0.01 ± 0.01 a | 0.007 ± 0.003 a | 0 b | | Leaf Removal West | 0.005 ± 0.001 a |
| | STD-UV-B | 0.009 ± 0.004 b | 0.007 ± 0.002 | 0.003 ± 0.003 b | 0.002 ± 0.001 b | 0 b | | STD-UV-B | 0.002 ± 0.001 b |
| | LRW-UV-B | 0.01 ± 0.004 a | 0.01 ± 0.004 | 0.005 ± 0.002 b | 0.001 ± 0.0009 b | 0.01 ± 0.008 a | | LRW-UV-B | 0.005 ± 0.001 a |
| Significance | | ** | ns | * | *** | *** | Significance | | ** |
| 76 | Standard (Control) | 0.006 ± 0.002 bc | 0.04 ± 0.06 a | 0.01 ± 0.009 a | 0.17 ± 0.006 a | nd | 76 | Standard (Control) | 0.011 ± 0.002 a |
| | Leaf Removal West | 0.003 ± 0.0005 c | 0.03 ± 0.01 a | 0.006 ± 0.002 ab | 0 b | nd | | Leaf Removal West | 0.006 ± 0.001 a |
| | STD-UV-B | 0.008 ± 0.004 b | 0.009 ± 0.004 b | 0.001 ± 0.002 cb | 0 b | nd | | STD-UV-B | 0.002 ± 0.001 c |
| | LRW-UV-B | 0.01 ± 0.004 a | 0.04 ± 0.009 a | 0 c | 0 b | nd | | LRW-UV-B | 0.007 ± 0.002 b |
| Significance | | *** | *** | *** | * | ns | Significance | | ns |
| 90 | Standard (Control) | 0.008 ± 0.004 a | 0.05 ± 0.01 a | 0.01 ± 0.001 a | 0.02 ± 0.01 a | nd | 90 | Standard (Control) | 0.014 ± 0.003 a |
| | Leaf Removal West | 0.02 ± 0.002 b | 0.05 ± 0.01 a | 0.009 ± 0.00 b | 0 b | nd | | Leaf Removal West | 0.009 ± 0.001 b |
| | STD-UV-B | 0.01 ± 0.003 a | 0 b | 0.001 ± 0.001c | 0 b | nd | | STD-UV-B | 0.002 ± 0.001 c |
| | LRW-UV-B | 0 b | 0.05 ± 0.01 a | 0 c | 0 b | nd | | LRW-UV-B | 0.006 ± 0.001 b |
| Significance | | ** | *** | *** | *** | ns | Significance | | ns |
| 116 | Standard (Control) | 0.03 ± 0.01 a | 0.09 ± 0.02 a | 0.03 ± 0.01 a | 0.03 ± 0.008 a | nd | 116 | Standard (Control) | 0.028 ± 0.007 a |
| | Leaf Removal West | 0 c | 0.08 ± 0.005 a | 0.005 ± 0.00b | 0 | nd | | Leaf Removal West | 0.011 ± 0.001 b |
| | STD-UV-B | 0.01 ± 0.004 b | 0 b | 0 b | 0 | nd | | STD-UV-B | 0.002 ± 0.001 c |
| | LRW-UV-B | 0 c | 0.08 ± 0.005 a | 0.005 ± 0.00b | 0 | nd | | LRW-UV-B | 0.012 ± 0.002 b |
| Significance | | *** | *** | *** | *** | ns | Significance | | *** |

Each value represents the mean of 5 replicates ± standard deviation of the concentration (mg/g skin) and content (mg/berry). Monomers: C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate. Dimers: B1, Ec-(4β-8-)Cat; B2, Ec-(4β-8-Ec). Treatments: STD (Shaded/Control); LRW (Leaf Removal West); STD with decreased UV-B radiation (STD-UV-B); LRW with decreased UV-B radiation (LRW-UV-B). Significance (*, ** and *** indicate significance at $p \leq 0.05, 0.01, 0.001$ respectively; nd: not detected; ns: not significant).

Addendum 7. Concentration and content of monomeric and dimeric skin flavan-3-ols in 2011/2012.

| Concentration (mg/g skin) | | | | | | Content (mg/berry) | | | |
|---------------------------|--------------------|-----------------|------------------|------------------|------------------|--------------------|---------------------|--------------------|--|
| DAA | Treatment | C | EC | ECG | B1 | B2 | DAA | Treatment | Skin monomeric and dimeric flavan-3-ols (mg/berry) |
| 26 | Standard (Control) | 0.103 ± 0.069 b | 0.007 ± 0.005 | 0.005 ± 0.002 | 0.007 ± 0.004 a | 0.22 ± 0.013 | 26 | Standard (Control) | 0.033 ± 0.047 |
| | Leaf Removal West | 0.126 ± 0.070 b | 0.010 ± 0.003 | 0.005 ± 0.001 | 0.009 ± 0.006 bc | 0.19 ± 0.009 | | Leaf Removal West | 0.11 ± 0.006 |
| | LR (-UV-B, 2xOp50) | 0.199 ± 0.028 a | 0.011 ± 0.001 | 0.005 ± 0.001 | 0.028 ± 0.006 a | 0.024 ± 0.009 | | LR (-UV-B, 2xOp50) | 0.019 ± 0.004 |
| | LR(-UV-B, 2xUHI) | 0.061 ± 0.030 b | 0.010 ± 0.002 | 0.006 ± 0.002 | 0.015 ± 0.006 b | 0.011 ± 0.004 | | LR(-UV-B, 2xUHI) | 0.052 ± 0.004 |
| Significance | | ** | ns | ns | *** | ns | Significance | | ns |
| 33 | Standard (Control) | 0.44 ± 0.025 | 0.008 ± 0.002 | 0.004 ± 0.001 | 0.007 ± 0.002 | 0.007 ± 0.003 | 33 | Standard (Control) | 0.005 ± 0.001 |
| | Leaf Removal West | 0.046 ± 0.039 | 0.008 ± 0.004 | 0.004 ± 0.002 | 0.006 ± 0.003 | 0.008 ± 0.005 | | Leaf Removal West | 0.005 ± 0.002 |
| | LR (-UV-B, 2xOp50) | 0.035 ± 0.018 | 0.006 ± 0.002 | 0.006 ± 0.002 | 0.005 ± 0.005 | 0.007 ± 0.002 | | LR (-UV-B, 2xOp50) | 0.004 ± 0.002 |
| | LR(-UV-B, 2xUHI) | 0.22 ± 0.014 | 0.008 ± 0.001 | 0.006 ± 0.005 | 0.005 ± 0.004 | 0.006 ± 0.002 | | LR(-UV-B, 2xUHI) | 0.072 ± 0.015 |
| Significance | | ns | ns | ns | ns | ns | Significance | | *** |
| 40 | Standard (Control) | 0.113 ± 0.074 a | 0.010 ± 0.002 a | 0.005 ± 0.002 | 0.019 ± 0.010 a | 0.009 ± 0.002 | 40 | Standard (Control) | 0.012 ± 0.008 |
| | Leaf Removal West | 0.127 ± 0.019 a | 0.010 ± 0.001 a | 0.005 ± 0.001 | 0.021 ± 0.005 a | 0.012 ± 0.001 | | Leaf Removal West | 0.013 ± 0.002 |
| | LR (-UV-B, 2xOp50) | 0.018 ± 0.005 b | 0.007 ± 0.001 b | 0.005 ± 0.001 | 0.008 ± 0.002 b | 0.007 ± 0.001 | | LR (-UV-B, 2xOp50) | 0.003 ± 0.001 |
| | LR(-UV-B, 2xUHI) | 0.043 ± 0.013 b | 0.010 ± 0.001 a | 0.007 ± 0.006 | 0.018 ± 0.006 a | 0.007 ± 0.002 | | LR(-UV-B, 2xUHI) | 0.115 ± 0.034 |
| Significance | | *** | *** | ns | * | *** | Significance | | *** |
| 47 | Standard (Control) | 0.078 ± 0.056 | 0.010 ± 0.003 b | 0.003 ± 0.001 | 0.024 ± 0.008 a | 0.012 ± 0.002 ab | 47 | Standard (Control) | 0.009 ± 0.004 |
| | Leaf Removal West | 0.112 ± 0.031 | 0.011 ± 0.001 b | 0.007 ± 0.005 | 0.027 ± 0.005 a | 0.014 ± 0.002 a | | Leaf Removal West | 0.012 ± 0.003 |
| | LR (-UV-B, 2xOp50) | 0.048 ± 0.036 | 0.008 ± 0.002 b | 0.007 ± 0.003 | 0.009 ± 0.006 b | 0.008 ± 0.002 c | | LR (-UV-B, 2xOp50) | 0.006 ± 0.004 |
| | LR(-UV-B, 2xUHI) | 0.056 ± 0.019 | 0.016 ± 0.004 a | 0.005 ± 0.001 | 0.026 ± 0.013 a | 0.010 ± 0.002 cb | | LR(-UV-B, 2xUHI) | 0.4666 ± 0.096 |
| Significance | | ns | ** | ns | * | ** | Significance | | *** |
| 54 | Standard (Control) | 0.138 ± 0.078 | 0.012 ± 0.004 | 0.007 ± 0.004 b | 0.038 ± 0.006 | 0.019 ± 0.003 | 54 | Standard (Control) | 0.012 ± 0.004 |
| | Leaf Removal West | 0.149 ± 0.033 | 0.012 ± 0.001 | 0.005 ± 0.002 b | 0.046 ± 0.003 | 0.022 ± 0.004 | | Leaf Removal West | 0.018 ± 0.004 |
| | LR (-UV-B, 2xOp50) | 0.156 ± 0.038 | 0.012 ± 0.003 | 0.015 ± 0.004 a | 0.037 ± 0.008 | 0.019 ± 0.005 | | LR (-UV-B, 2xOp50) | 0.018 ± 0.005 |
| | LR(-UV-B, 2xUHI) | 0.133 ± 0.026 | 0.016 ± 0.005 | 0.010 ± 0.005 ab | 0.035 ± 0.008 | 0.013 ± 0.003 | | LR(-UV-B, 2xUHI) | 0.628 ± 0.120 |
| Significance | | ns | ns | ** | ns | ns | Significance | | *** |
| 68 | Standard (Control) | 0.028 ± 0.034 | 0.010 ± 0.002 | 0.008 ± 0.006 | 0.011 ± 0.012 | 0.009 ± 0.005 b | 68 | Standard (Control) | 0.004 ± 0.001 |
| | Leaf Removal West | 0.030 ± 0.007 | 0.010 ± 0.004 | 0.013 ± 0.008 | 0.016 ± 0.003 | 0.042 ± 0.012 a | | Leaf Removal West | 0.011 ± 0.001 |
| | LR (-UV-B, 2xOp50) | 0.015 ± 0.007 | 0.011 ± 0.008 | 0.010 ± 0.004 | 0.011 ± 0.004 | 0.007 ± 0.002 b | | LR (-UV-B, 2xOp50) | 0.005 ± 0.001 |
| | LR(-UV-B, 2xUHI) | 0.11 ± 0.006 | 0.008 ± 0.003 | 0.006 ± 0.003 | 0.009 ± 0.006 | 0.004 ± 0.001 b | | LR(-UV-B, 2xUHI) | 0.470 ± 0.197 |
| Significance | | ns | ns | ns | ns | *** | Significance | | *** |
| 82 | Standard (Control) | 0.013 ± 0.004 | 0.018 ± 0.007 | 0.012 ± 0.004 ab | nd | 0.009 ± 0.004 b | 82 | Standard (Control) | 0.006 ± 0.001 |
| | Leaf Removal West | 0.014 ± 0.003 | 0.018 ± 0.005 | 0.017 ± 0.008 a | 0.013 ± 0.003 a | 0.034 ± 0.007 a | | Leaf Removal West | 0.010 ± 0.002 |
| | LR (-UV-B, 2xOp50) | 0.009 ± 0.003 | 0.015 ± 0.004 | 0.009 ± 0.003 b | 0.006 ± 0.001 b | nd | | LR (-UV-B, 2xOp50) | 0.005 ± 0.001 |
| | LR(-UV-B, 2xUHI) | 0.008 ± 0.007 | 0.010 ± 0.006 | nd | 0.005 ± 0.003 b | nd | | LR(-UV-B, 2xUHI) | 0.241 ± 0.060 |
| Significance | | ns | ns | *** | *** | *** | Significance | | *** |
| 96 | Standard (Control) | 0.008 ± 0.006 a | 0.018 ± 0.005 b | 0.012 ± 0.003 b | nd | 0.002 ± 0.005 | 96 | Standard (Control) | 0.005 ± 0.001 |
| | Leaf Removal West | nd | 0.053 ± 0.013 a | 0.015 ± 0.003 b | nd | nd | | Leaf Removal West | 0.009 ± 0.002 |
| | LR (-UV-B, 2xOp50) | 0.005 ± 0.002 a | 0.012 ± 0.003 b | 0.021 ± 0.007 a | 0.003 ± 0.001 a | nd | | LR (-UV-B, 2xOp50) | 0.005 ± 0.001 |
| | LR(-UV-B, 2xUHI) | nd | 0.020 ± 0.002 b | nd | nd | nd | | LR(-UV-B, 2xUHI) | 0.149 ± 0.025 |
| Significance | | *** | *** | *** | *** | ns | Significance | | *** |
| 110 | Standard (Control) | 0.010 ± 0.004 a | 0.019 ± 0.003 b | 0.019 ± 0.008 a | nd | nd | 110 | Standard (Control) | 0.006 ± 0.001 |
| | Leaf Removal West | nd | 0.050 ± 0.009 a | 0.009 ± 0.005 b | nd | nd | | Leaf Removal West | 0.008 ± 0.001 |
| | LR (-UV-B, 2xOp50) | 0.004 ± 0.001 b | 0.009 ± 0.002 c | 0.013 ± 0.002 ab | nd | nd | | LR (-UV-B, 2xOp50) | 0.003 ± 0 |
| | LR(-UV-B, 2xUHI) | nd | 0.020 ± 0.005 b | nd | nd | nd | | LR(-UV-B, 2xUHI) | 0.154 ± 0.026 |
| Significance | | *** | *** | *** | ns | ns | Significance | | *** |
| 130 | Standard (Control) | 0.002 ± 0.002 a | 0.017 ± 0.010 a | 0.017 ± 0.017 a | nd | nd | 130 | Standard (Control) | 0.004 ± 0.001 |
| | Leaf Removal West | nd | 0.003 ± 0.007 b | 0.009 ± 0.007 ab | nd | nd | | Leaf Removal West | 0.001 ± 0 |
| | LR (-UV-B, 2xOp50) | nd | 0.009 ± 0.003 ab | 0.004 ± 0.002 cb | nd | nd | | LR (-UV-B, 2xOp50) | 0.002 ± 0 |
| | LR(-UV-B, 2xUHI) | nd | 0.014 ± 0.005 a | nd | nd | nd | | LR(-UV-B, 2xUHI) | 0.119 ± 0.02 |
| Significance | | * | * | ** | ns | ns | Significance | | *** |

Each value represents the mean of 5 replicates ± standard deviation in concentration (mg/g skin) and content (mg/berry). Monomers: C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate. Dimers: B1, Ec-(4β-8-)Cat; B2, Ec-(4β-8-)Ec. Treatments: STD (Shaded/Control); LRW (Leaf Removal West); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05, 0.01, 0.001$ respectively; nd: not detected; ns: not significant).

