Effects of UV-B and Water Deficit on the Physiology and Chemical Composition of *Vitis vinifera* L. cv. Pinot noir

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Meng Sun

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Abstract

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by

Meng Sun

New Zealand Pinot noir has seen impressive growth in export sales in recent years. It is now second only to Sauvignon Blanc in production volume. There are four main Pinot noir regions in New Zealand: Central Otago, Waipara, Marlborough and Wairarapa. In these regions, the soil, climate and other conditions are suitable for Pinot noir growth. However, some environmental issues challenge Pinot noir growth in New Zealand, such as water deficit and UV-B. The mean annual rainfall in regions where Pinot noir is grown, is low, and long dry spells can occur, especially in summer. UV-B radiation in New Zealand is 30-40% higher than at similar latitudes in the Northern Hemisphere.

In this research, the aim was to determine effects of the separate and combined UV-B and water deficit on vine physiology and chemical composition of Pinot noir fruit. In the 2015-2016 and 2016-2017 vintages, two rows of Pinot noir grapevines in the West Vineyard at Lincoln University were chosen for the study. Treatments were a combination of leaf removal with plastic screens or shade cloth around the fruiting zone, and/or restricted irrigation. Grapevines and the fruit both responded to UV-B and water deficit. In comparison to exposure to natural UV-B in the vineyard, the potted vines were moved into a glasshouse

for preparation for the experiments in September, prior to budbreak. From October (fruit-set) to December (veraison), the grapevines were uniformly irrigated on a regular basis to soil capacity and were exposed to normal daylength hours in the glasshouse. From veraison, vines in treatments were exposed to supplemental UV-B interaction with restricted irrigation in a glasshouse.

The physiology of *Vitis vinifera* L. var. Pinot noir vines were altered by water deficit and UV-B. The combination of UV-B and water deficit changed the vine water status and leaf greenness in the glasshouse, but there was only a slight effect on berry parameters. However, UV-B exposure/exclusion interaction with water deficit did not affect vine water status and leaf greenness, even with no significant changes in fruit production capacity and °Brix in the vineyard.

Amino acids did not show consistent results in the two-year trial. In the glasshouse, amino acids were decreased by UV-B and water deficit in 2015-2016. However, there was no change in amino acids in response to UV-B in 2016-2017. The concentration of amino acids under water deficit was enhanced by its combination with UV-B. In the vineyard, there were no changes in fruit amino acids with UV-B exposure or exclusion over the two years, but the interaction of UV-B with water deficit significantly changed His, Val, Thr and Lys in 2016-2017.

Under UV-B and water deficit, vines were found to respond in both the glasshouse and vineyard trials. When berries were directly exposed to UV-B or water deficit, phenolic compounds accumulated to a greater degree in the berry skins. The combined stresses caused larger increases in phenolic composition than the individual stresses alone, such as skin anthocyanin contents in +UV-W (0.578 mg/berry) vs. -UV+W (0.398 mg/berry). A similar pattern was shown in the volatile compounds. For example, the concentration of hexanol was 1028.6 µg/L in +UV-W compared with 683.3 µg/L in -UV+W.

Overall, this study clearly demonstrates that UV-B exposure can interact with water deficit to increase the effects on physiological parameters of the vine and fruit composition to UV-B or water deficit alone. Water deficit can, potentially, increase additional responses to UV-B in both glasshouse and vineyard situations.

Keywords: *Vitis vinifera* L. var. Pinot noir, UV-B, water deficit, SPAD, carbon isotope ratio, leaf water potential, amino acids, phenolic composition, volatile compounds

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Abbreviations

ABA	abscisic acid
Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartate
˚C	degree centigrade
Chla	chlorophyll a
Chlb	chlorophyll b
Cys	cysteine
g, kg	gram, kilogram
Gln	glutamate
Glu	glutamine
Gly	glycine
GS-GOGAT	glutamine synthetase and glutamate synthase
His	histidine
HPLC	high performance liquid chromatography
HS-SPME GCMS	headspace solid-phase micro-extraction gas chromatograph mass spectrometry
Ile	isoleucine
Leu	leucine
LWP	leaf water potential
Lys	lysine
MCP	methylcellulose precipitation
Met	methionine
min	minute
PAR	photosynthetically active radiation
Phe	phenylalanine
Pro	proline
ROS	reactive oxygen species
TCA cycle	tricarboxylic acid cycle
TDR	time domain reflectometry
Thr	threonine
Trp	tryptophan
Tyr	tyrosine
Ser	serine
UV	ultraviolet
µM/L	µmol per liter of fresh grape juice
µg/L	µg per liter of fresh grape juice
Val	valine

Chapter 1

Introduction

In 2019, New Zealand's productive vineyard area reached 38,680 hectares and red varieties accounted for 7,876 hectares (https://www.nzwine.com/en/news-media/statistics-reports/new-zealand-winegrowers-annual-report/). A major red variety is Pinot noir, which comprises 5,625 hectares of that total, and which is predominantly grown in the cooler southerly regions of New Zealand: Marlborough, Nelson, Canterbury & Waipara, and Central Otago (http://www.nzwine.com/wine-styles/pinot-noir/). In Marlborough, the second largest planted variety in terms of area is Pinot noir, at 2,669 hectares. In that region, the eastern coastal aspect bestows cooling sea breezes and protective mountains, provide relief from extreme rain and wind. Average annual hours of sunshine and rainfall are 2,409 hours and 655 mm, so Pinot noir in this region displays red-fruited notes (cherry and raspberry) and fine tannins (https://www.nzwine.com/en/our-regions/marlborough/). The Canterbury and Waipara regions have an excellent reputation for elegant and expressive Pinot noir. The cool dry climate with high sunshine hours and a long growing season promotes full varietal expression. The protective Southern Alps ensure low rainfall, abundant sunshine and, often, very warm summers. The average annual sunshine is 2,100 hours and rainfall is 648 mm (https://www.nzwine.com/en/our-regions/canterbury-north-canterbury/). Central Otago is New Zealand's southernmost and highest elevation wine region. Pinot noir is a flagship variety in the region. High sunshine hours (2,025 hours on average, annually) and short, hot summers provide an environment that is, at times, brutal for vines. The dry autumns and overall low humidity are significant assets (360 mm average annual rainfall) (https://www.nzwine.com/en/our-regions/central-otago/).

Sunshine hours are relatively high in these New Zealand regions, at least 2000 hours annually. This can be compared with the classic Pinot noir producing region of Burgundy, where the average of annual sunshine is 1830 hours (http://www.regions-of-france.com/regions/burgundy/weather/). Also, in New Zealand, UV radiation levels are 30%-

40% higher compared to similar latitudes in the Northern Hemisphere (McKenzie et al., 1999; McKenzie et al., 2007; Seckmeyer and McKenzie, 1992). Long sunshine hours lead to grapevines' exposure to more intense UV-B in the regional plantings of Pinot noir. Therefore, high UV radiation in New Zealand, compared with other regions growing Pinot noir, could potentially induce changes in the grapevine physiology, the chemical composition of fruit and subsequent wine characteristics.

In New Zealand, the mean annual rainfall in the east of the South Island is low, and long dry spells can occur, especially in summer (https://www.niwa.co.nz/education-and-training/schools/resources/climate). The predominant regional plantings of Pinot noir in the South Island of New Zealand (Marlborough, Canterbury and Waipara and Central Otago) have annual rainfalls below 660 mm. According to a Ministry of the Environment report, climate change will reduce rainfall in the east of New Zealand (Ministry of the Environment, 2017). Thus, the decrease in rainfall has the potential to increase drought risk. Water deficit can cause changes in phenolic and aroma composition, fruit amino acids and vine growth in Pinot noir (to be reviewed in the next chapter).

Based on this knowledge, UV-B and water deficit are environmental issues for vineyard management in New Zealand. However, there has been only very limited research into the effects of a combination of UV-B radiation and water deficit on the vine physiology and the chemical composition of fruit. This study contributes to the understanding of the interaction between UV-B radiation exposure, water deficit, and the alteration of the vine physiology and the chemical composition of fruit in *Vitis vinifera* L. var. Pinot noir. In this study, Pinot noir vines in glasshouse and vineyard situations were subjected to different combinations of UV radiation and water deficit initiated from pre-veraison or veraison, and the effects on the composition of quality-related compounds in fruit measured. Finally, this research can give important information about Pinot noir to the NZ wine industry, according to an investigation into how grapes are affected by the naturally high levels of UV radiation and the likely occurrence of lower water availability in soils.

Chapter 2

Literature review

2.1 UV-B and its effects on plants

Solar radiation is the main energy supporting life on earth. Light supplies energy for plants via photosynthesis to produce carbohydrates and this, essentially, regulates plant growth and development. UV radiation is a significant component of sunlight and has been investigated as an environmental stress. UV can be divided by wavelength into UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (100-280 nm). UV-C and much of UV-B cannot penetrate the stratospheric ozone layer; therefore, the ozone layer protects life on earth from the most dangerous UV radiation. When a fraction of UV-B reaches the Earth's surface, as a highly energetic form of radiation, it can, however, cause damage to the biosphere (Jordan, 1996; Matsumi and Kawasaki, 2003). Human activities produce chlorofluorocarbon (CFC) and other organo-halogens, which caused the formation of the stratospheric ozone hole over the Antarctic (Farman et al., 1985). The destruction of the ozone layer allows increased penetration of UV-B in the biosphere (Jordan, 1996). There will be an effect later in the year when the ozone hole breaks up in November or early December, and ozone depleted air moves into surrounding areas in the southern hemisphere, including New Zealand (https://niwa.co.nz/news/antarctic-ozone-hole-near-record-levels). In 2019, ozone hole is the smallest on record since its discovery, ranging from 3.9 to 6.3 million square miles (https://www.nasa.gov/feature/goddard/2019/2019-ozone-hole-is-the-smallest-on-record-since-its-discovery).

The UV index (UVI) is a standard measurement of erythemal (sun-burn causing) UV intensity that gives a more objective measure than the old "time to burn". The scale is open-ended, but a UVI of greater than 10 is extreme and a UVI of less than 3 is low. For clear skies, the UVI depends mainly on the sun elevation angle and the ozone amount. The UVI also depends on cloud cover, sun-earth separation, altitude, pollution, and surface reflections

(e.g., snow cover) (https://www.niwa.co.nz/our-services/online-services/uv-ozone). In New Zealand, a UVI maximum summer value is generally about 12, but it can exceed 13 in the far North. The peak UVI in New Zealand is approximately 40% greater than at comparable latitudes in the Northern Hemisphere, due to differences in ozone, sun-earth separation and atmospheric pollution. The values increase in the morning, reach a peak at solar noon and decrease during the afternoon (McKenzie et al., 2007). In some researches, UV intensity and dose also have been used to measure ultraviolet B radiation (McKenzie et al., 2004; Sánchez-Pérez et al., 2019). The relationship between UVI and UV-B intensity is I_{UVB} = 18.9 × UVI (W/m^2). The expression for UV dose is $D = I^{4/3} \times t_e$ [$(W/m^2)^{4/3}$ s], including exposure time (t_e, s) and UV-B intensity (I, W/m^2) (Kiedron et al., 2007; McKenzie et al., 2004; Sánchez-Pérez et al., 2019). The increase in transmission of UV-B to the earth's surface is associated with damage to the biosphere due to stratospheric ozone depletion. UV-B causes changes in plant growth and development, regulation of primary and secondary metabolism and alterations in the molecular responses of plant cells (Jordan, 1996; Wargent and Jordan, 2013). The specific effects of UV-B on plants (Table 2.1) show changes in leaf area, loss of fresh and dry weight, the inhibition of photosynthesis (Jordan, 1996) and alterations in flowering and reproduction (Jansen, 2002; Jordan, 2002; Strid et al., 1990). In addition, it causes damage to DNA, proteins and lipids, changes in gene expression and pigment biosynthesis and produces antioxidants (Jordan, 2002; Jordan, 2017). However, plant defences have strategies to protect themselves from UV-B, while still allowing photosynthetically active radiation (PAR, 400-700 nm) through the modulation of plant sensitivity and photomorphogenesis in responses to UV-B (Escobar-Bravo et al., 2017). A high PAR level or a low UV-B/PAR ratio could cause the thicker leaves, leading to extend the pathlength for UV-B and increase the protective pigments within the epidermal cells (Jordan, 1996). Also, a high PAR level could ameliorate UV-B-induced down-regulation of mRNA for chloroplast protein (Hideg and Strid, 2017; Jordan, 1996; Mackerness et al., 1996).

Table 2.1 The specific effects of UV-B on plants

Component affected	Damage/Symptom	References
Morphology		
Flowering	Delayed flowering; Diminish lifetime flower production; Increase flower diameter and nectary volume	Dotto et al. (2018); Petropoulou et al. (2001); Sampson and Cane (1999)
Leaf	Decrease leaf area and leaf expansion; Increase leaf thickness, palisade thickness and hypodermal thickness	Hectors et al. (2010); Jansen (2002)
Shoot	Reduced stem elongation; Increase axillary branching and tillering	Furness et al. (2005); Jansen (2002)
Root	Increase allocation of biomass to roots	Bussell et al. (2012)
Assimilation partitioning	Reduce fresh and dry weight; Yield loss	Jordan et al. (1992); Jordan (1996)
Biological function		
Stomatal function	Reduced stomatal conductance	Nogués et al. (1999)
Pigments	Reduction in chlorophyll and carotenoids	Jordan et al. (1992)
Electron transport	Reduce oxidative capacity; D1 polypeptide turnover; Photoreduction of plastoquinone (PQ); Reduced the ratio of variable to total fluorescence yield; Lower apparent quantum yield of photosynthesis	Greenberg et al. (1989); Melis et al. (1992); Renger et al. (1989); Schultze and Bilger (2019); Sullivan et al. (1994); Sullivan and Teramura (1989)
CO_2 fixation	Reduced CO_2 uptake; Reduced Rubisco activity; Lower RUBP regeneration capacity	Sullivan and Teramura (1990); Strid et al. (1990)
Photosynthetically active effect (PAR)	High PAR reduces the extent of UV-B induced damage	Jansen et al. (2017); Jordan (1996); Mackerness et al. (1996)
Biochemistry		
Membranes	Peroxidation of lipid	Giordano et al. (2004)
Phytohormones	Photooxidation of IAA	Ros and Tevini (1995)
Secondary metabolism	Accumulation of phenylpropanoids and antioxidants; Accumulation of alkaloids, waxes and polyamines	Downey et al. (2006); Jansen et al. (2017); Schreiner et al. (2014); Schreiner et al. (2017)
Molecular biology		
UV-B photoreceptor	Activation of UVR8 (induced by low fluence UV-B)	Brown and Jenkins (2008); Falginella et al. (2012)
Gene expression	Up-regulation of genes of the phenylpropanoid pathway; Down-regulation of photosynthetic genes	Falginella et al. (2012); Jenkins (2017); Kennedy et al. (2012); Liu et al. (2015); Jordan et al. (1992); Jordan (1996); Mackerness et al. (1997); Strid et al. (1990)
DNA repair	Formation of cyclobutane-pyrimidine dimers (CPDs) and (6–4) photoproducts	Britt (1996); Britt (2004); Schmitz-Hoerner and Weissenböck (2003); Walbot (1999)

2.1.1 UV-B perception

Many photoreception mechanisms in plants, which monitor the light environment and regulate plant growth and development, have been studied. The identification and characterization of photoreceptions and photoreceptor molecules in plants depends on the absorption of different electromagnetic wavelengths (Briggs et al., 2001; Briggs and Olney, 2001; Kagawa, 2003; Quail et al., 1995; Yang et al., 2018). They can be divided into the red/far-red photoreceptor phytochrome and the blue/UV-A light receptors cryptochrome and phototropin (Briggs and Olney, 2001; Short and Briggs, 1994). However, UV-B perception and its signal pathways are far from being widely understood.

Research has shown that UV-B has a wide ranging impact on plant cells and gene expression and the different fluence rates, duration and wavelengths induced substantially different responses in plants (Jenkins and Brown, 2007). The effectiveness of UV wavelengths for photomorphogenesis had maxima of 295-300 nm (Jenkins, 2009; Jiang et al., 2012; Wellmann, 1976; Wellmann, 1983). In the latest research, the action spectra for photomorphogenic UV-B responses showed maximal photon effectiveness at 280-300 nm in Arabidopsis (Díaz-Ramos et al., 2018). Examples of photomorphogenic UV-B responses include the promotion of cotyledon opening, the inhibition of hypocotyl extension and the stimulation of flavonoid biosynthesis (Brown et al., 2005; Brown and Jenkins, 2008; Jenkins, 2009; Kalbina et al., 2008). These responses may be associated with a recently discovered component of a specific UV-B signalling pathway mediated by the UV-B photoreceptor UV RESISTANCE LOCUS8 (UVR8) (Jenkins, 2009; Rizzini et al., 2011; Wu et al., 2012).

UVR8 is a *β*-propeller protein with seven blade-shaped *β*-sheets with similar sequences to human REGULATOR OF CHROMATIN CONDENSATION1 (RCC1) (Rizzini et al., 2011; Wu et al., 2012), but UVR8 is unlikely to be a functional homologue of RCC1 (Brown et al., 2005). UVR8 is sensitive to wavelengths between 280-315 nm and this induces photomorphogenic responses in the plants (Brown and Jenkins, 2008; Yang et al., 2018). Normally, two molecules of UVR8 are in contact in the cytoplasm as a homodimer. Each UVR8 dimer has

arginine (Arg) across its interface with pyramids of tryptophan (Trp) to build a network of salt-bridges holding the monomers. Arginine residues, mainly Arg-286/338, make intramolecular cation-π interactions with Trp-285/233 to form a stable homo-dimeric interface. Also, Trp-285/233 strongly absorbs UV-B wavelengths as a UV-B chromophore (Jenkins, 2014a; Jenkins, 2014b). However, UV-B exposure disrupts the salt-bridges leading to the dissociation of dimers and the UVR8 dimers are then rapidly broken down into monomers and transported into the nucleus (Heilmann and Jenkins, 2013). Moreover, this monomerisation has a conformational change to expose the C-terminus of UVR8, which interacts with E3 ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) (Favory et al., 2009; Rizzini et al., 2011). The UVR8-COP1 complex controls the photomorphogenic UV-B response by UV-B perception, activation of gene expression and the acclimation of UV-B in plants (Rizzini et al., 2011; Yin et al., 2015). Meanwhile, UVR8 binds with chromatin inducing the ELONGATED HYPOCOTYL5 (HY5) and ELONGATED HYPOCOTYL5 HOMOLOG (HYH) (Brown et al., 2009; Brown and Jenkins, 2008; Díaz-Ramos et al., 2018; Yang et al., 2018). These are the transcription factors for photomorphogenic and specific defence responses to UV-B (Brown et al., 2005; Jordan et al., 2016).

In vivo, monomers re-associate to synthesise new dimers following UV-B exposure with purified UVR8 (Heilmann and Jenkins, 2013). The regeneration of UVR8 dimers is involved in REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2, which bind to the UVR8 C-terminal region (Cloix et al., 2012). The RUP1 and RUP2 proteins are negative regulators of the UVR8 pathway that replace COP1 to disrupt UVR8-COP1 interaction (Gruber et al., 2010; Yin and Ulm, 2017). This re-dimerisation maintains the balance and is not an excessive response in plants (Jenkins, 2014b).

2.1.2 UV-B signal transduction

The response to UV-B in plants can be identified into two types of UV-B signal pathways (Fig. 2.1). One is a non-specific UV-B signal pathway, also known as the high fluence UV-B

response (1.0 µmol/m^2.s and above), and the other is a specific UV-B signal pathway (the low fluence UV-B response, 0.1 µmol/m^2.s and below) (Rizzini et al., 2011; Wu et al., 2012).

Non-specific UV-B signal transduction pathway

Plants are exposed to high UV-B fluence rate through the non-specific UV-B signal pathway (Brown and Jenkins, 2008). This pathway overlaps with DNA damage signalling, reactive oxygen species (ROS) signalling and defence/wound signalling (Cloix et al., 2012; Jenkins and Brown, 2007). In responses to high UV-B fluence rate, the generation of excess reactive oxygen species (ROS) in plants is mediated by DNA damage signalling. DNA damage by short wavelength UV-B high energy in plants is induced by the formation of cyclobutane pyrimidine dimers (CPD) and pyrimidine [6-4] pyrimidone dimers (Britt, 1999; Britt, 2004).

ROS can act as signalling molecules in response to abiotic and biotic stresses and induce up-regulation and down-regulation of genes. In plants, UV-B dramatically increases accumulation of ROS, which can be involved in the regulation of gene expression (Apel and Hirt, 2004). The transcript levels of the LHCB1 gene encoding light harvesting complex binding proteins are decreased by high levels of UV-B in plants, especially the chlorophyll-binding protein of chloroplasts (Surplus et al., 1998). The damage to proteins involves photosynthetic electron transport, resulting in a reduction in the ability of excitation energy dissipation and excess ROS generation (Jansen et al., 1998). Moreover, some researchers posit that UV-B induces the ROS signal pathway by enzyme inhibition and ROS-scavenging systems (A-H-Mackerness et al., 2001; Dai et al., 1997). For example, the superoxide radical, which is a main form of ROS, can be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase activity (Barta et al., 2004).

UV-B stimulates gene expression from wounds/pathogens/defences signal pathways, including pathogenesis-related-1 (PR-1), defence-related and proteinase inhibitor genes (Jenkins, 2009). The signalling intermediates in response to UV-B in plants are ROS, jasmonic acids (JA), salicylic acid (SA) and ethylene (Jordan et al., 2016; Whitelam and Halliday, 2008). ROS generation is an early step in response to high levels of UV-B in *Arabidopsis thaliana* and

promotes the accumulation of SA, JA and ethylene, but the accumulation of SA is slower than for JA and ethylene. Further research found that transgenic NahG *A. thaliana* was unable to accumulate SA due to a reduction in the transcription of PR under UV-B treatment. In summary, UV-B down-regulates photosynthetic genes through ROS-dependent, but SA-independent, pathways and up-regulates PR genes through ROS- and SA-dependent pathways (A-H-Mackerness et al., 1999; Surplus et al., 1998).

Specific UV-B signal transduction pathway

Compared with the non-specific high fluence UV-B responses, photomorphogenic UV-B responses under specific low fluence UV-B can be characterized as including cotyledon opening, hypocotyl extension and flavonoid biosynthesis (Ballaré et al., 1995; Boccalandro et al., 2001; Brown and Jenkins, 2008; Suesslin and Frohnmeyer, 2003; Wellmann, 1976). Studies found that the stress/wound/defence signalling molecules were not mediations in response to the low fluence UV-B in plants (Jenkins and Brown, 2007; Kalbina et al., 2008). Although low fluence UV-B cannot stimulate the accumulation of JA, SA and ethylene or ROS, chalcone synthase (CHS) expression is induced by it (A-H-Mackerness et al., 1999). Low fluence UV-B induces gene expression of CHS that is involved in an increase in the activity of flavonoid biosynthetic pathway (Brown et al., 2009; Brown and Jenkins, 2008; Heilmann and Jenkins, 2013; Hofmann, 2012; Jenkins, 2009; Wu et al., 2012). It has been shown that a specific UV-B signalling pathway mediates the photomorphogenic UV-B response.

UVR8 acts as the specific UV-B photoreceptor for the low fluence UV-B signal transduction pathway. UVR8 interacts with transcription factors, such as COP1 and HY5, to mediate the photomorphogenic UV-B response (Binkert and Ulm, 2017; Brown and Jenkins, 2008; Jenkins, 2014a). In many cases, the UV-B responses can be divided into being dependent and independent of UVR8 (Brown and Jenkins, 2008). The UVR8-dependent pathway is involved in the flavonoid biosynthetic pathway by low fluence UV-B (Brown et al., 2005; Cloix et al., 2012). In *Arabidopsis* wild-types, UV-B induces the regulation of UVR8 gene expression for flavonoid biosynthesis, DNA repair and the generation of antioxidants, but there is no UV-B photomorphogenic response in *Arabidopsis uvr8* mutants (Brown et al., 2005; Favory et al.,

2009; Jenkins, 2014a). Rizzini et al. (2011) found that the *uvr8* mutants cannot interact with COP1, which reduced the UV-B photomorphogenic responses. Moreover, CHS expression decreased in the *uvr8-1* mutant, which reduced the level of flavonoid biosynthesis (Brown et al., 2005; Jenkins, 2014b). This evidence supports that UVR8 is a main mediator in the specific UV-B signal transduction pathway, inducing flavonoid biosynthesis.

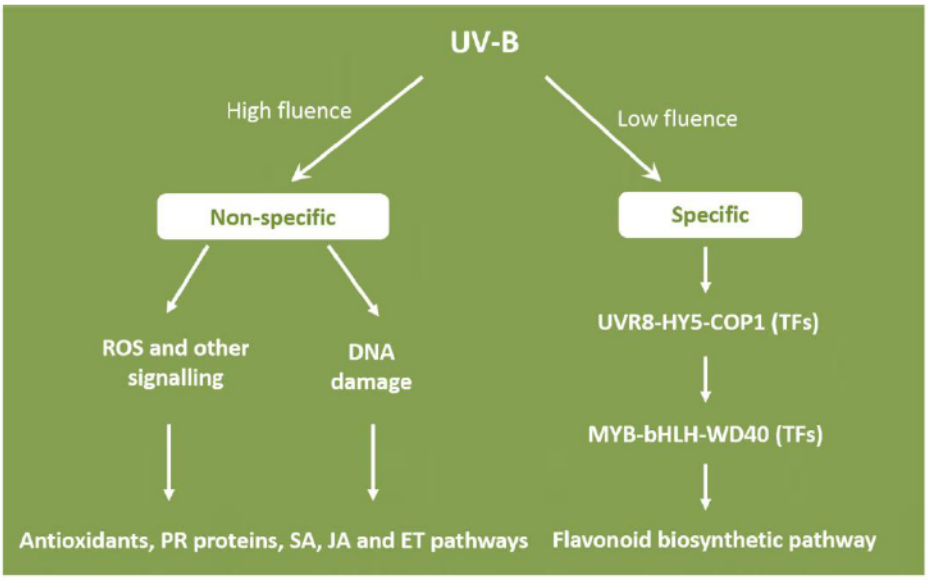


Figure 2.1 Schematic diagram to represent the non-specific and specific UV-B signal transduction pathway

ROS, reactive oxygen species; PR, pathogen-related; SA, salicylic acid; JA, jasmonic acid; ET, ethylene; UVR8, UV RESISTANCE LOCUS 8; HY5, ELONGATED HYPOCOTYL 5; COP1, CONSTITUTIVELY PHOTOMORPHOGENIC 1; TFs, transcription factors; MYB, myeloblastosis family; bHLH, basic helix-loop-helix domain protein; WD40, WD40-repeat protein (Liu et al., 2015); Figure 2.1.

2.1.3 Effects of UV-B on plants

Plants chronically exposed to sunlight have less UV-B related damage, due to the development of a range of mechanisms to protect themselves against it. Most UV-B radiation will be reflected by surface wax and absorbed by protecting compounds in the leaf epidermis. However, a small amount of UV-B can pass the anticlinal cell walls to damage the exposed mesophyll and palisade cells (Jordan, 1996). Plants' physiological and metabolic responses to UV-B radiation have recently been investigated. UV-B (0.45 W/m^2, 6h) caused a reduction of cell division in plants through accumulating damage from DNA (the formation

of cyclobutane pyrimidine dimers (CPD) and pyrimidine [6-4] pyrimidone dimers) (Jiang et al., 2011). Also, UV-B exposure (25 kJ/m^2.d) can affect cell expansion and proliferation in plants (Hectors et al., 2010; Wargent et al., 2009), because cell sizes increased with the decreased number of cells on the leaf surface, when *Arabidopsis* wild-types were exposed to UV-B (Hepworth and Lenhard, 2014; Wargent et al., 2009). Furthermore, UV-B increases the thickness of the mesophyll resulting in an increase in leaf thickness (Jansen et al., 2017). These consistent observations have also been shown in blueberry and birch leaves (Reyes-Díaz et al., 2016; Robson and Aphalo, 2012).

UV-B induces the accumulation of secondary metabolites in plants. The phenolic composition of plants, especially flavonoids, can be dramatically increased due to the regulation of the flavonoid biosynthetic genes by low fluence UV-B irradiation (see above) (Jenkins, 2014a; Liu et al., 2015; Rizzini et al., 2011). In plants, UV-B also affects the synthesis of carotenoids, which are tetraterpenoids (one class of isoprenoid composition) and precursors to aroma compounds (Schreiner et al., 2017). Carotenoids, as photosynthetic pigments, are involved in photoprotection and react with free radicals, such as ROS and superoxide (Edge and Truscott, 2018; Jansen et al., 1998). Moreover, UV-B causes an increase in carotenes and xanthophylls, which are associated with non-photochemical quenching, to protect photosynthetic machinery against over excitation and ROS (Jansen et al., 1998; Taiz et al., 2015), but carotenoid biosynthesis is impeded by chronic UV-B (Jansen et al., 2008). Finally, high fluence UV-B stimulates non-specific signal transduction by the up-regulation of genes through JA, SA and the ethylene pathway (see above) (Jordan et al., 2016; Whitelam and Halliday, 2008). Therefore, the different fluence of UV-B induce the different signalling transduction pathways intermediating secondary metabolites in plants.

2.2 Water deficit and its effects on plants

In plants, water is the most abundant resource to support growth and cell functions, and accounts for the largest percentage of cellular volume (Pallardy, 2010). About 97% of water uptake into plants is lost to the atmosphere, predominantly by transpiration. About 2% is

used for cell volume or cell expansion, and 1% is used for metabolism, such as photosynthesis (Taiz et al., 2015). Therefore, water is a key resource potentially limiting agricultural and horticultural productivity (Manavalan and Nguyen, 2012). A water deficit or drought involves insufficient water availability resulting in the limitation of plant growth (Bohnert and Jensen, 1996). Drought can be divided into three classes, agricultural drought, meteorological drought and hydrological drought. Meteorological drought is a period of insufficient precipitation that can lead to agricultural and hydrological drought. Agricultural drought is associated with precipitation shortages, differences between actual and potential evapotranspiration, soil water deficits and reduced groundwater or reservoir levels. Hydrological drought focuses on the effects of periods of precipitation (including snowfall) shortfalls on surface or subsurface water supply (i.e., streamflow, reservoir and lake levels, groundwater) (Vicente-Serrano et al., 2012; Wang et al., 2011). In addition, high evapotranspiration (high temperature, low relative humidity and high wind) and poor irrigation management are two other main reasons for drought (Palmer, 1965; Tate and Gustard, 2000).

2.2.1 Water stress signal perception and second messengers

In stress signal transduction, many receptors are found to sense stress. Water stress causes an increase in the concentration of cytosolic Ca^{2+} (Knight, 1999; Sanders et al., 1999). This indicates that the Ca^{2+} influx through ligand-sensitive Ca^{2+} channels is one of sensors for water stress (Xiong et al., 2002). Moreover, ligands, such as cyclic ADP ribose, nicotinic acid adenine dinucleotide phosphate and inositol polyphosphates, act as a second messenger to induce internal Ca^{2+} release in plant cells (Schroeder et al., 2001).

Phospholipids are not only the backbones of cell membranes, but also are precursors of the second messengers. This is because water deficit stimulates the expression of phosphoinositide-specific phospholipase C (PI-PLC) that contributes to an increase in phosphatidylinositol 4,5-bisphosphate (PIP_2) (Xiong et al., 2002). PIP_2 is then hydrolysed into diacylglycerol and inositol 1,4,5-trisphosphate (IP_3), which are the second messengers for

the activation of protein kinase C and Ca^{2+} release, respectively, under water stress (Schroeder et al., 2001; Shinozaki and Yamaguchi-Shinozaki, 1997). Phospholipase D (PLD) also participates in water stress signal transduction, which can hydrolyse phospholipids into phosphatidic acid (PA) (Kopka et al., 1998). In guard cells, PLD activity contributes to ABA-induced stomatal closure and leads to the generation of PA in response to drought. However, excess PLD activity can act against stress tolerance, because PA, a non-bilayer lipid, can influence cell membrane curvature resulting in destabilising membranes (Jacob et al., 1999; Wang, 1999). Furthermore, water stress stimulates the accumulation of ROS and these act as signals for inducing ROS scavengers against the oxidative damage in plants (Krasensky and Jonak, 2012; Xiong et al., 2002). So, ROS are intermediate signals for the activation of Ca^{2+} channels in guard cells, abscisic acids (ABA) in mediating the expression of the antioxidant Catalase 1 (*CAT1*) gene and stomatal closure (Guan et al., 2000).

2.2.2 Water stress signal transduction

ABA is an important signalling intermediate that controls gene expression in response to stresses, but some genes are not controlled by ABA. Therefore, two systems, ABA-dependent and ABA-independent pathways, are involved in drought-induced genes (Figure 2.2) (Ahuja et al., 2010; Shinozaki and Yamaguchi-Shinozaki, 1997; Shinozaki and Yamaguchi-Shinozaki, 2007).

Many genes are regulated by endogenous ABA under water stress, such as zeaxanthin epoxidase gene (*ZEP*), molybdenum cofactor sulfurase gene (*MCSU*) and aldehyde oxidase gene (*AAO3*) (Cheng et al., 2002; Valliyodan and Nguyen, 2006; Zhu, 2002). Two ABRE motifs are major *cis*-acting elements controlling ABA-responsive gene expression (*RD29B*) (Sakuma et al., 2002). Two basic leucine zipper (bZIP) transcription factors, ABRE-binding protein (AREB)/ABRE-binding factors (ABF), can bind to ABRE to activate ABA-dependent gene expression (Choi et al., 2000; Uno et al., 2000). ABA also mediates the induction of the drought-induced *RD22* gene, which is bound in other important transcriptional factors (MYC and MYB proteins) in the ABA-dependent pathway (Abe et al., 2003; Abe et al., 1997). After

the accumulation of endogenous ABA, these two proteins are synthesised, as this defines their roles in the later phase stress responses (Shinozaki and Yamaguchi-Shinozaki, 2007). Furthermore, this identifies a drought-induced *RD26* gene encoding a NAC transcription factor. RD26 protein has transcriptional activity in the nucleus (Fujita et al., 2004).

Another signal transduction response to a water deficit is the ABA-independent pathway. The *RD29A/COR78/LT178* gene is induced by water stress, including the two major *cis*-acting elements, ABRE (ABA-dependent regulation) and DRE (dehydration-responsive element)/CRT (Yamaguchi-Shinozaki and Shinozaki, 1994; Yamaguchi-Shinozaki and Shinozaki, 2005). Transcription factors belonging to the ERF/AP2 family that bind to DRE/CRT elements are isolated and called CBF/DREB1 and DREB2. Only DREB2 function relates to drought-responsive gene expression. Dehydration stress induces *DREB2* homologues' expression, in particular, *DREB2A* and *DREB2B* at high levels (Liu et al., 1998; Nakashima et al., 2000; Sakuma et al., 2002). Therefore, DREB2A and DREB2B are considerate as the major transcription factors in the drought-responsive function (Sakuma et al., 2002). However, *DREB2A* overexpression in transgenic plants does not reduce plant growth and enhance the stress tolerance. This indicates that the DREB2A protein is involved in post-translational activation, such as phosphorylation, to improve stress responses (Sakuma et al., 2006). Moreover, NAC is another class of transcription factor, which regulates gene expression not only in ABA-dependent, but also in ABA-independent pathways. The *ERD1* is a NAC family member, which is induced by dehydration and up-regulation in response to drought (Agarwal and Jha, 2010; Fujita et al., 2004; Tran et al., 2004).