Addendum 8. The mean monomer/dimer and skin tannin concentration and content in 2010/2011 and 2011/2012.

| Treatment | Monomer and dimer skin concentration (mg/g skin) | Monomer and dimer skin content (mg/berry) | Total skin tannin concentration (mg/g skin) | Total skin tannin content (mg/berry) |
|--------------------|--|---|---|--------------------------------------|
| 2010/2011 | | | | |
| Standard (Control) | 0.093 a | 6.67 a | 0.010 a | 0.668 a |
| Leaf Removal West | 0.065 b | 7.06 a | 0.006 b | 0.693 a |
| STD-UV-B | 0.039 c | 6.54 a | 0.003 c | 0.634 a |
| LRW-UV-B | 0.065 b | 4.78 b | 0.006 b | 0.448 b |
| Significance | *** | * | *** | * |
| 2011/2012 | | | | |
| Standard (Control) | 0.090 ab | 5.591 | 0.010 a | 0.509 b |
| Leaf Removal West | 0.116 a | 6.372 | 0.010 a | 0.588 a |
| LR (-UV-B, 2xOp50) | 0.086 b | 6.117 | 0.007 ab | 0.566 ab |
| LR(-UV-B, 2xUHI) | 0.068 b | 6.237 | 0.005 b | 0.533 ab |
| Significance | * | ns | * | * |

Each value represent the mean of 5 replicates at 8 sampling dates in 2010/2011 and 10 sampling dates in 2011/2012. Means in columns followed by a different letter are significantly different within one season. Monomers: C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate. Dimers: B1, Ec-(4 β -8-)Cat; B2, Ec-(4 β -8-Ec. Treatments: STD (Shaded/Control); LRW (Leaf Removal West); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Addendum 9. Concentration and content of flavonols in 2010/2011.

| DAA | Treatment | Concentration (mg/g skin) | | | | Total flavonol content (mg/berry) |
|-----|---------------------|---------------------------|-----------------------|---------------------|-----------------------|-----------------------------------|
| | | Quercetin-rutinoside | Quercetin-galactoside | Quercetin-glucoside | Quercetin-glucuronide | |
| 13 | Standard (Control) | 0.01 ± 0 | 0.00 ± 0 | 0.01 ± 0 | 0.16 ± 0.03 b | 0.011 ± 0.004 a |
| | Leaf Removal West | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.03 ± 0.01 | 0.28 ± 0.08 a | 0.0003 ± 0 b |
| | STD-UV-B | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.03 ± 0.03 | 0.21 ± 0.10 ab | 0.014 ± 0.005 a |
| | LRW-UV-B | 0.01 ± 0 | 0.00 ± 0 | 0.01 ± 0 | 0.16 ± 0.03 b | 0.0003 ± 0 b |
| | Significance | <i>ns</i> | <i>ns</i> | <i>ns</i> | * | *** |
| 17 | Standard (Control) | 0.01 ± 0.01 | 0.01 ± 0 | 0.01 ± 0.01 | 0.23 ± 0.06 | 0.015 ± 0.004 a |
| | Leaf Removal West | 0.03 ± 0.02 | 0.01 ± 0.01 | 0.05 ± 0.05 | 0.33 ± 0.25 | 0.0 ± 0 b |
| | STD-UV-B | 0.02 ± 0 | 0.01 ± 0 | 0.03 ± 0.01 | 0.25 ± 0.05 | 0.017 ± 0.005 a |
| | LRW-UV-B | 0.01 ± 0.01 | 0.01 ± 0 | 0.01 ± 0.01 | 0.23 ± 0.06 | 0.0000 ± 0 b |
| | Significance | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** |
| 22 | Standard (Control) | 0.01 ± 0 b | 0.00 ± 0 b | 0.01 ± 0 b | 0.15 ± 0.03 b | 0.010 ± 0.002 a |
| | Leaf Removal West | 0.03 ± 0.02 a | 0.01 ± 0.01 a | 0.06 ± 0.05 a | 0.38 ± 0.16 a | 0 ± 0 b |
| | STD-UV-B | 0.01 ± 0.01 b | 0.00 ± 0 b | 0.02 ± 0.01 b | 0.14 ± 0.07 b | 0 ± 0 b |
| | LRW-UV-B | 0.01 ± 0 b | 0.00 ± 0 b | 0.01 ± 0 b | 0.15 ± 0.03 b | 0.010 ± 0.006 a |
| | Significance | ** | ** | ** | *** | *** |
| 48 | Standard (Control) | 0.03 ± 0.01 b | 0.01 ± 0 b | 0.05 ± 0.02 | 0.35 ± 0.08 b | 0.038 ± 0.011 a |
| | Leaf Removal West | 0.05 ± 0.01 a | 0.03 ± 0 a | 0.12 ± 0.03 | 0.54 ± 0.13 a | 0.0 ± 0 c |
| | STD-UV-B | 0.01 ± 0.01 c | 0.00 ± 0 c | 0.02 ± 0.01 | 0.16 ± 0.05 c | 0.016 ± 0 b |
| | LRW-UV-B | 0.01 ± 0 c | 0.01 ± 0 c | 0.03 ± 0.01 | 0.15 ± 0.04 bc | 0.0 ± 0.006 c |
| | Significance | *** | *** | *** | *** | *** |
| 62 | Standard (Control) | 0.02 ± 0.01 b | 0.04 ± 0.02 a | 0.25 ± 0.07 b | 0.34 ± 0.07 b | 0.055 ± 0.019 a |
| | Leaf Removal West | 0.04 ± 0.01 a | 0.06 ± 0.02 a | 0.36 ± 0.14 a | 0.63 ± 0.19 a | 0 ± 0 c |
| | STD-UV-B | 0.01 ± 0.01 b | 0.00 ± 0 b | 0.07 ± 0.02 c | 0.15 ± 0.05 c | 0.024 ± 0.007 b |
| | LRW-UV-B | 0.01 ± 0 b | 0.01 ± 0.01 b | 0.08 ± 0.03 c | 0.15 ± 0.05 c | 0 ± 0 c |
| | Significance | *** | *** | *** | *** | *** |
| 76 | Standard (Control) | 0.01 ± 0 b | 0.04 ± 0 b | 0.26 ± 0.02 b | 0.32 ± 0.03 b | 0.083 ± 0.021 a |
| | Leaf Removal West | 0.04 ± 0.02 a | 0.09 ± 0.02 a | 0.52 ± 0.11 a | 0.59 ± 0.11 a | 0 ± 0 c |
| | STD-UV-B | 0.01 ± 0 b | 0.00 ± 0.01 c | 0.09 ± 0.01 c | 0.19 ± 0.04 c | 0.034 ± 0.004 b |
| | LRW-UV-B | 0.01 ± 0 b | 0.00 ± 0 c | 0.10 ± 0.03 c | 0.20 ± 0.07 c | 0 ± 0 c |
| | Significance | *** | *** | *** | *** | *** |
| 90 | Standard (Control) | 0.01 ± 0 b | 0.04 ± 0 | 0.26 ± 0.02 b | 0.32 ± 0.03 b | 0.085 ± 0.005 a |
| | Leaf Removal West | 0.03 ± 0.01 a | 0.09 ± 0.03 | 0.51 ± 0.21 a | 0.56 ± 0.15 a | 0.00 ± 0 c |
| | STD-UV-B | 0.01 ± 0.01 b | 0.00 ± 0 | 0.08 ± 0.02 c | 0.24 ± 0.08 bc | 0.043 ± 0.012 b |
| | LRW-UV-B | 0.01 ± 0.01 b | 0.00 ± 0 | 0.07 ± 0.02 c | 0.18 ± 0.05 c | 0.00 ± 0 c |
| | Significance | ** | *** | *** | *** | *** |
| 116 | Standard (Control) | 0.01 ± 0.01 b | 0.04 ± 0.02 b | 0.24 ± 0.09 b | 0.28 ± 0.07 b | 0.08 ± 0.02 a |
| | Leaf Removal West | 0.02 ± 0 a | 0.10 ± 0.02a | 0.63 ± 0.14 a | 0.49 ± 0.06 a | 0.0 ± 0 c |
| | STD-UV-B | 0.00 ± 0 c | 0.00 ± 0 c | 0.05 ± 0.01 c | 0.13 ± 0.04 c | 0.024 ± 0.005 b |
| | LRW-UV-B | 0.01 ± 0 c | 0.00 ± 0 c | 0.06 ± 0.02 c | 0.15 ± 0.02 c | 0.0 ± 0 c |
| | Significance | *** | *** | *** | *** | *** |

Each value represents the mean of 5 replicates ± standard deviation in concentration (mg/g skin) and content (mg/berry). Treatments: STD (Shaded/Control); LRW (Leaf Removal West); STD-UV-B (STD with decreased UV-B radiation); LRW-UV-B (LRW with decreased UV-B radiation). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Addendum 10. Concentration and content of flavonols in 2011/2012.

| DAA | Treatment | Concentration (mg/g skin) | | | | Total flavonol content (mg/berry) |
|-----|---------------------|---------------------------|-----------------------|---------------------|-----------------------|-----------------------------------|
| | | Quercetin-rutinoside | Quercetin-galactoside | Quercetin-glucoside | Quercetin-glucuronide | |
| 26 | Standard (Control) | 0.01 ± 0.01 b | 0.01 ± 0 b | 0.02 ± 0.01 b | 0.20 ± 0.08 b | 0.015 ± 0.008 b |
| | Leaf Removal West | 0.03 ± 0.01 a | 0.01 ± 0 a | 0.04 ± 0.01 a | 0.34 ± 0.06 a | 0.027 ± 0.006 a |
| | LR (-UV-B, 2xOp50) | 0.00 ± 0 b | 0.002 ± 0.001 c | 0.01 ± 0 b | 0.12 ± 0.01 c | 0.009 ± 0.002 b |
| | LR(-UV-B, 2xUHI) | 0.01 ± 0 b | 0.003 ± 0.002 bc | 0.01 ± 0.01 b | 0.14 ± 0.04bc | 0.009 ± 0.003 b |
| | Significance | *** | *** | *** | *** | *** |
| 33 | Standard (Control) | 0.02 ± 0.01 b | 0.01 ± 0 b | 0.03 ± 0.01 b | 0.24 ± 0.05 b | 0.020 ± 0.005 b |
| | Leaf Removal West | 0.06 ± 0.02 a | 0.03 ± 0.01 a | 0.07 ± 0.02 a | 0.55 ± 0.12 a | 0.053 ± 0.012 a |
| | LR (-UV-B, 2xOp50) | 0.01 ± 0 c | 0.003 ± 0.001 b | 0.02 ± 0.01 b | 0.13 ± 0.05 c | 0.010 ± 0.004 b |
| | LR(-UV-B, 2xUHI) | 0.01 ± 0 c | 0.003 ± 0.001 b | 0.02 ± 0 b | 0.13 ± 0.04 c | 0.012 ± 0.004 b |
| | Significance | *** | *** | *** | *** | *** |
| 40 | Standard (Control) | 0.05 ± 0.02 b | 0.02 ± 0.01 b | 0.06 ± 0.02 b | 0.39 ± 0.13 b | 0.039 ± 0.015 b |
| | Leaf Removal West | 0.10 ± 0.01 a | 0.04 ± 0 a | 0.12 ± 0.01 a | 0.70 ± 0.07 a | 0.071 ± 0.007 a |
| | LR (-UV-B, 2xOp50) | 0.01 ± 0.01 c | 0.01 ± 0 c | 0.02 ± 0.01 c | 0.16 ± 0.06 c | 0.015 ± 0.005 c |
| | LR(-UV-B, 2xUHI) | 0.01 ± 0.01 c | 0.004 ± 0 c | 0.02 ± 0.01 c | 0.15 ± 0.04 c | 0.014 ± 0.004 c |
| | Significance | *** | *** | *** | *** | *** |
| 47 | Standard (Control) | 0.04 ± 0.01 b | 0.02 ± 0 b | 0.06 ± 0.01 b | 0.36 ± 0.06 b | 0.036 ± 0.005 b |
| | Leaf Removal West | 0.11 ± 0.01 a | 0.04 ± 0 a | 0.15 ± 0.04 a | 0.87 ± 0.11 a | 0.084 ± 0.008 a |
| | LR (-UV-B, 2xOp50) | 0.01 ± 0 c | 0.003 ± 0 c | 0.01 ± 0 c | 0.11 ± 0.02 c | 0.010 ± 0.001 c |
| | LR(-UV-B, 2xUHI) | 0.01 ± 0 c | 0.004 ± 0 c | 0.02 ± 0.01 c | 0.14 ± 0.03 c | 0.012 ± 0.002 c |
| | Significance | *** | *** | *** | *** | *** |
| 54 | Standard (Control) | 0.06 ± 0.02 b | 0.03 ± 0.01 b | 0.11 ± 0.03 b | 0.55 ± 0.17 b | 0.052 ± 0.018 b |
| | Leaf Removal West | 0.12 ± 0.01 a | 0.06 ± 0 a | 0.17 ± 0.04 a | 0.96 ± 0.07 a | 0.099 ± 0.008 a |
| | LR (-UV-B, 2xOp50) | 0.01 ± 0.01 c | 0.01 ± 0 c | 0.02 ± 0.01 c | 0.18 ± 0.10 c | 0.017 ± 0.009 c |
| | LR(-UV-B, 2xUHI) | 0.01 ± 0 c | 0.01 ± 0 c | 0.03 ± 0 c | 0.16 ± 0.02 c | 0.015 ± 0.001 c |
| | Significance | *** | *** | *** | *** | *** |
| 68 | Standard (Control) | 0.03 ± 0.01 b | 0.05 ± 0.01 b | 0.21 ± 0.06 b | 0.47 ± 0.11 b | 0.086 ± 0.042 b |
| | Leaf Removal West | 0.08 ± 0.01 a | 0.09 ± 0.03 a | 0.41 ± 0.12 a | 0.94 ± 0.13 a | 0.212 ± 0.037 a |
| | LR (-UV-B, 2xOp50) | 0.01 ± 0.01 c | 0.02 ± 0.01 c | 0.10 ± 0.07bc | 0.28 ± 0.15 c | 0.081 ± 0.036 b |
| | LR(-UV-B, 2xUHI) | 0.02 ± 0 c | 0.02 ± 0.01 c | 0.04 ± 0.05 c | 0.10 ± 0.06 d | 0.082 ± 0.024 b |
| | Significance | *** | *** | *** | *** | *** |
| 82 | Standard (Control) | 0.03 ± 0.01 b | 0.06 ± 0.03 b | 0.21 ± 0.13 b | 0.50 ± 0.23 b | 0.107 ± 0.041 b |
| | Leaf Removal West | 0.09 ± 0.03 a | 0.15 ± 0.02 a | 0.56 ± 0.21 a | 1.25 ± 0.46 a | 0.20 ± 0.035 a |
| | LR (-UV-B, 2xOp50) | 0.01 ± 0.01 b | 0.05 ± 0.04 b | 0.19 ± 0.11 b | 0.41 ± 0.13 b | 0.058 ± 0.027 c |
| | LR(-UV-B, 2xUHI) | 0.02 ± 0.01 b | 0.05 ± 0.02 b | 0.24 ± 0.07 b b | 0.46 ± 0.07 | 0.030 ± 0.010 c |
| | Significance | *** | *** | *** | *** | *** |
| 96 | Standard (Control) | 0.02 ± 0.01 b | 0.07 ± 0.03 b | 0.36 ± 0.16 b | 0.33 ± 0.11 b | 0.107 ± 0.041 b |
| | Leaf Removal West | 0.04 ± 0 a | 0.14 ± 0.03 a | 0.68 ± 0.15 a | 0.68 ± 0.06 a | 0.200 ± 0.035 a |
| | LR (-UV-B, 2xOp50) | 0.01 ± 0 c | 0.04 ± 0.01 c | 0.15 ± 0.10 c | 0.27 ± 0.12bc | 0.058 ± 0.027 |
| | LR(-UV-B, 2xUHI) | 0.01 ± 0 c | 0.02 ± 0.01 c | 0.09 ± 0.06 c | 0.17 ± 0.06 c | 0.030 ± 0.010 c |
| | Significance | *** | *** | *** | *** | *** |
| 110 | Standard (Control) | 0.01 ± 0 b | 0.06 ± 0.02 b | 0.30 ± 0.09 b | 0.31 ± 0.03bc | 0.084 ± 0.019 b |
| | Leaf Removal West | 0.04 ± 0.01 a | 0.13 ± 0.05 a | 0.73 ± 0.14 a | 0.63 ± 0.12 a | 0.205 ± 0.042 a |
| | LR (-UV-B, 2xOp50) | 0.01 ± 0 b | 0.05 ± 0.01 b | 0.25 ± 0.05 b | 0.40 ± 0.06 b | 0.083 ± 0.016 b |
| | LR(-UV-B, 2xUHI) | 0.01 ± 0 b | 0.005 ± 0 c | 0.18 ± 0.14 b | 0.30 ± 0.06 c | 0.054 ± 0.023 b |
| | Significance | *** | *** | *** | *** | *** |
| 130 | Standard (Control) | 0.01 ± 0.01 b | 0.04 ± 0.01 b | 0.29 ± 0.09 b | 0.27 ± 0.06 b | 0.080 ± 0.020 b |
| | Leaf Removal West | 0.02 ± 0 a | 0.09 ± 0.02 a | 0.52 ± 0.06 a | 0.40 ± 0.07 a | 0.149 ± 0.017 a |
| | LR (-UV-B, 2xOp50) | 0.007 ± 0bc | 0.03 ± 0.01 b | 0.17 ± 0.07 c | 0.20 ± 0.05bc | 0.054 ± 0.021 c |
| | LR(-UV-B, 2xUHI) | 0.005 ± 0 c | 0.00 ± 0 c | 0.11 ± 0.05 c | 0.17 ± 0.04 c | 0.038 ± 0.009 c |
| | Significance | *** | *** | *** | *** | *** |