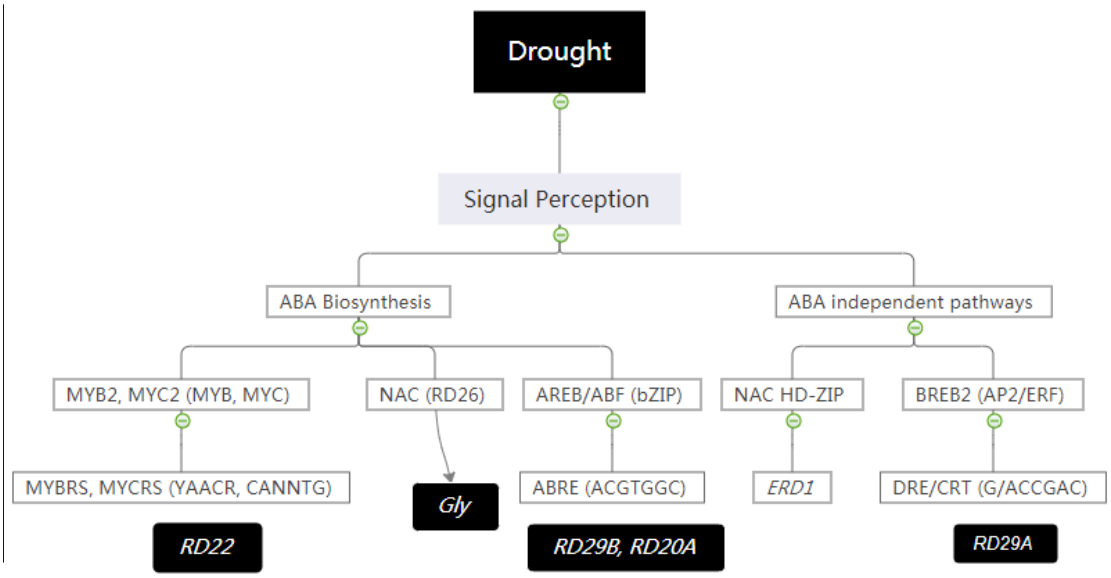


Figure 2.2 Transcriptional regulatory networks of abiotic stress signals and gene expression

Signal transduction pathways exist in drought responses: three are ABA dependent and two are ABA independent (Shinozaki and Yamaguchi-Shinozaki, 2007)(Figure 2).

2.2.3 Effects of water deficit on plants

With global warming, stronger water deficiencies are likely to appear more frequently and impact plant growth and development, resulting in the disruption of cropping development and reductions of breeding stock and yields in the fields (Mickelbart et al., 2015), as plants depend on drought resistance mechanisms to improve their drought tolerance. Drought resistance can be divided into desiccation postponement (the ability to sustain tissue drought tolerance or hydration at high water potential), desiccation tolerance (the ability to sustain tissue drought tolerance or hydration at low water potential) and drought escape (the ability to complete the life cycles and maintain the reproduction before the beginning of water deficit) (Manavalan and Nguyen, 2012). Meanwhile, most plants respond to water deficit by dehydration avoidance or dehydration tolerance to maintain their biological functions (Bray, 1997). Dehydration avoidance is the ability to maintain high plant water status through increasing water uptake or decreasing water loss under water deficit, so plants have large and deep root systems to absorb water from soil or close stomata to decrease transpiration (Kramer and Boyer, 1995). Moreover, plant physiological

characteristics and metabolic activities contribute to dehydration tolerance, such as osmotic adjustment, ROS production and the changes of biochemical metabolites (Reddy et al., 2004).

Vitis vinifera has been generally classified as a "drought avoiding" species through its control of stomatal conductivity, which manages transpiration rate (Chaves et al., 2010; Poni et al., 2014). Based on the physiological classification, stomatal behaviour in response to drought can be divided into isohydric and anisohydric genotypes (Schultz, 2003; Tardieu and Simonneau, 1998). The isohydric stomatal behaviour ('pessimists') would modify the growth and physiology for the conservation of current resources, while the anisohydric stomatal behaviour ('optimists') use all the resources available (Schultz, 2003; Tardieu and Simonneau, 1998). Two stomatal behaviours in response to water stress attribute the sensitivity of stomata to ABA concentration in the xylem sap. The isohydric genotype has higher ABA concentration in the xylem sap than the anisohydric genotype. There is an evidence of a midday increase in the expression of key genes involved in ABA biosynthetic pathway, higher in leaves of the isohydric genotype than in the anisohydric genotype (Chaves et al., 2010; Soar et al., 2006). In previous research, stomatal behaviour in Pinot noir is anisohydric when water stress is applied at pre-veraison and is isohydric when it is applied at post-veraison (Lovisolo et al., 2010; Poni et al., 1993). Additionally, the environmental conditions can convert stomatal behaviours as isohydric or anisohydric in the same variety (Lovisolo et al., 2010).

Leaf water potential (LWP) is an index indicating water status of a whole plant, so the reduction of LWP is the primary response to a low relative soil water status and humidity of the atmosphere, leading to a rapid decrease in cellular dehydration (Taiz et al., 2015). In addition, plants control their stomatal apertures to avoid water loss through transpiration (Hale and Orcutt, 1987). Leaf water potential drops in anisohydric cultivars under restricted soil water status or high atmospheric demand, whereas isohydric cultivars maintain their leaf water potential above a certain threshold, regardless of soil water status or atmospheric water demand. Based on this knowledge, vines behave as isohydric in which LWP rarely

drops below -1.5 MPa, regardless of soil water availability (Lovisolo et al., 2010). Isohydric cultivars also include decreases in stomatal conductance and the transpiration rate for keeping the vine water status constant (Lovisolo et al., 2010; Schultz, 2003). In Mejias-Barrera (2016) study, there was no difference between the control and the reduced irrigation treatment in stem water potential, but the stomatal conductance was decreased by the reduced irrigation. Thus, it suggested that the stomatal behaviour of Pinot noir is isohydric in the Waipara region (Canterbury).

A secondary effect of water deficit is osmotic adjustment, which is the intracellular accumulation of organic solutes, such as proline (Pro) and osmotin (Delauney and Verma, 1993; Shinozaki and Yamaguchi-Shinozaki, 2007). Osmotic adjustment maintains cell turgor with decreasing water potential and sustains stomatal conductance and photosynthesis at a low water potential. The purpose of osmotic adjustment in response to water deficit is to absorb more water from the soil, induce root growth and delay leaf abscission and death (Hsiao et al., 1976).

Furthermore, ROS act as signalling intermediates to regulate the response of plant growth to water deficit (Huang et al., 2012), because ROS induce oxidative damage under water stress. In plants, ROS are hydrogen peroxide (H_2O_2), superoxide and singlet oxygen in chloroplasts, mitochondria and peroxisomes that leads to damage of cellular membranes and photosynthesis, and potentially cell death. For the protection of plant tissues, ROS scavenging mechanisms induce superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and ascorbate peroxidase (APX) under water stress (Manavalan and Nguyen, 2012). Therefore, water deficit induces different changes in physiological characteristics and metabolites in plants.

2.3 Effects of UV-B interaction with water deficit on plants

Interactions between UV-B exposure and water deficit in plants has been the subject of research for about 30 years (Alexieva et al., 2001; Balakumar et al., 1993; Bandurska et al.,

2013; Tian and Lei, 2007). Water deficit plays an important role in crop qualitative and quantitative parameters and dramatically affects the responses induced by UV-B radiation (Balakumar et al., 1993; Jordan, 1996). For example, in *Nerium oleander*, there is no trend for lower plant leaf area under enhanced UV-B radiation, but the combination of UV-B radiation and water deficit dramatically reduces the leaf area (Drilias et al., 1997). Similar results are shown in plant height, leaf mass and leaf number. In another study, barley leaf hydration level decreases to a similar level as in first action of water deficit, but the response is earlier under enhanced UV-B and water deficit (Bandurska and Cieślak, 2013). Thus, effects of UV-B radiation on growth depend on the water status.

Sullivan and Teramura (1990) studied, under well-watered conditions, the enhanced UV-B decreased photosynthetic capacity, plant weight and leaf area in soybean. UV-B interaction with water deficit did not increase the responses compared with the individual stress, which was in agreement with Martinez-Luscher et al. (2015) research of grapevines. Either UV-B radiation or water deficit affects plant gas exchange and photosynthetic pigments (Jordan, 1996). This indicates that water deficit is a significant limitation to gas exchange because of the reduction of stomatal conductance, compared with the effects of UV-B radiation on its own or in combination with water deficit in grapevine leaves (Martinez-Luscher et al., 2015). In addition, water deficit could delay cell division, resulting in the decrease in soybean growth. Reduced growth was identified as a means of UV-B protection, when soybean was damaging under UV-B during cell division. Therefore, water deficit may be opposite to UV-B damage (Sullivan and Teramura, 1990).

A number of alterations have been reported in the literature about changes in plant secondary metabolism under UV-B radiation interaction with water stress. They cause the generation of ROS and H_2O_2, which induces the synthesis of JA, SA and ethylene, the accumulation of flavonoids and the synthesis of amino acids and proteins (Bandurska et al., 2013). For instance, in *A. thaliana*, water deficit increases the accumulation of sugar and soluble phenolics, while the combination of drought and UV-B radiation (5.5 KJ/m^2 per day) cause the inhibition of soluble sugar accumulation and a decrease in osmotic adjustment

(Poulson et al., 2006). In wheat and peas, UV-B and water deficit increase catalase activity and flavonoid levels to reduce oxidative damage (Alexieva et al., 2001; Feng et al., 2007). Similar results in tobacco show that UV-B interaction with water deficit cause the overexpression of aldose/aldehyde reductase involving the detoxification of lipid peroxidation and the reduction in H_2O_2 levels (Hideg et al., 2003). In barley seedlings, UV-B and water deficit cause an increase in SA concentration (Bandurska and Cieślak, 2013). As UV-B induced cross resistance to drought increases, the protective mechanism of SA may improve leaf water status through osmotic adjustment and stomatal closure, and up-regulate the activity of the antioxidant system (Gao et al., 2004; Saruhan et al., 2012). Thus, the combination of UV-B radiation and water deficit modifies the plant morphology and physiology, but the responses of plants to stresses depend on the species (Jordan, 1996).

2.4 Measurable physiological responses in Pinot noir

Leaves are the primary site of the light reactions of photosynthesis and the source of carbohydrate biosynthesis. Carbohydrate and water will be transferred to other tissues, primarily those that act as sinks, to support vine growth and berry development (Jordan et al., 2016; Lemoine et al., 2013; Palliotti et al., 2011; Taiz et al., 2015). Also, leaves are the location of nitrogen assimilation, fatty acid biosynthesis and the synthesis of volatile compounds (Schreiner et al., 2014; Schreiner et al., 2012; Wallsgrove et al., 1979). Given the role leaves play in the functioning of the vine, one would expect there would be measurable responses to changes in plant water availability and/or UV exposure.

2.4.1 Leaf chlorophyll content

Chlorophyll is a characteristic green pigment in plant leaves which enables them to capture energy from sunlight (Taiz et al., 2015). Plant photosynthetic organisms contain two types of chlorophylls, bluish green chlorophyll a (Chl *a*) and yellowish green chlorophyll b (Chl *b*). The content of Chl *a* in leaves is three times higher than Chl *b* (Palta, 1990). In leaf tissues, chlorophyll is found between the protein and lipid layers of the chloroplast lamellae. The

chlorophyll molecules include porphyrin and phytol, which are bound to the protein and lipid layers, respectively (Staehelin, 2003; Taiz et al., 2015). The absorption spectrum of chlorophyll shows strong absorption in blue light (about 430 nm) and red light (about 660 nm) (Taiz et al., 2015). Chlorophyll content in leaf tissues is influenced by environmental stresses, such as high sunlight, water stress, cold and salinity (Chaves et al., 2002; Giri et al., 2003; Larsson et al., 1998). The SPAD 502 Chlorophyll Meter (Konica Minolta Inc., Japan) is a hand-held device and can be rapidly and accurately used for the non-destructive evaluation of 'leaf greenness'. It measures transmittance by leaves at 650 nm (red light), which is highly absorbed by chlorophyll, and 940 nm (infrared light), which is not absorbed (Hoel and Solhaug, 1998; Martínez and Guiamet, 2004). Furthermore, the SPAD 502 Chlorophyll Meter is developed to monitor not only the chlorophyll content of vine leaves but also indirectly the nitrogen content of vine leaves (Maas and Dunlap, 1989; Minolta, 1989). However, there are disadvantages of SPAD meter measurements. It can measure only one spot on each measurement (Murdock et al., 2004). In addition, the values of SPAD meter measurement are affected by changing growth or environmental conditions that could lead to a redistribution of chloroplasts and uneven distribution in mesophyll cells (Nauš et al., 2010). Therefore, measurements of multiple spots of the same leaf and several leaves of each plant must be taken to get a reliable average (Ling et al., 2011).

2.4.2 Leaf water potential

Leaf water potential (LWP) is a sample indicator of leaf water status. LWP is measured using a pressure chamber, which can assess the water status of plants, and plays a role as a functional model for stomatal conductance of plants. A time-domain reflectometer (TDR) can be used to estimate the soil water content. When the results of TDR are used in combination with leaf water potential, vine water status can be evaluated at different soil moisture statuses and under different water application regimes. This result can also be correlated with vegetative and reproductive growth and yield (Centeno et al., 2010).

2.4.3 Carbon isotope ratio in grapevines

As with water, carbon dioxide (CO_2) is an important element of carbohydrate production through photosynthesis in plants. So, measurements of abundant stable isotope carbon atoms in plant tissues is a way to evaluate useful information about photosynthesis (Taiz et al., 2015). In nature, ^{12}C and ^{13}C are two stable carbon isotopes, and the lighter ^{12}C accounts for 98.93%, in comparison with 1.07% for the heaver ^{13}C (Brugnoli and Farquhar, 2000). In the atmosphere, the lighter carbon isotope in CO_2 molecules (^{12}C) is preferentially fixed by photosynthesis to plant tissues (Farquhar et al., 1989). Due to an effective diffusion fractionation factor, $^{12}CO_2$ has a greater diffusion rate than $^{13}CO_2$ across stomatal pores. In the carboxylation step, $^{12}CO_2$ is preferentially utilised to $^{13}CO_2$ by ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), because rubisco has higher reactivity to ^{12}C than to ^{13}C (Farquhar et al., 1989). In grapevine tissues, the $^{13}C/^{12}C$ ($\delta^{13}C$) is determined by the gradient of CO_2 concentration between atmospheric and intercellular spaces (C_i/C_a), which is influenced by environmental stresses, in particular, drought (Farquhar et al., 1982; Gaudillère et al., 2002). $\delta^{13}C$ in the whole plant is dominated by the assimilation and diffusion of CO_2 into leaves, but internal metabolism and partitioning of primary assimilates may produce differences in $\delta^{13}C$ among plant organs, particularly deciduous woody species, such as grapevines (Brugnoli and Farquhar, 2000; Leavitt and Long, 1985). The $\delta^{13}C$ value of leaf tissue shows not only C_i/C_a and environmental influences of the growing season, but also reflects carbon assimilation and allocation (Gaudillère et al., 2002). Numerous researches have shown that the environmental factors, such as increased temperatures (O'Leary, 1988), atmospheric CO_2 concentrations (Policy et al., 1993) and soil water content (Livingston et al., 1999), influenced stable isotope composition in plant tissues. For grapevines, sucrose containing $\delta^{13}C$ (isotope composition) is translocated from leaves to grapes and converted into fructose and glucose, during ripening (Leavitt and Long, 1985). Thus, the investigation of $\delta^{13}C$ in leaves and mature grapes targets leaf photosynthetic carbon isotopic discrimination (Centritto et al., 2009; Chaves et al., 2003).

2.5 Chemical composition in Pinot noir fruit

There are abundant chemicals found in grapes, such as phenolic compounds, amino acids and volatile compounds. The chemical composition of grapes at harvest is a contributor to fruit quality characteristics (Keller, 2015).

2.5.1 Amino acids

Amino acids and their biosynthesis are important for all living things. Amino acids are the subunits for proteins and enzymes and are also nitrogen and energy sources for yeast and bacterial metabolism (Bender, 2012; Nelson et al., 2008). In viticulture, amino acids in grapes are precursors of aromatic compounds being metabolised to higher alcohols, aldehydes, organic acids, phenols and lactones (Keller, 2015).

Nitrogen (N) is an essential element for amino acid biosynthesis and flows between environmental and life forms. In the soil, nitrogen content can reach from 1% to 6% and atmospheric nitrogen occupies 78%. There are four main processes in the nitrogen cycle. The first process is nitrogen fixation by plant material and bacteria to produce organic nitrogen, as in legumes, rhizobia and symbiosis. Secondly, ammonification converts organic nitrogen to ammonia. Next, is nitrification where plants absorb nitrate ions (NO_3^-) from the soil solution. Finally, urea from animal urine and urea fertilisers can also transfer in ammonium ions (NH_4^+). For grapevines, NO_3^- is the main form for nitrogen assimilation and is absorbed by roots and converted into organic nitrogen compounds, such as amino acids. The first step in nitrogen assimilation is the reduction of nitrate to nitrite in the cytosol, being catalysed by nitrate reductase (NR). Nitrite is a highly reactive, potentially toxic ion. It can be reduced by nitrite reductase (NiR) to NH_4^+ from the cytosol into chloroplasts in leaves and plastids in roots. In grape leaves, NH_4^+ is assimilated into amino acid via glutamine synthetase (GS) and glutamate synthase (GOGAT) (Fig. 2.3) (Andrews et al., 2013; Lam et al., 1996). Grapevine roots have the capacity to take up NO_3^-, NH_4^+ and amino acids (Keller et al., 2001). There are two main types of glutamine synthetase: GS1 (in the cytosol of all

grapevine organs and in the phloem companion cells) and GS2 (in the plastids of photosynthetic tissues and roots). The cytosolic GS1 is central to ammonium assimilation in the roots, whereas the leaf mesophyll is the predominant site for GS2 from ammonium assimilation (Keller, 2015). So, grapevine roots, shoots, leaves and berries can assimilate nitrate to amino acids, and metabolically active glutamine (Gln) is the major nitrogen transport compound in the xylem (Loulakakis and Roubelakis-Angelakis, 2001).

In grape berries, Gln is converted into other amino acids by aminotransferases, such as Pro and Arg, which occupy the greatest percentages of total amino acids in grapes (Stines et al., 2000). Arg can be used by yeast, but while Pro cannot be used in wine fermentation, it can serve to protect cells from excessive osmotic stress (Long et al., 2012). Pro and Arg biosynthetic pathways are from glutamate (Glu) via many enzymes (Fig. 2.4). Glu is converted into glutamate-semialdehyde (GSA) via pyrroline-5-carboxylate synthetase (P5CS), and automatically transforms to pyrroline-5-carboxylate (P5C). Pyrroline-5-carboxylate reductase (P5CR) catalyses P5C to Pro. The proline biosynthetic pathway occurs in the cytosol and chloroplasts. The proline catabolic pathway happens in mitochondria via proline dehydrogenase (PHD) and P5C dehydrogenase (P5CDH). Furthermore, proline can be synthesised from ornithine by ornithine-delta-aminotransferase (OAT) to GSA and P5C. Also, arginine biosynthesis is from ornithine via ornithine transcarbamylase (OTC) to produce citrulline intermediates. The activities of argininosuccinate synthase (AS) and argininosuccinate lyase (AL) catalyse citrulline to Arg (Szabados and Savouré, 2010).

The total amino acids concentration in grapes increases from veraison to harvest. At harvest, they can account for over 90% of the nitrogen content in musts (Bell and Henschke, 2005). The concentration of Pro in grape juices increases during ripening and reaches a peak pre-harvest, from there, it can slowly reduce until harvest, whereas Arg concentration rises from veraison to harvest (Berdeja et al., 2014; Stines et al., 2000). The high concentrations of Pro, threonine (Thr), glycine (Gly), serine (Ser), alanine (Ala), and methionine (Met) in wines are involved in the sweet taste, whereas arginine, lysine (Lys), histidine (His), phenylalanine (Phe), valine (Val) have a relatively bitter taste. Glutamine (Gln), glutamate (Glu), asparagine

(Asn) and aspartate (Asp) have an umami taste. The concentrations and components of amino acids in grapes are influenced by cultural conditions, rootstock/scion combination, vine management, vineyard location and growing season (Bell and Henschke, 2005; Hufnagel and Hofmann, 2008).

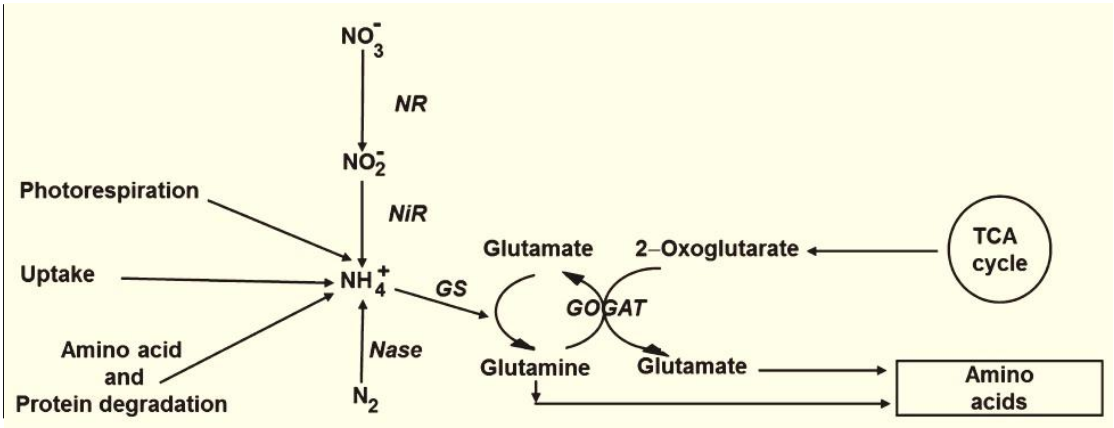


Figure 2.3 The assimilation of nitrogen (N) in higher plants

The main enzymes involved are indicated in italics: NR=nitrate reductase; NiR=nitrite reductase; Nase=nitrogenase; GS=glutamine synthetase; GOGAT =glutamate synthase. The ultimate source of inorganic N available to the plant is ammonium, which is incorporated into organic molecules in the form of glutamine and glutamate through the combined action of the two enzymes GS and GOGAT in the plastid or chloroplast. The precise route by which 2-oxoglutarate is synthesised from the tricarboxylic acid (TCA) cycle in the mitochondria is discussed (Andrews et al., 2013)(Figure 1).

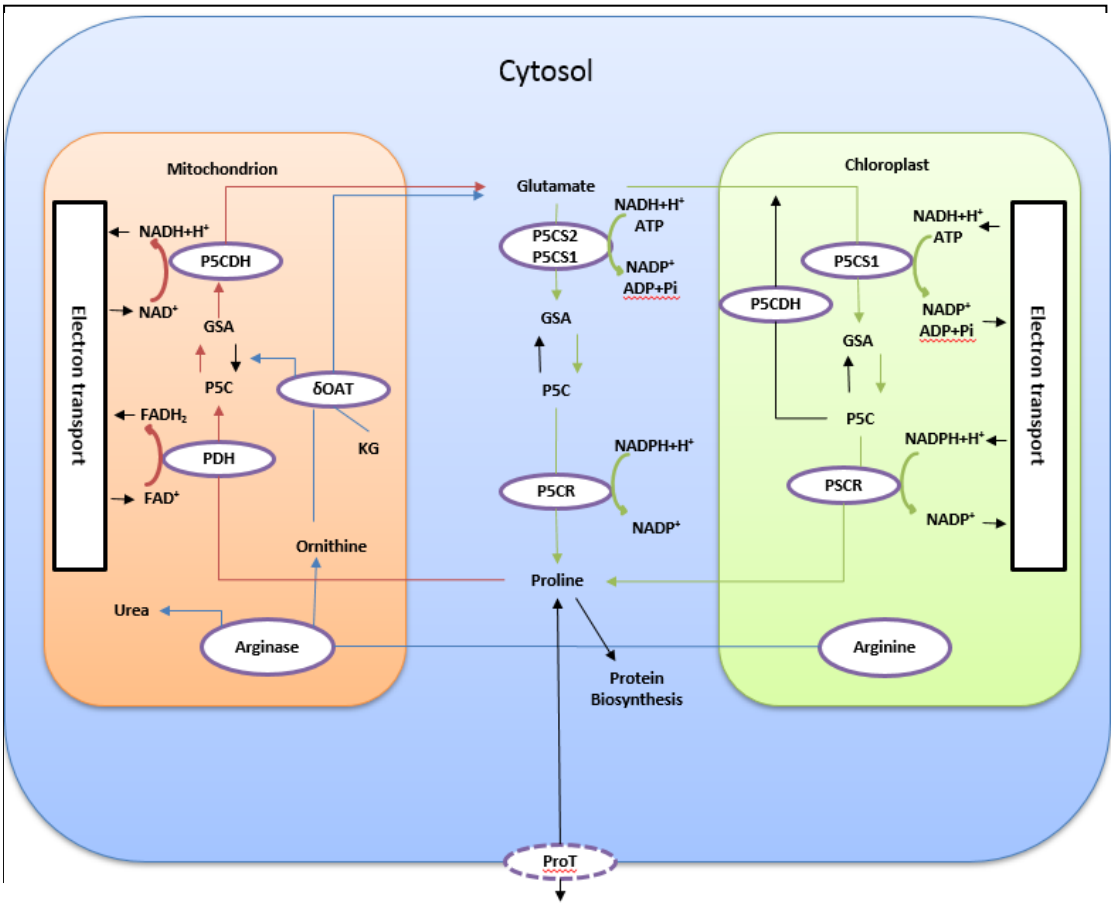


Figure 2.4 Proposed model for proline metabolism in higher plants.

The biosynthetic pathway is marked with green lines, the catabolic pathway with red lines and the ornithine pathway with blue lines. Abbreviations: BAC, basic amino acid transporter involved in arginine and ornithine exchange; Glu, glutamate; G/P, mitochondrial glutamate/proline antiporter; KG, alpha-ketoglutarate; P, mitochondrial proline transporter; Pi, inorganic phosphate; ProT, plasma membrane proline transporter; ?, predicted transporters (Szabados and Savouré, 2010)(Figure 1).

2.5.2 Phenolic composition

Phenolic compounds are a class of the most important plant secondary metabolites and significantly contribute to grape and wine quality. They are made up of six carbon atoms, with one or more hydroxyl groups or derivatives of this basic structure. The hydroxyl groups and unsaturated double bonds lead to phenolic oxidation. Phenolics are divided into non-flavonoids and flavonoids in grapes. Flavonoids are present in high concentrations in grapes (Conde et al., 2007; Kennedy et al., 2006). These are C6-C3-C6 polyphenolic compounds that have a heterocyclic C ring (a three-carbon chain) between two hydroxylated benzene rings. According to the oxidation of the C ring, flavonoids in grapes are divided into three major classes: flavonols, flavan-3-ols and anthocyanins (Conde et al., 2007).

The biosynthesis of flavonoids is via a number of enzyme steps in plants. In Fig. 2.5, the flavonoid biosynthetic pathway resulting in three major classes of flavonoids in grapevines: flavonols, anthocyanins and flavan-3-ols (this class is also referred to as tannins with respect to grapes and wine). This pathway starts from the deamination of phenylalanine by the enzyme phenylalanine ammonia lyase (PAL) via cinnamate-4-hydroxylase (C4H) to 4-coumaryl-CoA. The condensation reaction of one molecule of 4-coumaryl-CoA and three molecules of malonyl-CoA is via chalcone synthase (CHS) to produce chalcone. Chalcone isomerase (CHI) isomerises chalcone to flavanones. Flavanones are converted to dihydroflavonols by flavanone 3-hydroxylase (F3H). Dihydroflavonols are the central intermediates in the pathway. Flavonol synthase (FLS) catalyses dihydroflavonols to flavonols. For the biosynthesis of anthocyanins, dihydroflavonol reductase (DFR) reduces dihydroflavonols to leucoanthocyanidins. Leucoanthocyanidin dioxygenase (LDOX) catalyses leucoanthocyanidins to anthocyanidins. Anthocyanidins are bound to glucose molecules via UDP glucose-flavonoid 3-O-glucosyl transferase (UFGT) producing stable anthocyanins. Proanthocyanidins are converted from anthocyanidins by anthocyanidin reductase (ANR) or leucoanthocyanidins by leucoanthocyanidins reductase (LAR). Flavan-3-ols and their

polymers (proanthocyanidins) bind with themselves to generate large condensed tannins (Gomez et al., 2011; He et al., 2010; Hrazdina et al., 1984).

Flavonols, which can act as UV protectants and antioxidants, are a ubiquitous class of flavonoids in grape skins and the cell walls of seeds, but not in the pulp (Teixeira et al., 2013). The three main flavonols are kaempferol, myricetin and quercetin. Flavonol profiles vary in different grape varieties. In red grapes, the main flavonols are 3-O-glucosides, 3-O-galactosides and the 3-O-glucuronides of kaempferol, myricetin, quercetin, isorhamnetin, laricitrin and syringetin. Flavonol synthesis primarily begins at an early stage of berry development through to veraison (Downey et al., 2003).

Anthocyanins are dominant pigments in red grape skins, and include delphinidin, cyanidin, petunidin, peonidin and malvidin. For stable forms of pigments, the glycosylated anthocyanins, such as delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside and malvidin-3-O-glucoside, can be the primary form in grapes. In addition, most varieties can create acetyl and *p*-coumaryl glucoside derivatives (Revilla et al., 2013). Accumulation of anthocyanins occurs from veraison to ripening in red grape skins.

Flavan-3-ols are another important class of flavonoid compound in berry skins and seeds (Downey et al., 2003). Flavan-3-ol monomers in grapes are catechin, epicatechin, gallocatechin, epigallocatechin and catechin-3-O-gallate (Kennedy et al., 2001). The polymeric structure of flavan-3-ols are referred to as proanthocyanidins or tannins. Tannins can be composed of chains of almost identical subunits. Skin tannins (4 to more than 100 subunits) tend to be longer than seed tannins (2 to 20 subunits) (Keller, 2015). The highest level of catechins is in grape skins with epicatechin and epigallocatechin providing most of the extension subunits, while epicatechins are the major flavan-3-ol in grape seeds. Skin and seed tannins are synthesised from fruit set to veraison or before anthocyanin accumulation and then decline post veraison. The soluble flavan-3-ols and tannins are associated with

grape quality and contribute to bitterness and astringency in grape skins and wines (Conde et al., 2007).

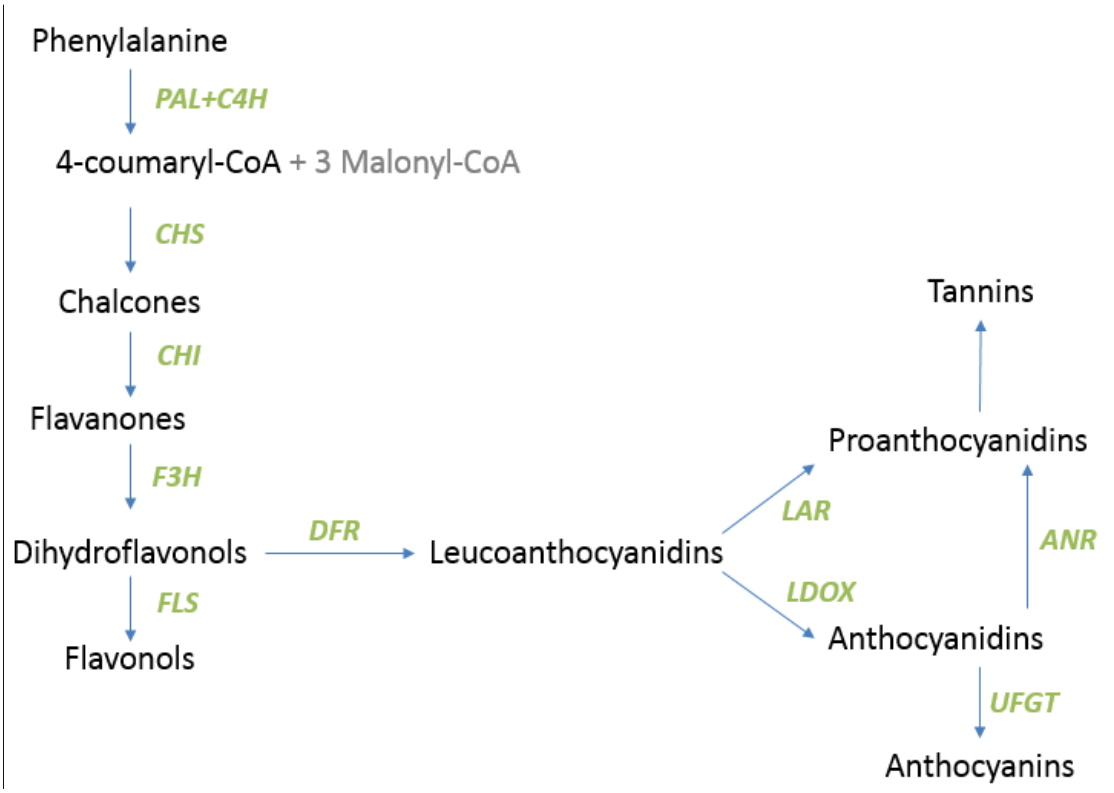


Figure 2.5 Schematic diagram to represent the flavonoid biosynthetic pathway of grapevines

Resulting in three major classes of flavonoids: flavonols, anthocyanins and tannins (from L. Liu's PhD thesis, Figure 2.3). Phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C4H), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavonol synthase (FLS), leucoanthocyanidin reductase (LAR), anthocyanidin reductase (ANR), leucoanthocyanidin dioxygenase (LDOX), dihydroflavonol 4-reductase (DFR), flavonoid glucosyltransferase (UFGT).

2.5.3 Aroma compounds

Grape volatile compounds are relevant to grape berries and the quality of wine aroma produced during ripening. These aroma compounds and their precursors of wine quality are established by secondary metabolites during the second growth phase (González-Barreiro et al., 2015). Some odour compounds are stored in grapes as water-soluble glycosides or combined with amino acids (Ribéreau-Gayon et al., 2006). In these forms, the compounds

cannot be detected by smell until glycosidases and peptidases release the volatile compounds from the water-soluble forms to the wine aroma (Loscos et al., 2009).

All volatile grape terpenoids are mono-, sesqui-, or norisoprenoid terpenes produced from the simple isoprene building block, isopentenyl pyrophosphate. There are two parallel pathways to the synthesis of a wide variety of terpenoids. The mevalonate pathway is limited to produce sesquiterpenes in the cytosol, and the deoxy-xylulose 5-phosphate/methyl-erythritol 4-phosphate (DOXP/MEP) pathway produces monoterpenoids, carotenoids and other compounds in the plastids (Luan and Wüst, 2002; Schwab et al., 2008). Terpenes are a family of compounds in grapes of which approximately 40 have been identified, such as linalool, geraniol, citronellol and nerol (Creasy and Creasy, 2018). They are associated with the smells of tropical fruits, rose, orange and so on. The monoterpenes are primarily found in two main forms in most grapes: as free volatile terpenes and as potential volatile terpenes. The free form of terpenes has different concentrations in different parts of berries; for example, a higher concentration of geraniol in the skin than in the juice. The potential volatile terpenes in the glycosylated forms are not odour-active, which are more common than the free volatile terpenes in grapes (Ribéreau-Gayon et al., 2006). During vinification, the bound form of terpenes can be hydrolysed to the free form by yeast-driven glycosidase activity and the acidic conditions found during fermentation.

Norisoprenoids are commonly derived from carotenoids in the plastids and play an important role in the volatile compound make-up of grapes to protect them from oxidative and photo damage (Baumes et al., 2002). Norisoprenoids include *β*-damascenone (notes: rose, dried fruit and tropical flowers) and *β*-ionone (notes: violet, raspberry and flora) in Pinot noir, Riesling and Cabernet Sauvignon grapes. Degradation of carotenoids can directly form free norisoprenoids or glycosylated forms, as found with terpenes, where the volatile aglycones can be released during fermentation (Carlomagno et al., 2016).

Another group of compounds are found in berry skins and mesocarp: C_6 compounds (C_6-aldehydes and C_6-alcohols). These are characteristic aroma compounds that are formed by

enzymatic oxidation of unsaturated lipids during ripening. They contribute to the 'fresh green', 'grassy' and 'herbaceous' aroma of grape berries (Keller, 2015).

2.6 Factors affecting the vine physiology and chemical composition of Pinot noir

Environmental factors affect the vine physiology and chemical composition of grapes. It has been shown that UV radiation or/and vineyard irrigation management can significantly alter vine physiology and fruit qualities (Acevedo-Opazo et al., 2010; Berdeja et al., 2014; Del-Castillo-Alonso et al., 2016; Martínez-Lüscher et al., 2014a).

2.6.1 UV radiation

Vine Physiology

The responses of vine leaves to UV-B exposure depends on vine development, their positions in terms of arrangement on the trellis and varieties (Dokoozlian and Kliewer, 1996; Grifoni et al., 2008; Núñez-Olivera et al., 2006). Most of UV-B radiation is reflected by surface wax and absorbed by protective compounds in the leaf epidermis. However, a small part of UV-B can pass through anticlinal cell walls to damage the exposed mesophyll and palisade cells (Figure 2.2). Lafontaine et al. (2004) found a high level of UV-B radiation led to losses in total chlorophyll and decreases in the ratio of Chl *a* and Chl *b* in Riesling leaves. Meanwhile, in a few studies, UV-B had a negative effect on $\delta^{13}C‰$ in plants, because UV-B radiation decreases the activity of ribulose-1,5-bisphosphate carboxylation/oxygenase (Rubisco) and stomatal conductance, resulting in reducing photosynthetic rate (Kakani et al., 2003). Finally, UV-B induces a reduction in photosynthetic rate leading to decreases of yields in vineyards (Doupis et al., 2016; Khudyakova et al., 2017).

Amino acids

Although amino acids are very important to wine grapes, there is little known about sunlight regulation of amino acids in grapevines, particularly UV-B radiation regulation. Over two

years research on Riesling grapes, the ambient UV-B caused a reduction in the concentration of total amino acids, compared with the excluded UV-B treatment (Schultz et al., 1998). In contrast, there were no effects of UV-B radiation on total free amino acids in Tempranillo grapes (Martínez-Lüscher et al., 2014b). Similar results were observed in Sauvignon blanc grapes (Gregan et al., 2012), but leaf removal treatments resulted in a decrease in the amino acids in berries. The different results presented were due to the varieties (Riesling vs. Sauvignon blanc and Tempranillo), treatments (potted trials vs. vineyard trials) and leaves (leaf removal vs. no leaf removal). In vineyard trials, leaves around the fruiting zone were removed from the excluding UV-B or UV-A screens. The most predominant change in the accumulation of amino acids was caused by the presence of leaves over the fruiting zone, where retaining the leaves resulted in higher concentrations in the berries at harvest (Ryona et al., 2008). These results indicate that Gln is the predominant amino acid in berries during their development and is the major form of organic nitrogen to be transported from leaves into berries via phloem. Leaf removal then directly decreases Gln, which is a precursor for biosynthesis of other amino acids in grape berries, including Pro and Arg via Glu (Stines et al., 2000). For individual amino acids, there are consistent decreases in the concentration of Pro and Arg in grapes (Gregan et al., 2012; Martínez-Lüscher et al., 2014b; Schultz et al., 1998). UV-B radiation damages the Calvin cycle, which reduces the supply of carbon skeletons for the synthesis of Pro and Arg (Forde and Lea, 2007; Schultz et al., 1998).

In grapevine vegetative and reproductive tissues, Pro can be synthesised from Glu via P5SC and P5CR and from ornithine (Orn) via the activity of δ-ornithine aminotransferase (OAT) and P5CR (Stines et al., 2000). Stress-induced accumulation of Pro occurs predominantly through Glu rather than Orn (Ashraf et al., 2018). Light promotes the P5CS1 gene to stimulate the accumulation of Pro and causes PDH gene repression. This indicates that light upregulates proline biosynthesis, while proline catabolism is activated in the dark (Stines et al., 1999; Szabados and Savouré, 2010).

Phenolic composition

As previous studies have shown, flavonol concentrations in grape skins are significantly affected by environmental elements, particularly UV radiation (Liu et al., 2018; Schreiner et al., 2014). In Sauvignon blanc berries, the concentrations of quercetin and kaempferol glycosides (quercetin-3-O-galactosides, quercetin-3-O-glucuronides and kaempferol-3-O-glucosides) dramatically increase in response to UV-B exposure. Similar results (kaempferol-3-O-glucosides, quercetin-3-O-galactosides, quercetin-3-O-glucuronides, quercetin-3-O-rutinoside and quercetin-3-O-glucopyranoside) were shown in Tempranillo in vineyard trials (Del-Castillo-Alonso et al., 2016). The biosynthesis of flavonols has been investigated at the molecular level and gene expression results suggested that UV-B up-regulated signal transduction HY5. HY5 induced the up-regulation of complex transcription factors, MYB/WD40/bHLH, with flavonol synthase genes (FLS) in the flavonoid pathway in response to UV-B in the vineyard (Cortell and Kennedy, 2006; Liu et al., 2015).

Anthocyanins are dramatically influenced by environmental factors, such as light/UV-radiation, temperature and water conditions (Cook et al., 2015; Ojeda et al., 2002; Yamane et al., 2006). The response to light/UV-B is not consistent and varies between varieties of grapevines. Shiraz berries have no significant difference in anthocyanin content between shading and light exposure treatments. Expression of the gene encoding for UFGT increases after veraison and is similar in both shaded and exposed berries (Downey et al., 2004). However, UV-B results in an increase in anthocyanins in Tempranillo, because high UV-B up-regulates genes encoding flavonoid glucosyltransferase (UFGT) (Martínez-Lüscher et al., 2014a; Price et al., 1995).

Flavan-3-ol monomers and tannins are in grape seeds and skins. In grape seeds, flavan-3-ol monomers and tannins are the most stable phenolic compounds under abiotic stresses (Teixeira et al., 2013). In grape skins and seeds, two LAR genes involved in the proanthocyanidin biosynthesis have different patterns of expression. LAR and ANR contribute to the accumulation of proanthocyanidins, so the regulation of genes encoding for ANR and LAR affects the concentration and composition of proanthocyanidins (Bogs et

al., 2005; Schreiner et al., 2012; Zhang et al., 2013). Shading reduces the transcription of LAR and ANR genes in the skins and has no effect on the seeds during the stage of proanthocyanidin accumulation (Xu et al., 2015). In the recent research, UV-B increased the concentration of catechin and procyanidin B in the skins of Tempranillo (Del-Castillo-Alonso et al., 2016) and Malbec (Berli et al., 2011), compared with the shading treatments. In contrast, there were no effects of shading on seed tannin concentrations in Pinot noir (Cortell and Kennedy, 2006) and Shiraz (Downey et al., 2004).

Volatile compounds

In previous studies, the level of terpenes was shown to be influenced by UV-B radiation (Gil et al., 2012; Skinkis et al., 2010). UV-B caused an increase in the concentrations of limonene and geraniol in Malbec berries, for example (Gil et al., 2013). This suggested that UV-B induced the generation of reactive oxygen species (ROS), so the biosynthesis of terpenes protected grapes from oxidative damage (Berli et al., 2010; Grassmann et al., 2005; Lee et al., 2005). Moreover, UV-B activates the expression of terpene synthase (TPS) genes resulting in terpene synthesis (Gil et al., 2012; Pontin et al., 2010). In berry skins, carotenoids in UV-B treatments had lower concentrations than in the control at harvest (Schultz et al., 1998). The results indicated that UV-B induced the degradation of carotenoids in fruit. In higher plants, the synthesis of carotenoids is controlled by blue light and UV receptors. UV-B up-regulates the carotenoid biosynthesis pathway (Schreiner et al., 2012; Schreiner et al., 2017). High UV-B intensity increases hexanal, but there is no effect on the alcohols 3-hexen-1-ol and 3-methyl pentanol (Gil et al., 2013). It has been shown that UV-B induces the oxidation and transcript abundance of lipoxygenases (LOX), and causes a breakdown of these fatty acids to C_6 compounds (Giordano et al., 2004; Podolyan et al., 2010).

2.6.2 Water deficit

Vine Physiology

Water deficit decreases the total chlorophyll concentration, due to the reduction in Chl *a* and Chl *b* concentrations. Bertamini et al. (2006) showed that Riesling vine leaves had lower

Chl *(a+b)* level under a water deficit than well-watered (1.59 g/kg and 2.47 g/kg, respectively). This suggested that plant cell dehydration led to damage in chloroplast membranes, alteration of cell turgor, distortion of lamellae vesiculation and phospholipids disorders (Bertamini et al., 2006; Mullet and Whitsitt, 1996; Reddy et al., 2004).

Meanwhile, water deficit leads to stomatal closure, and there can be a rapid decrease in stomatal conductance in response to the low relative soil water status (Centeno et al., 2010; Padgett-Johnson et al., 2003; Williams and Araujo, 2002). However, Pinot noir, which behaves as an isohydric cultivar, rarely has LWP values below -1.5, regardless of the soil water status (Lovisolo et al., 2010; Mejias-Barrera, 2016). Due to a curvilinear correlation between stomatal conductance and net photosynthesis, it can be concluded that stomatal closure reduces photosynthesis under water deficit (Hale and Orcutt, 1987). At the carboxylation step, a decrease in CO_2 uptake in grapevine leaves affects photosynthesis, and also $^{12}CO_2$ is preferentially utilised to $^{13}CO_2$ (Farquhar et al., 1989). In grapevine tissues, the gradient of CO_2 concentration between atmospheric and intercellular spaces (C_i/C_a) is influenced by drought. This is because stomatal closure leads to an increase in the relative ratio of intercellular $^{13}CO_2$ because assimilation of intercellular $^{12}CO_2$ is the preferred substrate of Rubisco during carboxylation (Farquhar et al., 1982; Gaudillère et al., 2002; Taiz et al., 2015). Therefore, plants grown under water stress tend to have more positive carbon isotope ratios ($\delta^{13}C$) (Gaudillère et al., 2002). For grapevine leaves and grape juice it has been observed that well-watered vines had lower $\delta^{13}C$ than those in the water stress treatment (respectively, -28.20‰ and -24.50‰) (Santesteban et al., 2012).

In vineyards, an increase in soil water availability to grapevines through irrigation can stimulate an increase in berry weight and vine yield (Acevedo-Opazo et al., 2010). By contrast, yield is decreased under a water deficit due to a reduction in net photosynthesis and stomatal closure (Escalona et al., 2000). Several studies have shown that water deficit caused a direct decline of yield in vineyards (Grimes and Williams, 1990; Intrigliolo et al., 2016). The yield can be increased by increasing irrigation. For example, the yield of Cabernet

Sauvignon in 75% crop evapotranspiration (ETc) treatment was 26% more than vines in a non-irrigated treatment (Intrigliolo et al., 2016).

Amino acids

Water deficit can increase the concentration of total free amino acids as well as some individual amino acids in grapevine leaves and berries, such as Pro, Arg, Gln, Ala and gamma-aminobutyrate (GABA) (Bertamini et al., 2006). Berdeja et al. (2014) reported water stress increased the concentrations of amino acids in Pinot noir berries, particularly Arg and Pro, due to the decomposition of leaf proteins. Amino acids were released in large numbers and then transferred into the berries. Pro in grape leaves plays a role as an osmoticum, which assists tissues to be relatively tolerant to a water deficit (Patakas et al., 2005), and also can act as an antioxidant. An increase in proline accumulation is associated with an increase in transcript abundance for σ 1-pyrroline-5-carboxylate synthetase (P5CS) (Deluc et al., 2009). P5SC controls proline biosynthesis, which is a reductive pathway using nicotinamide adenine dinucleotide phosphate (NADPH) and generating $NADP^+$ (Jiménez et al., 2013). Water deficit could also induce an increase in the synthesis and maintenance of the low ratio of NADPH:$NADP^+$ to reduce the accumulation of NADPH, resulting from the inhibition of the Calvin cycle (Allan et al., 2008). Furthermore, water deficit induces the stomatal closure resulting in decreasing the photosynthetic capacity to supply insufficient O_2 for respiration in mitochondrion. The carbon skeletons of amino acids can act as precursors or intermediates in the TCA cycle. Therefore, water deficit may reduce the catabolism of amino acids as alternative respiratory substrates for contributing to ATP production and mitochondrial metabolism (Hildebrandt et al., 2015; Hochberg et al., 2015).

Phenolic composition

An increased flavonol contents with decreasing vine water status has been observed in grapes. This result shows that flavonol synthase (FLS) catalyses the reaction from dihydroflavonol to flavonol in flavonol biosynthesis as the transcript abundance of FLS4 increases throughout berry development under water deficit (Castellarin et al., 2007; Deluc et al., 2009).

Water deficit can also stimulate the accumulation of anthocyanins in red grape varieties. For example, water deficit increased the anthocyanin concentration (per berry weight) (Roby et al., 2004) and the anthocyanin content (per berry) (Castellarin et al., 2007) in Cabernet Sauvignon. Previous studies on this cultivar stated that skin anthocyanin concentrations increases with the reduction in berry sizes, but there are no corresponding increases between anthocyanin content and berry fresh weight (Hardie and Considine, 1976; Roby et al., 2004). Water deficit increases relative skin mass, resulting in increasing anthocyanin concentration per berry weight (Roby et al., 2004). In addition, increases in skin anthocyanin concentration or content under a long-term water deficit may induce berry shrivelling resulting in an osmotic effect to enhance anthocyanin synthesis (Martinez-Luscher et al., 2015). Furthermore, water deficit increases the accumulation of B-ring trihydroxylated anthocyanins by increasing the expression of flavonoid 3',5'-hydroxylase (F3'5'H) and flavonoid 3'-hydroxylase (F3'H) (Castellarin et al., 2007).

Skin tannins have been investigated with respect to water supplementation. Water deficit increased the contents of skin tannin in Cabernet Sauvignon (Kennedy et al., 2002; Roby et al., 2004). These results suggest that water deficit induces the biosynthesis up-regulation of LAR expression in grape skins resulting in the accumulation of skin tannins (Castellarin et al., 2007; Martínez-Lüscher et al., 2017). Also, the berry development changes in plastids of pericarp cells which are important in polyphenol metabolism. Water deficits influence on plastid development, leading to stimulate the polymerization of proanthocyanidins to form tannins in berry skins (Roby et al., 2004).

Volatile composition

Moderate water stress can enhance aroma compounds in white and red grapes (Deluc et al., 2009). Song et al. (2012) reported that water deficit caused an increase in the concentration of geraniol in Merlot grapes, because water deficit induced oxidative stress resulting in an increase in the accumulation of terpenes. In addition, water deficit increased the level of *β*-damascenone which was an important aroma compound for strongly enhancing the overall fruity character and reducing vegetative notes in Pinot noir (Bindon et al., 2007). This result

suggested that the increase in *β*-damascenone reflected to the changes in the carotenoid profile. Water deficit potentially increased the operation of the xanthophyll cycle (thermal dissipation mechanism) to increase carotenoids as antioxidants for the reduction in ROS. Water deficit also increases the activity of carotenoid cleavage dioxygenase (CCD), resulting in the synthesis of carotenoids (Bindon et al., 2007; Deluc et al., 2009). Carotenoids can be degraded into *β*-damascenone during ripening, so the increase in carotenoids can stimulate the accumulation of *β*-damascenone under water deficit (Alem et al., 2018; Chen et al., 2017). C_6 compound concentrations decreased with a reduction in irrigation level in Merlot fruit. This is because water stress causes an increase in the berry sugar level, and the decreases in C_6 compounds increase with the berry maturity (Mendez-Costabel et al., 2014).

2.6.3 UV radiation interaction with water deficit

There has been much research undertaken about the effects of UV-B interacting with water deficit in plants, but only a few concerning grapevines. In Tempranillo leaves, the combination of UV-B and water deficit caused a small reduction in the relative water content and an increase in the concentrations of Chl *(a+b)*, compared with the control. It has been shown that the combined stresses significantly decreased stomatal conductance and the de-epoxidation stage of the xanthophyll cycle (Martinez-Luscher et al., 2015).

For phenolic composition, Martínez-Lüscher et al. (2014a) found that UV-B up-regulated FLS1, UFGT and F3H, while F3H and OMT2 were up-regulated by water deficit in Tempranillo berries. The combination of UV-B and water deficit was observed to significantly affect the profile of flavonol hydroxylation in grapes in comparison with the control, because of the competition of FLS, F3'H and F3'5'H for the same flavonol substrate.

With regard to amino acids, Pinot noir as a cultivar is known as a preferential accumulator of Arg and Pro, which are the dominant N storage compounds in grapes (Stines et al., 2000). Pro accumulation can be enhanced under water stress with elevated UV-B. Several studies indicate there are the alterations in amino acid concentrations under water deficit or UV

radiation in pea and wheat (Alexieva et al., 2001; Balakumar et al., 1993), but no information has been reported on Pinot noir's response to UV-B radiation with its interaction with water deficit.

UV-B or water deficit influences the volatile composition in berries as defence and stress responses. Both stresses are involved in the metabolism of fatty acids, isoprenoids and carotenoids and the process of photosynthesis. UV-B or water deficit can stimulate the activity of LOX to produce monoterpenoids and carotenoids from fatty acids in berries (Deluc et al., 2009; Schreiner et al., 2017).

Research hypotheses and objectives:

Based on a review of the literature, there has been no previous study of the response of Pinot noir to the combination of UV radiation and water deficit. Consequently, there are a number of significant areas of research that are important to investigate.

The general hypotheses of this research are:

1) The combination of UV radiation and water deficit will change vine physiological indexes, including SPAD, leaf water potential (LWP), carbon isotope ratio and berry parameters (°Brix, pH and TA). An intensive UV-B could potentially enhance the susceptibility of vine physiological indexes to water deficit in Pinot noir.

2) The increases in amino acids will occur under UV-B interaction with water deficit depending on different amino acid families, but the combined stresses may change the accumulation of amino acids induced by water deficit alone.

3) Phenolic compounds during berry development will be increased by UV-B and then be altered by the combination of UV-B and water deficit. Water deficit will strongly influence UV-B-induced increases in phenolic composition of Pinot noir fruit.

4) UV-B radiation and water deficit will significantly affect the accumulation of significant volatile compounds in Pinot noir fruit at harvest.

The objectives of this research are:

1) To investigate the responses of Pinot noir vine physiology to UV-B radiation without water deficit, water deficit without UV-B, and interactions of UV-B and water deficit during berry development;

2) To investigate the response of Pinot noir grapes to UV-B radiation without water deficit, water deficit without UV-B, and interactions of UV-B and water deficit on the chemical composition at harvest by considering important quality-related compounds in the fruit;

3) To investigate UV-B induced changes in grapevine with/without water deficit in the vineyard environment, compared to in a controlled environment cabinet (glasshouse).

Chapter 3

Materials and methods

3.1 Experimental design

3.1.1 Sites and materials

This study was conducted over two growing seasons (2015-2016 and 2016-2017) in the Horticulture Nursery and the West Vineyard at Lincoln University. Pinot noir clone 115 cuttings were collected in August 2013 and rooted on a heating pad before being transferred to 20 L pots and grown outdoors at the Nursery. The vines were pruned in the dormant season of 2014 to one cane on which two shoots, but no fruit, were allowed to grow. In 2015 and 2016, the vines were pruned similarly and grown with fruit. The potting mix was 80% composted bark and 20% pumice with fertilisation (Osmocote Exact 16-3.9-9.1, horticultural lime and Hydraflo).

In the field trial, the vineyard was located at 43°39'S, 172°28'E which is considered a cool climate area. The Pinot noir vines (clone 777 on 3309 rootstock) were planted in 1999 in a north-south row orientation with 1.2 m between vines and 2.5 m between rows. Vines were trained with two bilaterally-opposed canes in a vertical shoot positioned system (VSP).

All the grapes were harvested by hand in April 2016 and 2017.

3.1.2 Treatments

Glasshouse trial

The grapevines were moved into the glasshouse for preparation for the experiments in September, prior to budbreak. From October (fruit-set) to December (veraison), the grapevines were uniformly irrigated on a regular basis to soil capacity and were exposed to normal daylength hours in the glasshouse. All clusters were harvested in February.

UV-B treatment

Vines of similar leaf area and crop weight were divided into two groups of 18 vines each. In each group treatments were applied from veraison to harvest (Table 3.1): (i) UV-B control treatment (-UV): the vines were moved into the glasshouse; (ii) UV-B treatment (+UV): the vines were put in the same glasshouse, but UV was supplied by UVB-313 UV fluorescent tubes (Q-Lab Company, Westlake, OH, USA). The fluence rates of UV-B (280–313 nm) were measured by a UVB Biometer model 501 radiometer (Solar Light Company, Glenside, PA, USA). The glasshouse was maintained to the following specifications: 28°C/18°C, day/night, humidity 70–80% and, in the UV-area, the intensity of UV-B was kept at UVI-6 for 8 h/d (9:00-17:00). The relationship between UVI and UV-B intensity is $I_{UVB} = 18.9 \times UVI$ (W/m^2). The expression for UV dose is $D = I^{4/3} \times t_e$ [$(W/m^2)^{4/3}$ s], including exposure time (t_e, s) and UV-B intensity (I, W/m^2) (Kiedron et al., 2007; McKenzie et al., 2004; Sánchez-Pérez et al., 2019).

Water treatment

Vines were exposed to a water treatment in combination with the UV-B treatment. Both UV-B treatment groups were divided into two with two irrigation levels, each one consisting of nine vines (Table 3.1). There was a: (i) well-watered control treatment where vines are regularly irrigated to soil capacity (+W); and a (ii) water-deficit treatment where vines received half that amount of water (-W) (see below). Soil in the water deficit treatment was dry to the touch at re-watering and the grapes had visible shrivelling. Time domain reflectometry (TDR) (Hydrosense™, Campbell Scientific, Inc) were used to evaluate the percentage of substrate soil moisture for each pot.

Table 3.1 Glasshouse treatments (Three vines in a block).

Water treatment	UV-B treatment	Natural light
Well-watered	+W+UV (9 vines)	+W-UV (9 vines)
Water deficit	-W+UV (9 vines)	-W-UV (9 vines)

Field trial

The trial design, in 2016, was three UV-B treatments in eight replicated blocks (four blocks in two rows) (Table 3.2). An irrigation treatment was planned for 2016, but due to rainfall was not able to be imposed. In 2017, two water deficit treatments combined with three UV-B treatments in eight replicated blocks (four blocks in two rows) (Table 3.3). All vines were randomly selected in the vineyard, and buffer vines were used to avoid the impact of UV-B and water treatments on each vine.

Table 3.2 Vineyard experimental design in 2016.

SC: Shade cloth treatment; LR: Leaf removal treatment; PETG: Polyethylene terephthalate screen treatment.

Treatment (52 row)	Rep	Treatment (53 row)	Rep
	Buffer vines		Buffer vines
LR	R1	SC	R3
	Buffer vines		Buffer vines
SC	R1	LR	R3
	Buffer vines		Buffer vines
PETG	R1	PETG	R3
	Buffer vines		Buffer vines
LR	R1	SC	R3
	Buffer vines		Buffer vines
SC	R1	LR	R3
	Buffer vines		Buffer vines
PETG	R1	PETG	R3
	Buffer vines		Buffer vines
SC	R2	LR	R4
	Buffer vines		Buffer vines
PETG	R2	PETG	R4
	Buffer vines		Buffer vines
LR	R2	SC	R4
	Buffer vines		Buffer vines
SC	R2	LR	R4
	Buffer vines		Buffer vines
PETG	R2	PETG	R4
	Buffer vines		Buffer vines
LR	R2	SC	R4
	Buffer vines		Buffer vines

Table 3.3 Vineyard experimental design in 2017.

SC: Shade cloth treatment; LR: Leaf removal treatment; PETG: Polyethylene terephthalate screen treatment; WW: Well-watered; WD: Water deficit; I: Pre-veraison; II: Veraison.

Treatment (52 row)	Rep	Treatment (53 row)	Rep
	Buffer vines		Buffer vines
WW + LR II	R1	WD + SC I	R3
	Buffer vines		Buffer vines
WW + SC II	R1	WD + LR I	R3
	Buffer vines		Buffer vines
WW + PETG II	R1	WD + PETG I	R3
	Buffer vines		Buffer vines
WD + LR II	R1	WW + SC I	R3
	Buffer vines		Buffer vines
WD + SC II	R1	WW + LR I	R3
	Buffer vines		Buffer vines
WD + PETG II	R1	WW + PETG I	R3
	Buffer vines		Buffer vines
WW + SC I	R1	WD + LR II	R3
	Buffer vines		Buffer vines
WW + PETG I	R1	WD + PETG II	R3
	Buffer vines		Buffer vines
WW + LR I	R1	WD + SC II	R3
	Buffer vines		Buffer vines
WD + SC I	R1	WW + LR II	R3
	Buffer vines		Buffer vines
WD + PETG I	R1	WW + PETG II	R3
	Buffer vines		Buffer vines
WD + LR I	R1	WW + SC II	R3
	Buffer vines		Buffer vines
WD + SC II	R2	WD + PETG I	R4
	Buffer vines		Buffer vines
WD + LR II	R2	WD + LR I	R4
	Buffer vines		Buffer vines
WD + PETG II	R2	WD + SC I	R4
	Buffer vines		Buffer vines
WW + SC II	R2	WW + PETG I	R4
	Buffer vines		Buffer vines
WW + LR II	R2	WW + LR I	R4
	Buffer vines		Buffer vines
WW + PETG II	R2	WW + SC I	R4
	Buffer vines		Buffer vines
WW + LR I	R2	WW + PETG II	R4
	Buffer vines		Buffer vines
WW + PETG I	R2	WW + SC II	R4
	Buffer vines		Buffer vines
WW + SC I	R2	WW + LR II	R4
	Buffer vines		Buffer vines
WD + LR I	R2	WD + PETG II	R4
	Buffer vines		Buffer vines
WD + PETG I	R2	WD + SC II	R4
	Buffer vines		Buffer vines
WD + SC I	R2	WD + LR II	R4

UV treatment

Grapevines across two rows were divided into six groups (each group including four replications), in 2016, and twelve groups (each group including four replications), in 2017, each vine with visually similar leaf area and crop load. UV-B exclusion was achieved using the method of Gregan et al. (2012) and Liu et al. (2018). A-frame-mounted transparent screens (240 cm × 60 cm) containing UV-B exclusion materials were placed over individual vines to cover the fruiting zone of the test vine and buffer on either side. In each group of vines, the following treatments were applied from veraison to harvest in 2016 (Table 3.4) and from pre-veraison to harvest in 2017 (I) and from veraison to harvest in 2017 (II) (Table 3.3): (i) shade cloth treatment (SC): leaves around the fruiting zone were removed and clusters were covered by shade cloth (Ultra-Pro 70% shadecloth, Cosio Industries Ltd); (ii) leaf removal treatment (LR): all leaves and lateral shoots were removed in the bunch zone leaving clusters fully exposed; (iii) PETG (glycol-modified polyethylene terephthalate, Mulford Plastics, Christchurch New Zealand): all leaves and lateral shoots were removed in the bunch zone and clusters were covered by a PETG screen. In all treatments leaves in the fruiting zone were removed to maintain the same leaf areas across treatments.

Water treatment

TDR rods (40 cm) (16 rods) were installed into the soil to evaluate soil moisture in each block. Every UV-B treatment was divided into two groups with two irrigation levels and each one consisted of four vines from pre-veraison to harvest, in 2017 (Table 3.4): (i) no irrigation treatment; (ii) standard irrigation treatment.

Table 3.4 Vineyard treatments in 2017

	Shade cloth (S)		leaf removal (L)		UV screens (P)	
	Pre-veraison to harvest (I)	Veraison to harvest (II)	Pre-veraison to harvest (I)	Veraison to harvest (II)	Pre-veraison to harvest (I)	Veraison to harvest (II)
No irrigation treatment (WD)	DS I (4 vines)	DS II (4 vines)	DL I (4 vines)	DL I (4 vines)	DP I (4 vines)	DP I (4 vines)
Standard irrigation treatment (WW)	WS I (4 vines)	WS II (4 vines)	WL II (4 vines)	WL II (4 vines)	WP II (4 vines)	WP II (4 vines)

3.1.3 Sample collection

Glasshouse trial

Glasshouse experiments were carried out on potted vines (36 vines) from veraison (12 weeks post bud burst) to harvest (17 weeks post bud burst). Sampling time points were selected at veraison, 1-week post-veraison, 2-weeks post-veraison, 3-weeks post-veraison, 4-weeks post-veraison, 5-weeks post-veraison and 6-weeks post-veraison (harvest). At each time point, samples from three blocks (3 vines in one block) were randomly collected from the control treatment and UV-B or/and water deficit treatments before 9:00 and immediately stored in a walk-in freezer (-20℃). Ten berries from each block (2 clusters per vine × 1-2 berries per cluster) were collected from different sites (top, medium and bottom) of clusters for the analysis of phenolic composition. At harvest, sample collection of 10 berries (2 clusters per vine × 1-2 berries per cluster), 20 berries (2 clusters per vine × 3-4 berries per cluster) and 40 berries (2 clusters per vine × 6-7 berries per cluster) from each block were used for the analysis of berry parameters, amino acids and volatile composition, respectively.

Field trial

For the UV and water deficit trials in the Lincoln University Research Vineyard in 2015-2016 and 2016-2017, whole berries were collected at five stages of development: 1-week veraison (13 weeks post bud burst); 2-weeks veraison; 3-weeks veraison; 4-weeks veraison; 5-weeks veraison (harvest, 17 weeks post bud burst), in 2015-2016. In 2016-2017, eight sampling time points were taken throughout berry development: -4-weeks veraison (berries at pea-size); -2-weeks veraison; veraison (12 weeks post bud burst); 1-week veraison; 2-weeks veraison; 3-weeks veraison; 4-weeks veraison; 5-weeks veraison (harvest, 17 weeks post bud burst). At each time point, samples from four replicates were randomly collected from the control treatment and UV-B or/and water deficit treatment and immediately stored in a walk-in freezer (-20℃). Ten berries from each replicate were randomly collected from different sides of clusters for the analysis of phenolic composition. At harvest, sample collection of 10 berries, 20 berries and 40 berries per replicate from each treatment were

taken for the analysis of berry parameters, amino acids and volatile composition, respectively.

3.2 Measurement of physiological indices in vines

3.2.1 Leaf chlorophyll content

Six fully developed leaves per vine from four replicates at the top, middle, bottom and both sides of the canopies were randomly selected to measure for relative chlorophyll content, using a SPAD-502 Plus meter (Konica Minolta Co., Ltd, Osaka, Japan) from veraison to harvest in the 2016-2017 season. All the values were from six leaf averages to get one value per vine. The SPAD value measured the leaf transmittance in two wavelengths 650 nm and 940 nm (Uddling et al., 2007).

3.2.2 Leaf water potential

Leaf water potential (MPa) was determined from one healthy and fully expanded leaf per replicate in the vineyard (one vine in one replicate) and in the glasshouse (one group in one replicate) at harvest in the 2016-2017 season, randomly selected from those close to the clusters. Measurements were performed near solar noon, using a pressure chamber (Model 3000; Soil Moisture Equipment Corporation, Santa Barbara, CA, USA) (Boyer, 1967; Williams and Araujo, 2002).

3.2.3 Time domain reflectometry (TDR)

In the glasshouse, TDR (Hydrosense™, Campbell Scientific, Inc) was used to evaluate the percentage of substrate soil moisture for each pot and recorded as volumetric water content (%) as a measure of soil water status.

In the vineyard, TDR rods (40 cm) were inserted into the soil at the start of the trial and were used to measure soil moisture by the Model 6050X TRASE System I. The instrument was

used to evaluate the percentage of substrate soil moisture for Rows 52 and 53, and recorded as volumetric water content (%) as a measure of soil water status in four replicates.

3.2.4 Carbon isotope ratio in leaf dry matter and grape juice

Six leaves per replicate were randomly collected from the top, middle and bottom of canopies at harvest and ground to the fine powder after freeze-drying. Four milligrams of freeze-dried leaf powder were used for carbon isotope composition measurement. Fifty frozen berries (-20℃) per replicate were left to stand in a plastic bag at room temperature before the berries were gently crushed using plastic rods to produce grape juice for carbon isotope composition measurement.

Carbon isotope composition (δ^{13}C‰) was analysed by EA-IRMS (Elemental Analyser Isotope Ratio Mass Spectrometry), using a Sercon GSL elemental analyser (Crewe, UK), and a Sercon 20-22 IRMS (Isotope Ratio Mass Spectrometer). Samples were analysed in duplicate at a rate of one in eight. δ^{13}C‰ was referenced to Vienna-Pee Dee Belemnite standard (V-PDB) and was calculated as proposed by Farquhar and Richards (1984):

$$\delta 13C(‰) = \frac{Rs - Rb}{Rb} \times 1000$$

where *Rs* was the $^{13}C/^{12}C$ ratio of the sample and *Rb* was the $^{13}C/^{12}C$ ratio of the PBD Standard.

3.2.5 Pruning weight

At the end of each vintage and after vine leaf fall, pruning weight was measured to quantify the accumulation of the effects of the treatments on grapevines. In the vineyard, each vine was individually cane-pruned, and the weight of all removed parts determined *in situ* using a digital scale.

3.3 Chemical analysis

3.3.1 °Brix, pH and titratable acidity in grape juice

Fruit °Brix, TA and the pH of the grape juice were measured using the method of Iland et al. (2000):

Frozen berries were left to stand in test tubes and defrost to room temperature (20℃) before processing. The berries then were gently crushed with a plastic rod. A small volume of juice from the berries was used to measure °Brix using a digital refractometer (PAL-1 ATAGO, Tokyo, Japan).

The rest of the juice was pooled into beakers. Grape juice pH was measured by a Suntex pH/mV/temperature meter (SP-701; Suntex, Taiwan) with a Eutech Instruments probe (EC 620133; Eutech Instruments Pte Ltd, Singapore). Before the analyses, two standard buffer solutions of pH 4.0 and 7.0 were used to calibrate the pH meter.

Titratable acidity (TA) was determined by titration to pH 8.2 using 0.1 mol/L NaOH (LabServ, 97% min; Biolab (Australia) Ltd.). TA was measured on 10 mL of juice for the samples. NaOH (0.1 mol/L) was carefully added into the grape juice under constant stirring using a burette and the volume (mL) used for titration until pH 8.2 was recorded and used for calculations:

$$Titratableacidity\left({}^{gL}/_{as}\,H2T\right) = 75 \times molarity\ of\ NaOH \times Titrevalue(mL) \div Volume\ of\ juice(mL)$$

3.3.2 Amino acids analysis

The frozen berries were ground with liquid nitrogen in mortars, transferred into tubes and centrifuged for 5 min at 1960 g. The supernatant was diluted with deionised water (1:4) in a new tube. The grape juice samples were filtered through a 0.45 µmol/L nylon syringe filter into an HPLC glass vial and capped tightly. An internal standard, γ-aminobutyric acid (γ-GABA), was added to a final concentration of 100 µmol/L. For inline-derivatisation of the

primary amino acids, ơ-phthaldialdehyde was used as a fluorescence derivative; iodoacetic acid/mercaptopropionic acid was used to increase cysteine sensitivity, and 9-fluorenylmethyl chloroformate was a fluorescence derivative for proline.

The method of chromatography followed Gregan et al. (2012). The samples were injected into an HPLC system (Hewlett-Packard Agilent 1100 series, Waldbronn, Germany) with a 250 × 4.6 mm, 5 µm Prodigy C18 column (Phenomenex). Data were analysed using the Chemstation (Agilent) chromatography data system. The mobile phase consisted of two solvents: solvent A (0.01 mol/L Na2HPO4 with 0.8% tetrahydrofuran, adjusted to pH 7.5 with H3PO4) and solvent B (20% solvent A, 40% methanol, 40% acetonitrile). The gradient programme was 0 min, 0% B; 14 min, 40% B; 22 min, 55% B; 27 min, 100% B; 35 min, 100% B; 36 min, 0% B, with a flow rate of 1 mL/min. For detection, a fluorescence detector with an excitation at 335 nm and emission at 440 nm. At 25 min, the detector was switched to a second channel (excitation at 260 nm and emission at 315 nm) to detect proline. Amino acids were identified by their retention time and their concentrations were calculated in parallel to calibrate the internal amino acid standard (γ-GABA, 100 µmol/L).

3.3.3 Skin total phenolic compounds and skin anthocyanins analysis

Grape anthocyanins were extracted and analysed following the procedures described by Bonada et al. (2015) and Iland et al. (2000). Skins were separated from the pulp of berries using tweezers and scalpels. Skins were extracted in 20 mL conical flasks containing 10 mL of 50% v/v ethanol. Flasks were filled with nitrogen before being sealed to prevent oxidation. The flasks were then placed into a warm bath shaker (100 rpm, 22°C) for 24 h in the dark. The extracts were pooled into centrifuge tubes and centrifuged for 5 min at 1960 g. One millilitre of the collected extract was added to 10 mL of 1 mol/L HCl.

Measurements at 280 nm were carried out on a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan), using UV semi-micro 1.5 mL disposable cuvettes. The results were reported on the content of total phenolic substances per berry:

$$Skin\ phenolic\ substances\left({}^{au}/_{berry}\right) = \text{Abs}(280\text{nm}) \times DF \times EV \times 0.001$$

Measurements at 520 nm were carried out on a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan), using 1.5 mL disposable cuvettes. The results were reported in per milligram of malvidin-3-glucose equivalents per berry:

$$Skin\ anthocyanins\left({}^{mg}/_{berry}\right) = Abs(520\text{nm}) \div 500 \times DF \times EV$$

where DF was the dilution factor of the extract in 1 mol/L HCl and EV was the extracted volume after maceration with 50% ethanol. The value of 500 was based on a previous report that estimated the extinction coefficient of malvidin-3-glucose in g/100 mL of solution.