Each value represents the mean of 5 replicates (±) standard deviation in concentration (mg/g skin) and content (mg/berry). Treatments: STD (Shaded/Control); LRW (Leaf Removal West); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Addendum 11. The mean flavonol concentration and content in 2010/2011 and 2011/2012.

| Treatment | Flavonol concentration (mg/g skin) | Flavonol content (mg/berry) |
|--------------------|------------------------------------|-----------------------------|
| 2010/2011 | | |
| Standard (Control) | 0.43 b | 0.047 b |
| Leaf Removal West | 0.84 a | 0.091 b |
| STD-UV-B | 0.24 c | 0.023 c |
| LRW-UV-B | 0.23 c | 0.00 d |
| Significance | *** | *** |
| 2011/2012 | | |
| Standard (Control) | 0.59 b | 0.06 b |
| Leaf Removal West | 1.23 a | 0.12 a |
| LR (-UV-B, 2xOp50) | 0.35 c | 0.04 c |
| LR(-UV-B, 2xUHI) | 0.29 c | 0.03 c |
| Significance | *** | *** |

Each value represent the mean of 5 replicates at 8 sampling dates in 2010/2011 and 10 sampling dates in 2011/2012. Means in columns followed by a different letter are significantly different within one season. Treatments: STD (Shaded/Control); LRW (Leaf Removal West); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Addendum 12. Anthocyanin concentration (mg/g skin) and content evolution in Cabernet Sauvignon in the 2010/2011 season.

| DAA | Treatment | Concentration | | | | | | | | | | | Total anthocyanins (mg/berry) | |
|---------------------|--------------------|---------------|--------------|----------------|-------------|--------------------|--------------------|-------------------|-------------------|------------------------|------------------------|-----------------------|-------------------------------|-----------------------|
| | | Delph-3-gluc | Petun-3-gluc | Peon-3-gluc | Malv-3-gluc | Delph-3-acetylgluc | Petun-3-acetylgluc | Peon-3-acetylgluc | Malv-3-acetylgluc | Delph-3-coumaroylg luc | Petun-3-coumaroylg luc | Peon-3-coumaroylg luc | | Malv-3-coumaroylg luc |
| 48 | Standard (Control) | 0.01 ± 0 a | 0.01 ± 0 | 0.01 ± 0 a | 0.02 ± 0.01 | nd | nd | nd | 0.01 ± 0.01 | nd | nd | nd | nd | 0.005 ± 0 a |
| | Leaf Removal West | 0.0038 ± 0 ab | nd | 0.003 b | 0.01 ± 0.01 | nd | nd | nd | 0.01 ± 0.01 | nd | nd | nd | nd | 0.003 ab |
| | STD-UV-B | 0.001 b | nd | 0.001 b | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.0007 b |
| | LRW-UV-B | 0.001 b | nd | 0.001 b | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.0008 b |
| Significance | | * | ns | *** | ns | ns | ns | ns | ns | ns | ns | ns | ns | * |
| 62 | Standard (Control) | 0.13 ± 0.05 | 0.11 ± 0.03 | 0.08 ± 0.03 | 0.44 ± 0.14 | 0.04 ± 0.02 | 0.05 ± 0.02 | 0.04 ± 0.01 | 0.25 ± 0.08 | 0.01 ± 0 | 0.02 ± 0 | 0.02 ± 0 a | 0.09 ± 0.03 | 0.14 ± 0.05 |
| | Leaf Removal West | 0.14 ± 0.04 | 0.11 ± 0.03 | 0.09 ± 0.02 | 0.43 ± 0.11 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.23 ± 0.05 | 0.01 ± 0 | 0.02 ± 0 | 0.03 ± 0 a | 0.08 ± 0.02 | 0.13 ± 0.03 |
| | STD-UV-B | 0.08 ± 0.04 | 0.08 ± 0.03 | 0.06 ± 0.03 | 0.41 ± 0.10 | 0.03 ± 0.02 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.27 ± 0.07 | 0.01 ± 0 | 0.01 ± 0 | 0.02 ± 0 b | 0.09 ± 0.02 | 0.12 ± 0.03 |
| | LRW-UV-B | 0.11 ± 0.03 | 0.11 ± 0.02 | 0.06 ± 0.02 | 0.41 ± 0.11 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.26 ± 0.08 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 b | 0.07 ± 0.02 | 0.12 ± 0.03 |
| Significance | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | *** | ns | ns |
| 76 | Standard (Control) | 0.09 ± 0.02 b | 0.09 ± 0.01 | 0.07 ± 0.01 | 0.61 ± 0.06 | 0.03 ± 0.01 b | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.39 ± 0.04 cb | 0.01 ± 0 | 0.02 ± 0 | 0.02 ± 0 b | 0.16 ± 0.03 | 0.20 ± 0.01 |
| | Leaf Removal West | 0.10 ± 0.02 b | 0.10 ± 0.01 | 0.08 ± 0.01 | 0.65 ± 0.03 | 0.03 ± 0 b | 0.04 ± 0 | 0.03 ± 0 | 0.38 ± 0.03 c | 0.01 ± 0 | 0.02 ± 0 | 0.03 ± 0 b | 0.16 ± 0.01 | 0.20 ± 0.02 |
| | STD-UV-B | 0.11 ± 0.05 b | 0.11 ± 0.04 | 0.06 ± 0.02 | 0.67 ± 0.17 | 0.04 ± 0.02 a | 0.04 ± 0.02 | 0.03 ± 0.01 | 0.48 ± 0.11 ab | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 b | 0.18 ± 0.04 | 0.21 ± 0.04 |
| | LRW-UV-B | 0.17 ± 0.06 a | 0.17 ± 0.03 | 0.07 ± 0.01 | 0.79 ± 0.15 | 0.05 ± 0.01 ab | 0.04 ± 0.01 | 0.04 ± 0 | 0.52 ± 0.07 a | 0.02 ± 0 | 0.02 ± 0 | 0.04 ± 0.01 a | 0.18 ± 0.07 | 0.24 ± 0.04 |
| Significance | | * | ns | ns | ns | * | ns | ns | * | ns | ns | *** | ns | ns |
| 90 | Standard (Control) | 0.11 ± 0.02 | 0.10 ± 0.02 | 0.07 ± 0.01 | 0.72 ± 0.07 | 0.03 ± 0.01 | 0.04 ± 0.01 a | 0.03 ± 0.01 | 0.46 ± 0.02 b | 0.01 ± 0 | 0.02 ± 0 | 0.02 ± 0 c | 0.19 ± 0.62 | 0.24 ± 0.02 a |
| | Leaf Removal West | 0.07 ± 0.03 | 0.07 ± 0.02 | 0.06 ± 0.01 | 0.60 ± 0.11 | 0.02 ± 0.01 | 0.03 ± 0.01 b | 0.03 ± 0 | 0.38 ± 0.07 c | 0.01 ± 0 | 0.01 ± 0 | 0.02 ± 0.01 bc | 0.17 ± 0.03 | 0.19 ± 0.04 b |
| | STD-UV-B | 0.11 ± 0.05 | 0.11 ± 0.03 | 0.06 ± 0.01 | 0.71 ± 0.11 | 0.03 ± 0.02 | 0.04 ± 0.01 a | 0.03 ± 0 | 0.48 ± 0.03 a | 0.02 ± 0.02 | 0.02 ± 0 | 0.03 ± 0 b | 0.18 ± 0.02 | 0.24 ± 0.03 a |
| | LRW-UV-B | 0.07 ± 0 | 0.07 ± 0 | 0.05 ± 0.01 | 0.73 ± 0.06 | 0.02 ± 0 | 0.03 ± 0 a | 0.03 ± 0 | 0.52 ± 0.06 ab | 0.01 ± 0 | 0.02 ± 0 | 0.04 ± 0.01 a | 0.18 ± 0.02 | 0.22 ± 0.01 ab |
| Significance | | ns | ns | ns | ns | ns | * | ns | *** | ns | ns | *** | ns | * |
| 116 | Standard (Control) | 0.04 ± 0.02 | 0.05 ± 0.02 | 0.04 ± 0.01 ab | 0.48 ± 0.06 | 0.01 ± 0 | 0.02 ± 0.01 | 0.02 ± 0 | 0.30 ± 0.03 | 0.00 ± 0 | 0.01 ± 0 | 0.01 ± 0 b | 0.14 ± 0.02 | 0.16 ± 0.02 |
| | Leaf Removal West | 0.06 ± 0.01 | 0.07 ± 0.01 | 0.05 ± 0.01 a | 0.53 ± 0.07 | 0.01 ± 0 | 0.02 ± 0 | 0.02 ± 0 | 0.28 ± 0.05 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 b | 0.14 ± 0.01 | 0.15 ± 0.03 |
| | STD-UV-B | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0 b | 0.50 ± 0.09 | 0.01 ± 0 | 0.02 ± 0 | 0.02 ± 0 | 0.33 ± 0.07 | 0.01 ± 0.01 | 0.01 ± 0 | 0.02 ± 0.01 ab | 0.13 ± 0.04 | 0.16 ± 0.02 |
| | LRW-UV-B | 0.05 ± 0.01 | 0.05 ± 0.01 | 0.03 ± 0.01 b | 0.50 ± 0.06 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0 | 0.33 ± 0.03 | 0.01 ± 0 | 0.01 ± 0 | 0.02 ± 0.01 a | 0.13 ± 0.01 | 0.16 ± 0.02 |
| Significance | | ns | ns | *** | ns | ns | ns | ns | ns | ns | ns | * | ns | ns |

Each value represents the mean of 5 replicates (±) standard deviation of the concentration (mg/g skin) and content of the total anthocyanins (mg/berry). STD (Shaded/Control); LRW (Leaf Removal West); STD-UV-B (STD with decreased UV-B radiation); LRW-UV-B (LRW with decreased UV-B radiation). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Addendum 13. Anthocyanin concentration (mg/g skin) and content evolution in Cabernet Sauvignon in the 2011/2012 season.