3.3.4 Skin and seed tannins analysis

Skins and seeds were separated from the pulp of berries using tweezers and scalpels. Skins were extracted into 20 mL conical flasks containing 10 mL of 50% v/v ethanol. Freeze-dried seeds from 10 berries were ground in mortars. The seed powder was extracted into 20 mL conical flasks containing 10 mL of 50% v/v aqueous ethanol. Flasks were filled with nitrogen before being sealed to prevent oxidation. The flasks were placed into a warm bath shaker (100 rpm, 22°C) for 24 h in the dark. The extracts were transferred into centrifuge tubes and then centrifuged for 5 min at 1960 *g* (Sarneckis et al., 2006).

Before the analyses, epicatechin was used as a standard for each batch of samples. Aqueous (–)-epicatechin (Sigma-Aldrich E1753) solutions (10, 25, 50, 75, 100, 150 mg/L epicatechin) were used to establish a standard curve for reporting tannin absorbance. All A_{280} (tannin) values were reported in mg/L or g/L epicatechin equivalents of the original sample (Figure 3.1).

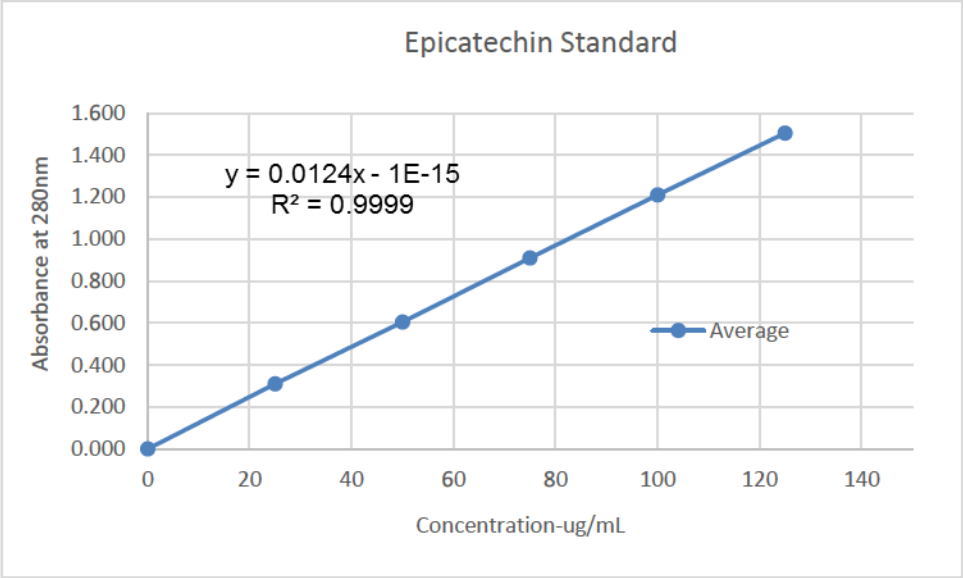


Figure 3.1 Epicatechin equivalent calibration curve

Skin and seed tannins were measured by the methylcellulose precipitation (MCP) tannin assay using the 1 mL assay in 1.5 mL disposable tubes (Sarneckis et al., 2006). For the treatment samples, 0.3 mL of methylcellulose solution (0.04% w/v, 1500 cP viscosity at 2%, M-0387, Sigma-Aldrich, USA) was added to 0.1 mL of skin or seed extract solution. After 3 minutes, 0.2 mL of saturated ammonium solution (Sigma-Aldrich, Auckland) was added into the mixed solution and made up to 1 mL with deionised water. The solution was mixed well, left to stand for 10 min, then centrifuged at 8936 *g* for 5 min (Table 3.5). Measurements at 280 nm were carried out on a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan), using UV (methacrylate) semi-micro 1.5 mL disposable cuvettes. For the control samples, 0.2 mL of saturated ammonium solution was added to 0.1 mL of the extract solutions and made up to final volume 1 mL with deionised water (Table 3.5). The solution was mixed well, stood for 10 min, then centrifuged at 8936 *g* for 5 min and measured at 280 nm.

Table 3.5 Volumes of sample and reagents for MCP tannin assay for grape extractions

	Sample (mL)	MCP (mL)	$(NH_4)_2SO_4$ (mL)	Water (mL)
Treatment	0.1	0.3	0.2	0.4
Control	0.1	0	0.2	0.7

A_{280nm} of the tannin in the sample solutions can be calculated by subtracting A_{280nm}(treatment) from A_{280nm}(control). Epicatechin solution was calculated by epicatechin equivalent calibration curve, ranging from 0 mg/L to 150 mg/L. The dilution factor for the skin or seed extract solutions was 10. The conversion to mg/g and mg/berry in seeds and skins from mg/L in the extract is shown below:

$$Tannin\ content\ of\ seeds\ or\ skins(mg/berry) = \frac{[Tannin]e \times Ve}{No.}$$

[Tannin]e = tannins concentration in extraction (mg/L epicatechin eq.)

Ve = final volume of extraction (L)

No. = initial number of berry samples

3.3.5 Volatile compounds analysis

The analysis of six C_6 and monoterpene volatile compounds in Pinot noir juice (Table 3.6) was determined using an automated HS-SPME GCMS (Headspace Solid-Phase Micro-Extraction Gas Chromatograph Mass Spectrometry) technique, based on the work of Canuti et al. (2009); Dennis et al. (2012); Fan et al. (2010); Fang and Qian (2012) and Yuan and Qian (2016). This adapted method utilised three synthetic deuterated internal standards; namely, hexanal (d_{12}) and hexyl (d_{13}) alcohol and linalool (d_3) all obtained from CDN isotopes (Sci Vac Pty Ltd, Australia). Eleven non-deuterated standards were used to generate standard curves for quantitative analysis. E-2-Hexenal was obtained from Acros Organics while all other non-deuterated standards were obtained from commercial supplier Sigma–Aldrich.

Table 3.6 Deuterated and non-deuterated standards for six C_6 and five monoterpene volatile compounds in Pinot noir juice.

Compound	ISTD ID No	RT (mins)	Target ion (m/z)	Confirming Ions (m/z, % of target)	Calibration Range ^ (µg/L)	CAS No
d_{12} hexanal	ISTD 1	7.78	64	48 (140.2), 46 (92.6)	-	1219803-74-3
n-Hexyl d_{13} Alcohol	ISTD 2	10.12	64	50 (45.2), 46 (44.1)	-	16416-34-5
d_3 linalool	ISTD 3	12.31	96	124 (25.9), 139 (10.1), 58 (16.8)	-	1216673-02-7
Hexanal	1	7.85	44	41 (77.8), 56 (75.2)	0-1048.6	66-25-1
(E)- 2-Hexenal	2	9.23	41	55 (74.4), 39 (59.5)	0-1517.1	6728-26-3
1-Hexanol	2	10.26	56	43 (64.5), 55 (51.3)	0-824.1	111-27-3
(E)- 3-Hexen-1-ol	2	10.33	67	82 (58.1), 100 (3.8)	0-23.4	928-97-2
(Z)- 3-Hexen-1-ol	2	10.53	41	67 (78.2), 55 (38.8)	0-265.4	928-96-1
(E)- 2-Hexen-1-ol	2	10.70	57	41 (50), 39 (20.5)	0-513.3	928-95-0
Linalool	3	12.35	93	12 (28.0), 136 (8.8)	0-8.6	78-70-6
Citronellol	3	15.44	138	82 (468.2), 95 (397.3), 109 (138.2)	0-8.2	7540-51-4
α- terpineol	3	14.59	93	121 (75.8), 136 (60.9), 81 (61.36)	0-6.3	10482-56-1
Nerol	3	16.06	68	123 (28.9), 139 (18.1), 136 (11.4)	0-7.3	106-25-2
Geraniol	3	16.88	84	93 (122.3), 123 (98.9)	0-13.3	106-24-1

^ All samples were diluted 2-fold with 0.2 mol/L citrate buffer, hence concentrations obtained were multiplied by this factor accordingly.

3.4 Statistical analyses

Statistical analysis was undertaken using IBM SPSS Statistics 22. The data were subjected to an independent-sample T-test and two/three-factor analyses (ANOVA) to partition the variance into the main effects (UV-B and water deficit; UV-B, water deficit and time) and the interaction among them. In the case of significant interactions among factors, treatments were compared using the least significant difference (LSD) at the 5% level ($P< 0.05$).

Chapter 4

Effects of UV radiation on the vine physiology and chemical composition of Pinot noir fruit

4.1 Introduction

UV-B radiation (280-315 nm) in New Zealand is up to 30%-40% higher than similar latitudes in the Northern Hemisphere (Lubin and Jensen, 1995; McKenzie et al., 2006; McKenzie et al., 1999). It is considered as an environmental stress, which affects plant growth and development (Jansen et al., 1998). Several studies have reported that UV-B caused damage to important functional molecules, such as DNA, protein and lipids, and the accumulation of ROS in plants, which were involved in endogenous growth processes (see table 2.1) (Jordan, 1996; Jordan, 2002; Jordan, 2011). Moreover, UV-B-induced responses for grapevines relate to canopy management through leaf removal (Jordan, 2017; Liu et al., 2015). Plucking leaves around the fruiting zone directly increases sun exposure to the clusters. Thus, it induces potential issues: 1) the alteration of vine physiology and metabolism in response to UV-B, which results in qualitative and quantitative changes in fruit; and 2) chemical compounds in the clusters being changed in response to direct sun exposure (Jordan, 2017). In this chapter, changes in the vine physiology and chemical composition of the fruit in Pinot noir in response to UV radiation are investigated.

4.2 Results

4.2.1 Glasshouse trials

Effects of UV-B radiation on physiology of Pinot noir vines

In the glasshouse, grapevines were divided into two groups: UV-B treated (+UV+W) and non-UV-B treated (-UV+W) to investigate how UV-B exposure affected the physiological indices. Figure 4.1a showed the trend of soil volumetric water content of potted vines in the

glasshouse from veraison to harvest, in 2016-2017, and that the UV-B treatment had no statistically significant effect on it. In general, the leaf SPAD level (Fig. 4.2b) declined during ripening and UV-B caused a significant reduction in leaf SPAD at weeks 3, 5 and 6. After one week of UV-B treatment, SPAD declined substantially and then showed a parallel trend to the control. At harvest, UV-B caused a significant decrease in SPAD, by 17.0%, compared to the control. There were no significant differences in the leaf water potential or the carbon isotope ratio of juice between treatments (Table 4.1), but UV-B increased (made less negative) the carbon isotope ratio in the leaves.

Table 4.1 also showed berry parameters measured at harvest in both glasshouse experiments. In 2015-2016, there was no significant effect on °Brix, TA and pH. In the following year, UV-B caused an increase in °Brix and pH, while there was a reduction in TA compared with the control. In comparing the results of the two experiments, °Brix and TA were very close and had no statistical differences between the two years, but a significant difference was shown for pH between the two trials.

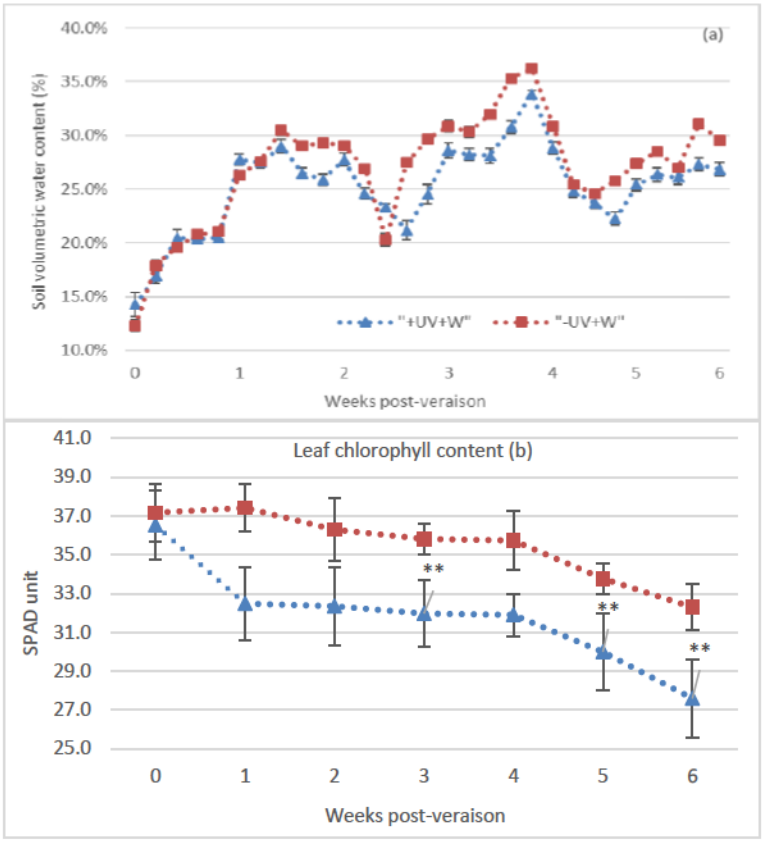


Figure 4.1 In 2016-2017 glasshouse trials: the soil volumetric content (%) of potted vines from veraison to harvest (a); effects of UV-B radiation on leaf chlorophyll content (SPAD unit) in Pinot noir from veraison to harvest (b).

Data showed mean ± standard error of the mean of three replicates. *P-values* for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level (*, *P<0.05*; **, *P<0.01*). +UV, UV-B treatment, -UV, no UV-B treatment, +W, well-watered. The blue line is UV-B treatment; the red line is the control.

Table 4.1 Effects of UV-B radiation on leaf water potential, $\delta^{13}C$‰ of leaf and juice and berry parameters in Pinot noir at harvest in 2015-2016 and 2016-2017 glasshouse trials.

		+UV+W	-UV+W	P_{UV}
2015-2016	°Brix	21.0	21.0	n.s
	TA(g/L)	5.4	6.6	n.s
	pH**	3.69	3.76	n.s
2016-2017	°Brix	21.0	20.1	0.041
	TA(g/L)	6.1	7.3	0.014
	pH**	3.41	3.23	0.002
	Leaf water potential (MPa)	-0.98	-0.94	n.s
	Leaf ^{13}C vs V-PDB ‰	-28.27	-29.07	0.001
	Juice ^{13}C vs V-PDB ‰	-29.16	-28.82	n.s

** indicates a significant difference between 2015-2016 and 2016-2017, *P*<0.01.

Data showed mean ± standard error of the mean of three replicates. *P*-values for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level; n.s, no significant difference. +UV, enhanced UV-B, -UV, no UV-B treatment, +W, well-watered.

Effects of UV-B radiation on the chemical composition of Pinot noir fruit

Amino acids

The amino acid concentrations were measured from samples collected at harvest in 2015-2016 and 2016-2017, as shown in Table 4.2, and the percentages of each amino acid compared to the total was presented in Table 4.3. Compared with the control, the UV treatment significantly affected the concentrations of amino acids in 2015-2016 and 2016-2017. There was no significant effect from UV on the percentages of each amino acid in total amino acids over the two years, except for Glu and Asn in 2015-2016 and Glu, His, Phe Thr, Ile and Ser in 2016-2017.

In 2015-2016, the control (-UV+W) had a total amino acid concentration of 8961 µM, but the UV-B treatment (+UV+W) dramatically decreased this value to half that amount. The most abundant amino acids were Arg and Pro, reaching 2176 µM and 1765 µM in -UV+W, respectively, and 976 µM and 891 µM in +UV+W, respectively. For the α-ketoglutarate and pyruvate families, concentrations of these amino acids in +UV+W were lower than in the control. Similar results were shown in the shikimate (aromatic), aspartate and 3-phosphoglycerate families. However, Cys was not detected in 2015-2016, but was in the following year. In 2016-2017, +UV+W caused significant increases in the concentrations of His, Tyr, Leu, Ile, Gly and total amino acids, but the rest of the amino acids were affected. The concentration of total amino acids was 2101 µM in +UV+W, higher than the 1869 µM in -UV+W. Moreover, there were significant differences in amino acids (except for His, Gly, Ile and Leu) between the two experiments, but with no consistent pattern. The UV treatment caused a decrease in all amino acids in 2015-2016 but led to either no change or a slight increase in 2016-2017, so there was no consistent response over the two growing seasons.

In Table 4.3, the percentages of individual amino acids were seen to be similar in both experiments. The largest values were found for Arg and Pro, which accounted for nearly 50% of the total. In 2015-2016, the UV treatment caused an increase in Glu by 2% and a reduction in Asn to 0.5%. There was no effect of UV in the pyruvate family in 2015-2016 or 2016-2017. In 2016-2017, the largest change was shown in Thr, which the UV treatment

decreased by 1.7%. There were also decreases in Glu, Phe and Ser under UV. His and Ile increased by 0.1% under UV-B. Again, no consistent effects occurred over the two growing seasons.

Table 4.2 Effects of UV-B radiation on amino acids in Pinot noir berries at harvest in 2015-2016 and 2016-2017 glasshouse trials.

Amino Acid (μM)		2015-2016			2016-2017		
		+UV+W	-UV+W	P_{UV}	+UV+W	-UV+W	P_{UV}
α-ketoglutarate							
Proline**	Pro	891	1765	0.002	487	321	n.s
Arginine**	Arg	976	2176	0.032	622	608	n.s
Glutamate**	Glu	331	532	0.001	100	100	n.s
Glutamine**	Gln	158	380	0.001	45	43	n.s
Histidine**	His	84	174	0.023	16	11	0.012
Shikimate (aromatic)							
Phenylalanine**	Phe	34	70	0.005	17	18	n.s
Tryptophan**	Trp	35	59	n.s	13	13	n.s
Tyrosine	Tyr	4	9	0.002	7	5	0.028
Pyruvate							
Leucine**	Leu	56	150	0.002	33	25	0.032
Valine**	Val	93	203	0.004	17	20	n.s
Alanine**	Ala	692	1532	0.004	336	297	n.s
Aspartate							
Aspartate**	Asp	174	363	0.015	54	51	n.s
Asparagine	Asn	5	49	0.039	15	13	n.s
Threonine**	Thr	283	713	0.002	161	176	n.s
Isoleucine	Ile	27	86	0.002	25	20	0.018
Methionine**	Met	11	23	0.005	5	4	n.s
Lysine	Lys	17	55	n.s	19	18	n.s
3-phosphoglycerate							
Cysteine	Cys	N.A	N.A	N.A	2	3	n.s
Serine**	Ser	242	571	<0.001	116	113	n.s
Glycine	Gly	11	49	0.008	12	10	0.014
Total**		4124	8961	0.002	2101	1869	0.017

** indicates a significant difference between 2015-2016 and 2016-2017, $P<0.01$; * indicates a significant difference between 2015-2016 and 2016-2017, $P<0.05$.

Data showed mean ± standard error of the mean of three replicates. *P*-values for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level; n.s, no significant difference. +UV, enhanced UV-B, -UV, no UV-B treatment, +W, well-watered.

Table 4.3 Effects of UV-B radiation on the percentages of each amino acid in total amino acids in Pinot noir berries at harvest in 2015-2016 and 2016-2017 glasshouse trials.

Amino Acid (μM)	2015-2016			2016-2017		
	+UV+W	-UV+W	P_{UV}	+UV+W	-UV+W	P_{UV}
α-ketoglutarate						
Pro	21.6%	19.7%	n.s	23.2%	17.2%	n.s
Arg**	23.7%	24.3%	n.s	29.6%	32.5%	n.s
Glu*	8.0%	5.9%	0.009	4.8%	5.4%	0.049
Gln**	3.8%	4.2%	n.s	2.1%	2.3%	n.s
His**	2.0%	1.9%	n.s	0.7%	0.6%	0.015
Shikimate (aromatic)						
Phe	0.8%	0.8%	n.s	0.8%	1.0%	0.035
Trp	0.8%	0.7%	n.s	0.6%	0.7%	n.s
Tyr**	0.1%	0.1%	n.s	0.3%	0.3%	n.s
Pyruvate						
Leu	1.4%	1.7%	n.s	1.6%	1.3%	n.s
Val**	2.3%	2.3%	n.s	0.8%	1.1%	n.s
Ala	16.8%	17.1%	n.s	16.0%	15.9%	n.s
Aspartate						
Asp**	4.2%	4.1%	n.s	2.6%	2.7%	n.s
Asn*	0.1%	0.5%	0.045	0.7%	0.7%	n.s
Thr	6.9%	8.0%	n.s	7.7%	9.4%	0.007
Ile*	0.7%	1.0%	n.s	1.2%	1.1%	0.041
Met	0.3%	0.3%	n.s	0.2%	0.2%	n.s
Lys**	0.4%	0.6%	n.s	0.9%	1.0%	n.s
3-***phosphoglycerate***						
Cys	N.A	N.A	N.A	0.1%	0.2%	n.s
Ser	5.9%	6.4%	n.s	5.5%	6.0%	0.004
Gly	0.3%	0.5%	n.s	0.6%	0.5%	n.s

** indicates a significant difference between 2015-2016 and 2016-2017, $P<0.01$; * indicates a significant difference between 2015-2016 and 2016-2017, $P<0.05$.

Data showed mean ± standard error of the mean of three replicates. *P*-values for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level; n.s, no significant difference. +UV, enhanced UV-B, -UV, no UV-B treatment, +W, well-watered.

Phenolic composition

To investigate the effect of UV treatments on Pinot noir fruit, samples collected from veraison to harvest in 2015-2016 and 2016-2017 were analysed for skin total phenolics, skin anthocyanins, skin tannins and seed tannins.

In the 2015-2016 glasshouse trials, the skin anthocyanin contents went as high as 0.5 mg/berry during ripening in the control (-UV+W) and the UV treatment (+UV+W) (Fig. 4.2a). At 1-week post-veraison, the contents of skin anthocyanin were 0.026 mg/berry and 0.057 mg/berry in -UV+W and +UV+W, respectively, and increased from there onwards. -UV+W and +UV+W reached peaks at 4 and 5-weeks post-veraison, respectively. At harvest, +UV+W had increased skin anthocyanins by 36.3%, compared to the control.

The contents of skin total phenolics from veraison to harvest in the two treatments firstly decreased and then increased (Fig. 4.2b). In +UV+W, the contents of skin total phenolics increased from 0.275 au/berry at the initial measurement to 0.376 au/berry at harvest, and -UV+W went from 0.200 au/berry to 0.308 au/berry, respectively. At harvest, +UV+W caused a significant increase in skin total phenolics compared to -UV+W.

Skin and seed tannin contents fluctuated from veraison to harvest with no difference between treatments (Fig. 4.2c/d). The overall trend for skin tannins was a reduction during ripening. From the first sampling date to harvest, skin tannins decreased by 0.290 mg/berry in +UV+W and by 0.125 mg/berry in -UV+W. From 1-week to 4-weeks post-veraison, +UV+W had higher contents of skin tannin than -UV+W, but then the effect swapped and +UV+W ended up a little higher at harvest. Seed tannins were initially significantly influenced by UV-B but then remained similar to the control berries during ripening. +UV+W started at 3.157 mg/berry at veraison climbing to 6.535 mg/berry at harvest, and -UV+W started from 2.848 mg/berry and climbed to 5.518 mg/berry.

In 2016-2017, skin anthocyanin contents increased during ripening in the control and treatment, with both reaching their peak at 4-weeks post-veraison and 6-weeks post-veraison (harvest), respectively (Fig. 4.3a). Compared with -UV+W, +UV+W had higher

contents of skin anthocyanin after two weeks of the treatment. The skin anthocyanin contents in +UV+W peaked at 0.702 mg/berry at 4-weeks post-veraison and then decreased to 0.606 mg/berry at harvest, while -UV+W had the peak at 0.461 mg/berry at harvest.

As shown in Figure 4.3b, -UV+W sharply increased skin total phenolics to 0.366 au/berry after one week of the experiment and then decreased to 0.214 au/berry at 3-weeks post-veraison but increased again to 0.306 au/berry until harvest. +UV+W increased skin total phenolics during ripening, while the skin total phenolics peaked 5-weeks post-veraison at 0.423 au/berry and were 0.397au/berry at harvest. +UV+W significantly affected the skin total phenolics from 1-week post-veraison to harvest, compared to the control. After two weeks of UV treatment, the changes in contents of skin total phenolics showed a parallel trend to that of the control.

A bimodal curve for skin tannins was shown from veraison to harvest in +UV+W, while the data went up and then slowly decreased in -UV+W (Fig. 4.3c). UV treatment had lower contents of skin tannin from veraison to 3-weeks post-veraison and higher contents from 4-weeks to 6-weeks post-veraison (harvest) than the control (Fig. 4.3c). The trend of skin tannins in -UV+W showed the substantial decrease from 0.929 mg/berry to 0.464 mg/berry during ripening. UV-B caused two peaks of skin tannins at 2-week (1.024 mg/berry) and 4-week (1.105 mg/berry) post-veraison and had 0.713 mg/berry at harvest.

In Figure 4.3d, the trend showed an initial increase in seed tannins from veraison and then an eventual decline to harvest. The net increase in seed tannins under UV-B was from 2.787 mg/berry to 5.273 mg/berry and, in -UV+W, from 3.160 mg/berry to 5.086 mg/berry. There were significant differences between +UV+W and -UV+W at all sampling dates, but no significant differences at harvest.

In general, the trends of skin anthocyanin, skin total phenolics and seed tannin contents in +UV+W and -UV+W showed substantial increases during ripening in 2015-2016 and 2016-2017. The seed tannin contents showed the net decreases during ripening in +UV+W of two seasons and -UV+W of 2015-2016, but the net increase in skin tannins contents was

presented in -UV+W of 2016-2017. Compared with 2015-2016, 2016-2017 had higher contents of skin anthocyanin and lower contents of skin tannin at harvest. Also, the magnitude of the treatment differences in 2016-2017 was larger than in 2015-2016. These results indicated that harvest date and *Botrytis* were related to the accumulation of pigments.

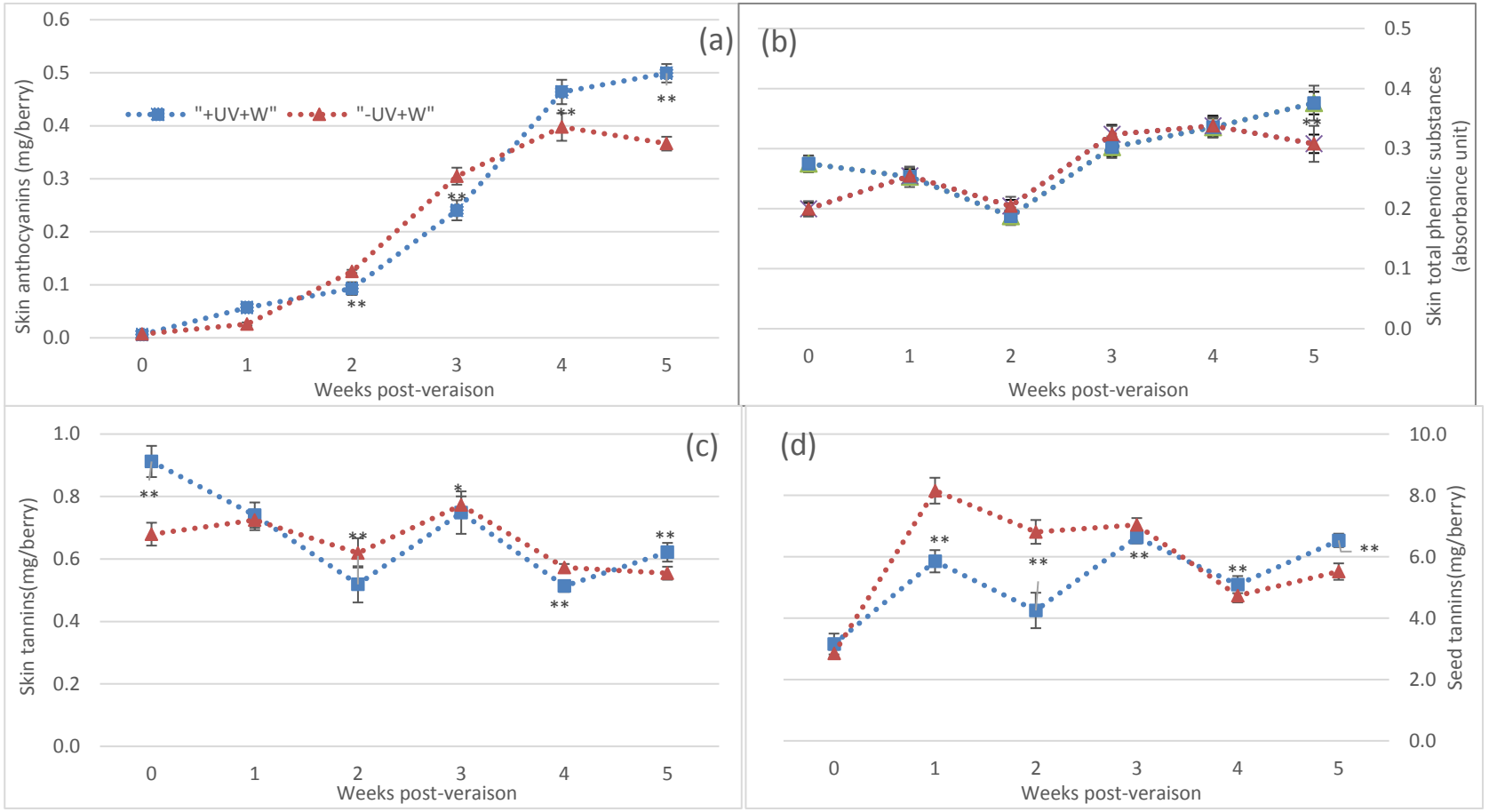


Figure 4.2 Effects of UV-B radiation on (a) skin anthocyanins, (b) skin total phenolic substances, (c) skin tannins and (d) seed tannins in Pinot noir berries during ripening in 2015-2016 glasshouse trials.

Data showed mean ± standard error of the mean of three replicates. *P*-values for statistical significance compared the different treatments according to Independent-sample T-test and LSD test at the 5% level (*, $P<0.05$, **, $P<0.01$). The blue line is the UV treatment; the red line is the control.

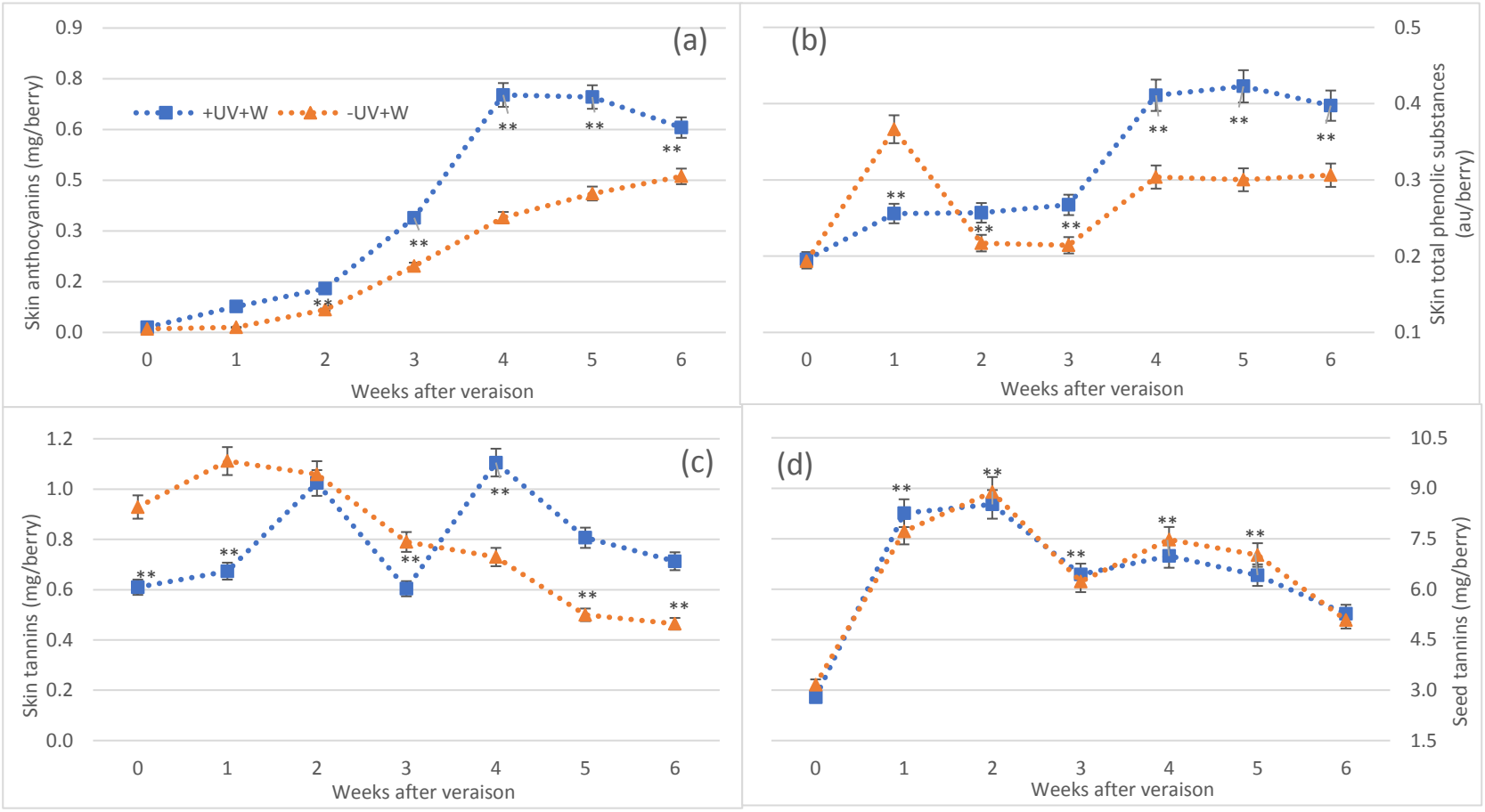


Figure 4.3 Effects of UV-B radiation on (a) skin anthocyanins, (b) skin total phenolic substances, (c) skin tannins and (d) seed tannins in Pinot noir berries during ripening in 2016-2017 glasshouse trials.

Data showed mean ± standard error of the mean of three replicates. *P*-values for statistical significance compared the different treatments according to Independent-sample T-test and LSD test at the 5% level (*, $P<0.05$, **, $P<0.01$). The blue line is the UV treatment; the red line is the control.

Volatile compounds

Table 4.4 illustrated the results from the glasshouse trials for C_6 aldehydes, C_6 alcohols and free monoterpenes of Pinot noir juice. The UV treatment had no effect on C_6 aldehydes (hexanal and E-2-hexenal) over the two trial years. In C_6 alcohols, the UV treatment significantly decreased hexanol and (Z)-3-hexenol in 2015-2016, but no difference was found the following year. (E)-3-hexenol and (E)-2-hexenol were not different between treatments in either year, but treatments in 2016-2017 had twice the concentrations of (E)-3-hexenol and (E)-2-hexenol compared to the 2015-2016 trial. UV effect on free monoterpenes was not consistent across the two experiments, except that *α*-terpineol decreased with UV. The UV treatment caused a significant reduction in linalool, citronellol, nerol and geraniol in 2015-2016, but not in 2016-2017.

Table 4.4 Effects of UV-B radiation on volatile compounds in Pinot noir juice at harvest in 2016 and 2017 glasshouse trials.

Volatile compounds (μg/L)	2015-2016			2016-2017		
	+UV+W	-UV+W	P_{UV}	+UV+W	-UV+W	P_{UV}
C_6 aldehydes						
Hexanal*	334.2	317.1	n.s	223.1	193.5	n.s
(E)-2-hexenal**	177.3	157.3	n.s	119.6	128.8	n.s
C_6 alcohols						
Hexanol**	276.3	322.5	0.017	751.8	683.3	n.s
(E)-3-hexenol**	6.8	7.2	n.s	11.9	11.2	n.s
(Z)-3-hexenol	23.1	31.2	0.006	29.3	30.0	n.s
(E)-2-hexenol**	133.3	172.6	n.s	529.5	571.3	n.s
Free monoterpenes						
Linalool*	1.7	2.0	0.022	1.5	1.5	n.s
α-terpineol*	1.5	2.3	0.044	1.0	1.2	0.016
Citronellol	1.0	1.4	0.010	1.0	1.1	n.s
Nerol	2.5	3.2	0.037	3.0	2.7	n.s
Geraniol*	14.1	18.0	0.011	13.5	13.3	n.s

** indicates a significant difference between 2015-2016 and 2016-2017, $P<0.01$; * indicates a significant difference between 2015-2016 and 2016-2017, $P<0.05$.

Data showed mean ± standard error of the mean of three replicates. *P*-values for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level; n.s, no significant difference. +UV, enhanced UV-B, -UV, no UV-B treatment, +W, well-watered.

4.2.2 Vineyard trials

Effects of UV-B exposure/exclusion on the physiology in Pinot noir vines

In the vineyard, vine physiological traits were measured - leaf chlorophyll content, leaf water potential (LWP), several berry-related parameters and vine crop load. In Figure 4.4a, the trend for leaf chlorophyll content (estimated through SPAD readings) showed a decrease from -4-weeks to veraison and from veraison to harvest in the shading (WSI/II), UV-B exclusion (WPI/II) and UV-B exposure (WLI/II) treatments, but there were no significant differences between treatments. Figure 4.4b indicated that early imposition of the treatments caused a significant decrease in LWP by veraison, but there were no effects of the UV treatments at veraison or harvest.

In the 2015-2016 vintage, the UV-B exposure, UV-B exclusion and shading results are presented in Table 4.5. Compared with shading (SC), UV-B exposure (LR) and UV-B exclusion (PETG) caused increased in °Brix at harvest, but there were no effects on TA and pH. In the 2016-2017 season, an early treatment was imposed pre-veraison (pea-size berries). Under these conditions, there was a significant reduction of TA in the UV-B exposure (WLI/II) and UV-B exclusion (WPI/II) treatments in comparison with the shading (WSI/II) treatments. In leaves, WLI/II had the highest (least negative) carbon isotope ratio (-28.71‰ and -28.30‰) and WLI/II and WPI/II resulted in less negative carbon isotope ratios in juice (Table 4.5). The early imposition of UV-B treatments resulted in higher yields than in the later impositions. The pruning weight gave a hint of the changes between treatments. According to pruning weights and yields, the Ravaz Index showed the lowest value in WLI/II.

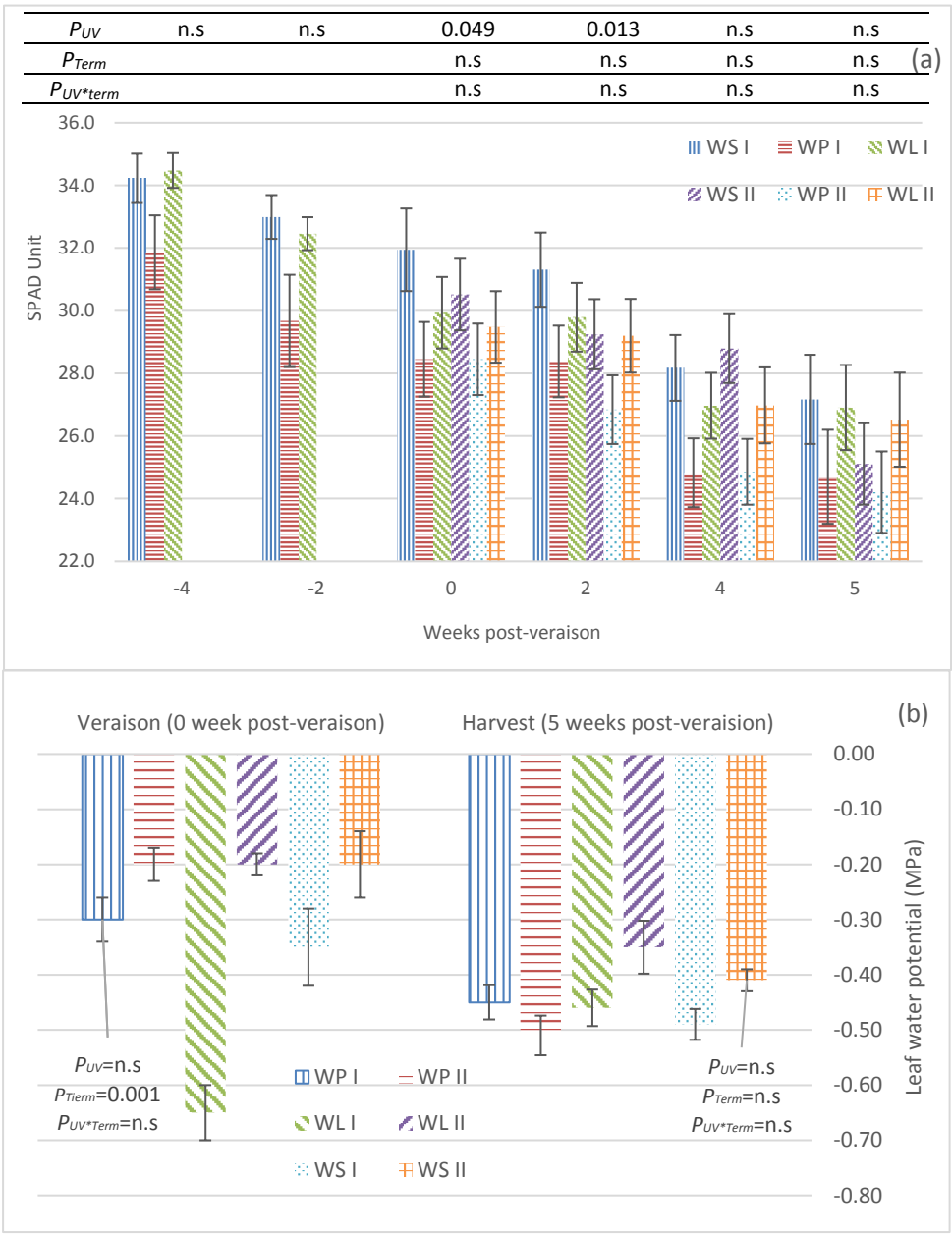


Figure 4.4 Effects of UV-B radiation on leaf chlorophyll content (SPAD unit) (a) and leaf water potential (b) in Pinot noir from pre-veraison or veraison to harvest in 2016-2017 vineyard trials.

Data showed mean ± standard error of four replicates. W, well-water; P, PETG screen, L, UV-B exposure, S, shade cloth; Ⅰ, UV treatment at pre-veraison; Ⅱ, UV treatment at veraison. *P* for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at the 5% level. P_{UV}, UV effects averaged across time treatments; P_{Term}, pre-veraison/veraison set-up effects averaged across UV treatments; $P_{UV*Term}$, pre-

Table 4.5 Effects of UV-B exposure and exclusion on berry parameters, $\delta^{13}C‰$ of leaf and juice, yield, pruning weight and Ravaz Index in Pinot noir at harvest in 2015-2016 and 2016-2017 vineyard trials.

Treatment	°Brix	TA(g/L)	pH	Vine yield (kg)	Pruning weight (kg)	Ravaz Index	Leaf ^{13}C vs V-PDB ‰	Juice ^{13}C vs V-PDB ‰
SC	[a]20.5	8.6	3.53	2.58	0.82	3.38		
LR	[b]21.6	8.6	3.58	2.47	0.66	4.28		
PETG	[b]21.6	8.2	3.59	2.31	0.72	3.32		
P-value	0.028	n.s	n.s	n.s	n.s	n.s		
WS I	16.4	11.0	3.57	4.37	0.87	5.19	-28.88	-28.47
WS II	17.1	11.4	3.65	3.43	0.62	5.55	-29.13	-28.11
WL I	16.6	10.7	3.55	3.24	0.92	3.47	-28.71	-27.53
WL II	18.2	10.4	3.76	1.60	1.21	1.98	-28.30	-26.53
WP I	16.6	10.2	3.59	4.32	0.94	4.81	-28.90	-27.56
WP II	16.4	10.5	3.62	2.97	0.71	4.23	-29.16	-27.74
P_{UV}	n.s	0.036	n.s	n.s	n.s	0.020	0.004	<0.001
P_{Term}	n.s	n.s	n.s	0.034	n.s	n.s	n.s	0.040
$P_{UV*Term}$	n.s	n.s	n.s	n.s	n.s	n.s	n.s	0.046

Data showed mean ± standard error of four replicates from samples at harvest in 2015-2016 and 2016-2017.

P-value, significance of light exposure effect according to One-way ANOVA and LSD test at the 5% level. Different letters indicate significant differences at $P<0.05$; n.s, no significance; PETG, PETG screen, LR, UV-B exposure, SC, shade cloth.

P for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at the 5% level. P_{UV}, UV effects averaged across time treatments; P_{UV}, UV effects averaged across time treatments; P_{Term}, pre-veraison/veraison set-up effects averaged across UV treatments; $P_{UV*Term}$, pre-veraison/veraison set-up effects depend on UV treatments and UV effects depend on time treatments; significant difference at P<0.05; n.s, no significance; W, well-watered; P, PETG; L, LR; S, SC; Ⅰ,UV treatment at pre-veraison ; Ⅱ, UV treatment at veraison.

Effects of UV-B exposure/exclusion on chemical composition of Pinot noir fruit

Amino acids

In the 2015-2016 and 2016-2017 vintages, Pinot noir berries from the vineyard trials were taken at harvest for amino acid analyses by HPLC. Table 4.6 illustrated the results of the amino acid concentrations, and the percentages of the total concentration in 2015-2016. There were no significant differences between treatments for most of the amino acids on a concentration basis, except for Pro. The different treatments had a prominent influence on the concentration of Pro. In the UV-B exposure treatment (LR), the fruit fully exposed to UV-B had the highest Pro concentration, at 2474 µM, whereas the shading treatment (SC) had the lowest, at 1827 µM, in grape juice. Pro in the UV-B exclusion treatment (PETG) was intermediate, at 2222 µM. This result indicated that UV-B exposure can apparently increase Pro in berries. The percentages of His, Thr and Pro were significantly affected by UV-B exposure and exclusion. Compared to SC (11.2%), LR caused an increase in Pro, to 16.1%. With respect to SC, no consistent significant effects from the LR and PETG treatments were found in the percentages of His and Thr. His was increased by PETG and reduced by LR, while both LR and PETG treatments caused increases in Thr.

In 2016-2017, there were no statistically significant effects on amino acids (Table 4.7). Compared to WSI/II, WLI/II significantly increased Pro, Val, Lys, Ser and Gly and decreased Met. UV-B exclusion treatments (WPI/II) caused significant increases in Pro, Val Lys and Gly and reductions in Met and Ser. The time factor had no statistically significant effect on the percentages of individual amino acids, except for Pro (Table 4.8). The earlier UV-B exclusion treatment (WPI) had a higher concentration of Pro than the later one (WPII). However, the shading treatments (WSI/II) and UV-B exposure treatments (WLI/II) had higher Pro concentration in the late imposition than in the early imposition.

Table 4.6 Effects of UV-B exposure/exclusion on amino acids and the percentages of each amino acid in total amino acids in Pinot noir berries at harvest in 2015-2016 vineyard trials.

Amino Acid (μM)	Treatment			*P* value	Treatment			*P* value
	SC	LR	PETG		SC	LR	PETG	
α-ketoglutarate								
Pro	[a]1827	[b]2474	[ab]2222	0.025	[a]11.2%	[b]16.1%	[ab]15.6%	0.007
Arg	6225	5949	5444	n.s	38.2%	39.5%	38.1%	n.s
Glu	246	211	222	n.s	1.5%	1.4%	1.6%	n.s
Gln	1855	856	866	n.s	11.4%	5.7%	6.1%	n.s
His	283	217	253	n.s	[b]1.7%	[a]1.4%	[b]1.8%	0.011
Shikimate (aromatic)								
Phe	443	341	315	n.s	2.7%	2.3%	2.2%	n.s
Trp	144	121	123	n.s	0.9%	0.8%	0.9%	n.s
Tyr	29	20	22	n.s	0.2%	0.1%	0.2%	n.s
Pyruvate								
Leu	515	444	451	n.s	3.2%	2.9%	3.2%	n.s
Val	332	289	284	n.s	2.0%	1.9%	2.0%	n.s
Ala	1647	1489	1482	n.s	10.1%	9.9%	10.4%	n.s
Aspartate								
Asp	281	261	252	n.s	1.7%	1.7%	1.8%	n.s
Asn	101	52	67	n.s	0.6%	0.3%	0.5%	n.s
Thr	1157	1166	1153	n.s	[a]7.1%	[ab]7.7%	[b]8.1%	0.033
Ile	368	309	301	n.s	2.3%	2.1%	2.1%	n.s
Met	89	73	71	n.s	0.5%	0.5%	0.5%	n.s
Lys	66	64	63	n.s	0.4%	0.4%	0.4%	n.s
3-phosphoglycerate								
Cys	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A
Ser	656	669	661	n.s	4.0%	4.4%	4.6%	n.s
Gly	29	32	35	n.s	0.2%	0.2%	0.2%	n.s
Total	16305	15052	14282	n.s				

Data showed the mean of four replicates from samples at harvest in 2015-2016. *P-values* for statistical significance comparing the different treatments according to One-way ANOVA and LSD test at the 5% level. Different letters indicate significant differences at $P<0.05$; n.s, no significance; N.A, not available.

Table 4.7 Effects of UV-B exposure/exclusion on amino acids in Pinot noir berries at harvest in 2016-2017 vineyard trials.

Amino Acid (μM)	Treatments						*P*		
	WP Ⅰ	WP Ⅱ	WL Ⅰ	WL Ⅱ	WS Ⅰ	WS Ⅱ	P_{UV}	P_{Term}	$P_{UV*Term}$
α-ketoglutarate									
Pro	892	748	820	1332	506	516	<0.001	0.023	n.s
Arg	3066	3349	3357	2854	3314	3256	n.s	n.s	n.s
Glu	263	262	270	389	271	276	n.s	n.s	n.s
Gln	2279	2261	2132	3419	2416	2180	n.s	n.s	n.s
His	164	148	158	192	119	117	n.s	n.s	n.s
Shikimate (aromatic)									
Phe	493	465	490	582	440	477	n.s	n.s	n.s
Trp	118	114	99	155	94	69	n.s	n.s	n.s
Tyr	57	52	54	77	47	52	n.s	n.s	n.s
Pyruvate									
Leu	420	372	417	577	383	397	n.s	n.s	n.s
Val	313	272	302	526	791	776	<0.001	n.s	n.s
Ala	1521	1612	1809	2749	1881	1895	n.s	n.s	n.s
Aspartate									
Asp	234	242	223	242	191	218	n.s	n.s	n.s
Asn	63	65	66	97	75	67	n.s	n.s	n.s
Thr	1147	1199	1252	1375	1089	1061	n.s	n.s	n.s
Ile	344	304	343	460	298	309	n.s	n.s	n.s
Met	81	40	13	173	167	169	<0.001	n.s	n.s
Lys	38	40	40	50	27	31	0.015	n.s	n.s
3-phosphoglycerate									
Cys	9	12	10	10	11	6	n.s	n.s	n.s
Ser	775	728	874	1113	798	741	0.037	n.s	n.s
Gly	34	28	36	56	34	30	0.026	n.s	n.s
Total	12312	12314	12764	16429	12951	12646	n.s	n.s	n.s

Data showed mean ± standard error of four replicates from samples at harvest in 2016-2017.

P for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at the 5% level. P_{UV}, UV effects averaged across time treatments; P_{Term}, pre-veraison/veraison set-up effects averaged across UV treatments; $P_{UV*Term}$, pre-veraison/veraison set-up effects depend on UV treatments and UV effects depend on time treatments; significant difference at $P<0.05$; n.s, no significance; W, well-watered; P, PETG; L, LR; S, SC; Ⅰ,UV treatment at pre-veraison ; Ⅱ, UV treatment at veraison.

Table 4.8 Effects of UV-B exposure/exclusion on the percentages of each amino acid in total amino acids in Pinot noir berries at harvest in 2016-2017 vineyard trials.

Amino Acid (μM)	Treatments						*P*		
	WP Ⅰ	WP Ⅱ	WL Ⅰ	WL Ⅱ	WS Ⅰ	WS Ⅱ	P_{UV}	P_{Term}	$P_{UV*Term}$
α-ketoglutarate									
Pro	7.2%	6.1%	6.4%	8.1%	3.9%	4.1%	0.027	n.s	n.s
Arg	24.9%	27.2%	26.3%	17.4%	25.6%	25.8%	n.s	n.s	n.s
Glu	2.1%	2.1%	2.1%	2.4%	2.1%	2.2%	n.s	n.s	n.s
Gln	18.5%	18.4%	16.7%	20.8%	18.7%	17.2%	n.s	n.s	n.s
His	1.3%	1.2%	1.2%	1.2%	0.9%	0.9%	<0.001	n.s	n.s
Shikimate (aromatic)									
Phe	4.0%	3.8%	3.8%	3.5%	3.4%	3.8%	n.s	n.s	n.s
Trp	1.0%	0.9%	0.8%	0.9%	0.7%	0.5%	n.s	n.s	n.s
Tyr	0.5%	0.4%	0.4%	0.5%	0.4%	0.4%	n.s	n.s	n.s
Pyruvate									
Leu	3.4%	3.0%	3.3%	3.5%	3.0%	3.1%	n.s	n.s	n.s
Val	2.5%	2.2%	2.4%	3.2%	6.1%	6.1%	<0.001	n.s	n.s
Ala	12.4%	13.1%	14.2%	16.7%	14.5%	15.0%	0.004	n.s	n.s
Aspartate	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%			
Asp	1.9%	2.0%	1.7%	1.5%	1.5%	1.7%	<0.001	n.s	0.016
Asn	0.5%	0.5%	0.5%	0.6%	0.6%	0.5%	n.s	n.s	n.s
Thr	9.3%	9.7%	9.8%	8.4%	8.4%	8.4%	0.015	n.s	n.s
Ile	2.8%	2.5%	2.7%	2.8%	2.3%	2.4%	n.s	n.s	n.s
Met	0.7%	0.3%	0.1%	1.1%	1.3%	1.3%	0.049	n.s	n.s
Lys	0.3%	0.3%	0.3%	0.3%	0.2%	0.2%	<0.001	n.s	n.s
3-phosphoglycerate									
Cys	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	n.s	n.s	n.s
Ser	6.3%	5.9%	6.8%	6.8%	6.2%	5.9%	0.002	n.s	n.s
Gly	0.3%	0.2%	0.3%	0.3%	0.3%	0.2%	0.024	n.s	n.s

Data showed mean ± standard error of four replicates from samples at harvest in 2016-2017.

P for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at the 5% level. P_{UV}, UV effects averaged across time treatments; P_{Term}, pre-veraison/veraison set-up effects averaged across UV treatments; $P_{UV*Term}$, pre-veraison/veraison set-up effects depend on UV treatments and UV effects depend on time treatments; significant difference at $P<0.05$; n.s, no significance; W, well-watered; P, PETG; L, LR; S, SC; Ⅰ,UV treatment at pre-veraison ; Ⅱ, UV treatment at veraison.

Phenolic composition

To determine the effects of UV-B exposure and exclusion on Pinot noir fruit, samples were taken during ripening from both vintages and analysed for skin total phenolics, skin anthocyanins, skin tannins and seed tannins. For the 2015-2016 vintage, skin anthocyanins on a per berry basis were shown in Figure 4.5a. Values for the shading treatment (SC) remained relatively stable during ripening and peaked at 4-weeks post veraison. The UV-B exposure treatment (LR) and UV-B exclusion (PETG) had similar patterns with SC. Compared with SC, skin anthocyanin contents were significantly increased by the UV-B exposure treatment (LR) and UV-B exclusion (PETG) from 2-weeks to 5-weeks post-veraison (harvest). At harvest, LR and PETG had 0.561 and 0.422 mg/berry, respectively, compared to 0.374 mg/berry in SC.

The contents of skin total phenolics in SC, PETG and LR increased to the peaks at 4-weeks post-veraison and then declined (Fig. 4.5b). LR had higher skin total phenolics contents than SC from 2-weeks to 5-weeks post-veraison. Once exposed, skin total phenolics values for UV-B (LR) fruit were higher compared to those under PETG, with a rise from 3-weeks to 5-weeks post-veraison. The skin total phenolics contents were higher in LR (0.334 au/berry), compared with PETG (0.252 au/berry) and SC (0.254 au/berry) at harvest. These results indicated that the major increases in skin total phenolics and anthocyanins were because of the effect of UV-B.

During ripening, the contents of skin tannin in the treatments presented net decreases (Fig. 4.5c). SC caused decreases in skin tannin contents from 1-weeks to 4-weeks post-veraison and then increased at harvest. Skin tannins in LR went down after one week of the treatment and up in the following two weeks and decreased at harvest. Skin tannin contents in PETG increased to the peak at 2-weeks post-veraison and declined until harvest. The reduction in skin tannins for SC was 0.358 mg/berry from 1-week to 5-weeks post-veraison (harvest), while the losses for LR and PETG were 0.226 mg/berry and 0.300 mg/berry, respectively.

Seed tannins through their development also illustrated increases and then decreases in the treatments (Fig. 4.5d). The peaks of the seed tannin contents in treatments showed at 2-weeks post-veraison. PETG had the highest contents of seed tannin than SC and LR from 2-weeks to 4-weeks post-veraison, but the effects on seed tannins swapped at harvest. The contents of seed tannins in PETG was the lowest than SC and LR. The net increase in seed tannins was from 5.484 mg/berry at 1-week post-veraison to 9.471 mg/berry at 5-weeks post-veraison in SC. With respect to SC, LR and PETG significantly decreased the contents of seed tannin with 8.450 mg/berry and 7.694 mg/berry, respectively, at 5-weeks post-veraison.

In the 2016-2017 vintage, the skin anthocyanin contents in all treatments significantly increased from 0-week (veraison) to 5-weeks post-veraison (harvest) and peaked at 4-weeks post-veraison (Fig. 4.6a). In comparison to the shading treatments (WSI/II), the UV-B exposure treatments (WLI/II) and UV-B exclusion (WPI/II) had significantly higher contents of skin anthocyanin at all stages of development. At harvest, WLI/II and WPI/II had increased skin anthocyanins to 0.356/0.446 mg/berry and 0.281/0.242 mg/berry, respectively.

The contents of skin total phenolics from -4-weeks post-veraison to 5-weeks post-veraison in the treatments were variable (Figure 4.6b). At harvest, consistent UV-B exposure (WLI/II) responses were observed in skin total phenolics. The time of the UV-B exposure treatment (WLI/II) caused significant increases in skin total phenolics (0.261/0.292 au/berry), whereas skin total phenolics were increased by the earlier UV-B exclusion treatment (WPI) and were reduced by the late UV-B exclusion (WPII), compared with the shading treatments (WSI/II).

In Figure 4.6c, the contents of skin tannin increased and then reduced in all treatments from -4 weeks post-veraison to 5 weeks post-veraison. There were no significant differences between different light environmental treatments from -4/0-weeks to 4-weeks post-veraison. At harvest, WPI/II (0.689/0.540 mg/berry) significantly increased the contents of skin tannins and reduced skin tannins in WLI/II (0.511/0.414 mg/berry), compared to WSI/II (0.531/0.524 mg/berry). Early LR increased skin tannins from veraison to harvest, though the

fell back to around the same levels by veraison at harvest. Early applied PETG, however, caused decreases in skin tannins from -2-weeks to 2-weeks post-veraison and increases to similar contents of skin tannin by veraison at harvest. The early treatments significantly affected skin tannin contents from 1-weeks to 3-weeks post-veraison.

In Figure 4.6d, the values for seed tannins fluctuated from -4-weeks to 5-weeks post-veraison. There were significant effects of UV on seed tannin contents at 1-week and 2-weeks post-veraison, but no effect from any treatment at 5-weeks post-veraison (harvest). Seed tannin contents in WPI/II (4.811/4.921 mg/berry) at 1-week post-veraison were lower than WLI/II (5.223/5.102 mg/berry) and WSI/II (5.723/5.078 mg/berry), while WLI/II had lower seed tannin contents at 2-weeks post-veraison.

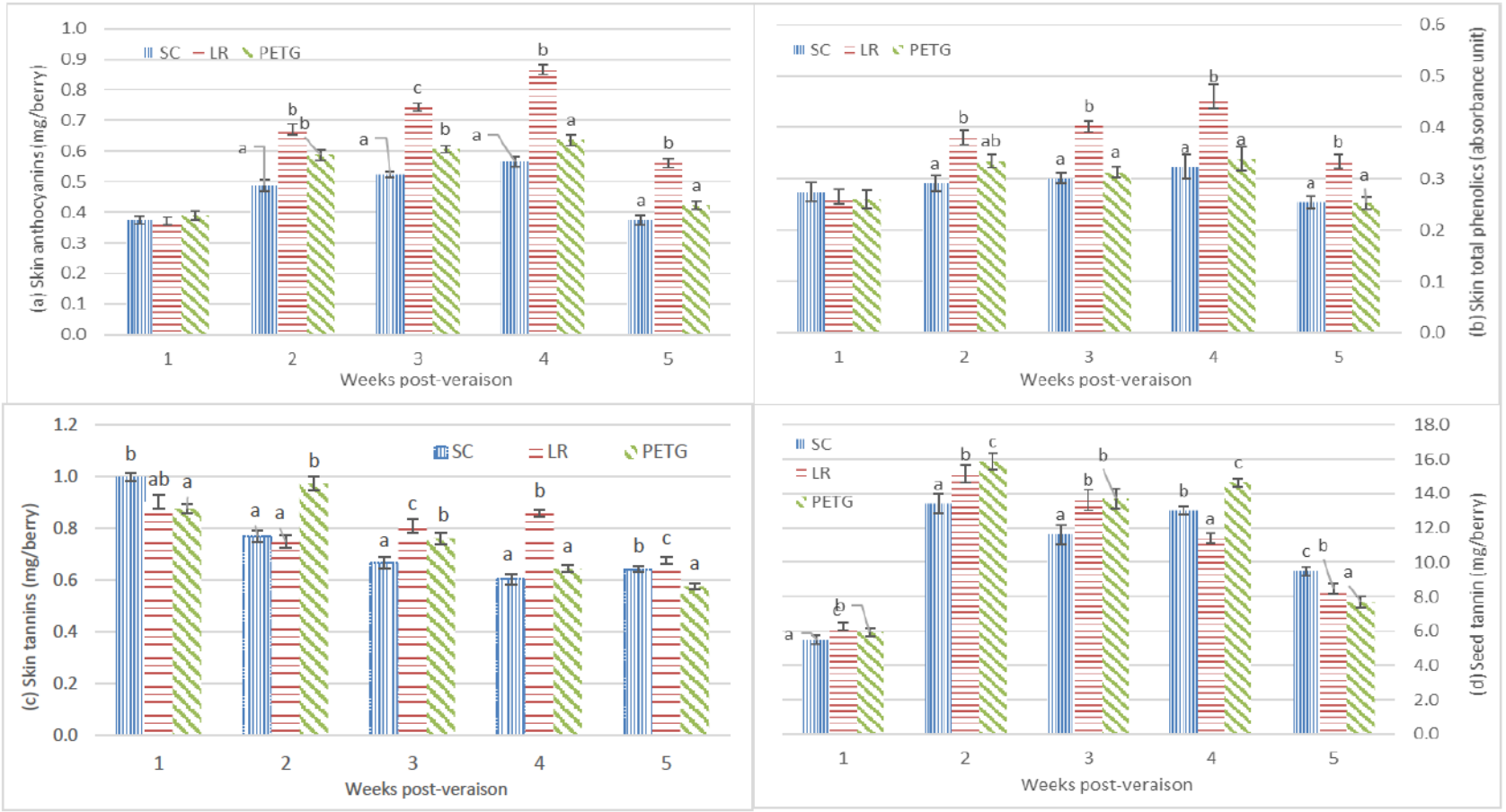
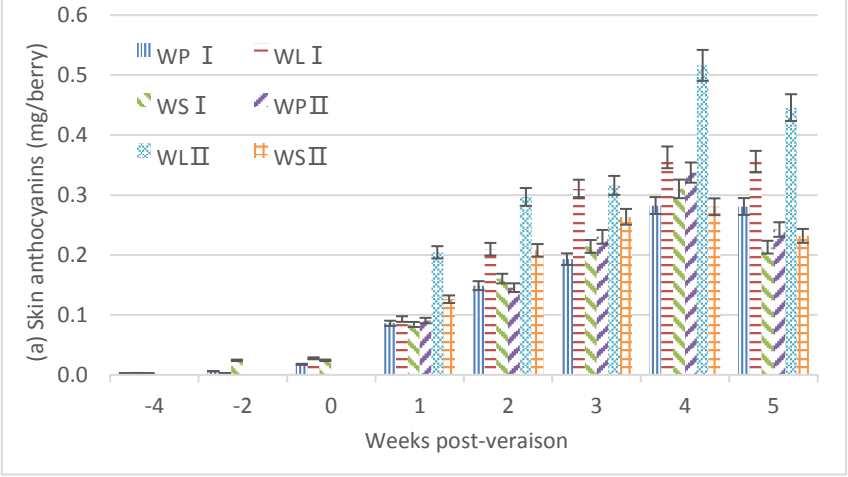


Figure 4.5 Effects of UV-B exposure/exclusion on (a) skin anthocyanins, (b) skin total phenolic substances, (c) skin tannins and (d) seed tannins in Pinot noir berries during ripening in 2015-2016 vineyard trials.

Data showed mean ± standard error of four replicates. *P*-values for statistical significance compared the different treatments according to One-way ANOVA and LSD test at the 5% level. Different letters indicate significant differences at $P<0.05$.

P_{UV}	n.s	0.001	n.s	n.s	n.s	n.s	0.010	0.006
P_{Term}				n.s	n.s	n.s	n.s	n.s
$P_{UV*term}$				n.s	n.s	n.s	n.s	n.s



<0.001	0.001	n.s	0.003	0.028	n.s	<0.001	0.005	P_{UV}
			n.s	n.s	n.s	n.s	n.s	P_{Term}
			n.s	n.s	n.s	n.s	n.s	$P_{UV*term}$

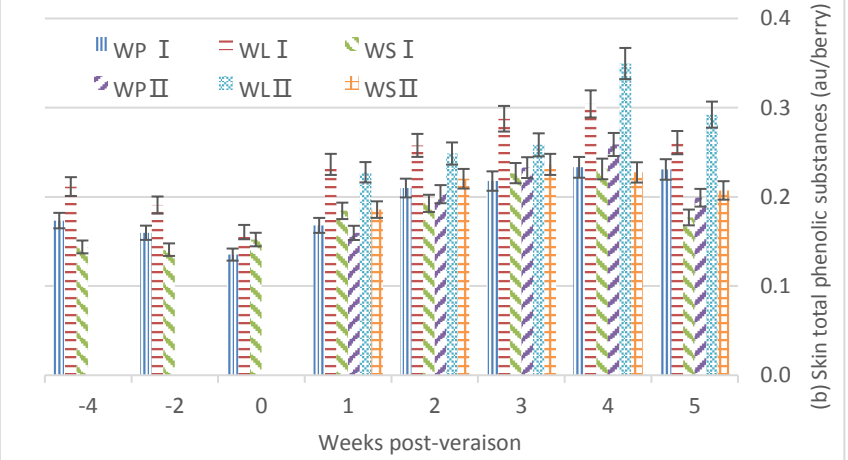
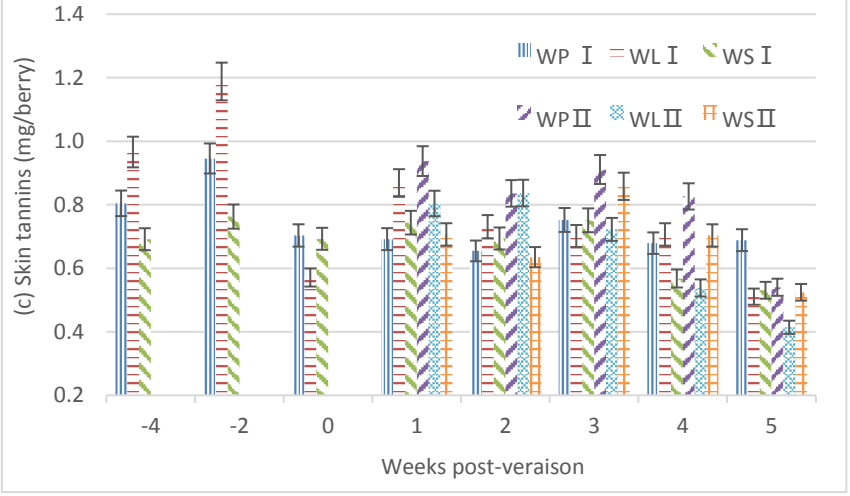


Figure 4.6 Effects of UV-B exposure/exclusion on (a) skin anthocyanins and (b) skin total phenolic substances in Pinot noir berries from pre-veraison/veraison to harvest in 2016-2017 vineyard trials.

Data showed mean ± standard error of four replicates. W, well-water; P, PETG screen, L, UV-B exposure, S, shade cloth; Ⅰ, UV treatment at pre-veraison; Ⅱ, UV treatment at veraison. *P* for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at the 5% level. P_{UV}, UV effects averaged across time treatments; P_{Term}, pre-veraison/veraison set-up effects averaged across UV treatments; $P_{UV*Term}$, pre-veraison/veraison set-up effects depend on UV treatments and UV effects depend on time treatments.

P_{UV}	n.s	n.s	n.s	n.s	n.s	n.s	n.s	0.012
P_{Term}				<0.001	0.021	0.001	n.s	n.s
$P_{UV*term}$				n.s	n.s	n.s	n.s	n.s



<0.001	n.s	n.s	0.001	0.048	n.s	n.s	n.s	P_{UV}
			n.s	n.s	n.s	n.s	n.s	P_{Term}
			n.s	n.s	n.s	n.s	n.s	$P_{UV*term}$

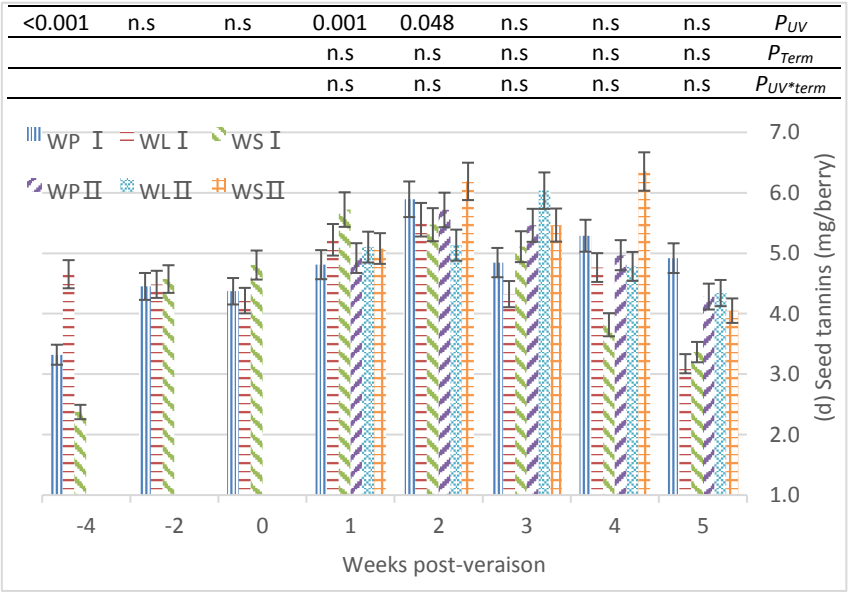


Figure 4.7 Effects of UV-B exposure/exclusion on (c) skin tannins and (d) seed tannins in Pinot noir berries from pre-veraison/veraison to harvest in 2016-2017 vineyard trials.

Data showed mean ± standard error of four replicates. W, well-water; P, PETG screen, L, UV-B exposure, S, shade cloth; Ⅰ, UV treatment at pre-veraison; Ⅱ, UV treatment at veraison. *P* for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at the 5% level. P_{UV}, UV effects averaged across time treatments; P_{Term}, pre-veraison/veraison set-up effects averaged across UV treatments; $P_{UV*Term}$, pre-veraison/veraison set-up effects depend on UV treatments and UV effects depend on time treatments.

Volatile composition

In the 2015-2016 vineyard trials, there were no statistically significant effects from UV-B exposure and exclusion on volatile compounds in Pinot noir juice (Table 4.9). In the following year, the treatments did not significantly affect the C_6 aldehyde family at harvest. At the harvest sampling in the UV-B exposure (WLI/II) and UV-B exclusion (WPI/II) treatments, (Z)-3-hexenol concentrations were 74.0/45.1 μg/L and 90.5/76.6 μg/L, respectively, compared with 98.2/89.5 μg/L in WSI/II. Significant decreases in (E)-2-hexenol were induced by WPI/II and WLI/II in Pinot noir juice, and the earlier treatments had higher concentrations of (E)-2-hexenol than the late treatments, except for WP. WPI/II caused significant increases in α-terpineol and nerol, and WLI/II reduced α-terpineol, but increased nerol at harvest, compared with WSI/II. Meanwhile, the earlier treatments had higher concentrations of nerol than the late treatments.

Table 4.9 Effects of UV-B exposure/exclusion on volatile compounds in Pinot noir juice at harvest in 2015-2016 and 2016-2017 vineyard trials.