| DAA | Treatment | Concentration | | | | | | | | | | | | Total anthocyanin (mg/berry) |
|---------------------|---------------------|----------------|----------------|----------------|----------------|--------------------|--------------------|-------------------|-------------------|-----------------------|-----------------------|----------------------|----------------------|------------------------------|
| | | Delph-3-gluc | Petun-3-gluc | Peon-3-gluc | Malv-3-gluc | Delph-3-acetylgluc | Petun-3-acetylgluc | Peon-3-acetylgluc | Malv-3-acetylgluc | Delph-3-coumaroylgluc | Petun-3-coumaroylgluc | Peon-3-coumaroylgluc | Malv-3-coumaroylgluc | |
| 68 | Standard (Control) | 0.05 ± 0.04 | 0.07 ± 0.04 | 0.04 ± 0.02 | 0.48 ± 0.16 a | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.27 ± 0.06 | 0.01 ± 0 | 0.01 ± 0 | 0.01 ± 0 | 0.11 ± 0.02 a | 0.08 ± 0.03 |
| | Leaf Removal West | 0.08 ± 0.1 | 0.04 ± 0.02 | 0.04 ± 0.02 | 0.20 ± 0.11 b | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.11 ± 0.06 | nd | 0.01 ± 0. | 0.01 ± 0.01 | 0.04 ± 0.02 b | 0.06 ± 0.03 |
| | LR & (-UV-B,2xOp50) | 0.05 ± 0.04 | 0.05 ± 0.04 | 0.03 ± 0.03 | 0.26 ± 0.31 ab | 0.02 ± 0.02 | 0.02 ± 0.02 | 0.01 ± 0.01 | 0.17 ± 0.21 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.05 ± 0.07 ab | 0.07 ± 0.08 |
| | LR & (-UV-B,2xUHI) | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.13 ± 0.06 b | 0.01 ± 0 | 0.01 ± 0.01 | 0.01 ± 0 | 0.08 ± 0.04 | nd | nd | nd | 0.02 ± 0.01 b | 0.03 ± 0.01 |
| Significance | | <i>ns</i> | <i>ns</i> | <i>ns</i> | * | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | * | <i>ns</i> |
| 82 | Standard (Control) | 0.10 ± 0.02 b | 0.09 ± 0.02 b | 0.07 ± 0.01 ab | 0.56 ± 0.07 a | 0.04 ± 0.01 b | 0.04 ± 0.01 b | 0.04 ± 0.01 ab | 0.38 ± 0.05 b | 0.01 ± 0 b | 0.02 ± 0 | 0.02 ± 0 | 0.13 ± 0.02 a | 0.16 ± 0.01 b |
| | Leaf Removal West | 0.10 ± 0.02 b | 0.08 ± 0.02 b | 0.07 ± 0.02 b | 0.41 ± 0.08 b | 0.03 ± 0.01 b | 0.03 ± 0.01 b | 0.03 ± 0.01 b | 0.23 ± 0.04 b | 0.01 ± 0 | 0.02 ± 0 | 0.02 ± 0 | 0.09 ± 0.02 a | 0.16 ± 0.01 b |
| | LR & (-UV-B,2xOp50) | 0.16 ± 0.03 a | 0.13 ± 0.03 a | 0.09 ± 0.02 a | 0.66 ± 0.11 a | 0.06 ± 0.01 a | 0.06 ± 0.01 a | 0.04 ± 0.01 a | 0.41 ± 0.07 a | 0.02 ± 0 a | 0.02 ± 0 | 0.02 ± 0.01 | 0.14 ± 0.02 a | 0.22 ± 0.02 a |
| | LR & (-UV-B,2xUHI) | 0.08 ± 0.03 b | 0.09 ± 0.02 b | 0.06 ± 0.01 b | 0.60 ± 0.08 a | 0.03 ± 0.01 b | 0.04 ± 0.01 b | 0.03 ± 0.01 b | 0.40 ± 0.06 b | 0.01 ± 0 b | 0.02 ± 0 | 0.02 ± 0 | 0.15 ± 0.02 a | 0.16 ± 0.01 b |
| Significance | | *** | ** | * | ** | * | *** | * | *** | * | <i>ns</i> | <i>ns</i> | *** | *** |
| 96 | Standard (Control) | 0.03 ± 0.02 ab | 0.05 ± 0.02 ab | 0.03 ± 0.01 a | 0.33 ± 0.07 | 0.01 ± 0.01 ab | 0.02 ± 0.01 ab | 0.01 ± 0 a | 0.19 ± 0.02 a | 0.003 ± 0 ab | 0.01 ± 0 a | 0.01 ± 0 a | 0.07 ± 0.01 | 0.11 ± 0.02 a |
| | Leaf Removal West | 0.03 ± 0.01 ab | 0.04 ± 0.02 ab | 0.03 ± 0.01 a | 0.31 ± 0.09 | 0.01 ± 0 b | 0.02 ± 0 ab | 0.01 ± 0 a | 0.15 ± 0.04 ab | 0.003 ± 0 ab | 0.01 ± 0 a | 0.01 ± 0 a | 0.07 ± 0.01 | 0.10 ± 0.02 a |
| | LR & (-UV-B,2xOp50) | 0.05 ± 0.02 a | 0.06 ± 0.02 a | 0.04 ± 0.02 a | 0.38 ± 0.11 | 0.02 ± 0.01 a | 0.02 ± 0.01 a | 0.01 ± 0.01 a | 0.18 ± 0.05 a | 0.01 ± 0 a | 0.01 ± 0 a | 0.01 ± 0 a | 0.07 ± 0.02 | 0.11 ± 0.03 a |
| | LR & (-UV-B,2xUHI) | 0.02 ± 0.01 b | 0.03 ± 0.01 b | 0.01 ± 0.01 b | 0.23 ± 0.07 | 0.004 ± 0 b | 0.01 ± 0 b | 0.01 ± 0 b | 0.12 ± 0.03 b | 0.002 ± 0 b | 0.003 ± 0 b | 0.003 ± 0 b | 0.05 ± 0.01 | 0.05 ± 0.01 b |
| Significance | | * | * | * | <i>ns</i> | * | * | * | * | * | ** | ** | <i>ns</i> | ** |
| 110 | Standard (Control) | 0.10 ± 0.04 b | 0.09 ± 0.02 b | 0.06 ± 0.01 | 0.61 ± 0.03 | 0.03 ± 0.01 b | 0.03 ± 0.01 b | 0.02 ± 0 b | 0.34 ± 0.03 ab | 0.01 ± 0 b | 0.02 ± 0 b | 0.02 ± 0 b | 0.14 ± 0.02 cb | 0.18 ± 0.02 a |
| | Leaf Removal West | 0.13 ± 0.04 b | 0.12 ± 0.03 b | 0.20 ± 0.27 | 0.49 ± 0.27 | 0.03 ± 0.01 b | 0.04 ± 0.01 b | 0.03 ± 0.01 b | 0.29 ± 0.04 b | 0.01 ± 0 b | 0.02 ± 0 b | 0.02 ± 0 a | 0.13 ± 0.02 c | 0.20 ± 0.04 a |
| | LR & (-UV-B,2xOp50) | 0.21 ± 0.05 a | 0.18 ± 0.04 a | 0.11 ± 0.01 | 0.85 ± 0.13 | 0.06 ± 0.01 a | 0.06 ± 0.01 a | 0.04 ± 0.01 a | 0.40 ± 0.09 a | 0.02 ± 0 a | 0.02 ± 0 a | 0.02 ± 0 a | 0.18 ± 0.04 a | 0.25 ± 0.03 b |
| | LR & (-UV-B,2xUHI) | 0.11 ± 0.05 b | 0.11 ± 0.03 b | 0.06 ± 0.02 | 0.70 ± 0.08 | 0.03 ± 0.01 ab | 0.04 ± 0.01 b | 0.03 ± 0.01 b | 0.37 ± 0.03 a | 0.01 ± 0 b | 0.02 ± 0 b | 0.02 ± 0 b | 0.18 ± 0.02 ab | 0.20 ± 0.02 a |
| Significance | | ** | ** | <i>ns</i> | * | ** | ** | ** | * | ** | * | ** | * | * |
| 130 | Standard (Control) | 0.05 ± 0.01 b | 0.06 ± 0.01 ab | 0.04 ± 0.01 a | 0.49 ± 0.07 a | 0.01 ± 0 b | 0.02 ± 0 b | 0.02 ± 0 a | 0.25 ± 0.05 a | 0.004 ± 0 b | 0.01 ± 0 a | 0.01 ± 0 a | 0.10 ± 0.01 a | 0.14 ± 0.02 a |
| | Leaf Removal West | 0.05 ± 0.02 bc | 0.05 ± 0.01 cb | 0.03 ± 0.01 ab | 0.33 ± 0.07 b | 0.01 ± 0 bc | 0.07 ± 0.02 a | 0.01 ± 0 b | 0.15 ± 0.04 b | 0.02 ± 0 a | 0.01 ± 0 bc | 0.01 ± 0 a | 0.07 ± 0.02 b | 0.12 ± 0.03 a |
| | LR & (-UV-B,2xOp50) | 0.08 ± 0.02 a | 0.08 ± 0.02 a | 0.04 ± 0.02 a | 0.46 ± 0.08 a | 0.02 ± 0 a | 0.02 ± 0 b | 0.01 ± 0 ab | 0.18 ± 0.04 b | 0.01 ± 0 b | 0.01 ± 0 b | 0.01 ± 0 a | 0.09 ± 0.02 ab | 0.13 ± 0.02 a |
| | LR & (-UV-B,2xUHI) | 0.04 ± 0.02 c | 0.04 ± 0.02 c | 0.02 ± 0.01 b | 0.35 ± 0.12 b | 0.01 ± 0.01 c | 0.01 ± 0.01 b | 0.01 ± 0.01 c | 0.19 ± 0.10 b | 0.002 ± 0 b | 0.01 ± 0 c | 0.035 ± 0 b | 0.08 ± 0.04 b | 0.09 ± 0.03 b |
| Significance | | *** | *** | * | ** | *** | *** | *** | ** | *** | ** | ** | * | ** |

Each value represents the mean of 5 replicates (±) standard deviation of the concentration (mg/g skin) and content of total anthocyanins (mg/berry). STD (Shaded/Control); LRW (Leaf Removal West); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; *ns*: not significant).

Addendum 14. The mean anthocyanin concentration and content in 2010/2011 and 2011/2012.

| Treatment | Anthocyanin concentration (mg/g skin) | Anthocyanin content (mg/berry) |
|--------------------|---------------------------------------|--------------------------------|
| 2010/2011 | | |
| Standard (Control) | 1.17 | 0.15 |
| Leaf Removal West | 1.12 | 0.14 |
| STD-UV-B | 1.18 | 0.15 |
| LRW-UV-B | 1.25 | 0.14 |
| Significance | ns | ns |
| 2011/2012 | | |
| Standard (Control) | 1.22 | 0.13 ab |
| Leaf Removal West | 1.11 | 0.12 ab |
| LR (-UV-B, 2xOp50) | 1.30 | 0.16 a |
| LR (-UV-B, 2xUHI) | 0.92 | 0.10 b |
| Significance | ns | * |

Each value represent the mean of 5 replicates at 8 sampling dates in 2010/2011 and 10 sampling dates in 2011/2012. Means in columns followed by a different letter are significantly different within one season. STD (Shaded/Control); LRW (Leaf Removal West); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively; ns: not significant).

Addendum 15. Compositional and structural characterisation of seed extracts during ripening in 2010/2011.

| DAA | Treatment | Terminal units ^a | | | Extension units ^a | | | mDP | %G ^b | avMM ^b | Proanthocyanidins |
|---------------------|--------------------|-----------------------------|--------------|--------------|------------------------------|--------------|--------------|-------------|-----------------|-------------------|-------------------|
| | | C | EC | ECG | C | EC | ECG | | | | |
| 13 | Standard (Control) | 79.63 ± 3.91 | 14.89 ± 3.09 | 5.46 ± 0.97 | 12.01 ± 1.59 | 86.95 ± 1.83 | 1.03 ± 0.30 | 5.9 ± 0.7 | 1.8 ± 0.5 a | 1726 ± 205 a | 11.2 ± 5.5 a |
| | Leaf Removal West | 83.71 ± 1.73 | 12.74 ± 1.38 | 3.53 ± 0.64 | 12.17 ± 0.43 | 87.1 ± 0.36 | 0.731 ± 1.25 | 5.5 ± 0.5 | 1.2 ± 0.25 b | 1611 ± 162 ab | 12.5 ± 2.6 a |
| | STD-UV-B | 88.98 ± 1.60 | 12.01 ± 1.00 | nd | 12.13 ± 0.90 | 88.79 ± 1.46 | nd | 5.0 ± 0.4 | 0 c | 1466 ± 106 b | 11.06 ± 5.5 a |
| | LRW-UV-B | 79.63 ± 3.91 | 14.89 ± 3.09 | 5.46 ± 0.97 | 12.01 ± 1.59 | 88.79 ± 1.46 | nd | 5.9 ± 0.7 | 1.8 ± 0.5 a | 1726 ± 205 a | 11.2 ± 2.4 a |
| <i>Significance</i> | | *** | ns | *** | ns | ns | ns | ns | ns | ns | ns |
| 17 | Standard (Control) | 81.52 ± 1.57 | 14.06 ± 1.23 | 4.40 ± 0.87 | 10.78 ± 0.58 | 88.2 ± 0.64 | 1.01 ± 0.49 | 8.1 ± 0.3 a | 1.4 ± 0.5 a | 2360 ± 99.3 a | 16.2 ± 2.2 a |
| | Leaf Removal West | 81.65 ± 1.37 | 14.18 ± 1.49 | 4.16 ± 0.50 | 11.23 ± 0.61 | 87.70 ± 0.40 | 1.06 ± 0.31 | 7.1 ± 0.6 b | 1.4 ± 0.3 a | 2081.2 ± 194 b | 14.6 ± 3.1 a |
| | STD-UV-B | 86.03 ± 0.83 | 13.31 ± 1.51 | 0.65 ± 0.96 | 11.11 ± 0.54 | 88.73 ± 0.54 | 0.1 ± 0.15 | 6.5 ± 0.9 b | 0.2 ± 0.2 b | 1896 ± 276 c | 13.2 ± 2.3 a |
| | LRW-UV-B | 81.52 ± 1.57 | 14.06 ± 1.23 | 4.4 ± 0.87 | 10.78 ± 0.58 | 88.73 ± 0.54 | 0.1 ± 0.15 | 8.1 ± 0.3 a | 1.4 ± 0.5 a | 2360 ± 99.3 a | 16.2 ± 2.2 a |
| <i>Significance</i> | | ns | ns | ns | ns | ns | ns | ** | ** | *** | ns |
| 22 | Standard (Control) | 72.71 ± 2.73 | 16.19 ± 1.39 | 11.09 ± 3.01 | 10.05 ± 0.14 | 86.08 ± 1.30 | 3.86 ± 1.37 | 9.2 ± 0.2 a | 4.6 ± 1.5 a | 2720 ± 92.7 a | 19.8 ± 0.8 a |
| | Leaf Removal West | 75.31 ± 2.37 | 16.68 ± 0.75 | 8 ± 3.01 | 10.26 ± 0.43 | 87.25 ± 0.80 | 2.48 ± 1.05 | 9.1 ± 0.4 a | 3.1 ± 1.1 a | 2692 ± 145.1 a | 17.6 ± 1.45 b |
| | STD-UV-B | 82.82 ± 2.19 | 18.05 ± 0.67 | nd | 11.36 ± 0.47 | 90.43 ± 4.02 | 0.1 ± 0.14 | 7.6 ± 0.2 b | 0.13 ± 0.12 b | 2192 ± 92.3 b | 11.48 ± 2.5 c |
| | LRW-UV-B | 72.71 ± 2.73 | 16.19 ± 1.39 | 11.09 ± 0.87 | 10.05 ± 0.14 | 90.43 ± 4.02 | 0.1 ± 0.14 | 9.2 ± 0.2 a | 4.6 ± 1.5 a | 2720 ± 92.7 a | 19.8 ± 0.8 a |
| <i>Significance</i> | | *** | ns | *** | ns | ** | * | *** | *** | *** | *** |
| 48 | Standard (Control) | 64.73 ± 3.24 | 31.08 ± 2.21 | 4.18 ± 5.07 | 12.08 ± 1.39 | 85.41 ± 2.06 | 2.5 ± 3.21 | 2.0 ± 0.7 b | 1.7 ± 0.9 a | 588.1 ± 205 b | 29.6 ± 3.4 b |
| | Leaf Removal West | 66.25 ± 1.97 | 28.54 ± 1.65 | 5.2 ± 0.76 | 10.37 ± 0.47 | 86.53 ± 0.59 | 3.09 ± 0.44 | 3.1 ± 0.3 a | 3.7 ± 0.5 b | 915.1 ± 97.7 a | 34.7 ± 2.5 a |
| | STD-UV-B | 67.08 ± 1.79 | 30.96 ± 1.74 | 1.94 ± 0.69 | 11.15 ± 0.97 | 87.94 ± 0.85 | 0.9 ± 0.301 | 2.7 ± 0.2 a | 1.3 ± 0.4 b | 791.4 ± 62.4 a | 27.46 ± 1.9 b |
| | LRW-UV-B | 67.01 ± 1.53 | 29.95 ± 1.11 | 3.02 ± 1.18 | 11.21 ± 0.62 | 87.94 ± 0.85 | 0.9 ± 0.301 | 3.0 ± 0.3 a | 2.02 ± 0.8 b | 876 ± 107.5 a | 28.1 ± 1.35 b |
| <i>Significance</i> | | ns | ns | ns | ** | ns | ns | *** | ** | * | ** |
| 62 | Standard (Control) | 58.57 ± 1.55 | 37.12 ± 1.49 | 4.3 ± 2.34 | 10.83 ± 0.53 | 85.56 ± 2.19 | 3.6 ± 2.22 | 3.8 ± 0.5 a | 3.8 ± 2.2 a | 1123.9 ± 157.9 a | 33.5 ± 4.6 a |
| | Leaf Removal West | 62 ± 3.00 | 34.5 ± 2.41 | 3.49 ± 0.94 | 11.56 ± 0.79 | 85.95 ± 0.74 | 2.48 ± 0.44 | 3.7 ± 0.5 a | 2.7 ± 0.5 a | 1108 ± 150 a | 26.2 ± 3.6 b |
| | STD-UV-B | 60 ± 3.35 | 39.22 ± 3.51 | 0.76 ± 0.46 | 12.03 ± 0.37 | 87.58 ± 0.21 | 0.38 ± 0.26 | 2.9 ± 0.2 b | 0.5 ± 0.3 b | 863.1 ± 72.4 b | 18.1 ± 2.6 c |
| | LRW-UV-B | 60.92 ± 0.70 | 37.74 ± 1.25 | 1.33 ± 1.42 | 12.36 ± 0.51 | 87.58 ± 0.21 | 0.38 ± 0.26 | 3.0 ± 0.3 b | 1.1 ± 1.1 b | 886.7 ± 101.8 b | 20.4 ± 2.5 c |
| <i>Significance</i> | | ns | * | ns | ns | ns | ns | ** | * | * | *** |