Treatment	C_6 aldehydes (μg/L)		C_6 alcohols (μg/L)				Free monoterpenes (μg/L)				
	Hexanal	(E)-2-hexenal	Hexanol	(E)-3-hexenol	(Z)-3-hexenol	(E)-2-hexenol	Linalool	α-terpineol	Citronellol	Nerol	Geraniol
SC	34.7	66.4	749.7	11.9	38.4	401.3	1.7	1.6	1.2	4.7	17.6
LR	44.5	67.8	671.0	11.7	43.1	404.5	1.7	1.4	1.0	4.8	16.9
PETG	33.8	71.1	754.3	11.6	41.5	446.2	1.7	1.6	1.0	5.1	18.2
P-value	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
WP I	29.2	28.3	1032.2	12.9	98.2	305.4	1.8	1.3	0.8	3.6	12.3
WP II	31.4	33.9	1062.2	14.9	89.5	156.1	1.7	1.1	1.0	4.4	13.8
WL I	37.6	37.4	1198.1	17.4	74.0	49.8	1.7	1.2	0.9	5.2	11.1
WL II	43.7	51.9	1064.8	15.7	45.1	11.7	1.8	1.3	1.2	8.5	13.3
WS I	31.1	56.6	1083.3	16.7	90.5	130.6	1.8	1.4	1.0	4.6	12.1
WS II	35.0	35.1	1118.2	17.4	76.6	148.9	1.8	1.4	1.0	4.8	12.7
P_{UV}	n.s	n.s	n.s	n.s	0.042	<0.001	n.s	0.012	n.s	<0.001	n.s
P_{Term}	n.s	n.s	n.s	n.s	n.s	<0.001	n.s	n.s	n.s	0.002	n.s
$P_{UV*Term}$	n.s	n.s	n.s	n.s	n.s	<0.001	n.s	n.s	n.s	0.013	n.s

Data showed mean ± standard error of four replicates from samples at harvest in 2015-2016 and 2016-2017.

P-value, significance of light exposure effect according to One-way ANOVA and a Fisher's LSD test at the 5% level. Different letters indicate significant differences at $P<0.05$; n.s, no significance; PETG, PETG screen, LR, UV-B exposure, SC, shade cloth.

P for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at the 5% level. P_{UV}, UV effects averaged across time treatments; P_{Term}, pre-veraison/veraison set-up effects averaged across UV treatments; $P_{UV*Term}$, pre-veraison/veraison set-up effects depend on UV treatments and UV effects depend on time treatments; significant difference at $P<0.05$; n.s, no significance; W, well-watered; P, PETG; L, LR; S, SC; Ⅰ,UV treatment at pre-veraison ; Ⅱ, UV treatment at veraison.

4.3 Discussion

4.3.1 Alteration of vine physiological factors as induced by UV-B radiation

In this study, the effects of UV-B on SPAD and the stable carbon isotope composition are related to grapevine photosynthesis. In the glasshouse, UV-B decreased SPAD value during ripening, compared with the control (Fig. 4.1b), which was consistent with Hoel and Solhaug (1998) and Núñez-Olivera et al. (2006). The environmental parameters (temperature and humidity) in the glasshouse were controlled by a thermostat during the trial period, so grapevines in the control and the UV-B treatment exactly received the same temperature over the whole trial period. The two layers of 125-micron clear natural polythene laid over the top of the glass resulted in a reduction of approximately 66% PAR and the exclusion of UV-A/B. The low ratio of PAR to UV-B may enhance the sensitivity of leaves to UV-B (Krizek, 2004) and showed changes in pigment composition and stomatal resistance (Jordan, 1996). It may be that the SPAD value depends on light transmittance of leaves, where decreases in SPAD value are associated with increases in light transmittance (Martínez and Guiamet, 2004). UV-B leads the chloroplasts to move to the periclinal cell walls with an increase in light transmittance as a result (Martínez and Guiamet, 2004), which could be associated with a decrease in SPAD in the glasshouse. However, there was no statistically significant effect of UV-B exclusion or UV-B exposure on SPAD in the vineyard in comparison with shading (Fig. 4.4a). That UV-B may have a reduction in leaf greenness in the glasshouse, but not in the vineyard, could be attributed to the ratio of PAR to UV-B. In the vineyard, high PAR penetrates through the outside cell layers to drive photosynthesis and protects the grapevines from damage of UV-B exposure (Krizek, 2004). Therefore, the high ratio of PAR to UV-B will lead to a smaller degree of damage compared to the low ratio (Hideg and Strid, 2017; Jordan et al., 2016). Furthermore, considering the limitation of a SPAD meter, although multiple measurements of SPAD have been taken in the glasshouse and vineyard treatments, the SPAD values may be influenced by a redistribution of chloroplasts and uneven distribution in mesophyll cells (Nauš et al., 2010).

The carbon isotope composition ratio ($\delta^{13}C‰$) was investigated with its response to UV-B in Pinot noir leaves and juice in the glasshouse and vineyard. UV-B significantly increased (made less negative) $\delta^{13}C‰$ of leaves in the glasshouse (Table 4.1) and vineyard (Table 4.5) and $\delta^{13}C‰$ of juice in the vineyard. $\delta^{13}C‰$ is related to the ratio of intercellular and atmospheric CO_2 concentrations (C_i/C_a). Therefore, these results align with those reporting UV-B causes stomatal closure resulting in a reduction in gas exchange between the outside and inside of the leaf, changing the amount of intercellular $^{13}CO_2$ that is directly involved in carboxylation to produce triose phosphate in the chloroplast stroma (Guehl et al., 1995; Taiz et al., 2015). The triose phosphate is transferred into the cytoplasm for the synthesis of sucrose in the berries (Gaudillère et al., 2002). Thus, ^{13}C can be incorporated into sucrose and changes in the carbon isotope composition can be measured in grape juice.

The Ravaz Index (fruit yield/pruning weight) is a useful parameter for reflecting the final capacity of vines given the management practices and also in evaluating vine balance (Howell, 2001). Vine balance helps to maintain productive yields, fruit quality and vine health (Kliewer and Dokoozlian, 2005). Yields and pruning weights in the two vintages were not affected by PETG, SC or LR (Table 4.5), but differences in yields were shown between the two treatment timings in 2016-2017. These changes may be due to bird damage not directly related to the treatments. Late timing treatments caused more colour accumulation (Fig. 4.6a) than early treatments in grape skins during ripening, which are more attractive to birds. The Ravaz Index is an effect driven by differences in yields and pruning weights. In the 2015-2016 and 2016-2017 vintages, there was no consistent effect of UV-B on the Ravaz Index (Table 4.5). This result is supported by Howell (2001), who stated that there were strong annual fluctuations in weather conditions during the growing season in cool climates. In 2016-2017 vintage, there was rainfall, again, during the ripening period, leading to fluctuate the yield and pruning weight. In addition, WLI/II had the lowest Ravaz Index than WSI/II and WPI/II, due to the decreases in yields in WLI/II.

4.3.2 The effects of UV-B radiation on amino acids in berries

Amino acids are a very important nitrogen source for grape fermentation. In grape leaves, NH_4^+ is assimilated into amino acids via glutamine synthetase (GS) and the glutamate synthase (GOGAT) pathway in chloroplasts (Lam et al., 1996). Glutamine (Gln) is biosynthesized via this pathway and is the major nitrogen transport compound from leaves to berries (Forde and Lea, 2007). Light and other radiation plays an important role in the assimilation of amino acids, but there is less known about regulation of the synthesis of various amino acids in grapevines (Bell and Henschke, 2005; Gregan et al., 2012). Earlier studies of amino acids in berries and the effect of UV radiation are limited and their results contradictory. Previous research has showed no effect of UV-B radiation on total free amino acids in Tempranillo and Sauvignon blanc grapes (Keller and Torres-Martinez, 2002; Martínez-Lüscher et al., 2014b), but UV significantly decreased the concentration of amino acids in Riesling (Schultz et al., 1998). The results of this two-year trial of Pinot noir fruit found that the effects of UV-B radiation on amino acid concentrations were contradictory in the glasshouse. There, berries in 2015-2016 had lower concentrations of amino acids under UV-B than in the control treatment. This could be because fruit suffered from *Botrytis* in the 2015-2016 glasshouse trial. Although we removed the unhealthy appearing clusters, pathogen-related (PR) proteins can be produced before infection is apparent, consuming amino acids in the vines. PR proteins are synthesized by the pathogen/wound/defence pathway and induced by biotic and abiotic stresses, including pathogens, UV radiation, salt and wounding (Azarkan et al., 2004; Dhekney et al., 2011; Linthorst and Van Loon, 1991). Therefore, UV-B decreased the amounts of six of the amino acids at harvest in 2015-2016 of the glasshouse trials.

However, UV-B caused little to a slight increase in amounts of amino acids in 2016-2017 glasshouse trial, consistent with the two years vineyard trials, except for Pro (Table 4.7). Pro as an abundant amino acid in berries was significantly changed in the vineyard trials, which may be related to the level of berry maturity. Pro was found in grape berries from the

vineyard with all fruit harvested at a similar level of maturity in both seasons. However, the shading treatments had lower levels of °Brix than the other treatments, and °Brix in 2015-2016 was higher than in 2016-2017 (Table 4.5). Pro evolution in berry development is confined to late ripening period, from around 4-weeks post-veraison (Bell and Henschke, 2005; Stines et al., 2000). With respect to the accumulation of Pro during ripening, similar results were reported by Garde-Cerdán et al. (2018), which showed significant Pro accumulation in grapes from post-veraison and the peak of proline concentrations at 25°Brix. Therefore, the lower level of Pro in grape juice could have been driven by the lower °Brix. Also, Pro in plant tissues mostly accumulates in response to osmotic stress (Downton and Loveys, 1978). At the late stages of ripening, the berry pulp could be affected by an increase in osmotic pressure with the increasing sugar concentration. Thus, it is possible that the accumulation of Pro occurs in response to this developmentally-imposed osmotic stress and plays an osmotic-protective role in the developing berry cells (Stines et al., 2000). In addition, in grapevine vegetative and reproductive tissues, ornithine can be transferred into Δ1-pyrroline-5-carboxylate (P5C) via the activity of δ-ornithine aminotransferase (OAT); and P5C is a precursor of proline (Stines et al., 2000). However, no evidence suggested that OAT under UV-B played a regulatory role in determining the Pro concentration in berries.

4.3.3 The phenolic composition in berries in response to UV-B radiation

The phenolic composition was analysed in Pinot noir fruit at different stages during ripening. The contents of skin anthocyanin and skin total phenolics showed developmental accumulation in Pinot noir berries from veraison to harvest in the glasshouse (Fig. 4.2a/b and 4.3a/b). At harvest, the UV-B treatment had significantly increased contents of skin anthocyanin and skin total phenolics in both 2015-2016 and 2016-2017. In the vineyard, compared with the shading treatment (SC), the light exposure treatment (LR) and UV-B exclusion treatment (PETG) caused an increase in skin anthocyanins and skin total phenolics during berry development (Fig. 4.5a/b), in particular, LR. In 2016-2017, consistent results in response to light/UV-B were shown in skin anthocyanins and skin total phenolics (Fig.

4.6a/b). These results were consistent with Del-Castillo-Alonso et al. (2016), Carbonell-Bejerano et al. (2014), Cortell and Kennedy (2006) and Pastore et al. (2013) who stated that UV-B radiation affected the biosynthesis of skin anthocyanins and skin total phenolics, due to the up-regulation of the genes encoding for flavonoid 3',5'-hydroxylase (F3'5'H) and flavonoid glucosyltransferase (UFGT) by UV-B (Falginella et al., 2012; Martínez-Lüscher et al., 2014a). Moreover, UV-B stimulates phenolic compounds in Pinot noir berry skins, which play a role as UV protectants/antioxidants (Teixeira et al., 2013). In addition, there was lower skin anthocyanins accumulation in the first glasshouse trial, compared to the second one, most likely because of *Botrytis* infection. These results were consistent with a previous finding that skin anthocyanins decreased drastically in grape skins of *Botrytis*-affected berries (Ky et al., 2012). During a *Botrytis* infection and grape tissue colonization, the mycelium grows at the fruit surface and within the skin tissue, leading to direct contact between the skin compounds, especially phenolics and fungal extracellular enzymes (Bollag and Leonowicz, 1984; Ky et al., 2012). These extracellular enzymes can catalyse the oxidation of phenolic compounds, including anthocyanins (Osman et al., 2007; Pezet, 1998). The following vintage was a challenging season with rainfall again (see Table 5.5), resulting in low °Brix further than the decreases in fruit phenolic composition. It could be explained that biosynthesis of anthocyanins appeared to highly depending on berry sugar levels, of which sugar levels are not only carbohydrate sources, but also involved in the stimulation of gene activities (Bobeica et al., 2015; Dai et al., 2014; Zheng et al., 2009). Sucrose can up-regulate *F3H* expression, coinciding with the enhancement of anthocyanin and phenolic composition levels (Dai et al., 2014; Solfanelli et al., 2006).

Tannins are polymeric structures of flavan-3-ols and can be composed of chains of almost identical subunits. Skin tannins are longer, on average, than seed tannins, but the amount of seed tannins is higher than skin tannins in grapes (Downey et al., 2003; Keller, 2015). In this study, the accumulation of skin and seed tannins during berry development fluctuated, but the overall trends showed an increase in skin tannins (Fig. 4.2c) and a reduction in seed tannins (Fig. 4.2d). The skin tannins in the 2015-2016 glasshouse trials were lower than in

2016-2017, probably due to *Botrytis* (see above). In the glasshouse, the control had lower skin tannins than the other treatments at harvest (Fig. 4.2c and 4.3c). In the vineyard, two vintages did have consistent results at harvest (Fig. 4.5c and 4.6c). These results suggested that tannins may have accumulated to a greater extent under UV radiation to protect grape skins, which could be attributed to the stimulation of ANR and LAR gene expression to regulate the synthesis of skin tannins (Bogs et al., 2005; Downey et al., 2004; Martínez-Lüscher et al., 2014a; Martínez-Lüscher et al., 2014b). The fluctuation of skin tannins during ripening may relate to the polymeric flavan-3-ols. This polymerization is dramatically influenced by environmental factors, such as UV-B, temperature and rainfall, and changed at different stages of berry development (Cortell and Kennedy, 2006; Downey et al., 2003; Kennedy et al., 2002). Thus, in 2016-2017 vintage, environmental factors at harvest may influence on the polymerization of tannins in berry skins resulting in not consistent results with two years glasshouse and the first vintage vineyard trials. There were no consistent effects of UV-B on seed tannins in either year in the glasshouse and vineyard. It could because only a small part of UV-B can pass the anticlinal cell walls to impact on sub-dermal tissues and, as UV-B cannot penetrate far into the berry, direct impact on seed tannins is unlikely (Jordan, 1996). Moreover, fruit in the 2015-2016 glasshouse trials suffered from *Botrytis*, but fungal development was mostly localized within berry skins, resulting in limited contact with the seeds (Ky et al., 2012).

4.3.4 Effects of UV-B radiation on volatile composition in berry juice

To investigate the effects of UV-B radiation on volatile composition in Pinot noir juice, samples were taken at harvest from the glasshouse and vineyard trials. The C_6 aldehydes, C_6 alcohols and monoterpenes are the most important terpene families responsible for fruity and floral aromas. There were no consistent patterns to the changes in volatile composition at harvest in the glasshouse and vineyard trials. C_6 compounds were not different in either year of the glasshouse and vineyard trials, except for a reduction in hexanol and (Z)-3-hexenol in the 2015-2016 glasshouse experiment, and (Z)-3-hexenol in the 2016-2017

vineyard experiment, under the UV-B treatments (Table 4.4 and 4.9). This suggests that the formation and degradation of C_6 compounds in berries in response to UV-B is not clear. Some alterations of C_6 compounds may be because UV-B induces the catabolism of fatty acids in cell membranes (Gil et al., 2013). Also, hexanal can be converted into other volatile compounds to play a role in defence signalling (Halitschke et al., 2004). UV-B caused significant reductions in free monoterpenes in the 2015-2016 glasshouse trials, but there was no response to UV-B in the 2016-2017 glasshouse trials. In the vineyard trials, there was no difference from UV-B on monoterpenes in either 2015-2016 or 2016-2017, except for α-terpineol and nerol, in 2016-2017. These results may align with the idea that the biosynthesis of monoterpenes in berry skins is via the 1-deoxy-D-xylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate (DOXP/MEP) pathway in plastids and the mevalonate pathway in cytosols. In these pathways, prenyl diphosphates are catalysed by terpene synthases (TPS) to produce an abundance of terpenes. UV-B stimulates TPS, leading to the synthesis of monoterpenes (Gil et al., 2012; Lücker et al., 2004; Pontin et al., 2010). Moreover, UV-B stimulated the production of ROS, which caused the generation of monoterpenes to protect grapes from oxidative damage (Berli et al., 2010; Grassmann et al., 2005; Lee et al., 2005). The stimulation of monoterpenoids by UV enhanced the high aroma and contributed to the characteristic fragrances (eg. Rose, lilac, pine and citrus) in grapes, which could be transferred into final wine (Keller, 2015).

4.4 Conclusion

The metabolites in Pinot noir berries were altered by UV radiation in both the glasshouse and vineyard experiments. In the glasshouse, the decreases in SPAD and the stable carbon isotope composition under UV-B conditions inferred to the reduction in grapevine photosynthesis, but there was no similar change in vines in the vineyard. The phenolic composition in berries changed depending on berry development. The skin total phenolics and anthocyanins in these berries showed an increasing pattern throughout the ripening period measured. Quantitative increases in skin total phenolics and anthocyanins were also

observed in response to UV-B radiation from 1-week post-veraison to harvest. At harvest, the increases in the contents of the skin total phenolics and anthocyanin were driven by UV radiation in both the two vintages of glasshouse and vineyard trials. However, there were inconsistent changes of skin and seed tannins through two years of glasshouse and vineyard trials. Skin tannin contents showed substantial decreases in the treatments during ripening. There are large differences of skin tannins under UV-B during mid-ripening, but then the effect swapped, and the UV ended up a little higher at harvest. In the vineyard, the UV-B exposure treatment had higher contents of skin tannin than shading and UV-B exclusion treatments during ripening in 2015-2016. However, in the following year, the changes in skin tannins were shown by different light environment only at harvest and by the timings of treatment from 1-week to 3-weeks post-veraison. UV radiation did not affect amino acids but caused slight increases in monoterpenoids in this study. Therefore, UV radiation may potentially affect fruit ripeness and crop load and stimulate the accumulation of phenolic compounds that may affect Pinot noir quality.

Chapter 5

Effects of water deficit on the vine physiology and chemical composition of Pinot noir fruit

5.1 Introduction

Many vineyards are in areas experiencing seasonal droughts and where soil and atmospheric water deficit limits yield and quality (Jackson and Lombard, 1993). Water deficit has multifaceted effects on grapevine growth and metabolism (Bertamini et al., 2006; Cramer et al., 2007b). *Vitis vinifera* has been generally classified as a "drought avoiding" species through the control of stomatal apertures to effectively control transpiration (Chaves et al., 2010; Poni et al., 2014). In response to drought, stomatal behaviour of grapevines can be physiologically classified into isohydric and anisohydric (Schultz, 2003; Tardieu and Simonneau, 1998). In Mejias-Barrera (2016) study, Pinot noir is an isohydric cultivar in the Canterbury region (cool climate) as the same region as this study. Leaf water potential (LWP) as one physiological indicator predominantly depends on stomatal behaviour in response to water deficit. At the carboxylation step, $^{12}CO_2$ is preferentially utilised to $^{13}CO_2$ by Rubisco under normal circumstances, because Rubisco has higher reactivity to ^{12}C than to ^{13}C (Farquhar et al., 1989). Stomatal constraints lead to a decrease in CO_2 uptake in grapevine leaves under water deficit. Thus, intercellular $^{13}CO_2$ is more likely to be directly involved in the substrate of Rubisco (Gaudillère et al., 2002; Santesteban et al., 2012). Therefore, the evaluation of the carbon isotope ratio in grapevines can reflect vine water status under water deficit.

Water deficit also changes the chemical composition (berry parameters, amino acids, phenolic composition and volatile compounds) in fruit (Koundouras et al., 2006; Loulakakis and Roubelakis-Angelakis, 2001; Song et al., 2012). Water deficit can increase the concentration of total free amino acids as well as some individual amino acids in grapevine

leaves and berries (Bertamini et al., 2006). In this study, phenolic compounds, including skin total phenolics, skin anthocyanins, skin tannins and seed tannins, were measured. These compounds in grapes can potentially influence the final wine characteristics such as colour, mouth-feel and antioxidant potential (Downey et al., 2006). Water deficit may increase skin total phenolics, skin anthocyanins and skin tannins during ripening (Castellarin et al., 2007; Kennedy et al., 2002; Martinez-Luscher et al., 2015). Volatile compounds (C_6 compounds and terpenoid) may have different changes under water deficit (Deluc et al., 2009). Water deficit increases the concentrations of terpenoids, but causes the decreases in C_6 compounds in previous researches (Mendez-Costabel et al., 2014; Song et al., 2012). This study relied on irrigation to change grapevine water status in the vineyard and glasshouse. The purpose of this chapter is to report on the effects of water deficit on the physiological and chemical responses in Pinot noir vines and fruit.

5.2 Results

5.2.1 Glasshouse trials

Effects of water deficit on vine physiology

The limited irrigation caused a reduction in the soil volumetric water content in the water deficit treatment (-UV-W) during ripening (Fig. 5.1a). -UV-W maintained the soil volumetric water content at around 10%, compared with about 30% in the control (-UV+W). This indicator was related to leaf water potential at harvest (Table 5.1). A water deficit significantly decreased (made more negative) the leaf water potential at harvest, with -1.38 MPa and -0.94 MPa in -UV-W and -UV+W, respectively. Also, significant increases (made less negative) were illustrated in both of leaf and juice carbon isotope ratios at harvest under water deficit (Table 5.1). As shown in Figure 5.1b, the SPAD level declined during ripening. After one week of -UV-W, the SPAD level declined substantially and then showed a parallel trend. At harvest, -UV-W caused a significant decrease in SPAD, compared to -UV+W.

As shown in Table 5.1, there were no consistent effects of water deficit on berry parameters. Water deficit did not affect °Brix, TA and pH in the 2015-2016 glasshouse trials. Similar results were shown in the 2016-2017 trials, but °Brix was increased by water deficit. In comparing the two years, the 2015-2016 trials had higher pH values than the 2016-2017 trials.

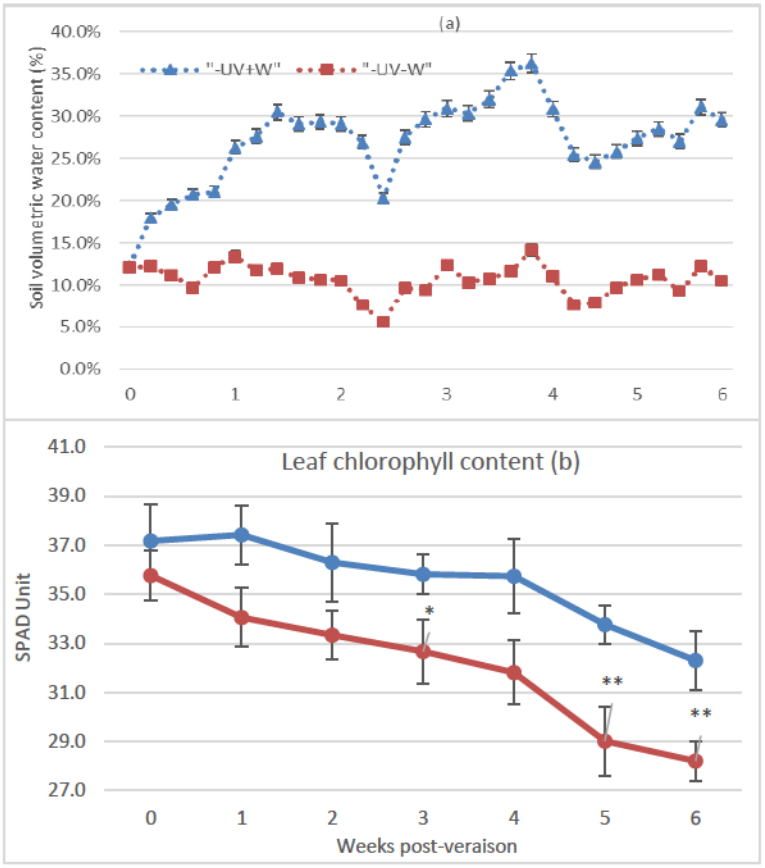


Figure 5.1 In 2016-2017 glasshouse trials: the soil volumetric content (%) of potted vines from veraison to harvest (a); and effects of water deficit on leaf chlorophyll content (SPAD unit) in Pinot noir from veraison to harvest (b).

Data showed ± standard error of the mean of three replicates. *P-values* for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level (**, $P<0.01$). -UV, normal light, +W, well-watered, -W, water deficit. The blue line is the well-watered treatment (control); the red line is water deficit treatment.

Table 5.1 Effects of water deficit on leaf water potential, $\delta^{13}C‰$ of leaf and juice and berry parameters in Pinot noir at harvest in 2015-2016 and 2016-2017 glasshouse trials.

		-UV-W	-UV+W	P_{UV}
2015-2016	°Brix	22.0	21.0	n.s
	TA(g/L)	6.4	6.6	n.s
	pH**	3.76	3.76	n.s
2016-2017	°Brix	21.7	20.1	0.005
	TA(g/L)	7.5	7.3	n.s
	pH**	3.23	3.23	n.s
	Leaf water potential (MPa)	-1.38	-0.94	<0.001
	Leaf ^{13}C vs V-PDB ‰	-28.36	-29.07	0.001
	Juice ^{13}C vs V-PDB ‰	-26.75	-28.77	0.001

** indicates a significant difference between 2015-2016 and 2016-2017, $P<0.01$.

Data showed ± standard error of the mean of three replicates. *P*-values for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level; n.s, no significant difference. -UV, normal light, +W, well-watered, -W, water deficit.

Effects of water deficit on the chemical composition

Amino acids

The HPLC analysis of amino acids in 2015-2016 and 2016-2017 revealed no consistent effects of water deficit on the concentration of total amino acids in the berries at harvest (Table 5.2). In 2015-2016, water deficit caused a slight decrease in the total amino acids compared to the control, due to notable decreases in the amino acid, Arg. Moreover, there were increases in Phe, Tyr and Gly under water deficit. In 2016-2017, there was a significant increase in the total amino acids in water deficit compared to the control, which was attributed to increases in the individual amino acids, except for Glu, Asp and Lys. Pro, one of the most abundant amino acids, had 511 µM in -UV-W and 321 µM in -UV+W. There were significant differences in the concentrations of amino acids between the two years. The overall amino acid concentrations in 2015-2016 were higher than in 2016-2017.

The percentages of each amino acid of the total in Pinot noir juice at harvest were illustrated in Table 5.3. The largest values in 2015-2016 and 2016-2017 were Arg and Pro, which together accounted for nearly 40%, in 2015-2016, and 50%, in 2016-2017, of the total. In 2015-2016, -UV-W did not affect the percentages of amino acids in the pyruvate family, but caused significant increases in Pro, Phe, Trp, Tyr, Asp and Gly. In 2016-2017, there were changes in the percentages under water deficit, except for Glu, Val, Ala, Met, Lys, Cys and Gly. Compared to -UV+W, -UV-W increased Pro by 4.6% and decreased Arg by 7.1%. Also, significant changes in the percentages of individual amino acids occurred between 2015-2016 and 2016-2017, including Arg, Glu, Gln, His, Tyr, Leu, Val, Asp, Thr, Lys and Ser. The percentages of Arg changed dramatically between the two years but remained at higher levels in 2016-2017 compared to 2015-2016.

Table 5.2 Effects of water deficit on amino acids in Pinot noir berries at harvest in 2015-2016 and 2016-2017 glasshouse trials.

Amino Acid (μM)	2015-2016			2016-2017		
	-UV-W	-UV+W	P_{water}	-UV-W	-UV+W	P_{water}
α-ketoglutarate						
Pro**	1869	1765	n.s	511	321	0.002
Arg**	1324	2176	0.010	629	608	n.s
Glu**	485	532	n.s	99	100	n.s
Gln**	417	380	n.s	56	43	<0.001
His**	150	174	n.s	15	11	0.003
Shikimate (aromatic)						
Phe**	101	70	0.002	19	18	n.s
Trp**	69	59	n.s	27	13	0.003
Tyr*	22	9	0.012	8	5	0.006
Pyruvate						
Leu**	163	150	n.s	31	25	<0.001
Val**	178	203	n.s	45	20	n.s
Ala**	1238	1532	n.s	357	297	0.001
Aspartate						
Asp**	309	363	n.s	50	51	n.s
Asn	60	49	n.s	19	13	0.011
Thr**	540	713	n.s	183	176	n.s
Ile	118	86	n.s	28	20	<0.001
Met**	31	23	n.s	11	4	n.s
Lys	39	55	0.028	17	18	n.s
3-phosphoglycerate						
Cys	N.A	N.A	N.A	4	3	0.021
Ser**	517	571	n.s	130	113	0.002
Gly	67	49	0.002	13	10	0.031
Total**	7698	8961	n.s	2254	1869	0.002

** and * indicates a significant difference between 2015-2016 and 2016-2017, $P<0.01$ and $P<0.05$.
Data showed ± standard error of the mean of three replicates. *P*-values for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level; n.s, no significant difference. -UV, normal light, +W, well-watered, -W, water deficit.

Table 5.3 Effects of water deficit on the percentages of each amino acid in total amino acids in Pinot noir berries at harvest in 2015-2016 and 2016-2017 glasshouse trials.

Amino Acid (μM)	2015-2016			2016-2017		
	-UV-W	-UV+W	P_{water}	-UV-W	-UV+W	P_{water}
α-ketoglutarate						
Pro	24.3%	19.7%	0.030	22.7%	17.2%	0.001
Arg**	17.2%	24.3%	0.007	27.9%	32.5%	<0.001
Glu*	6.3%	5.9%	n.s	4.4%	5.4%	0.004
Gln**	5.4%	4.2%	n.s	2.5%	2.3%	n.s
His**	1.9%	1.9%	n.s	0.7%	0.6%	0.015
Shikimate (aromatic)						
Phe	1.3%	0.8%	0.035	0.8%	1.0%	0.001
Trp	0.9%	0.7%	0.048	1.2%	0.7%	<0.001
Tyr*	0.3%	0.1%	0.012	0.4%	0.3%	0.038
Pyruvate						
Leu**	2.1%	1.7%	n.s	1.4%	1.3%	0.023
Val*	2.3%	2.3%	n.s	2.0%	1.1%	n.s
Ala	16.1%	17.1%	n.s	15.8%	15.9%	n.s
Aspartate						
Asp**	4.0%	4.1%	n.s	2.2%	2.7%	0.002
Asn	0.8%	0.5%	0.021	0.9%	0.7%	0.004
Thr**	7.0%	8.0%	n.s	8.1%	9.4%	<0.001
Ile	1.5%	1.0%	n.s	1.3%	1.1%	0.002
Met	0.4%	0.3%	n.s	0.5%	0.2%	n.s
Lys*	0.5%	0.6%	n.s	0.8%	1.0%	n.s
3-phosphoglycerate						
Cys	N.A	N.A	N.A	0.2%	0.2%	n.s
Ser*	6.7%	6.4%	n.s	5.8%	6.0%	0.037
Gly	0.9%	0.5%	0.017	0.6%	0.5%	n.s

** and * indicates a significant difference between 2015-2016 and 2016-2017, $P<0.01$ and $P<0.05$.

Data showed ± standard error of the mean of three replicates. *P*-values for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level; n.s, no significant difference. -UV, normal light, +W, well-watered, -W, water deficit.

Phenolic composition

In the 2015-2016 and 2016-2017 glasshouse trials, Pinot noir berries were collected during ripening for analysis of their phenolic composition (Fig. 5.2 and 5.3), including skin total anthocyanins, skin total phenolics, and skin and seed tannins.

In 2015-2016, the accumulations of skin anthocyanins were from 0 to 0.473/0.366 mg/berry during ripening in -UV-W and -UV+W (Fig. 5.2a). After one week of the treatment, the skin anthocyanin reached 0.026 mg/berry and 0.036 mg/berry in -UV+W and -UV-W, respectively, and then the two treatments' values substantially increased. The contents of skin anthocyanin reached their peak at 4-weeks post-veraison, at 0.398 mg/berry in -UV+W and 0.473 mg/berry in -UV-W. At harvest, -UV-W increased skin anthocyanins, by 0.107 mg/berry, compared to -UV+W.

During ripening, the overall trend of skin total phenolics was an increase (Fig. 5.2b). The skin total phenolics in both treatments remained stable for several weeks then increased again to become stable with only small difference between the treatments for the rest of the growing season. In -UV-W, the contents of skin total phenolics decreased from veraison to 2-weeks post-veraison and increased in the following weeks. The net increases in skin total phenolics contents were 0.108 au/berry in -UV+W and 0.120 au/berry in -UV-W. At harvest, skin total phenolics contents in the -UV-W and -UV+W were 0.357 and 0.308 au/berry, respectively. -UV-W significantly increased the contents of skin total phenolics, compared to -UV+W.

In Figure 5.2c, the trends of skin tannins were that of reductions during ripening, with only minor changes but no effect of the treatment on the amounts. There were significant effects of water deficit on skin tannins from veraison to 5-weeks post-veraison (harvest), except for 4-weeks post-veraison. -UV+W presented a bimodal curve of skin tannins during ripening and peaked at 1-week and 3-weeks post-veraison. -UV-W caused the decreases from veraison to 2-weeks post-veraison, the increase in the following week and decreases until harvest. Skin tannins decreased by 0.181 mg/berry in -UV-W and 0.125 mg/berry in -UV+W.

At harvest, skin tannins under water deficit (0.594 mg/berry) significantly increased, compared with the control (0.555 mg/berry).

Seed tannins were not influenced by water deficit during ripening, which showed an initial upward trend but then the content remained relatively stable, with few consistent differences between treatments (Fig. 5.2d). -UV-W accumulated from 2.848 mg/berry at veraison to 6.161 mg/berry at harvest, and -UV+W was from 2.848 mg/berry to 5.518 mg/berry. -UV+W and -UV-W caused the sharp increases in seed tannin contents after one week of the treatment and then decreases until 4-weeks post-veraison. The slight increases were at the last week.

In 2016-2017, skin anthocyanins in -UV+W and -UV-W increased from veraison to harvest (6-weeks post-veraison) (Fig. 5.3a). The skin anthocyanins were sharply accumulated in -UV+W and -UV-W from 2-weeks post-veraison. Compared with -UV+W, -UV-W had a visible higher contents of skin anthocyanin after two weeks of the treatment. At harvest, the skin anthocyanin contents were 0.585 mg/berry in -UV-W, compared to 0.461 mg/berry in -UV+W.

In Figure 5.3b, skin total phenolics sharply increased from veraison to 1-week post-veraison and dropped in the following week, but then showed upward trends and maintained relatively stable in -UV+W and -UV-W from 2-weeks post-veraison to 6-weeks post-veraison (harvest). The contents of skin total phenolics in -UV-W had a net increase of 0.103 mg/berry, compared with 0.113 mg/berry in -UV+W during ripening. -UV-W significantly affected the skin total phenolic substances from veraison to 4-weeks post-veraison, but there was no statistically significant difference in skin total phenolic substances between them at harvest.

The overall trend of the skin tannin content in -UV-W increased after one-week treatment and then decreased over the following weeks (Fig. 5.3c). The different pattern was the bimodal curve as shown in the control (-UV+W). Two peaks of skin tannins were at 2- and 4-weeks post-veraison in -UV+W. At 1- and 3-weeks post-veraison, -UV-W had higher skin

tannin content than -UV+W, and the effect of water deficit on skin tannins swapped from 4-weeks to harvest. At harvest, the contents of skin tannin were 0.709 mg/berry in -UV-W and 0.709 mg/berry in -UV+W.

In Figure 5.3d, after two weeks of dramatic increases, seed tannins went down in -UV+W and -UV-W from 2-weeks post-veraison. -UV-W still decreased until harvest, but -UV+W went up from 3- to 4-weeks post-veraison and down again at last two weeks. -UV-W was associated with a net increase in seed tannins from 2.943 mg/berry to 4.838 mg/berry, and the net change for -UV+W was from 3.160 mg/berry to 5.086 mg/berry. There were significant differences between treatments from veraison to 5 weeks post-veraison, but no significant differences at harvest.

Compared with 2015-2016, 2016-2017 had higher contents of skin anthocyanins at harvest. Also, the range of values between the two treatments in 2016-2017 were larger than in 2015-2016. Harvest time and *Botrytis* may be related to these differences in the accumulation of pigments.