Addendum 15 (cont.)

| | | | | | | | | | | | |
|---------------------|--------------------|---------------|--------------|--------------|--------------|--------------|--------------|-------------|---------------|------------------|---------------|
| 76 | Standard (Control) | 55.58 ± 3.05 | 40.38 ± 3.08 | 4.03 ± 4.19 | 11.33 ± 0.90 | 84.64 ± 4.81 | 4.02 ± 5.56 | 4.3 ± 0.9 | 4.05 ± 5.31 a | 1275 ± 328 a | 24.1 ± 8.2 a |
| | Leaf Removal West | 58.42 ± 3.24 | 39.14 ± 3.01 | 2.43 ± 0.39 | 12.36 ± 0.52 | 86.39 ± 0.97 | 1.23 ± 0.58 | 4.2 ± 0.2 | 1.5 ± 0.48 a | 1226 ± 60.3 a | 18 ± 3.6 ab |
| | STD-UV-B | 55.86 ± 2.71 | 43.46 ± 2.69 | 0.66 ± 0.45 | 12.21 ± 0.69 | 87.44 ± 0.67 | 0.34 ± 0.148 | 3.6 ± 0.3 | 0.42 ± 0.18 a | 1049 ± 92.8 a | 16.8 ± 3.2 b |
| | LRW-UV-B | 56.08 ± 1.35 | 42.61 ± 1.93 | 1.29 ± 1.39 | 12.03 ± 0.53 | 87.44 ± 0.67 | 0.34 ± 0.148 | 3.8 ± 0.3 | 0.92 ± 1.04 a | 1111.8 ± 155.1 a | 17.8 ± 3.5 ab |
| <i>Significance</i> | | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | * | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> |
| 90 | Standard (Control) | 51.33 ± 1.22 | 39.49 ± 0.86 | 9.16 ± 1.11 | 9.23 ± 0.21 | 76.98 ± 0.24 | 13.78 ± 0.34 | 7.0 ± 0.5 a | 13.1 ± 0.46 a | 2159.4 ± 163 a | 35.2 ± 2.9a |
| | Leaf Removal West | 55.08 ± 2.62 | 40.16 ± 1.70 | 4.75 ± 1.81 | 11.08 ± 0.55 | 85.65 ± 1.64 | 3.26 ± 1.70 | 4.9 ± 0.3 b | 3.5 ± 1.6 b | 1462.4 ± 120.4 b | 23.7 ± 2.7 b |
| | STD-UV-B | 55.96 ± 2.75 | 43.16 ± 2.88 | 0.86 ± 0.47 | 12.02 ± 0.58 | 87.52 ± 0.69 | 0.44 ± 0.28 | 3.8 ± 0.2 c | 0.5 ± 0.31 c | 1117.6 ± 61.2 c | 19.4 ± 3.8 c |
| | LRW-UV-B | 56.965 ± 2.26 | 42.17 ± 2.14 | 0.86 ± 0.26 | 12.43 ± 0.77 | 87.52 ± 0.69 | 0.44 ± 0.28 | 3.6 ± 0.2 c | 0.48 ± 0.1 c | 1060.6 ± 61.1 c | 16.2 ± 2.1 c |
| | | <i>ns</i> | <i>ns</i> | * | * | *** | *** | *** | *** | *** | *** |
| 116 | Standard (Control) | 50.51 ± 1.90 | 39.26 ± 2.03 | 10.21 ± 1.98 | 9.23 ± 0.44 | 76.63 ± 0.66 | 14.13 ± 0.48 | 8.0 ± 0.9 a | 13.6 ± 0.5 a | 2478 ± 290 a | 49.1 ± 10.2 a |
| | Leaf Removal West | 54.4 ± 3.07 | 40.93 ± 1.47 | 4.66 ± 2.33 | 11.37 ± 0.26 | 84.7 ± 2.39 | 3.92 ± 2.46 | 5.3 ± 0.3 b | 3.9 ± 2.4 b | 1561.3 ± 98.9 b | 28.1 ± 4.3 b |
| | STD-UV-B | 51.66 ± 1.11 | 46.9 ± 1.21 | 1.42 ± 0.68 | 12.06 ± 0.37 | 87.17 ± 0.33 | 0.75 ± 0.44 | 4.4 ± 0.5 c | 0.86 ± 0.4 c | 1276 ± 169.2 c | 18.7 ± 2.1 c |
| | LRW-UV-B | 52.94 ± 1.18 | 45.82 ± 1.14 | 1.23 ± 0.61 | 12.04 ± 0.54 | 87.17 ± 0.33 | 0.75 ± 0.44 | 4.3 ± 0.5 c | 0.79 ± 0.4 c | 1251.0 ± 73.4 c | 21.5 ± 4.0 bc |
| <i>Significance</i> | | <i>ns</i> | *** | *** | *** | *** | *** | *** | *** | *** | *** |

Each value represents the mean of 5 replicates (±) standard deviation in units of mg/g seed tannin extract. STD (Shaded/Control); LRW (Leaf Removal West); STD-UV-B (STD with decreased UV-B radiation); LRW-UV-B (LRW with decreased UV-B radiation). ^aPercent composition of proanthocyanidin subunits (in moles) C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate. mDP, mean degree of polymerization; %G, percentage galloylation; avMM, average molecular mass; nd, not detected;. Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively, ns: not significant).

Addendum 16. Compositional and structural characterisation of seed extracts during ripening in 2011/2012.

| DAA | Treatment | Terminal units ^a | | | Extension units ^a | | | mDP | %G ^b | avMM ^b | Proanthocyanidins |
|---------------------|--------------------|-----------------------------|------------|------------|------------------------------|-------------|------------|---------------|-----------------|-------------------|-------------------|
| | | C | EC | ECG | C | EC | ECG | | | | |
| 26 | Standard (Control) | 54.8 ± 2.9 | 11.9 ± 1.0 | 33.3 ± 2.0 | 8.6 ± 0.7 | 77 ± 1.5 | 14.4 ± 0.9 | 7.8 ± 0.8 a | 16.8 ± 0.9 ab | 2450 ± 257.4 a | 31.3 ± 3.7 a |
| | Leaf Removal West | 56.3 ± 0.5 | 11.4 ± 0.4 | 32.3 ± 0.7 | 8.1 ± 0.4 | 77.6 ± 0.3 | 14.3 ± 0.4 | 7.4 ± 0.2 a | 16.7 ± 0.3 b | 2347.2 ± 66.0 a | 28.6 ± 1.4 a |
| | LR (-UV-B, 2xOp50) | 54.2 ± 1.4 | 10.6 ± 1.1 | 31.2 ± 7.2 | 8.3 ± 0.6 | 71.9 ± 8.8 | 14 ± 3.9 | 7.5 ± 0.6 a | 17.9 ± 1.1 a | 2353.5 ± 173.2 a | 28.8 ± 2.5 a |
| | LR (-UV-B, 2xUHI) | 53.7 ± 4.4 | 11.6 ± 1.0 | 29.4 ± 6.6 | 8.3 ± 0.9 | 73.1 ± 9.5 | 12.7 ± 2.9 | 7.9 ± 0.5 a | 16.5 ± 0.7 b | 2485 ± 150.9 a | 29.9 ± 3.3 a |
| <i>Significance</i> | | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> |
| 33 | Standard (Control) | 49.6 ± 2.5 | 13.3 ± 1.4 | 37.2 ± 2.1 | 8.9 ± 0.6 | 77.6 ± 1.2 | 13.5 ± 0.9 | 7.6 ± 0.9 a | 16.6 ± 0.9 ab | 2384 ± 7.2 a | 21.2 ± 7.2 a |
| | Leaf Removal West | 52.3 ± 1.0 | 13.0 ± 1.6 | 34.8 ± 1.1 | 9.2 ± 0.5 | 77.7 ± 0.4 | 13.1 ± 0.6 | 7.5 ± 0.2 a | 15.9 ± 0.7 b | 2342.6 ± 51.0 a | 23.3 ± 3.6 a |
| | LR (-UV-B, 2xOp50) | 51.8 ± 1.2 | 12.1 ± 0.4 | 36.1 ± 0.8 | 9.8 ± 0.4 | 75.8 ± 0.7 | 14.4 ± 0.4 | 6.8 ± 0.3 a | 17.6 ± 0.4 a | 2145.4 ± 83.3 a | 23.0 ± 2.8 a |
| | LR (-UV-B, 2xUHI) | 51.7 ± 2.5 | 12.7 ± 1.8 | 32.0 ± 8.8 | 9.7 ± 0.9 | 77.4 ± 1.5 | 12.8 ± 0.6 | 7.5 ± 0.6 a | 15.9 ± 0.8 b | 2330.2 ± 178.7 a | 25.3 ± 3.3 a |
| <i>Significance</i> | | <i>ns</i> | <i>ns</i> | * | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | * | <i>ns</i> | <i>ns</i> |
| 40 | Standard (Control) | 46.9 ± 1.1 | 16.0 ± 0.8 | 37.2 ± 0.6 | 9.4 ± 0.5 | 78.8 ± 0.5 | 11.9 ± 0.4 | 7.1 ± 0.3 b | 15.4 ± 0.3 a | 2218.9 ± 84.5 b | 24.7 ± 3.3 b |
| | Leaf Removal West | 50.7 ± 1.2 | 13.8 ± 0.6 | 35.5 ± 1.1 | 9.6 ± 0.3 | 79.2 ± 0.6 | 11.2 ± 0.7 | 7.5 ± 0.2 a | 14.4 ± 0.5 c | 2330.9 ± 68.8 a | 26.2 ± 0.7 b |
| | LR (-UV-B, 2xOp50) | 46.6 ± 0.7 | 15.8 ± 1.0 | 37.6 ± 0.4 | 9.8 ± 0.2 | 79.1 ± 0.5 | 11.1 ± 0.4 | 7.0 ± 0.2 b | 14.9 ± 0.3 b | 2181.5 ± 80.9 bc | 27.4 ± 0.8 ab |
| | LR (-UV-B, 2xUHI) | 49.4 ± 1.1 | 15.4 ± 0.7 | 35.2 ± 1.0 | 9.9 ± 0.3 | 79.4 ± 0.4 | 10.7 ± 0.2 | 6.8 ± 0.2 b | 14.3 ± 0.3 c | 2130.5 ± 54.8 c | 29.7 ± 1.7 a |
| <i>Significance</i> | | * | <i>ns</i> | * | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | *** | *** | • |
| 47 | Standard (Control) | 48.8 ± 2.9 | 19.8 ± 0.7 | 31.4 ± 2.5 | 9.6 ± 0.2 | 78.6 ± 0.5 | 11.8 ± 0.4 | 4.5 ± 0.6 b | 16.2 ± 0.2 ab | 1406.2 ± 202.5 b | 40.7 ± 6.1 a |
| | Leaf Removal West | 48.8 ± 1.5 | 18.7 ± 1.3 | 32.5 ± 2.3 | 9.3 ± 0.3 | 79 ± 0.3 | 11.7 ± 0.4 | 5.2 ± 0.6 a | 15.7 ± 0.3 bc | 1637 ± 190.9 a | 35.7 ± 4.3 bc |
| | LR (-UV-B, 2xOp50) | 46.4 ± 1.1 | 20.2 ± 1.7 | 33.4 ± 2.2 | 9.9 ± 0.3 | 78.1 ± 0.45 | 12 ± 0.3 | 4.8 ± 0.5 ab | 16.4 ± 0.3 a | 1506.9 ± 156.8 ab | 32.0 ± 2.6 b |
| | LR (-UV-B, 2xUHI) | 51.4 ± 4.2 | 17.1 ± 2.4 | 28.9 ± 0.8 | 9.4 ± 0.6 | 79.2 ± 0.3 | 11 ± 0.4 | 4.6 ± 0.3 b | 15.3 ± 0.4 c | 1436 ± 107.2 b | 39.1 ± 2.1 ab |
| <i>Significance</i> | | * | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | <i>ns</i> | <i>ns</i> | *** | <i>ns</i> | * |
| 54 | Standard (Control) | 62.5 ± 2.0 | 33.6 ± 2.4 | 4.2 ± 3.5 | 11.4 ± 1.5 | 85.9 ± 1.4 | 1.6 ± 1.6 | 3.2 ± 0.3 b | 2.3 ± 2.2 b | 949.1 ± 81.4 b | 25.3 ± 4.5 |
| | Leaf Removal West | 66 ± 3.7 | 31.5 ± 3.4 | 2.4 ± 1.4 | 11.3 ± 0.6 | 87.9 ± 0.6 | 0.8 ± 0.6 | 3.2 ± 0.3 b | 1.3 ± 0.8 b | 945.6 ± 78.6 b | 19.5 ± 4.9 |
| | LR (-UV-B, 2xOp50) | 64.4 ± 1.6 | 33.6 ± 1.5 | 2.0 ± 1.0 | 12.5 ± 0.8 | 87 ± 0.6 | 0.5 ± 0.3 | 3.4 ± 0.35 ab | 0.9 ± 0.5 b | 994.3 ± 102.8 b | 22.1 ± 9.5 |
| | LR (-UV-B, 2xUHI) | 60.1 ± 9.9 | 25.2 ± 5.0 | 10.3 ± 9.8 | 10.4 ± 1.7 | 79.7 ± 8.5 | 4.3 ± 4.6 | 3.6 ± 0.2 a | 7.5 ± 7.2 a | 1096.3 ± 99.1 a | 35.0 ± 12.4 |
| <i>Significance</i> | | * | <i>ns</i> | * | * | <i>ns</i> | <i>ns</i> | * | * | * | ** |
| 68 | Standard (Control) | 58.7 ± 3.0 | 37.8 ± 3.4 | 3.5 ± 1.4 | 11.9 ± 1.2 | 86.5 ± 0.8 | 1.6 ± 0.9 | 3 ± 0.2 a | 2.2 ± 1.0 a | 885.3 ± 67.4 a | 23.0 ± 2.8 b |
| | Leaf Removal West | 59.2 ± 3.7 | 37.8 ± 3.5 | 3.0 ± 1.9 | 12.1 ± 1.0 | 69.2 ± 38.2 | 1.4 ± 1.1 | 3.1 ± 0.3 a | 1.9 ± 1.3 a | 876.9 ± 82.4 a | 23.1 ± 5.3 b |
| | LR (-UV-B, 2xOp50) | 63.4 ± 4.3 | 30 ± 1.6 | 2.7 ± 1.2 | 12.1 ± 1.2 | 77.8 ± 8 | 1.3 ± 0.6 | 2.5 ± 0.2 b | 2.7 ± 1.4 a | 736.3 ± 75.3 b | 26.6 ± 4.4 b |
| | LR (-UV-B, 2xUHI) | 63.1 ± 1.6 | 33.4 ± 1.8 | 3.6 ± 2.2 | 13.1 ± 0.6 | 85.4 ± 0.9 | 1.5 ± 1.1 | 3.1 ± 0.3 a | 2.1 ± 1.4 a | 878.4 ± 92.7 a | 25.5 ± 4.2 |
| <i>Significance</i> | | * | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | * | <i>ns</i> | | |

Addendum 16 (cont.)

| | | | | | | | | | | | |
|---------------------|--------------------|------------|-------------|-----------|------------|------------|-----------|--------------|--------------|-------------------|---------------|
| 82 | Standard (Control) | 57.5 ± 1.7 | 39.3 ± 1.7 | 3.1 ± 1.2 | 12.2 ± 0.6 | 86.3 ± 0.4 | 1.5 ± 0.6 | 3.6 ± 0.4 b | 1.9 ± 0.7 a | 1066 ± 111.9 b | 22.0 ± 4.0 a |
| | Leaf Removal West | 58.8 ± 1.5 | 38.8 ± 2.0 | 2.4 ± 1.1 | 11.2 ± 1.2 | 86.9 ± 2.2 | 1.9 ± 1.6 | 4 ± 0.5 b | 2.0 ± 1.6 a | 1170.3 ± 145.2 b | 24.0 ± 4.5 a |
| | LR (-UV-B, 2xOp50) | 58.5 ± 2.3 | 38.6 ± 2.9 | 2.9 ± 1.0 | 12.3 ± 0.6 | 86.2 ± 0.7 | 1.5 ± 0.6 | 3.6 ± 0.1 b | 1.9 ± 0.7 a | 1060.5 ± 43.7 b | 21.8 ± 2.1 a |
| | LR (-UV-B, 2xUHI) | 58.3 ± 1.9 | 37.6 ± 3.3 | 3.5 ± 1.9 | 11.6 ± 0.7 | 84 ± 5.7 | 1.7 ± 1.2 | 4.5 ± 0.3 a | 2.5 ± 1.6 a | 1320.8 ± 87.3 a | 21.7 ± 1.6 a |
| Significance | | ns | ns | ns | ns | ns | ns | *** | ns | ** | ns |
| 96 | Standard (Control) | 56.0 ± 3.5 | 41.6 ± 3.1 | 2.3 ± 1.0 | 12.5 ± 1.1 | 86.1 ± 0.5 | 1.3 ± 0.7 | 4.1 ± 0.4 b | 1.5 ± 0.7 ab | 1194.3 ± 125 b | 18.9 ± 2.8 ab |
| | Leaf Removal West | 56.8 ± 1.5 | 39.5 ± 2.2 | 3.7 ± 1.7 | 10.7 ± 0.9 | 87 ± 1.7 | 2.4 ± 1.5 | 4.9 ± 0.2 a | 2.6 ± 1.5 a | 1433.3 ± 62.9 a | 21.6 ± 3.0 a |
| | LR (-UV-B, 2xOp50) | 58.4 ± 1.5 | 40.3 ± 2.9 | 2.0 ± 1.0 | 12.9 ± 0.7 | 87.9 ± 7.3 | 1.0 ± 1.7 | 4.1 ± 0.2 b | 1.2 ± 0.8 b | 1163.4 ± 69.0 b | 17.6 ± 1.9 bc |
| | LR (-UV-B, 2xUHI) | 58.0 ± 2.1 | 39.4 ± 2.6 | 2.6 ± 0.9 | 12.2 ± 0.6 | 86.5 ± 0.9 | 1.3 ± 0.6 | 4.7 ± 0.3 a | 1.5 ± 0.6 ab | 1373.3 ± 81.2 a | 15.0 ± 3.2 c |
| Significance | | ns | ns | ns | *** | ns | ns | *** | ns | *** | ** |
| 110 | Standard (Control) | 54.7 ± 2.1 | 41.4 ± 3.5 | 3.9 ± 2.1 | 11.8 ± 0.6 | 85.7 ± 1.4 | 2.4 ± 1.9 | 4.6 ± 0.4 a | 2.8 ± 1.9 a | 1340 ± 143.2 a | 25.1 ± 4.2 a |
| | Leaf Removal West | 55.9 ± 0.7 | 40.1 ± 2.0 | 4.0 ± 1.9 | 10.9 ± 0.7 | 86.9 ± 1.5 | 2.1 ± 1.2 | 4.8 ± 0.1 a | 2.5 ± 1.3 a | 1416.7 ± 32.5 a | 22.6 ± 1.3 ab |
| | LR (-UV-B, 2xOp50) | 57.9 ± 3.6 | 41.0 ± 3.9 | 1.7 ± 1.2 | 13.6 ± 0.7 | 88.2 ± 4.6 | 0.8 ± 0.8 | 3.9 ± 0.1 b | 0.6 ± 0.3 b | 1142.3 ± 81.8 b | 18.6 ± 3.0 b |
| | LR (-UV-B, 2xUHI) | 56.3 ± 1.4 | 41.5 ± 1.7 | 2.2 ± 0.7 | 12.7 ± 0.5 | 86.4 ± 0.6 | 0.8 ± 0.5 | 4.6 ± 0.2 a | 1.1 ± 0.5 ab | 1335.4 ± 80.2 a | 20.0 ± 2.2 b |
| Significance | | ns | ns | ns | *** | ns | ns | ** | ns | ** | * |
| 130 | Standard (Control) | 54.9 ± 2.2 | 41.8 ± 1.8 | 2.8 ± 1.0 | 11.9 ± 0.3 | 84.4 ± 3.6 | 1.8 ± 0.9 | 4.4 ± 0.2 b | 2.1 ± 0.8 a | 1300 ± 76 b | 20.3 ± 3.1 a |
| | Leaf Removal West | 56.4 ± 1.7 | 40.6 ± 1.5 | 3.0 ± 1.6 | 12.3 ± 1.0 | 85.7 ± 1.2 | 1.9 ± 1.3 | 4.5 ± 0.6 ab | 2.1 ± 1.4 a | 1313.9 ± 171.9 ab | 16.0 ± 4.5 ab |
| | LR (-UV-B, 2xOp50) | 60.8 ± 3.7 | 37.4 ± 32.6 | 3.1 ± 0.7 | 12.2 ± 1.2 | 85.8 ± 9.0 | 4.1 ± 4.2 | 4.4 ± 0.6 b | 2.1 ± 0.6 a | 1280.4 ± 170.7 b | 18.3 ± 2.2 ab |
| | LR (-UV-B, 2xUHI) | 54.6 ± 1.7 | 42.0 ± 2.4 | 3.4 ± 1.5 | 11.6 ± 0.5 | 86.3 ± 0.9 | 2.1 ± 1.2 | 5.1 ± 0.4 a | 2.3 ± 1.2 a | 1494.1 ± 135 a | 15.4 ± 2.3 b |
| Significance | | * | ns | ns | ns | ns | ns | ns | ns | ns | * |

Each value represents the mean of 5 replicates (±) standard deviation in units of mg/g seed tannin extract.). STD (Shaded/Control); LRW (Leaf Removal West); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). ^aPercent composition of proanthocyanidin subunits (in moles) C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate. mDP, mean degree of polymerization; %G, percentage galloylation; avMM, average molecular mass; nd, not detected;. Significance (*, ** and *** indicate significance at p ≤ 0.05, 0.01, 0.001 respectively, ns: not significant).

Addendum 17. Compositional and structural characterisation of skin extracts during ripening in 2010/2011.

| DAA | Treatment | Terminal units ^a | | | Extension units ^a | | | | mDP | %G | % P | avMM | Proanthocyanidins |
|---------------------|--------------------|-----------------------------|--------------|-----------|------------------------------|--------------|-------------|---------------|---------------|---------------|-----------|---------------------|-------------------|
| | | C | EC | ECG | C | EC | ECG | EGC | | | | | |
| 13 | Standard (Control) | 94.0 ± 5.5 | 5.95 ± 5.55 | nd | 2.52 ± 0.19 | 45.52 ± 1.19 | 0.15 ± 0.09 | 51.802 ± 1.39 | 22.5 ± 2.3 | 0.15 ± 0.1 a | 49.5 b | 6735.8 ± 701 a | 12.1 ± 6.1 a |
| | Leaf Removal West | 95.8 ± 5.66 | 4.13 ± 5.66 | nd | 2.65 ± 0.47 | 41.39 ± 2.4 | 0.08 ± 0.07 | 55.86 ± 2.76 | 22.4 ± 5.2 | 0.08 ± 0.1 b | 53.3 a | 6661.4 ± 1541 a | 11 ± 3.4 a |
| | STD-UV-B | 94.96 ± 4.6 | 5.03 ± 4.66 | nd | 2.52 ± 0.19 | 45.52 ± 1.19 | 0.15 ± 0.09 | 51.8 ± 1.39 | 22.8 ± 2.4 | 0.10 ± 0.1ab | 49.5 b | 6754 ± 712 a | 7.3 ± 4.1 a |
| | LRW-UV-B | 94.0 ± 5.5 | 5.95 ± 5.55 | nd | 2.52 ± 0.19 | 45.52 ± 1.19 | 0.15 ± 0.09 | 51.8 ± 1.39 | 22.5 ± 2.3 | 0.15 ± 0.1 ab | 49.5 b | 6735.8 ± 701 a | 12.1 ± 6.1 a |
| Significance | | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | ns | <i>ns</i> | <i>ns</i> | <i>ns</i> |
| 17 | Standard (Control) | 91.4 ± 0.46 | 8.53 ± 0.46 | nd | 1.93 ± 0.16 | 41.4 ± 1.49 | 0.20 ± 0.07 | 56.45 ± 1.61 | 35.3 ± 1.85 a | 0.16 ± 0.1 ab | 54.9 a | 10497 ± 555.7 a | 20.5 ± 2.5 a |
| | Leaf Removal West | 100 | nd | nd | 2.81 ± 0.39 | 37.5 ± 2.86 | nd | 59.67 ± 2.96 | 21.2 ± 3.5 b | 0 ± 0 c | 56.8 a | 6302 ± 1049 b | 4.7 ± 1.9 c |
| | STD-UV-B | 97.8 ± 4.8 | 2.16 ± 4.83 | nd | 2.1 ± 0.29 | 39.4 ± 2.57 | 0.05 ± 0.08 | 58.30 ± 2.84 | 31.6 ± 8.13 a | 0.05 ± 0.1bc | 56.4 a | 9416.7 ± 2428.3 a | 12.6 ± 3.2 b |
| | LRW-UV-B | 91.4 ± 0.46 | 8.53 ± 0.46 | nd | 1.93 ± 0.16 | 41.4 ± 1.49 | 0.20 ± 0.07 | 56.45 ± 1.61 | 35.3 ± 1.85 a | 0.16 ± 0.1 ab | 54.8 a | 10497 ± 555.7 a | 20.5 ± 2.5 a |
| Significance | | <i>ns</i> | <i>ns</i> | <i>ns</i> | * | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | *** | <i>ns</i> | ** | *** |
| 22 | Standard (Control) | 100 | nd | nd | 2.58 ± 0.52 | 41.6 ± 1.30 | 0.41 ± 0.11 | 55.39 ± 1.18 | 20.5 ± 4.3 | 0.4 ± 0.1 a | 52.6 b | 6089.7 ± 1301.4 a | 6.5 ± 3.2 a |
| | Leaf Removal West | 100 | nd | nd | 2.43 ± 0.25 | 39.0 ± 1.78 | 0.03 ± 0.07 | 58.49 ± 1.97 | 24 ± 2.9 | 0.03 ± 0.1 c | 56 a | 7140.2 ± 869 a | 7.7 ± 2.0 a |
| | STD-UV-B | 86.9 ± 2.12 | 13 ± 2.12 | nd | 2.55 ± 0.36 | 42.2 ± 1.5 | 0.24 ± 0.07 | 54.99 ± 1.84 | 22.7 ± 3.1 | 0.23 ± 0.1 b | 52.5 b | 6742.2 ± 934 a | 8.4 ± 3.5 a |
| | LRW-UV-B | 100 | nd | nd | 2.58 ± 0.52 | 41.6 ± 1.30 | 0.41 ± 0.11 | 55.39 ± 1.18 | 20.5 ± 4.3 | 0.4 ± 0.1 a | 52.6 b | 6089.7 ± 1301.4 a | 6.5 ± 3.2 a |
| Significance | | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | ** | *** | <i>ns</i> | <i>ns</i> |
| 48 | Standard (Control) | 95.9 ± 1.0 | nd | nd | 1.50 ± 0.18 | 40.9 ± 0.85 | 0.44 ± 0.08 | 57.12 ± 0.92 | 38.8 ± 2.0 b | 0.53 ± 0.1 a | 55.6 b | 11546.5 ± 610 b | 18.4 ± 2.3 a |
| | Leaf Removal West | 89.4 ± 1.7 | 10.5 ± 1.7 | nd | 1.76 ± 0.29 | 35.8 ± 3.47 | 0.45 ± 0.84 | 61.97 ± 2.46 | 38.2 ± 4.1 b | 0.44 ± 0.1 a | 60.3 a | 11397.8 ± 1272.6 b | 16.6 ± 5.3 ab |
| | STD-UV-B | 100 | nd | nd | 1.72 ± 0.18 | 39.6 ± 3.04 | 0.14 ± 0.08 | 58.46 ± 3.17 | 42.1 ± 5.1 ab | 0.14 ± 0.1 a | 57.1 b | 12523.6 ± 1540.7 ab | 13.4 ± 3.2 b |
| | LRW-UV-B | 78.1 ± 32.7 | 2.3 ± 3.2 | nd | 1.32 ± 0.56 | 32.7 ± 16.56 | 0.24 ± 0.18 | 46.74 ± 22.63 | 44.7 ± 2.8 a | 0.31 ± 0.1 a | 56.3 b | 13300.8 ± 846 a | 17.1 ± 2.1 ab |
| Significance | | ** | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | * | * | <i>ns</i> | * | * | <i>ns</i> |
| 62 | Standard (Control) | 57.7 ± 11.4 | 23.4 ± 7.95 | nd | 1.70 ± 0.23 | 40.7 ± 4.76 | 0.47 ± 0.70 | 57.34 ± 5.01 | 23.7 ± 6.5 c | 1.5 ± 1.4 a | 54.8 a | 7086.2 ± 1909 c | 7.1 ± 1.9 b |
| | Leaf Removal West | 70.2 ± 4.8 | 22.8 ± 3.16 | nd | 1.71 ± 0.13 | 41.36 ± 1.26 | 1.7 ± 0.08 | 55.75 ± 1.36 | 43.9 ± 4.5 a | 1.3 ± 0.1 ab | 54.5 a | 13127.6 ± 1368.0 a | 13.9 ± 2.2 a |
| | STD-UV-B | 68.6 ± 7.2 | 21.6 ± 7.49 | nd | 1.78 ± 0.24 | 41.8 ± 0.36 | 0.30 ± 0.06 | 56.11 ± 0.32 | 33.5 ± 8.3 b | 0.60 ± 0.1 ab | 54.3 a | 9975.2 ± 2482.3 b | 9.5 ± 2.8 b |
| | LRW-UV-B | 89.7 ± 2.12 | 8.07 ± 18.06 | nd | 1.77 ± 0.14 | 40.4 ± 1.35 | 0.19 ± 0.07 | 57.48 ± 1.22 | 42.1 ± 1.6 a | 0.44 ± 0.1 b | 56.2 a | 12543.5 ± 481.6 a | 8.3 ± 2.0 b |
| Significance | | * | * | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | <i>ns</i> | *** | * | <i>ns</i> | *** | ** |