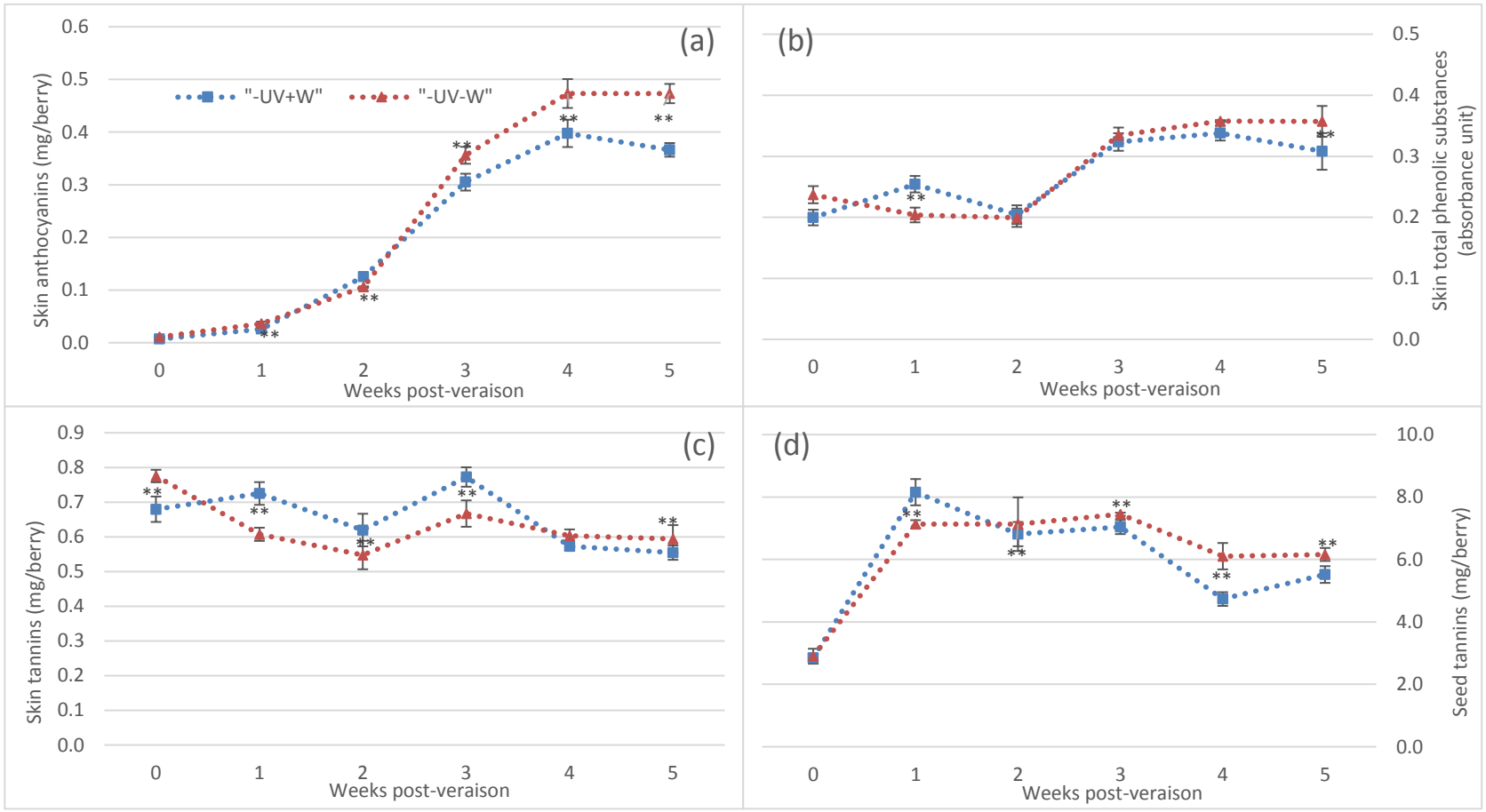


Figure 5.2 Effects of water deficit on (a) skin anthocyanins, (b) skin total phenolic substances, (c) skin tannins and (d) seed tannins in Pinot noir berries during ripening in 2015-2016 glasshouse trials.

Data showed ± standard error of the mean of three replicates. *P-values* for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level (**, *P*<0.01). -UV, normal light, +W, well-watered, -W, water deficit. The blue line is the control; the red line is water deficit treatment.

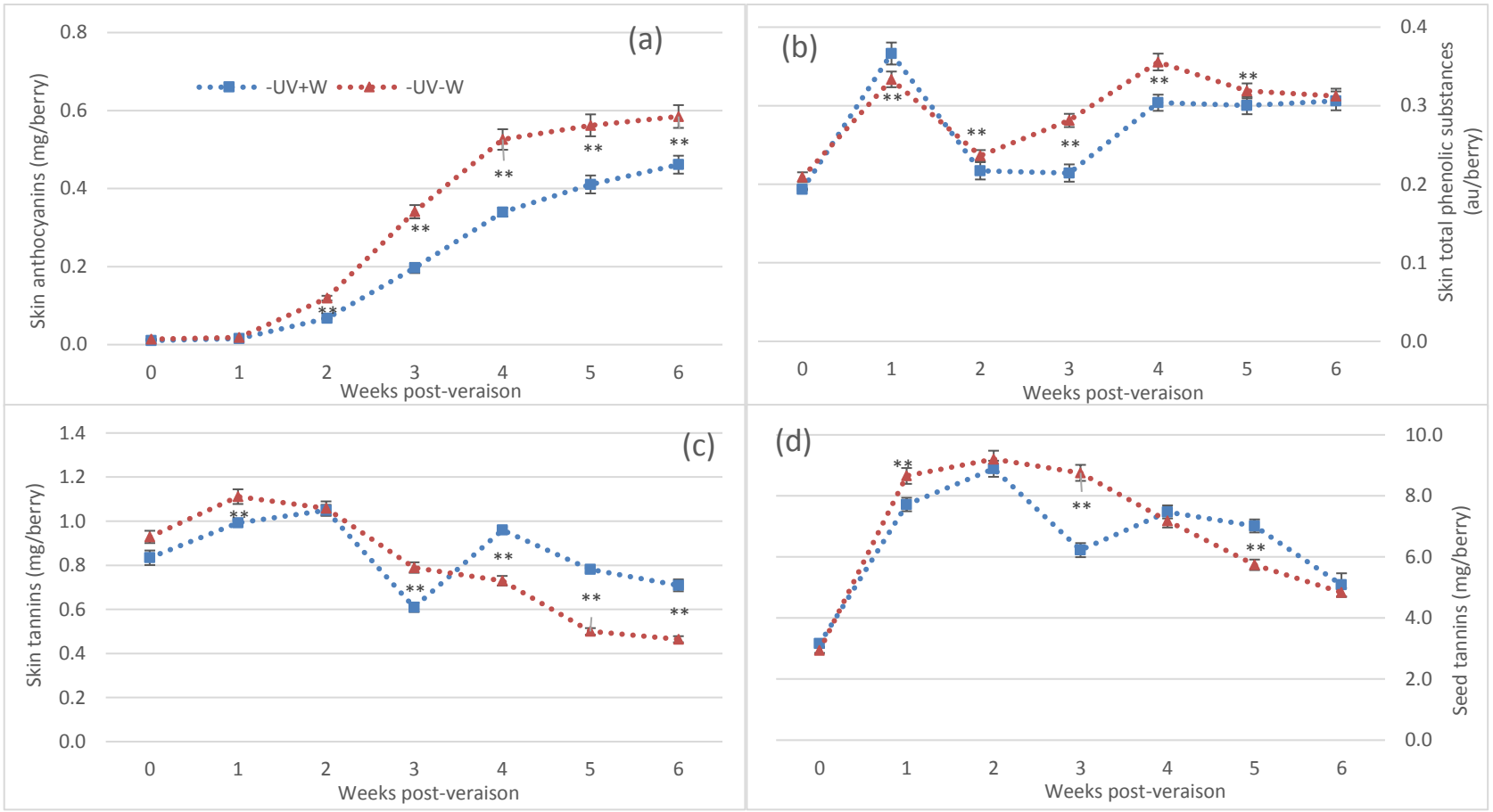


Figure 5.3 Effects of water deficit on (a) skin anthocyanins, (b) skin total phenolic substances, (c) skin tannins and (d) seed tannins in Pinot noir berries during ripening in 2016-2017 glasshouse trials.

Data showed ± standard error of the mean of three replicates. *P-values* for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level (**, *P*<0.01). -UV, normal light, +W, well-watered, -W, water deficit. The blue line is the control; the red line is water deficit treatment.

Aroma composition

Table 5.4 illustrated the results from the glasshouse trials for C_6 aldehydes, C_6 alcohols and free monoterpenes of Pinot noir juice. In 2015-2016, there were no significant effects of water deficit on C_6 aldehydes and C_6 alcohols, except for (Z)-3-hexenol with 20.2 μg/L and 31.2 μg/L in -UV-W and -UV+W, respectively. -UV-W significantly decreased free monoterpenes. In the following year, -UV-W did not cause significant changes in volatile composition, except for (E)-2-hexenal and nerol. With respect to -UV+W, -UV-W caused a reduction in (E)-2-hexenal and an increase in nerol. There was no consistent effect of water deficit on the volatile composition between the 2015-2016 and 2016-2017 trials. The concentrations of C_6 aldehydes in 2015-2016 were higher than in 2016-2017, while C_6 alcohols levels in 2015-2016 were lower than in 2016-2017.

Table 5.4 Effects of water deficit on volatile compounds in Pinot noir juice at harvest in 2016 and 2017 glasshouse trials.

Volatile compounds (μg/L)	2015-2016			2016-2017		
	-UV-W	-UV+W	P_{Water}	-UV-W	-UV+W	P_{Water}
C_6 aldehydes						
Hexanal**	367.7	317.1	n.s	169.0	193.5	n.s
(E)-2-hexenal**	140.4	157.3	n.s	96.7	128.8	0.025
C_6 alcohols						
Hexanol**	334.3	322.5	n.s	646.2	683.3	n.s
(E)-3-hexenol**	6.2	7.2	n.s	11.8	11.2	n.s
(Z)-3-hexenol	20.2	31.2	0.001	29.6	30.0	n.s
(E)-2-hexenol**	180.6	172.6	n.s	562.2	571.3	n.s
Free monoterpenes						
Linalool	1.5	2.0	0.001	1.5	1.5	n.s
α-terpineol*	1.4	2.3	0.024	1.2	1.2	n.s
Citronellol	0.9	1.4	0.003	1.1	1.1	n.s
Nerol	3.0	3.2	n.s	3.4	2.7	0.039
Geraniol	12.6	18.0	0.002	14.3	13.3	n.s

** and * indicates a significant difference between 2015-2016 and 2016-2017, $P<0.01$ and $P<0.05$.

Data showed ± standard error of the mean of three replicates. *P*-values for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level; n.s, no significant difference. -UV, normal light, +W, well-watered, -W, water deficit.

5.2.2 Vineyard trials

Effects of water deficit on vine physiology

The monthly rainfall and solar irradiance in the 2015-2016 and 2016-2017 vintages are illustrated in Table 5.5. The rainfall in the 2015-2016 vintage was high from January to March, resulting in no differences in soil volumetric water contents between the irrigation treatments in the vineyard. The 2016-2017 vintage was also a challenging season with abundant rainfall after veraison, so there was no significant difference in soil volumetric water content during ripening (Table 5.5 and Fig. 5.4a), though there was before veraison.

Values for the soil volumetric water content were significantly different from -4 weeks post-veraison to veraison between water treatments, but there were no significant effects of water deficit on SPAD or LWP (Fig. 5.4 b/c). At harvest, there were no significant effects of water deficit on berry parameters, crop load or the carbon isotope ratio in leaf tissue, but water deficit caused an increase in the carbon isotope ratio in juice (Table 5.6).

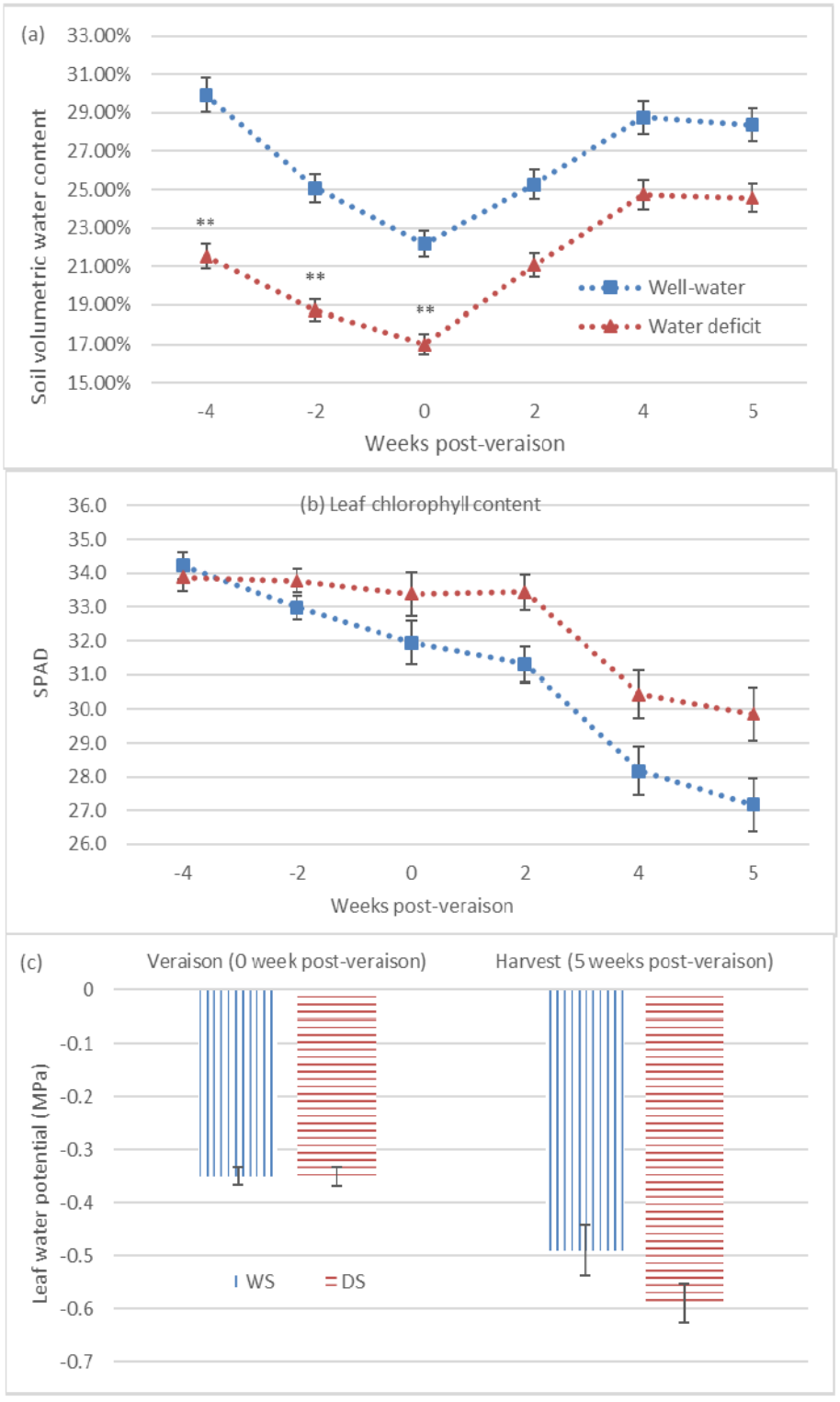


Figure 5.4 Effects of water deficit on: (a) soil volumetric water content (%), (b) leaf chlorophyll content (SPAD unit) and (c) leaf water potential (MPa) in Pinot noir from veraison to harvest in 2016-2017 vineyard trials.

Data showed mean ± standard error of four replicates. *P-values* for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level (**, $P<0.01$). WS, well-water

Table 5.5 Monthly rainfall and solar irradiance of the west vineyard in 2016 and 2017.

		Rainfall (mm)	Rad (MJ/m^2)
Monthly values for 1971-2000	Jan.	42	678.9
	Feb.	39	526.4
	Mar.	54	437.1
	Apr.	54	291.0
	Total	189	1933.4
2016	Jan.	107	578.5
	Feb.	24	600.2
	Mar.	34	460.0
	Apr.	10	325.7
	Total	175	1964.4
2017	Jan.	42	705.5
	Feb.	3	550.4
	Mar.	73	380.2
	Apr.	123	260.0
	Total	241	1896.1

Table 5.6 Effects of water deficit on berry parameters, δ^{13}C‰ of leaf and juice, yield, pruning weight and Ravaz Index in Pinot noir at harvest in 2016-2017 vineyard trials.

	WS	DS	$P_{\text{-value}}$
°Brix	16.7	16.8	n.s
TA (g/L)	11.2	10.7	n.s
pH	3.6	3.7	n.s
Yield (kg)	3.65	4.07	n.s
Pruning weight (kg)	0.82	0.90	n.s
Ravaz Index	4.52	4.22	n.s
Leaf ^{13}C vs V-PDB ‰	-28.88	-28.32	n.s
Juice ^{13}C vs V-PDB ‰	-28.47	-27.28	0.022

Data represent mean (n=4) from samples at harvest in 2016-2017. P_{value}, significance of water deficit effect according Independent-sample T-test and LSD test at the 5% level; n.s, no significance; WS, well-water with shade cloth, DS, water deficit with shade cloth.

Effects of water deficit on the chemical composition

Amino acids

Table 5.7 illustrated the results from vineyard trials for amino acids and the percentages of each amino acid of the total amino acids in 2016-2017. There were no significant differences in most amino acid concentrations between treatments, except for Pro, Phe, Trp, Leu, Ile, Lys and Cys. The water deficit treatment (DS) caused substantial decreases in the concentrations of Phe, Trp, Leu, Ile, Lys and Cys. In DS, the Pro concentration was 791 µM, compared to the well-watered treatment (WS) at 506 µM. Moreover, the percentages of Pro were significantly affected by water deficit. Compared to WS, DS caused an increase in Pro percentage from 3.9% in the control to 5.9%. With respect to WS, significant reductions of DS were in the percentages of Phe, Trp, Leu, Val, Ile, Lys and Cys.

Table 5.7 Effects of water deficit on amino acids and the percentages of each amino acid in total amino acids in Pinot noir berries at harvest in 2016-2017 vineyard trials.

Amino Acid (μM)	Treatments					
	DS	WS	P_{water}	DS	WS	P_{water}
α-ketoglutarate						
Pro	791	506	<0.001	5.9%	3.9%	0.001
Arg	3121	3314	n.s	23.2%	25.6%	n.s
Glu	294	271	n.s	2.2%	2.1%	n.s
Gln	2976	2416	n.s	22.2%	18.7%	n.s
His	115	119	n.s	0.9%	0.9%	n.s
Shikimate (aromatic)						
Phe	306	440	0.005	2.3%	3.4%	0.003
Trp	49	94	<0.001	0.4%	0.7%	<0.001
Tyr	43	47	n.s	0.3%	0.4%	n.s
Pyruvate						
Leu	288	383	0.006	2.1%	3.0%	0.005
Val	740	791	n.s	5.5%	6.1%	0.028
Ala	1973	1881	n.s	14.7%	14.5%	n.s
Aspartate						
Asp	230	191	n.s	1.7%	1.5%	n.s
Asn	86	75	n.s	0.6%	0.6%	n.s
Thr	1051	1089	n.s	7.8%	8.4%	n.s
Ile	201	298	0.002	1.5%	2.3%	<0.001
Met	186	167	n.s	1.4%	1.3%	n.s
Lys	39	27	0.003	0.3%	0.2%	0.003
3-phosphoglycerate						
Ser	892	798	n.s	6.6%	6.2%	n.s
Cys	5	11	0.004	0.0%	0.1%	0.001
Gly	41	34	n.s	0.3%	0.3%	n.s
Total	13428	12951	n.s			

Data represent mean (n=4) from samples at harvest in 2016-2017. P_{value}, significance of water deficit effect according Independent-sample T-test and LSD test at the 5% level; n.s, no significance; WS, well-water with shade cloth, DS, water deficit with shade cloth.

Phenolic composition

In the 2016-2017 vineyard trials, the phenolic composition of Pinot noir fruit was measured during ripening (Fig. 5.5). Rainfall, again, was an issue, and though it occurred later than in the previous season, the result was no difference in soil moisture after veraison. In Figure 5.5a, the sharp increases in skin anthocyanins were from veraison to 5 weeks post-veraison (harvest) in both DS and WS. In WS, the skin anthocyanins increased to the peak of 0.310 mg/berry at 4-weeks post-veraison and then decreased to 0.213 mg/berry at harvest. However, for DS there was a more rapid increase in the skin anthocyanins, followed by a slower rate of increase from 2-weeks post-veraison to its peak at 4-weeks post-veraison. From there, it decreased to 0.194 mg/berry, which was not significantly different from the control. The skin total phenolics had little change from -4-weeks post-veraison to 5-weeks post-veraison in WS and DS, though there was a peak in the latter treatment at 2-weeks post-veraison (Fig. 5.5b). There were slight decreases of skin tannins through the ripening period, with no consistent treatment-associated trend (Fig. 5.5c). In Figure 5.5a, b and c, there were no significant effects from water deficit on the skin anthocyanins, skin total phenolics or skin tannins at harvest. Also, the seed tannins fluctuated from -4-weeks to 5-weeks post-veraison, but hardly changed throughout the period in the two treatments aside from a peak at 4-weeks post-veraison in DS (Fig. 5.5d). At harvest, DS did not cause a statistic change in the seed tannins with respect to WS.

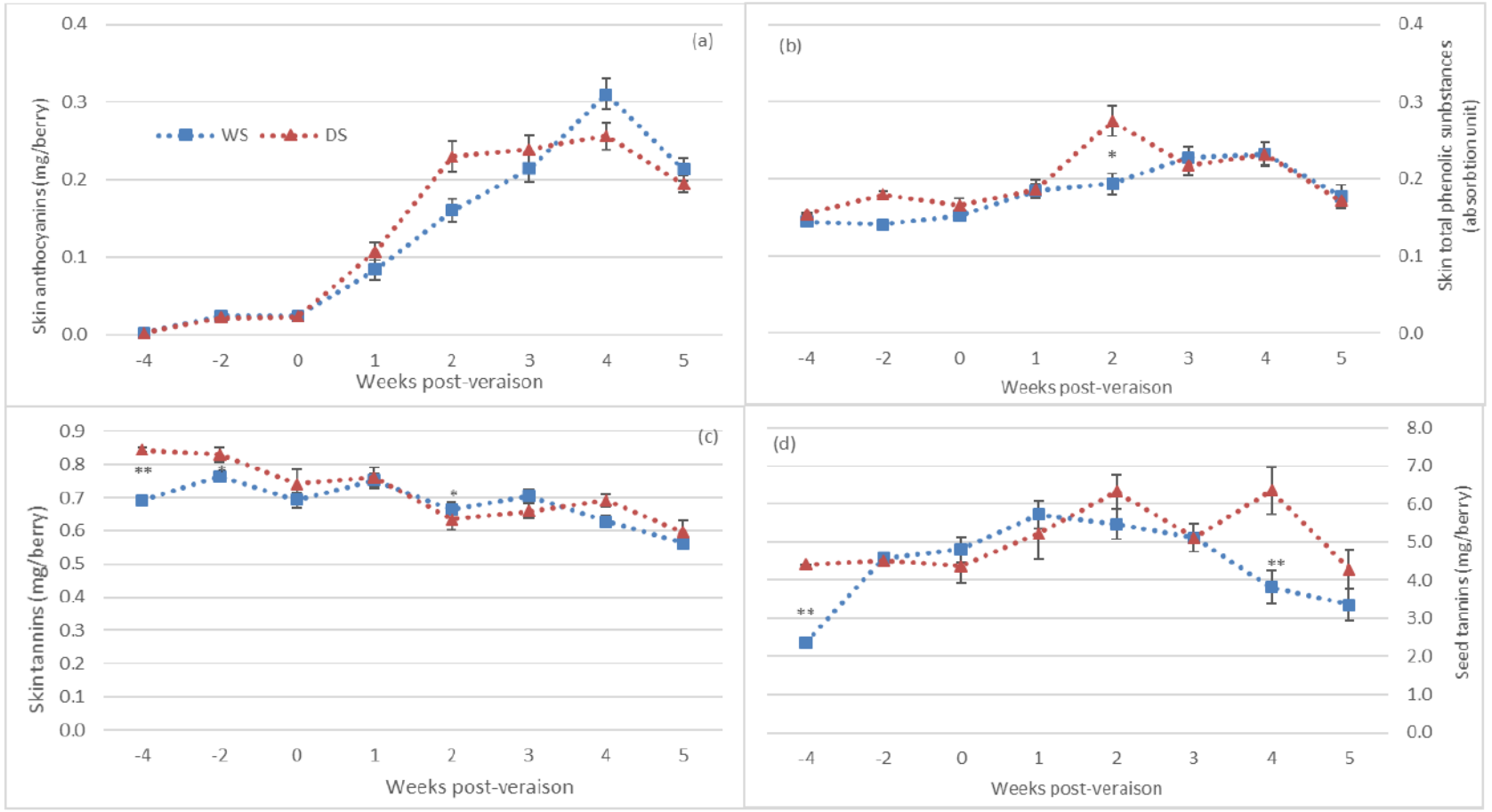


Figure 5.5 Effects of water deficit on: (a) skin anthocyanins, (b) skin total phenolic substances, (c) skin tannins and (d) seed tannins in Pinot noir berries during ripening in 2016-2017 vineyard trials.

Data showed mean ± standard error of four replicates. *P-values* for statistical significance comparing the different treatments according to Independent-sample T-test and LSD test at the 5% level (*, $P<0.05$; **, $P<0.01$). WS, well-water with shade cloth, DS, water deficit with shade cloth.

Volatile composition

Pinot noir fruit collected at harvest were used for analysis of volatile compounds (Table 5.8). In the composition of volatiles, C_6 aldehydes and C_6 alcohols accounted for the major parts. At harvest, water deficit caused a significant decrease of 36.9% in (E)-2-hexenal and an increase of 35.5% in (Z)-3-hexenol compared to the control. There was little effect of water deficit on free monoterpenes.

Table 5.8 Effects of water deficit on volatile compounds in Pinot noir juice at harvest in 2016-2017 vineyard trials.

Volatile compounds (μg/L)	Treatment		P_{water}
	WS	DS	
C_6 aldehydes			
Hexanal	31.1	30.4	n.s
(E)-2-hexenal	56.6	35.7	0.006
C_6 alcohols			
Hexanol	1083.3	936.8	n.s
(E)-3-hexenol	16.7	15.3	n.s
(Z)-3-hexenol	88.4	119.8	0.020
(E)-2-hexenol	206.3	214.3	n.s
Free monoterpenes			
Linalool	1.8	1.8	n.s
α-terpineol	1.4	1.3	n.s
Citronellol	1.0	1.0	n.s
Nerol	4.8	4.7	n.s
Geraniol	12.1	13.3	n.s

Data represent mean (n=4) from samples at harvest in 2016-2017. P_{value}, significance of water deficit effect according Independent-sample T-test and LSD test at the 5% level; n.s, no significance; WS, well-water with shade cloth, DS, water deficit with shade cloth.

5.3 Discussion

In the glasshouse, the environmental conditions were presented in the previous chapter. In the 2015-2016 vintage, rainfall leading up to, and during, the ripening period meant a water deficit was not able to be achieved. In 2016-2017, the water treatments were set up at -4-weeks post-veraison to allow for a potentially a greater amount of water deficit, but though a deficit was established pre-veraison, there was rainfall, again, during the ripening period (Table 5.5).

5.3.1 Alteration of vine physiological indices as induced by water deficit

Previous research has used measurements of leaf water potential (LWP) (Williams and Araujo, 2002), soil volumetric water content (Centeno et al., 2010), carbon isotope ratio of leaves and juice ($\delta^{13}C‰$) (Gaudillère et al., 2002; Santesteban et al., 2012) and SPAD (Zulini et al., 2005) to assess vine water status and water use efficiency. In this study, these physiological indicators showed that SPAD and LWP decreased with the reduction in soil volumetric water content, and leaf and juice $\delta^{13}C‰$ increased in the glasshouse (Fig. 5.1 and Table 5.1), but not in the vineyard, except for juice $\delta^{13}C‰$ (Fig. 5.4 and Table 5.6). These alterations of physiological results were consistent with Bajji et al. (2001), Flexas et al. (2000) and (Zulini et al., 2005).

Water deficit induces damage to chloroplast membranes and distortion of the lamellae vesiculation, resulting in decreasing Chl contents and leaf greenness (Bertamini et al., 2006; Fanizza et al., 1991). For Pinot noir as an isohydric cultivar, LWP rarely drops below -1.5 MPa, regardless of soil water availability (Lovisolo et al., 2010). In this study, water deficit slightly decreased the LWP, but LWP in the well-water and water deficit treatments of the glasshouse and vineyard were over -1.5 MPa.

At harvest, water deficit caused an increase in leaf $\delta^{13}C‰$ in the glasshouse and juice $\delta^{13}C‰$ was increased by water deficit in both the vineyard and the glasshouse (Table 5.1 and 5.6). $\delta^{13}C‰$ is related to the ratio of intercellular and atmospheric CO_2 concentrations

(C_i/C_a), and water stress is the main factor affecting this ratio. An increase in leaf $\delta^{13}C‰$ in the glasshouse indicates that water deficit induces stomatal closure resulting in more intercellular $^{13}CO_2$ uptake for photosynthesis at the carboxylation step, which is synthesized to sucrose with ^{13}C in leaves. However, the grapevines after veraison were not under water stress, so more $^{12}CO_2$ than $^{13}CO_2$ can be synthesised in the vineyard. Increases in juice $\delta^{13}C$ in the glasshouse and vineyard are explained by glucose containing ^{13}C being translocated from leaves to fruit without turnover during fruit ripening (Gaudillère et al., 2002; Santesteban et al., 2015). The capacity of $^{12}CO_2$ assimilation and glucose translocation also helps to explain the small differences in leaf $\delta^{13}C‰$ between the well-watered and water deficit vines at harvest in the vineyard.

5.3.2 Effects of water deficit on amino acids in berries

Under water deficit, amino acid profiles were obtained for Pinot noir berries from the glasshouse and vineyard at harvests in 2015-2016 and 2016-2017 (Table 5.2 and 5.7). A comparison of the potted vines versus vineyard vines could account, to some extent, for the contrasting results. In the 2015-2016 glasshouse trials, water deficit decreased the total amino acids concentration, while the opposite results were shown in the 2016-2017 glasshouse and vineyard trials. These results could have been induced by the *Botrytis* infection, as in 2015-2016 for glasshouse and in the berries in the water deficit treatment there was more *Botrytis* infection than in the control. *Botrytis* infected berries can reduce the concentration of total amino acids, compared to uninfected berries (Bell and Henschke, 2005).

The total amino acid concentration was increased by water deficit in both the 2016-2017 glasshouse and vineyard trials. These changes were attributed to the direct effect of water deficit on the increases in Pro. Pro is the primary free amino acid in Pinot noir juice and accounts for a large amount of the total amino acids. The concentration and percentage of Pro at harvest were higher in the water deficit treatments and lower in the well-watered treatments (Table 5.2 and 5.7), which were consistent with Krüger (2002) and Berdeja et

al. (2014). Pro functions as an antioxidant via ROS scavenging activity under water deficit (Berdeja et al., 2014). Also, Pro biosynthesis is a reductive pathway that requires NADPH for the reduction of glutamate to pyrroline-5-carboxylate (P5C), then produce Pro and generate $NADP^+$, which is controlled by *P5CS* and *P5CR* gene activations. Under water deficit, the overexpression of *P5CR* confirms that Pro biosynthesis and the generation of $NADP^+$ maintain a low NADPH:$NADP^+$ ratio to sustain the Calvin cycle (Berdeja et al., 2014; Szabados and Savouré, 2010). As stated above, this explained why the concentrations of total amino acids and Pro in the water deficit treatment were higher than in the well-watered treatment. Although Pro is a major amino acid in grapes, it is non-YAN.

Furthermore, there was a qualitative change in amounts of amino acids that substantially contributed to YAN, such as Gln and Trp. Under water deficit, Gln increased in the glasshouse and did not change in the vineyard, while Trp increased in the glasshouse and decreased in the vineyard. Gln is the major nitrogen transport compound in xylem and converted into other amino acids by aminotransferases in grapes (Roubelakis-Angelakis and Kliewer, 1992; Wang et al., 2017). Trp is a main amino acid in aromatic family and is produced by shikimate pathway in grapes. It not only is one of the precursors for auxin in plants but also can be degraded to the aroma compound methyl anthranilate (Wang and Luca, 2005). In the glasshouse trials, the environmental conditions were consistent between the water deficit treatment and the control. The increases in Gln and Trp under water deficit suggested that water deficit induced the oxidative stress response and the catabolism of protein in grapes (Grimplet et al., 2009; Reddy et al., 2004). Also, the increase in Gln suggested that water deficit caused the increases in the transcript abundance of *GS1* (Glutamine synthetase cytosolic isozyme 1) in grapes and GS catalysed the condensation of Glu and NH_4^+ to generation Gln (Cramer et al., 2007b; Forde and Lea, 2007). Trp is produced by the shikimate and phenylpropanoid pathway. Water deficit increased shikimate concentrations because of the significant increases in the transcript abundance of *3-deoxy-D-arabinoheptulosonate 7-phosphate synthase (DHPS), chorismate mutase* and *phenylalanine ammonia lyase* (Deluc et al., 2009; Wang et al., 2017). There was no significant effect of water deficit on Glu but there were decreases in Trp in the

vineyard. With respect to the glasshouse, vineyard trials could be influenced by other environmental elements. The rainfall after veraison caused no change in soil water content between the treatments. In other studies, the abundant water supplement resulted in an improvement of photosynthesis, because of an increase in stomatal conductance as well as upregulation of photosystem activities (Cramer et al., 2007b; Parry et al., 2002). In addition, the rainfall may not induce the changes in the transcript abundance of genes to influence on the generation of amino acids in grapes (Ferrandino and Lovisolo, 2014).

5.3.3 Phenolic composition in berries in response to water deficit

Application of water deficit from veraison/-4-weeks post-veraison to harvest had an influence on the levels of phenolic compounds in both the glasshouse (Fig. 5.2 and 5.3) and the vineyard (Fig. 5.5) experiments. This was in agreement with previous reports (Bindon et al., 2011; Herrera et al., 2015; Kennedy et al., 2002; Roby et al., 2004), which showed increases and changes in composition of total phenolics in response to water deficit in a wide range of species. Water deficit caused an increase in skin anthocyanins compared with the well-watered treatment during ripening in the glasshouse. Similar to previous studies (Castellarin et al., 2007; Ollé et al., 2011), water deficit after veraison must have stimulated the accumulation of B-ring di-hydroxylated and tri-hydroxylated anthocyanins and caused a slight increase in cyanidin and delphinine derivatives in Shiraz berries. This may also be accounted for by the regulation of the biosynthetic pathways that produce anthocyanins, with water stress-induced regulation taking place at veraison. In grape berry skins, the synthesis of anthocyanins was known to be altered by water deficit-induced transcriptional regulation. The water deficit response in grapes is to increase gene expression in these regulatory pathways: *F3H, LDOX, UFGT and GST* (Castellarin et al., 2007; Falginella et al., 2012). However, in the vineyard, there was no significant difference in the accumulation of skin anthocyanins between water treatments during fruit development and the low contents of skin anthocyanin in well-watered and water deficit treatments at harvest (Fig. 5.5a). It may be that the rainfall again after veraison led to no significant differences in vine water status between the treatments. Moreover, the rainfall

resulted in the low maturity of berries at harvest, and then the low level of glucose molecules that can be bound with anthocyanidins into stable anthocyanins (Keller, 2015; Roby and Matthews, 2004).

There were consistent results for the skin and seed tannin contents under water deficit in the vineyard (Fig. 5.5c and d) and glasshouse trials (Fig. 5.2 c/d and 5.3 c/d), except for the skin tannin contents in 2016-2017 glasshouse trials. Water deficit significantly increased the contents of skin tannin In the 2016-2017 glasshouse trials, but did not significantly affect the contents of seed tannin in the glasshouse and vineyard, which were consistent with results in Cabernet Sauvignon (Kennedy et al., 2002; Roby et al., 2004). There may be the effect of water deficit on a direct stimulation of biosynthesis. Water deficit can cause the direct stimulation of proanthocyanidins biosynthesis, such as through LAR expression in grape skins, while water deficit has not directly affected the synthesis of seed tannins (Bogs et al., 2005; Castellarin et al., 2007; Ollé et al., 2011). Also, water deficit can increase a greater of degree of polymerization of proanthocyanidins in berry skins, though not seeds (Ojeda et al., 2002). Therefore, the skin tannin contents can be increased by water deficit.