Addendum 17 (cont.)

| | | | | | | | | | | | | | |
|---------------------|--------------------|--------------|---------------|-----------|-------------|--------------|-------------|--------------|---------------|----------------|-----------|--------------------|---------------|
| 76 | Standard (Control) | 65.1 ± 7.39 | 13.03 ± 1.55 | nd | 1.72 ± 0.09 | 41.4 ± 2.27 | 0.25 ± 0.14 | 56.61 ± 2.36 | 23.3 ± 4.4 d | 1.2 ± 0.5 a | 54.1 b | 6974.8 ± 1302.3 c | 6.3 ± 1.3 c |
| | Leaf Removal West | 54.9 ± 5.2 | 31.1 ± 8.01 | nd | 1.66 ± 0.20 | 41.3 ± 2 | 1.09 ± 0.20 | 55.92 ± 2.37 | 31.9 ± 1.9 b | 1.5 ± 0.3 a | 54.1 b | 9539.6 ± 586.4 b | 11.7 ± 1.6 a |
| | STD-UV-B | 66.79 ± 5.21 | 23.42 ± 5.38 | nd | 1.93 ± 0.12 | 42.2 ± 1.8 | 0.27 ± 0.16 | 55.52 ± 1.84 | 27.6 ± 27.6 c | 0.62 ± 0.1 b | 53.5 b | 8213.6 ± 83.1 c | 9.3 ± 1.5 b |
| | LRW-UV-B | 80.9 ± 6.83 | 3.22 ± 7.2 | nd | 1.72 ± 0.13 | 39.6 ± 2.2 | 0.11 ± 0.03 | 58.39 ± 2.19 | 40.5 ± 2.9 a | 0.6 ± 0.3 b | 56.9 a | 12066.7 ± 880.6 a | 10.8 ± 1.7 ab |
| <i>Significance</i> | | *** | *** | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | <i>ns</i> | *** | ** | * | *** | ** |
| 90 | Standard (Control) | 62.15 ± 9.6 | 18.60 ± 10.5 | nd | 1.81 ± 0.19 | 37.8 ± 1.43 | 0.18 ± 0.09 | 60.21 ± 1.52 | 25 ± 3.5 b | 0.94 ± 0.11 ab | 57.8 a | 7483.6 ± 1057.2 b | 7.5 ± 0.8 b |
| | Leaf Removal West | 59.3 ± 3.91 | 29.1 ± 6.87 | nd | 1.87 ± 0.34 | 43.5 ± 7.8 | 0.29 ± 0.17 | 54.27 ± 8.36 | 24 ± 3.3 b | 0.77 ± 0.2 b | 51.9 a | 7151.2 ± 979.6 b | 10.1 ± 2.3 a |
| | STD-UV-B | 78.7 ± 7.18 | nd | nd | 1.88 ± 0.25 | 42.1 ± 1.05 | 0.38 ± 0.07 | 55.56 ± 1.19 | 35 ± 3.2 a | 0.81 ± 0.04 ab | 55.8 a | 10.436.7 ± 970.6 a | 8.7 ± 0.8 ab |
| | LRW-UV-B | 77.6 ± 1.7 | 29.12 ± 6.8 | nd | 1.76 ± 0.17 | 40.5 ± 2.31 | 0.36 ± 0.11 | 56.4 ± 1.74 | 38.6 ± 1.1 a | 0.97 ± 0.11 a | 54 a | 11525.2 ± 343.6 a | 8.7 ± 0.6 ab |
| <i>Significance</i> | | * | *** | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | * | <i>ns</i> | *** | <i>ns</i> |
| 116 | Standard (Control) | 67.0 ± 6.8 | 9.89 ± 5.67 | nd | 1.34 ± 0.12 | 40.11 ± 2.08 | 0.57 ± 0.27 | 57.95 ± 2.10 | 20.6 ± 2.1 c | 1.66 ± 0.2 a | 55.1 a | 6178.9 ± 620.5 c | 4.9 ± 0.4 c |
| | Leaf Removal West | 67.3 ± 10 | 18.74 ± 10.72 | nd | 1.59 ± 0.12 | 40.7 ± 2.62 | 0.36 ± 0.06 | 57.32 ± 2.74 | 33.1 ± 4.7 b | 0.8 ± 0.1 b | 55.6 a | 9875.1 ± 1386.1 b | 11 ± 1.6 a |
| | STD-UV-B | 83.7 ± 0.75 | nd | nd | 1.87 ± 0.17 | 44.6 ± 0.88 | 0.34 ± 0.11 | 53.16 ± 0.97 | 37 ± 3.9 ab | 0.7 ± 0.1 b | 51.7 b | 11015 ± 1158.7 | 7.8 ± 1.4 b |
| | LRW-UV-B | 79.5 ± 4.4 | 3.15 ± 7.05 | nd | 1.57 ± 0.36 | 42.6 ± 2.86 | 0.26 ± 0.07 | 57.6 ± 4.01 | 40.8 ± 4.5 a | 0.7 ± 0.1 b | 55.1 a | 12154 ± 1330.5 a | 8.0 ± 1.1 b |
| <i>Significance</i> | | <i>ns</i> | * | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | * | *** | *** | *** |

Each value represents the mean of 5 replicates (±) standard deviation in units of mg/g skin tannin extract. STD (Shaded/Control); LRW (Leaf Removal West); STD-UV-B (STD with decreased UV-B radiation); LRW-UV-B (LRW with decreased UV-B radiation). ^aPercent composition of proanthocyanidin subunits (in moles) C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate. mDP, mean degree of polymerization; %G, percentage galloylation; avMM, average molecular mass; nd, not detected;. Significance (*, ** and ***) indicate significance at $p \leq 0.05$, 0.01 , 0.001 respectively, *ns*: not significant).

Addendum 18. Compositional and structural characterisation of skin extracts during ripening in 2011/2012 vintage.

| DAA | Treatment | Terminal units ^a | | | Extension units ^a | | | | mDP | %G | %P | avMM | Proanthocyanidins |
|---------------------|--------------------|-----------------------------|------------|------------|------------------------------|------------|-----------|------------|---------------|---------------|--------------|--------------------|-------------------|
| | | C | EC | ECG | C | EC | ECG | EGC | | | | | |
| 26 | Standard (Control) | 89.4 ± 7.8 | 8.4 ± 5.8 | 1.3 ± 0.3 | 1.3 ± 0 | 42.7 ± 1.4 | 1.3 ± 0.1 | 54.7 ± 1.4 | 46.1 ± 11.9 a | 53.4 ± 1.6 a | 1.3 ± 0.1 bc | 13751.6 ± 3575.3 a | 22.6 ± 4.4 a |
| | Leaf Removal West | 94.4 ± 1.5 | 4.2 ± 2.1 | 1.4 ± 0.7 | 1.4 ± 0.1 | 41.8 ± 2.0 | 1.1 ± 0.1 | 55.3 ± 1.6 | 47.3 ± 6.6 a | 54.4 ± 1.9 a | 1.2 ± 0.2 c | 14105.8 ± 1995.3 a | 22.8 ± 2.2 a |
| | LR (-UV-B, 2xOp50) | 62.6 ± 2.3 | 4.8 ± 0.7 | 32.6 ± 2.6 | 1.4 ± 0.1 | 44.6 ± 1.4 | 1.1 ± 0.1 | 52.9 ± 1.5 | 28 ± 2.2 b | 51.0 ± 1.5 b | 2.2 ± 0.1 a | 8387.6 ± 653.4 b | 23.4 ± 2.1 a |
| | LR (-UV-B, 2xUHI) | 92.6 ± 1.2 | 5.6 ± 1.4 | 1.7 ± 0.3 | 1.5 ± 0.1 | 43.5 ± 0.9 | 1.4 ± 0.1 | 53.6 ± 1.0 | 51.7 ± 6.8 a | 52.5 ± 1.1 ab | 1.4 ± 0.1 b | 15429.4 ± 2024.2 a | 19.8 ± 2.7 a |
| <i>Significance</i> | | *** | ns | *** | ns | ns | ns | ns | ** | *** | * | ** | ns |
| 33 | Standard (Control) | 90.8 ± 1.3 | 7.3 ± 1.1 | 1.8 ± 0.3 | 1.3 ± 0.1 | 40.6 ± 1.8 | 1.2 ± 0.1 | 56.9 ± 2.0 | 55.7 ± 4.5 a | 55.9 ± 2.0 a | 1.2 ± 0.1 b | 16656.2 ± 1347.5 a | 17.1 ± 3.8 a |
| | Leaf Removal West | 91.2 ± 1.2 | 6.9 ± 1.7 | 1.9 ± 0.5 | 1.4 ± 0.2 | 40.3 ± 1.4 | 1.2 ± 0.1 | 57.1 ± 1.5 | 51.7 ± 6.9 a | 56.0 ± 1.3 a | 1.2 ± 0.1 b | 15447.5 ± 2043.8 a | 16.3 ± 4.7a |
| | LR (-UV-B, 2xOp50) | 53.7 ± 2.3 | 3.8 ± 0.5 | 42.5 ± 2.7 | 1.6 ± 0.1 | 41.6 ± 1.6 | 1.2 ± 0 | 55.7 ± 1.6 | 32 ± 1.7 b | 53.9 ± 1.6 a | 2.5 ± 0.1 a | 9619.9 ± 507.2 b | 14.9 ± 3.0 a |
| | LR (-UV-B, 2xUHI) | 90.4 ± 1.8 | 7.1 ± 0.4 | 2.5 ± 1.0 | 1.4 ± 0.2 | 41.0 ± 1.7 | 1.4 ± 0.1 | 56.2 ± 1.7 | 56.4 ± 7.7 a | 55.2 ± 1.8 a | 1.4 ± 0.1 b | 16848.1 ± 2315.1 a | 14.9 ± 3.4 a |
| <i>Significance</i> | | *** | ns | *** | ns | ns | ns | ns | *** | ns | *** | *** | *** |
| 40 | Standard (Control) | 91.3 ± 2.18 | 8.5 ± 1.6 | 1.6 ± 0.6 | 1.2 ± 0.1 | 39.5 ± 1.7 | 1.0 ± 0 | 58.3 ± 1.7 | 47.3 ± 0.7 b | 57.1 ± 1.7 a | 1.0 ± 0 b | 14124 ± 199.7 b | 24.5 ± 2.9 a |
| | Leaf Removal West | 90.6 ± 1.8 | 7.6 ± 1.7 | 1.7 ± 0.8 | 1.2 ± 0.1 | 39.3 ± 1.6 | 1.1 ± 0.1 | 58.4 ± 1.6 | 46.8 ± 2.5 b | 57.2 ± 1.6 a | 1.1 ± 0.1 b | 13978.2 ± 749.5 b | 23.4 ± 2.2 a |
| | LR (-UV-B, 2xOp50) | 53.0 ± 1.7 | 4.1 ± 1.4 | 42.9 ± 2.3 | 1.3 ± 0 | 40.5 ± 1.3 | 1.1 ± 0.1 | 57.1 ± 1.3 | 34.4 ± 1.6 c | 55.4 ± 1.2 a | 2.3 ± 0.2 a | 10341.4 ± 476.5 c | 16.2 ± 1.8 b |
| | LR (-UV-B, 2xUHI) | 91.9 ± 1.6 | 6.5 ± 1.4 | 1.6 ± 0.5 | 1.2 ± 0.1 | 40.0 ± 1.1 | 1.2 ± 0.1 | 57.6 ± 1.2 | 54.0 ± 3.0 a | 56.5 ± 1.3 a | 1.2 ± 0 b | 16127.3 ± 895.0 a | 17.9 ± 1.9 b |
| <i>Significance</i> | | *** | ns | *** | ns | ns | ns | ns | *** | ns | *** | *** | *** |
| 47 | Standard (Control) | 96.2 ± 1.0 | 3.2 ± 1.1 | 0.6 ± 0.1 | 1.4 ± 0.1 | 38.5 ± 1.0 | 0.5 ± 0.1 | 59.6 ± 1.0 | 51.5 ± 5.1 a | 58.5 ± 1.0 ab | 0.5 ± 0 c | 15362.9 ± 1532.6 a | 14.0 ± 2.5 ab |
| | Leaf Removal West | 95.7 ± 1.2 | 3.7 ± 1.3 | 0.7 ± 0.2 | 1.3 ± 0.1 | 37.3 ± 2.3 | 0.4 ± 0.2 | 61.0 ± 2.4 | 44.1 ± 3.6 b | 59.5 ± 2.2 a | 0.4 ± 0.2 c | 13164.0 ± 1078.8 b | 15.7 ± 2.5 a |
| | LR (-UV-B, 2xOp50) | 70.1 ± 11.2 | 2.3 ± 0.7 | 2.3 ± 0.3 | 1.4 ± 0.2 | 38.5 ± 1.6 | 0.5 ± 0.4 | 59.3 ± 1.8 | 36.6 ± 4.9 b | 57.9 ± 1.9 ab | 1.6 ± 0.4 a | 10972.0 ± 1466.0 c | 11.3 ± 1.9 b |
| | LR (-UV-B, 2xUHI) | 93.7 ± 1.1 | 4.2 ± 0.8 | 2.1 ± 0.9 | 1.3 ± 0.1 | 40.0 ± 1.7 | 1.2 ± 0.1 | 57.5 ± 1.8 | 55.1 ± 5.8 a | 56.5 ± 1.8 b | 1.2 ± 0.1 b | 16460.3 ± 1750.1 a | 14.2 ± 2.8 ab |
| <i>Significance</i> | | *** | ns | ns | ns | ns | *** | ns | ** | ns | *** | *** | ns |
| 54 | Standard (Control) | 97.0 ± 0.8 | 2.5 ± 0.9 | 0.5 ± 0.1 | 1.2 ± 0.1 | 36.4 ± 1.3 | 0.3 ± 0.1 | 62.0 ± 1.4 | 45.5 ± 2.2 a | 60.7 ± 1.4 a | 0.3 ± 0.1 b | 13576.9 ± 649.5 a | 18.1 ± 4.5 ab |
| | Leaf Removal West | 88.5 ± 4.0 | 10.8 ± 4.4 | 0.7 ± 0.4 | 1.3 ± 0.1 | 36.9 ± 1.0 | 0.4 ± 0.1 | 61.6 ± 1.0 | 40.1 ± 3.9 b | 60.1 ± 0.8 a | 0.3 ± 0.1 b | 11950.9 ± 1171.1 b | 19.8 ± 2.5 a |
| | LR (-UV-B, 2xOp50) | 87.2 ± 4.0 | 1.8 ± 0.8 | 14.5 ± 4.5 | 1.3 ± 0.1 | 36.8 ± 1.7 | 0.2 ± 0.1 | 61.6 ± 1.9 | 39.5 ± 1.5 b | 60.1 ± 1.8 a | 0.5 ± 0.1 a | 11772.3 ± 444.8 b | 18.4 ± 2.8 ab |
| | LR (-UV-B, 2xUHI) | 96.7 ± 0.5 | 2.6 ± 0.4 | 0.7 ± 0.3 | 1.2 ± 0.1 | 38.3 ± 2.0 | 0.4 ± 0 | 60.2 ± 2.2 | 48.6 ± 5.2 a | 59.0 ± 2.2 a | 0.3 ± 0.1 b | 14472.7 ± 1574.2 a | 15.6 ± 1.5 b |
| <i>Significance</i> | | ns | ns | *** | ns | ns | ns | ns | ** | ns | ** | ** | ns |

Addendum 18 (cont.)

| | | | | | | | | | | | | | |
|---------------------|--------------------|-------------|-------------|------------|-----------|------------|-----------|-------------|---------------|---------------|-------------|---------------------|---------------|
| 68 | Standard (Control) | 67.6 ± 3.3 | 28.3 ± 2.0 | 4.1 ± 1.5 | 1.2 ± 0.1 | 37.7 ± 0.5 | 0.7 ± 0.1 | 60.5 ± 0.6 | 39.4 ± 1.7 a | 58.9 ± 0.6 a | 0.8 ± 0.1 a | 11761.6 ± 486.4 a | 13.0 ± 1.3 ab |
| | Leaf Removal West | 72.7 ± 4.8 | 24.6 ± 4.6 | 2.8 ± 1.1 | 1.1 ± 0.1 | 37.8 ± 0.6 | 0.6 ± 0.2 | 60.5 ± 0.4 | 38.5 ± 4.2 ab | 58.9 ± 0.3 a | 0.6 ± 0.2 a | 11481.3 ± 1250.0 ab | 15.3 ± 0.6 a |
| | LR (-UV-B, 2xOp50) | 63.9 ± 9.3 | 19.3 ± 4.2 | 18.8 ± 4.1 | 1.5 ± 0.1 | 37.8 ± 2.0 | 0.2 ± 0.1 | 60.5 ± 1.9 | 32.9 ± 3.6 b | 58.7 ± 1.8 a | 0.8 ± 0.3 a | 9813.3 ± 1058.8 b | 12.6 ± 3.0 b |
| | LR (-UV-B, 2xUHI) | 75.2 ± 10.8 | 22.4 ± 9.9 | 2.4 ± 1.1 | 1.3 ± 0.1 | 37.7 ± 1.1 | 0.7 ± 0.1 | 60.3 ± 1.2 | 41.2 ± 5.7 a | 58.8 ± 1.2 a | 0.7 ± 0.2 a | 12303.1 ± 1676.7 a | 11.8 ± 1.1 b |
| Significance | | <i>ns</i> | <i>ns</i> | *** | *** | <i>ns</i> | *** | <i>ns</i> | * | <i>ns</i> | <i>ns</i> | * | <i>ns</i> |
| 82 | Standard (Control) | 76.0 ± 1.0 | 20.6 ± 1.1 | 3.4 ± 0.8 | 1.2 ± 0.1 | 37.8 ± 1.0 | 0.4 ± 0.1 | 60.7 ± 1.0 | 42.7 ± 1.3 a | 59.3 ± 1.0 a | 0.4 ± 0.1 b | 12729.7 ± 403.5 a | 13.9 ± 2.6 a |
| | Leaf Removal West | 76.8 ± 2.5 | 20.5 ± 2.5 | 2.7 ± 0.2 | 1.2 ± 0.1 | 37.5 ± 1.6 | 0.4 ± 0 | 60.9 ± 1.6 | 39.2 ± 2.5 b | 59.3 ± 1.6 a | 0.5 ± 0 b | 11682.9 ± 738.6 b | 14.1 ± 1.9 a |
| | LR (-UV-B, 2xOp50) | 65.3 ± 2.6 | 19.8 ± 2.6 | 14.9 ± 2.8 | 1.1 ± 0 | 38.4 ± 2.0 | 0.3 ± 0.1 | 60.1 ± 2.0 | 33.6 ± 2.4 c | 58.3 ± 1.9 a | 0.8 ± 0.2 a | 10033.1 ± 710.1 c | 14.1 ± 2.2 a |
| | LR (-UV-B, 2xUHI) | 74.7 ± 2.6 | 18.5 ± 2.2 | 6.8 ± 2.0 | 1.1 ± 0 | 37.2 ± 2.0 | 0.5 ± 0.1 | 61.1 ± 1.9 | 44.6 ± 1.4a | 59.7 ± 1.8 a | 0.7 ± 0.2 a | 13302.7 ± 401.5 a | 13.2 ± 0.9 a |
| Significance | | <i>ns</i> | <i>ns</i> | *** | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | *** | <i>ns</i> | ** | *** | <i>ns</i> |
| 96 | Standard (Control) | 86.0 ± 3.3 | 32.9 ± 18.4 | 7.9 ± 7.0 | 1.1 ± 0.1 | 40.1 ± 0.9 | 0.7 ± 0.1 | 58.1 ± 1.1 | 53.7 ± 5.0 a | 57.0 ± 1.0 a | 0.8 ± 0.1 a | 16031.4 ± 1488.8 a | 10.2 ± 1.7 a |
| | Leaf Removal West | 61.2 ± 4.4 | 35.1 ± 5.2 | 3.7 ± 1.2 | 1.1 ± 0 | 39.1 ± 0.9 | 0.6 ± 0.1 | 59.2 ± 0.9 | 34.3 ± 4.3 c | 57.5 ± 0.7 a | 0.7 ± 0.1 a | 10232.4 ± 1295.9 c | 9.6 ± 1.4 a |
| | LR (-UV-B, 2xOp50) | 51.3 ± 4.9 | 36.1 ± 4.2 | 12.6 ± 1.7 | 1.0 ± 0.1 | 38.1 ± 2.2 | 0.3 ± 0.1 | 60.7 ± 2.4 | 30.4 ± 5.2 c | 58.6 ± 2.5 a | 0.7 ± 0.2 a | 9068.2 ± 1559.4 c | 9.9 ± 1.6 a |
| | LR (-UV-B, 2xUHI) | 65.3 ± 5.6 | 30.7 ± 6.4 | 4.0 ± 1.5 | 1.0 ± 0.1 | 38.9 ± 0.8 | 0.5 ± 0.1 | 59.5 ± 0.8 | 42.4 ± 6.0 b | 58.1 ± 0.8 a | 0.6 ± 0.1 a | 12662.7 ± 1783.8 b | 10.7 ± 1.8 a |
| Significance | | * | *** | *** | <i>ns</i> | <i>ns</i> | *** | <i>ns</i> | *** | <i>ns</i> | Ns | *** | <i>ns</i> |
| 110 | Standard (Control) | 83.3 ± 3.3 | 11.4 ± 2.5 | 5.3 ± 1.4 | 1.2 ± 0.1 | 36.9 ± 1.1 | 0.6 ± 0 | 61.5 ± 1.1 | 46.8 ± 2.1 a | 60.1 ± 1.0 a | 0.6 ± 0.2 b | 13982.9 ± 633.5 a | 8.6 ± 1.2 c |
| | Leaf Removal West | 73.4 ± 13.4 | 18.9 ± 9.7 | 7.7 ± 7.0 | 1.3 ± 0.1 | 36.8 ± 1.1 | 0.6 ± 0 | 61.4 ± 1.1 | 40.6 ± 7.5 ab | 59.8 ± 1.3 ab | 0.8 ± 0.3 b | 12131.6 ± 2240.2 ab | 10.9 ± 0.9 b |
| | LR (-UV-B, 2xOp50) | 51.2 ± 7.6 | 32.0 ± 5.0 | 16.8 ± 6.1 | 1.2 ± 0 | 37.6 ± 1.5 | 0.6 ± 0.1 | 60.6 ± 1.3 | 29.7 ± 7.4 c | 58.4 ± 1.0 bc | 1.2 ± 0.4 a | 8872.2 ± 2202.4 c | 13.1 ± 1.7a |
| | LR (-UV-B, 2xUHI) | 63.8 ± 7.0 | 29.2 ± 8.6 | 7.0 ± 1.7 | 1.2 ± 0.1 | 38.6 ± 0.7 | 0.6 ± 0 | 59.6 ± 0.7 | 37.8 ± 4.8 bc | 58.0 ± 0.5 c | 0.8 ± 0.1 b | 11284.6 ± 1424.4 bc | 13.3 ± 1.0 a |
| Significance | | *** | * | *** | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | ** | * | * | ** | *** |
| 130 | Standard (Control) | 61.9 ± 1.9 | 31.7 ± 5.6 | 4.1 ± 0.9 | 1.2 ± 0 | 42.6 ± 1.6 | 0.7 ± 0 | 56 ± 1.5 | 34.9 ± 1.9 ab | 54.3 ± 1.4 a | 0.7 ± 0 b | 10393.2 ± 559.2 ab | 9.2 ± 0.6 ab |
| | Leaf Removal West | 72.3 ± 13.0 | 20.3 ± 9.5 | 7.4 ± 7.8 | 1.3 ± 0.1 | 41.7 ± 2.5 | 0.6 ± 0.1 | 56.5 ± 2.6 | 40.4 ± 9.0 a | 55.0 ± 2.6 a | 0.7 ± 0.3 b | 12030.6 ± 2684.6 a | 8.8 ± 1.2 a |
| | LR (-UV-B, 2xOp50) | 54.0 ± 8.0 | 32.2 ± 7.7 | 18.5 ± 4.7 | 1.3 ± 0.2 | 40.5 ± 3.4 | 0.5 ± 0.2 | 59.2 ± 12.5 | 28.6 ± 1.2 b | 54.4 ± 2.5 a | 1.2 ± 0.3 a | 8528 ± 351.3 b | 10.8 ± 1.6 bc |
| | LR (-UV-B, 2xUHI) | 54.0 ± 3.8 | 43.2 ± 4.0 | 2.8 ± 0.6 | 1.2 ± 0 | 41.8 ± 1.8 | 0.6 ± 0.1 | 57.1 ± 2.7 | 33.8 ± 2.3 ab | 54.8 ± 1.8 a | 0.7 ± 0.1 b | 10065.3 ± 683.7 ab | 8.5 ± 0.8 c |
| Significance | | *** | *** | *** | <i>ns</i> | <i>ns</i> | <i>ns</i> | <i>ns</i> | * | <i>ns</i> | * | * | *** |

Each value represents the mean of 5 replicates (\pm) standard deviation in units of mg/g skin tannin extract.) STD (Shaded/Control); LRW (Leaf Removal West); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone). ^aPercent composition of proanthocyanidin subunits (in moles) C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate; EGC, (-)-epigallocatechin. mDP, mean degree of polymerization; %G, percentage galloylation; avMM, average molecular mass; nd, not detected;. Significance (*, ** and *** indicate significance at $p \leq 0.05$, 0.01, 0.001 respectively, ns: not significant).

Addendum 19. Aroma compound concentrations (mg/L) of 2011/2012 Cabernet Sauvignon wines.

| Analytes | STD | LRW | LR (-UV-B, 2xOp50) | LR (-UV-B, 2xUHI) |
|--------------------------|--------|--------|--------------------|-------------------|
| Monoterpenes | | | | |
| Limonene | nd | nd | nd | 0.32 |
| Linalooloxide 1 | 15.03 | 19.89 | 12.92 | 29.81 |
| Linalooloxide 2 | 8.40 | 9.71 | nd | nd |
| Linalool | 6.23 | 4.90 | 5.01 | 47.31 |
| A-Terpeneol | nd | nd | nd | 4.40 |
| Citronellol | 10.25 | 8.31 | 7.78 | 5.65 |
| Nerol | 17.57 | 16.93 | 17.98 | 21.85 |
| Geraniol | 91.44 | 116.98 | 199.08 | 274.71 |
| B-Farnesol 1 | 1.73 | 0.70 | 201.92 | 238.15 |
| B-Farnesol 3 | 3.27 | 7.14 | 218.04 | 70.24 |
| Major volatiles | | | | |
| Ethyl Acetate | 84.18 | 62.73 | 88.01 | 85.51 |
| Methanol | 117.84 | 99.66 | 100.78 | 101.89 |
| Ethyl Butyrate | 0.25 | 0.23 | 0.25 | 0.30 |
| Propanol | 92.83 | 61.20 | 85.68 | 93.62 |
| Isobutanol | 48.86 | 37.64 | 45.26 | 43.95 |
| Isoamyl Acetate | 1.35 | 1.69 | 1.08 | 1.04 |
| Butanol | 2.75 | 2.45 | 2.39 | 2.87 |
| Isoamyl Alcohol | 495.79 | 436.92 | 452.19 | 467.71 |
| Ethyl Hexanoate | 0.48 | 0.54 | 0.53 | 0.55 |
| Pentanol | 0.13 | 0.07 | 0.12 | 0.13 |
| Acetoin | 9.51 | 12.07 | 6.12 | 6.25 |
| 3-Methyl-1-pentanol | 0.01 | 0.07 | nd | nd |
| Ethyl Lactate | 156.61 | 101.36 | 163.22 | 100.91 |
| Hexanol | 2.38 | 1.92 | 2.92 | 2.60 |
| 3-ethoxy-1-propanol | 8.15 | 3.50 | 7.39 | 8.42 |
| Ethyl Caprylate | 0.28 | 0.37 | 0.27 | 0.28 |
| Acetic Acid | 471.72 | 398.16 | 508.98 | 462.01 |
| Ethyl-3-hydroxybutanoate | 1.77 | 1.60 | 1.72 | 1.83 |
| Propionic Acid | 4.95 | 3.74 | 4.76 | 4.76 |
| Isobutyric Acid | 5.14 | 3.00 | 5.90 | 5.33 |
| Butyric acid | 0.25 | 0.27 | 0.25 | 0.31 |
| ethyl caprate | 0.22 | 0.45 | 0.09 | 0.31 |
| isovaleric acid | 5.45 | 3.37 | 5.65 | 5.23 |
| Diethyl succinate | 6.77 | 5.04 | 7.24 | 14.77 |
| Valeric Acid | 0.39 | 0.34 | 0.33 | 0.35 |
| 2-Phenylethyl Acetate | 0.47 | 0.43 | 0.41 | 0.40 |
| Ethyl phenylacetate | 0.18 | 0.43 | 0.00 | 0.00 |
| Hexanoic Acid | 2.11 | 2.28 | 2.35 | 2.33 |
| 2-Phenylethanol | 113.52 | 116.58 | 104.02 | 106.08 |
| Octanoic Acid | 1.58 | 1.84 | 1.48 | 1.52 |
| Decanoic Acid | 0.90 | 0.64 | 0.48 | 0.47 |

STD (Shaded/Control); LRW (Leaf Removal West); LR-UV-B, 2xOp50 (Leaf removal with decreased UV-B radiation and 2xOp50 UV-sheets added on both sides of the bunch zone); LR-UV-B, 2xUHI (Leaf removal with decreased UV-B radiation and 2xUHI UV-sheets added on both sides of the bunch zone).