5.3.4 Effects of water deficit on volatile composition in berry juice

Some important volatile compounds in grapes, including lipids, terpenoids (mono-, sesqui- and noriso-) and C_6 compounds (C_6 aldehydes and C_6 alcohols), were quantified in Pinot noir fruit (Brander et al., 1980; Fang and Qian, 2005; Miranda-Lopez et al., 1992). In this study, C_6 aldehydes, C_6 alcohols and free monoterpenes were measured in Pinot noir juice. The results showed water deficit reduced the concentrations of C_6 compounds, especially (E)-2-hexenal, in the glasshouse (Table 5.4) and the vineyard (Table 5.8). Previous research has linked C_6 compounds with fruit maturity, which demonstrated decreases in C_6 compounds with increases in fruit maturity (Coelho et al., 2006; García et al., 2003). This study was consistent with them, where the water deficit treatment increased °Brix with the reduction in concentrations of C_6 compounds at harvest, compared with the control. C_6

compounds also are involved in the catabolism of fatty acids, which contribute to herbaceous and green odours in grapes. Water deficit causes an increase in the transcript abundance of LOX, resulting in the increase in the catabolism of fatty acids to hydroperoxides. The hydroperoxides can be converted to C_6 aldehydes by hydroperoxide lyase (HPL). Water deficit also increases the transcript abundance of HPL. In the next steps of this pathway, the transcript abundance of *alcohol dehydrogenases* (*ADH*) and *alcohol acyl transferases* (*AAT*) genes are increased by water deficit, so C_6 aldehydes are converted to C_6 alcohols and then to volatile esters (Cramer et al., 2007a; Deluc et al., 2009). Therefore, more C_6 compounds under water deficit may be converted to volatile esters than under well-watered.

The free monoterpenes quantified in this study were linalool, *α*-terpineol, citronellol, nerol and geraniol, which had low concentrations in grapes. Monoterpenes are important to the aroma of many wines, but red varieties are not phenotypically characterized by high levels of terpenes (Qian et al., 2009; Robinson et al., 2013). Water deficit had little effect on free monoterpenoids in the glasshouse (Table 5.4) or the vineyard (Table 5.8). In accordance with other studies, water deficit had variable effects on the levels of monoterpenes, with either no effect or increasing the concentrations of some compounds (Grimplet et al., 2007; Ou et al., 2010).

5.4 Conclusion

In this chapter, the effects of water deficit on vine physiology and chemistry of Pinot noir fruit in glasshouse and vineyard trials were investigated. In the glasshouse, the results indicated that water deficit caused significant decreases in SPAD and leaf water potential and increases in the stable carbon isotope composition of leaves and juice, but not in the berry compositional parameters. The skin anthocyanins in these berries showed an increasing pattern throughout the ripening period measured. Quantitative changes in skin anthocyanins were also observed in response to water deficit. Skin total phenolics showed the substantial increases from veraison to harvest in the control and water deficit

treatment. Water deficit caused the significant increases in skin total phenolics at 1-week and 5-weeks post-veraison (harvest) in 2015-2016. The increases in skin total phenolics were from 1-week to 5-weeks post-veraison but not at 6-weeks post-veraison (harvest) under water deficit in 2016-2017. The skin tannin contents were driven by water in the glasshouse. Skin tannins were increased by water deficit from 1-week to 3-weeks post-veraison in 2015-2016, while the effects of water deficit on skin tannins swapped after 4-weeks of treatments and there was a dramatic increase in them at harvest in 2016-2017. However, in the 2015-2016 vineyard trial, rainfall during the ripening period meant a water deficit was not able to be achieved. In the following season, although water treatments were set up at grape pea-size to allow for a potentially larger water deficit, as it was a challenging season, again, suffering rainfall from March to April. Vine physiological indicators showed significant differences at the early berry development stage. Also, amino acids and phenolic compounds were not affected by water deficit in the vineyard. Furthermore, water deficit clearly did not affect the volatile compounds in this study.

Chapter 6

Effects of UV-B radiation interaction with water deficit on the vine physiology and chemical composition of Pinot noir fruit

6.1 Introduction

UV-B radiation has been proposed as an influential factor to alter grapevine fruits' chemical composition, including amino acids, phenolics and volatile compounds (Berdeja et al., 2014; Williams and Araujo, 2002). Also, imposing a moderate water deficit on vines through limiting irrigation is widely used for improving wine grape quality. For example, low water availability may stimulate fruit ripening and increase the concentration of specific secondary metabolites, such as Pro, anthocyanins and tannins (Martínez-Lüscher et al., 2014a; Stines et al., 1999). In New Zealand, Pinot noir regions suffer high UV radiation levels and low rainfall in summer. These environmental conditions could significantly affect the characteristics of Pinot noir fruit and wine. Therefore, the effects of UV-B radiation interaction with water deficit are important for scientific understanding of the changes in grapevine physiology and fruit chemical composition in Pinot noir, and even to provide valuable information for vineyard management. This chapter is reported the changes in grapevine physiological traits and fruit chemical compounds (amino acids, phenolic and volatile compounds), as a result of the combination of treatments and the individual treatments or the control.

6.2 Results

6.2.1 Glasshouse trials

Effects of UV-B radiation interaction with water deficit on vine physiology

The soil volumetric water content of potted vines from veraison to harvest in the glasshouse were presented in Fig. 6.1a. The water deficit treatments (-UV-W and +UV-W), were successful in reducing soil water compared to the well-watered treatments (+UV+W and -

UV+W). In +UV-W, the soil volumetric water content was maintained at around 10%, which was about half the value of soil volumetric water content in the control (-UV+W).

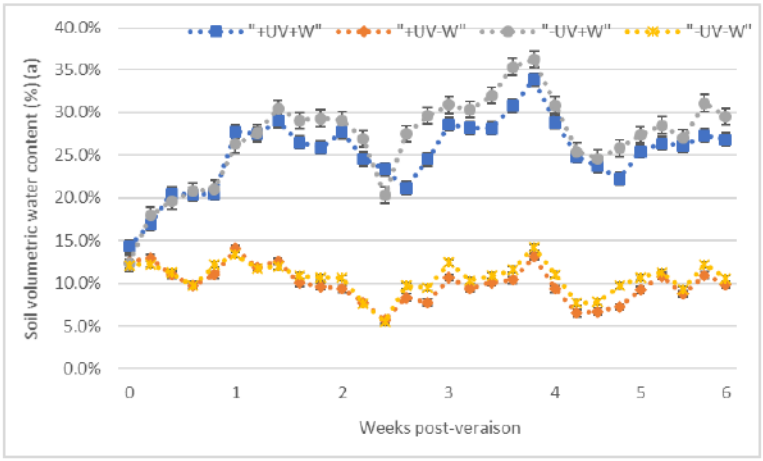
LWP directly reflected the soil water content and was decreased by water deficit, but not UV-B (Table 6.1). Under UV-B stress, LWP was -1.31 MPa in +UV-W compared to -UV+W at -0.94 MPa, and +UV+W at -0.98 MPa.

In all treatments, leaf SPAD decreased from veraison to harvest (Fig. 6.1b). SPAD sharply decreased after one week for all treatments, and then they showed a parallel trend. There was no significant difference between treatments after two weeks of veraison. At 3-, 4- and 6-weeks (harvest) post-veraison, under water deficit, +UV-W significantly decreased SPAD, compared to -UV-W. While, under UV-B, SPAD had more reduction in +UV-W than +UV+W at 3- and 5-weeks post-veraison.

UV-B interaction with water deficit (+UV-W) did not influence the carbon isotope ratio in leaves (Table 6.1). In the carbon isotope ratio of juice, UV-B caused a drop in the well-watered (+UV+W, -29.16‰) and water deficit (+UV-W, -27.26‰) treatments in comparison with their respective no UV-B treatments (-28.77‰ in -UV+W and -26.75‰ in -UV-W), while the water deficit treatments made the carbon isotope ratio of the juice less negative (Table 6.1).

In 2015-2016 and 2016-2017, berry parameters, including °Brix, pH and TA, were recorded at harvest (Table 6.1). There were no significant differences in °Brix or TA between treatments in 2015-2016 (Table 6.1), but there was for pH. Only UV-B (3.69 in +UV+W and 3.66 in +UV-W) significantly decreased pH compared with no UV-B treatments (3.76 in each of -UV+W and -UV-W). In 2016-2017, °Brix was influenced by water treatments. Well-watered treatments had a lower °Brix than the water deficit treatments. A significant difference in TA was shown between UV treatments, with UV-B causing a decrease. The combination of UV-B and water deficit resulted in a significant difference in pH between treatments. UV-B treatments significantly increased the pH compared with no UV-B treatments. +UV-W caused a smaller increase in pH than +UV+W. In comparing the two years of glasshouse

trials, only pH showed a statistically significant difference where, in 2015-2016, the values were higher than in 2016-2017.



P_{UV}	n.s	n.s	n.s	0.022	0.028	n.s	0.036
P_{Water}	n.s	n.s	n.s	0.019	n.s	0.017	n.s
$P_{UV*water}$	n.s	n.s	n.s	n.s	n.s	n.s	n.s

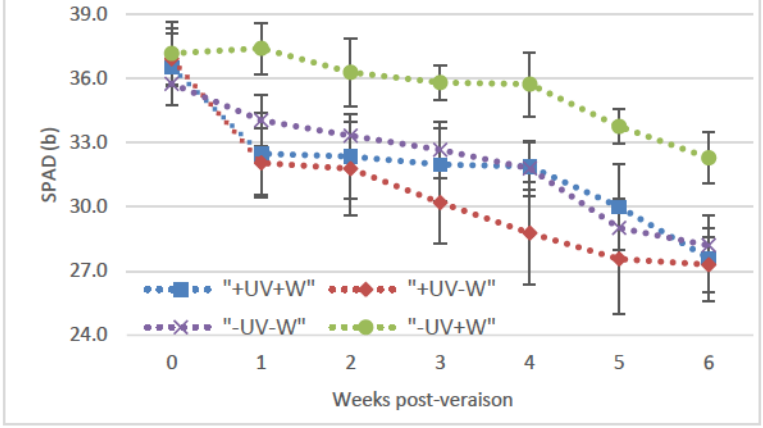


Figure 6.1 In 2016-2017 glasshouse trials: (a) the soil volumetric water content (%) of potted vines from veraison to harvest; (b) effects of UV-B and water deficit on leaf chlorophyll content (SPAD unit) in Pinot noir from veraison to harvest.

Data showed the mean of four replicates. *P*-values for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at 5% level; P_{UV}, UV effects averaged across water treatments; P_{water}, water effects averaged across UV treatments; $P_{UV*water}$, water effects depend on UV treatments and UV effects depend on water treatments; n.s, no significant difference. +W, well-watered, -W, water deficit; +UV, UV-B radiation, -UV, normal light.

Table 6.1 Effects of UV-B and water deficit on leaf water potential, $\delta^{13}C‰$ of leaf and juice and berry parameters in Pinot noir at harvest in 2015-2016 and 2016-2017 glasshouse trials.

		+UV+W	+UV-W	-UV+W	-UV-W	P_{UV}	P_{water}	$P_{UV*water}$
2015-2016	°Brix	21.0	19.1	21.0	22.0	n.s	n.s	n.s
	TA(g/L)	5.4	5.4	6.6	6.4	n.s	n.s	n.s
	pH**	3.69	3.66	3.76	3.76	0.037	n.s	n.s
2016-2017	°Brix	21.0	21.0	20.1	21.7	n.s	0.010	n.s
	TA(g/L)	6.1	6.6	7.3	7.5	<0.001	n.s	n.s
	pH**	3.41	3.30	3.23	3.23	0.001	n.s	0.040
	Leaf water potential (MPa)	-0.98	-1.31	-0.94	-1.38	n.s	<0.001	n.s
	Leaf ^{13}C vs V-PDB ‰	-28.27	-28.88	-29.07	-28.36	n.s	n.s	n.s
	Juice ^{13}C vs V-PDB ‰	-29.16	-27.26	-28.77	-26.75	0.001	<0.001	n.s

** indicates a significant difference between 2015-2016 and 2016-2017, $P<0.01$.

Data showed the mean ± standard error of three replicates from harvest in 2015-2016 and 2016-2017. *P*-values for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at 5% level; P_{UV}, UV effects averaged across water treatments; P_{water}, water effects averaged across UV treatments; $P_{UV*water}$, water effects depend on UV treatments and UV effects depend on water treatments; n.s, no significant difference. +W, well-watered, -W, water deficit; +UV, UV-B radiation, -UV, normal light.

Effects of UV-B radiation interaction with water deficit on chemical composition

Amino acids

Amino acids were measured in berries collected at harvest in two vintages (Table 6.2 and 6.3). In 2015-2016, treatments caused a reduction in the concentration of total free amino acids compared with the control (-UV+W) (Table 6.2). Amino acids, such as Arg, Glu, Ala, Asp, Thr and Gly, were decreased by UV-B or water deficit in comparison with the control. UV-B strongly decreased the magnitude of the water deficit-induced reduction in these amino acids. Trp concentration decreased by 41% in +UV+W but increased by 17% in -UV-W compared to -UV+W. +UV-W caused a smaller reduction in Trp than in -UV-W. The concentrations of Phe, Tyr and Met were increased by water deficit and decreased by UV-B. However, the water deficit effect with UV caused more reduction in Phe, Tyr and Met. Therefore, water deficit behaves in amino acids differently depending on the UV-B status.

In 2016-2017, there were apparent highly significant interactions between UV-B and water deficit for almost all the amino acids and the total amino acid concentration, except for Cys and Met (Table 6.3), therefore the total amino acid concentration was increased by UV-B or water deficit, and the effects of the individual stress were qualified by UV-B interaction with water deficit. The concentration of total amino acids was higher in the water deficit treatments than in the well-watered treatments, but the effect of water depended on the UV-B conditions. Overall, the total amino acid concentration was the highest at 4150 µM in +UV-W than in the other treatments. Also, the combination of UV-B and water deficit changed the concentration of the other amino acids. The most abundant amino acids were Pro, Arg and Ala, reaching over 300 µM in treatments. The concentrations of Pro and Ala under water deficit were higher in the +UV than -UV. There was also higher Pro and Ala concentrations in +UV+W than -UV+W. Arg was increased by 2% in +UV+W and 4% in -UV+W, compared to -UV+W. +UV-W enhanced an increase in Arg (1357 µM), which was double the concentration in -UV+W (608 µM).

Although there were significant differences in the concentrations of amino acids, the fruit showed no consistent alterations in amino acid levels compared to the control, in 2015-2016

or 2016-2017. In 2015-2016, +UV-W caused significant decreases in amino acids, while amino acids were increased in +UV-W in 2016-2017. The concentrations of total free amino acids were 2874 µM and 4150 µM in berries from +UV-W in 2015-2016 and 2016-2017, respectively, compared to -UV+W, with 8961 µM and 1869 µM in 2015-2016 and 2016-2017, respectively. Total amino acids in -UV+W were higher in 2015-2016 than in 2016-2017, while the opposite results for +UV-W were shown in 2015-2016 and 2016-2017. Thus, over both growing seasons, there were no consistent statistical interactions between UV-B and water deficit treatments.

In 2015-2016, there were effects of UV-B or water deficit on the percentages of Arg, Glu, Phe, Trp, Tyr, Val, Asn, Ile, Met, Ser and Gly (Table 6.4). Only Trp, Tyr, Val and Met were affected by an interaction between UV-B and water deficit. With respect to UV-B and no UV-B, there was a higher percentage of Arg under the well-watered conditions compared to a water deficit. The percentages of Trp under well-watered conditions were equal in the UV-B and no UV-B treatments, at about 1%. However, under water deficit, the percentages of Trp accounted for 2% in the UV treatment compared to 1% in the no UV treatment. The percentages of Tyr and Met under water deficit had a greater reduction due to +UV-B compared the reduction under well-watered.

However, in 2016-2017, the individual amino acid percentages showed significant changes in berries exposed to UV-B and water deficit together (Table 6.5). The α-ketoglutarate family accounted for the highest proportion of amino acids but, in terms of treatment effects, there was only an interaction between UV-B and water deficit on Arg. When vines were in the well-watered treatment, the percentage of Arg was larger under no UV-B than with UV-B. However, the value under -UV-W was 28% lower than 33% +UV-W. Other amino acid percentages in the shikimate (aromatic) and aspartate families were affected by the combined stresses but accounted for low percentages of the total. In comparing the two years, although there were large differences in the concentrations of amino acids, most of the percentage values were similar. Arg and Pro accounted for the largest percentages, with Arg making up around 20% in 2015-2016 and above 32% in 2016-2017.

Table 6.2 Effects of UV-B and water deficit on amino acids in Pinot noir berries at harvest in 2015-2016 glasshouse trials.

Amino Acid (μM)	+UV+W	+UV-W	-UV+W	-UV-W	P_{UV}	P_{water}	$P_{UV*water}$
α-ketoglutarate							
Pro	891	608	1765	1869	<0.001	n.s	n.s
Arg	976	494	2176	1324	<0.001	<0.001	n.s
Glu**	331	253	532	485	<0.001	0.010	n.s
Gln*	158	127	380	417	<0.001	n.s	n.s
His**	84	72	174	150	<0.001	n.s	n.s
Shikimate (aromatic)							
Phe	34	26	70	101	<0.001	0.006	<0.001
Trp	35	52	59	69	0.002	0.017	n.s
Tyr	4	0	9	22	<0.001	0.016	<0.001
Pyruvate							
Leu	56	46	150	163	<0.001	n.s	n.s
Val**	93	95	203	178	<0.001	n.s	n.s
Ala	692	490	1532	1238	<0.001	0.038	n.s
Aspartate							
Asp*	174	99	363	309	<0.001	0.012	n.s
Asn	5	15	49	60	<0.001	0.004	n.s
Thr	283	206	713	540	0.001	0.022	n.s
Ile	27	39	86	118	<0.001	0.024	n.s
Met	11	5	23	31	<0.001	n.s	0.050
Lys*	17	28	55	39	<0.001	n.s	0.001
3-phosphoglycerate							
Cys	N.A	N.A	N.A	N.A	N.A	N.A	N.A
Ser*	242	198	571	517	<0.001	n.s	n.s
Gly	11	20	49	67	<0.001	<0.001	n.s
Total*	4124	2874	8961	7698	<0.001	0.020	n.s

** and * indicates a significant difference between 2015-2016 and 2016-2017, P<0.01 and P<0.05.

Data showed the mean ± standard error of three replicates from harvest in 2015-2016 and 2016-2017. P-values for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at 5% level; P_{UV}, UV effects averaged across water treatments; P_{water}, water effects averaged across UV treatments; $P_{UV*water}$, water effects depend on UV treatments and UV effects depend on water treatments; n.s, no significant difference. +W, well-watered, -W, water deficit; +UV, UV-B radiation, -UV, normal light.

Table 6.3 Effects of UV-B and water deficit on amino acids in Pinot noir berries at harvest in 2016-2017 glasshouse trials.

Amino Acid (µM)	+UV+W	+UV-W	-UV+W	-UV-W	P_{UV}	P_{water}	$P_{UV*water}$
α-ketoglutarate							
Pro	487	825	321	511	0.001	0.001	0.007
Arg	622	1357	608	629	<0.001	<0.001	<0.001
Glu	100	141	100	99	<0.001	<0.001	<0.001
Gln	45	91	43	56	<0.001	<0.001	<0.001
His	16	37	11	15	<0.001	<0.001	<0.001
Shikimate (aromatic)							
Phe	17	52	18	19	<0.001	<0.001	<0.001
Trp	13	82	13	27	<0.001	<0.001	<0.001
Tyr	7	15	5	8	<0.001	<0.001	<0.001
Pyruvate							
Leu	33	87	25	31	<0.001	<0.001	<0.001
Val	17	69	20	45	0.031	<0.001	0.009
Ala	336	624	297	357	<0.001	<0.001	<0.001
Aspartate							
Asp	54	59	51	50	<0.001	n.s	0.026
Asn	15	33	13	19	<0.001	<0.001	<0.001
Thr	161	327	176	183	<0.001	<0.001	<0.001
Ile	25	86	20	28	<0.001	<0.001	<0.001
Met	5	8	4	11	n.s	0.002	n.s
Lys	19	28	18	17	<0.001	0.002	0.001
3-phosphoglycerate							
Cys	2	1	3	4	n.s	n.s	n.s
Ser	116	207	113	130	<0.001	<0.001	<0.001
Gly	12	22	10	13	<0.001	<0.001	<0.001
Total	2101	4150	1869	2254	<0.001	<0.001	<0.001

Table 6.4 Effects of UV-B and water deficit on the percentages of each amino acid in total amino acids in Pinot noir berries at harvest in 2015-2016 glasshouse trials.

Amino Acid (μM)	+UV+W	+UV-W	-UV+W	-UV-W	P_{UV}	P_{water}	$P_{UV*water}$
α-ketoglutarate							
Pro	21.6%	21.2%	19.7%	24.3%	n.s	n.s	n.s
Arg**	23.7%	17.2%	24.3%	17.2%	n.s	0.001	n.s
Glu*	8.0%	8.8%	5.9%	6.3%	0.015	n.s	n.s
Gln**	3.8%	4.4%	4.2%	5.4%	n.s	n.s	n.s
His**	2.0%	2.5%	1.9%	1.9%	n.s	n.s	n.s
Shikimate (aromatic)							
Phe*	0.8%	0.9%	0.8%	1.3%	n.s	0.012	n.s
Trp	0.8%	1.8%	0.7%	0.9%	0.004	0.002	0.017
Tyr**	0.1%	0.0%	0.1%	0.3%	<0.001	<0.001	<0.001
Pyruvate							
Leu	1.4%	1.6%	1.7%	2.1%	n.s	n.s	n.s
Val**	2.3%	3.3%	2.3%	2.3%	n.s	0.020	0.041
Ala*	16.8%	17.1%	17.1%	16.1%	n.s	n.s	n.s
Aspartate							
Asp**	4.2%	3.4%	4.1%	4.0%	n.s	n.s	n.s
Asn**	0.1%	0.5%	0.5%	0.8%	0.002	0.013	n.s
Thr	6.9%	7.2%	8.0%	7.0%	n.s	n.s	n.s
Ile	0.7%	1.3%	1.0%	1.5%	n.s	0.014	n.s
Met	0.3%	0.2%	0.3%	0.4%	0.038	n.s	0.031
Lys	0.4%	1.0%	0.6%	0.5%	n.s	n.s	n.s
3-phosphoglycerate							
Cys	N.A	N.A	N.A	N.A	N.A	N.A	N.A
Ser**	5.9%	6.9%	6.4%	6.7%	n.s	0.030	n.s
Gly	0.3%	0.7%	0.5%	0.9%	0.030	0.008	n.s

** and * indicates a significant difference between 2015-2016 and 2016-2017, $P<0.01$ and $P<0.05$.

Data showed the mean ± standard error of three replicates from harvest in 2015-2016 and 2016-2017. *P*-values for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at 5% level; P_{UV}, UV effects averaged across water treatments; P_{water}, water effects averaged across UV treatments; $P_{UV*water}$, water effects depend on UV treatments and UV effects depend on water treatments; n.s, no significant difference. +W, well-watered, -W, water deficit; +UV, UV-B radiation, -UV, normal light.

Table 6.5 Effects of UV-B and water deficit on the percentages of each amino acid in total amino acids in Pinot noir berries at harvest in 2016-2017 glasshouse trials.

	+UV+W	+UV-W	-UV+W	-UV-W	P_{UV}	P_{water}	$P_{UV*water}$
α-ketoglutarate							
Pro	23.2%	19.9%	17.2%	22.7%	n.s	n.s	n.s
Arg	29.6%	32.7%	32.5%	27.9%	n.s	n.s	0.008
Glu	4.8%	3.4%	5.4%	4.4%	<0.001	<0.001	n.s
Gln	2.1%	2.2%	2.3%	2.5%	0.001	n.s	n.s
His	0.7%	0.9%	0.6%	0.7%	<0.001	0.001	n.s
Shikimate (aromatic)							
Phe	0.8%	1.3%	1.0%	0.8%	0.002	<0.001	<0.001
Trp	0.6%	2.0%	0.7%	1.2%	<0.001	<0.001	<0.001
Tyr	0.3%	0.4%	0.3%	0.4%	n.s	0.003	0.024
Pyruvate							
Leu	1.6%	2.1%	1.3%	1.4%	<0.001	<0.001	0.002
Val	0.8%	1.7%	1.1%	2.0%	n.s	0.001	n.s
Ala	16.0%	15.0%	15.9%	15.8%	n.s	n.s	n.s
Aspartate							
Asp	2.6%	1.4%	2.7%	2.2%	<0.001	<0.001	<0.001
Asn	0.7%	0.8%	0.7%	0.9%	n.s	<0.001	0.020
Thr	7.7%	7.9%	9.4%	8.1%	<0.001	0.016	0.003
Ile	1.2%	2.1%	1.1%	1.3%	<0.001	<0.001	<0.001
Met	0.2%	0.2%	0.2%	0.5%	0.039	n.s	0.021
Lys	0.9%	0.7%	1.0%	0.8%	n.s	0.001	n.s
3-phosphoglycerate							
Cys	0.1%	0.0%	0.2%	0.2%	0.010	n.s	n.s
Ser	5.5%	5.0%	6.0%	5.8%	<0.001	<0.001	n.s
Gly	0.6%	0.5%	0.5%	0.6%	n.s	n.s	n.s

Phenolic composition

The combination of UV-B radiation and water deficit changed some aspects of phenolic composition in Pinot noir fruit from veraison to harvest in the 2015-2016 and 2016-2017 glasshouse trials (Fig. 6.2 and 6.3). In 2015-2016, the trend of skin total phenolics (on a per berry basis) in treatments was increasing from 2 to 5 weeks post-veraison (harvest) (Fig. 6.2a). The total skin phenolics in the combined stresses treatment (+UV-W) were then higher than other treatments at 4- and 5-weeks post-veraison, while the control (-UV+W) had the lowest content in the last two weeks. At harvest, the contents of skin total phenolics were 0.410 au/berry, 0.376 au/berry and 0.357 au/berry in +UV-W, +UV+W and -UV-W, respectively.

Skin anthocyanins accumulated from veraison to harvest (Fig. 6.2b). The combined stresses caused greater increases in skin anthocyanins than the individual stresses from 4- to 5-weeks post-veraison. At harvest, the skin anthocyanin contents in +UV-W, +UV+W and -UV-W reached their maximum values at 0.578 mg/berry, 0.499 mg/berry and 0.473 mg/berry, respectively, but skin anthocyanins in -UV+W peaked one week before harvest, with 0.398 mg/berry.

The contents of skin and seed tannin from veraison to harvest were variable (Fig.6.3a and b). In general, skin tannins showed only slight changes in all treatments from veraison to harvest. At harvest, +UV-W had the highest content of skin tannin at 1.367 mg/berry than in all the other treatments. The seed tannins had increases and then decreases in all treatments (Fig. 6.3b). From veraison to 3-weeks post-veraison, both UV treatments had lower contents of seed tannin. At harvest, +UV-W significantly increased the seed tannins by 0.506 mg/berry, compared with -UV+W, but the growth in value for seed tannins in +UV-W was less than 1.017 mg/berry in +UV+W and 0.643 mg/berry in -UV-W.

In 2016-2017, the accumulations of skin total phenolics were affected by both UV-B and water deficit (Fig. 6.4a). Compared with the control, the separated or combined UV-B and water deficit caused increases in skin total phenolics. At harvest, the content of skin total

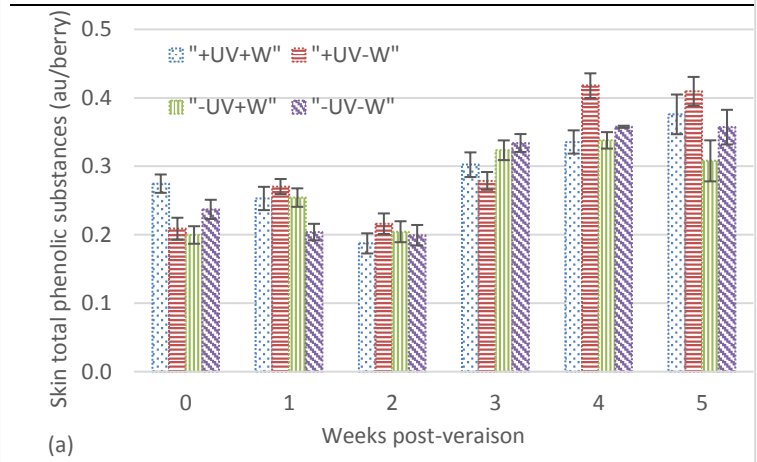
phenolics in +UV-W was 0.354 au/berry higher than the 0.312 au/berry in -UV-W but lower than the 0.306 au/berry in +UV+W.

The skin anthocyanins in the treatments accumulated from veraison to harvest and showed a sharp increase after 1-week post-veraison (Fig. 6.4b). With respect to the control, the individual or combined UV-B and water deficit significantly increased the skin anthocyanins from 2- to 6-weeks post-veraison. Both +UV+W and +UV-W had more skin anthocyanins than -UV-W from 4- to 6-weeks post-veraison. At harvest, the skin anthocyanin content in +UV-W was 0.679 mg/berry higher than the 0.606 mg/berry in +UV+W.

Skin tannins showed increases from veraison to 2-weeks post-veraison and substantial reductions from 3-weeks post-veraison to harvest in treatments and the control (Fig. 6.5a). With respect to -UV+W, +UV and -W treatments caused the reduction in skin tannins from veraison to 3-weeks post-veraison and then increased until harvest. At harvest, the skin tannin contents reached 0.596 mg/berry in +UV-W, which was lower than 0.713 mg/berry in +UV+W and 0.709 mg/berry in -UV-W, but higher than the 0.464 mg/berry in -UV+W. Seed tannins in the treatments showed increases and then the decreases during ripening (Fig. 6.5b). There were no consistent changes in seed tannin contents between treatments during ripening and no statistically significant differences between treatments at harvest.

Compared with 2015-2016, +UV-W had smaller changes in skin anthocyanins and total phenolics than -UV+W, in 2016-2017. In the combination of the two stresses, skin anthocyanins in 2015-2016 were lower than in 2016-2017, while the skin total phenolics in 2015-2016 were at higher levels than in 2016-2017. Skin and seed tannins showed higher contents in 2015-2016 than in 2016-2017. Also, there were few changes in skin tannins from veraison to harvest in 2015-2016, whereas decreases were present during ripening in 2016-2017.

P_{UV}	0.015	<0.001	n.s	0.001	<0.001	<0.001
P_{Water}	n.s	0.005	n.s	n.s	<0.001	<0.001
$P_{UV*water}$	<0.001	<0.001	n.s	0.041	<0.001	n.s



P_{UV}	n.s	<0.001	n.s	<0.001	<0.001	<0.001
P_{Water}	n.s	<0.001	<0.001	<0.001	<0.001	<0.001
$P_{UV*water}$	n.s	<0.001	<0.001	n.s	n.s	n.s

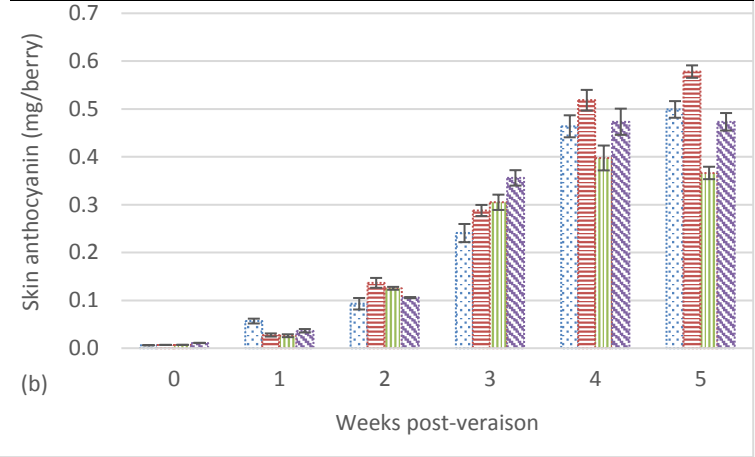


Figure 6.2 (a) and (b): Effects of UV-B and water deficit on skin total phenolic substances and skin anthocyanins in Pinot noir berries during ripening in 2015-2016 glasshouse trials.

Data showed the mean ± standard error of four replicates. *P*-values for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD at the 5% level; P_{UV}, UV effects averaged across water treatments; P_{water}, water effects averaged across UV treatments; $P_{UV*water}$, water effects depend on UV treatments and UV effects depend on water treatments; n.s, no significant difference. +W, well-watered, -W, water deficit; +UV, UV-B radiation, -UV, normal light.

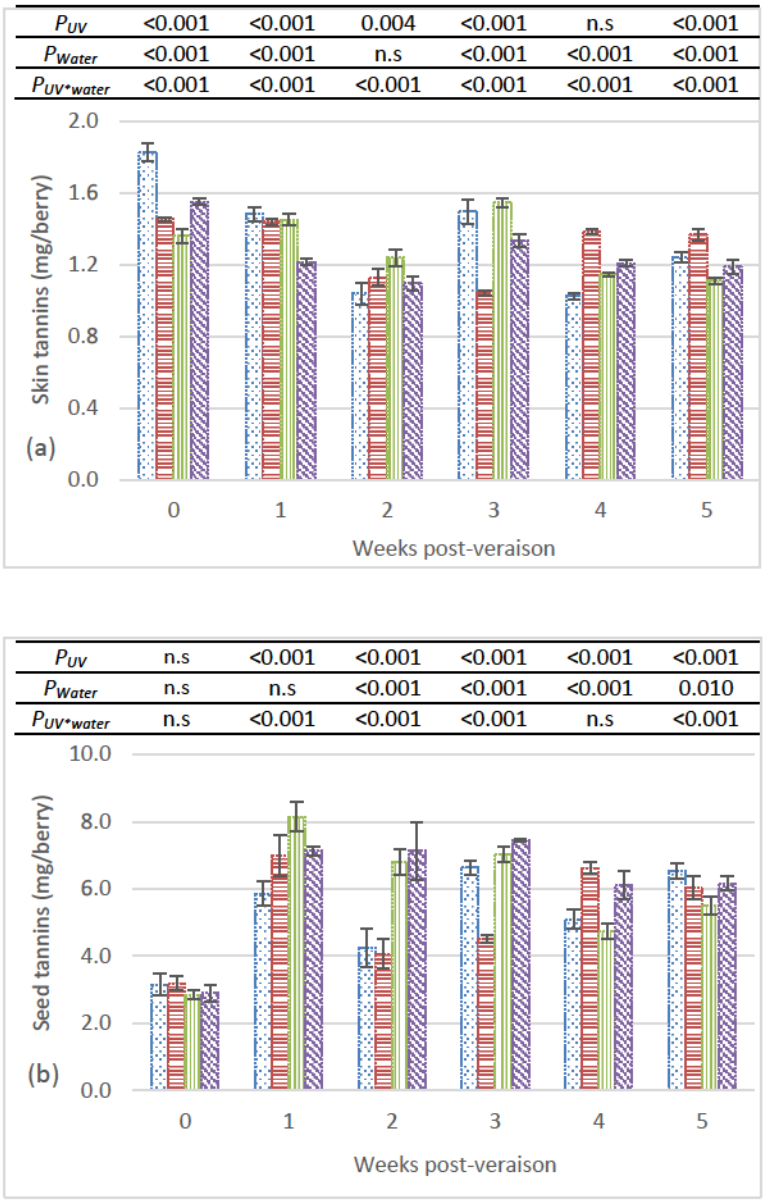
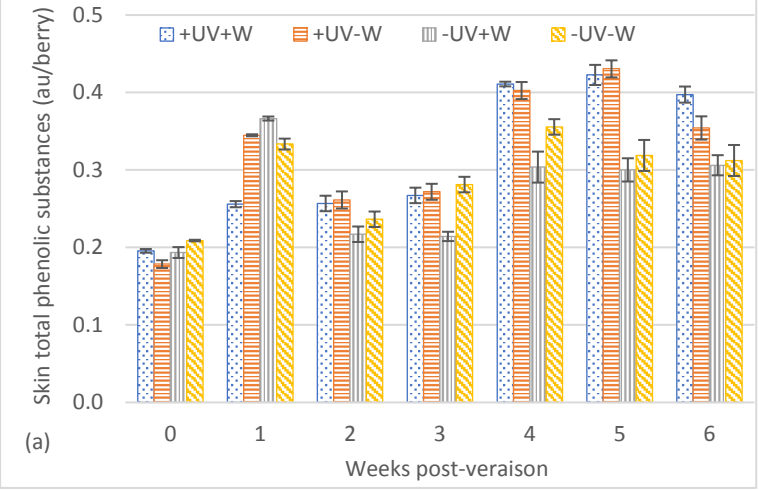


Figure 6.3 (a) and (b) Effects of UV-B and water deficit on skin tannins and seed tannins in Pinot noir berries during ripening in 2015-2016 glasshouse trials.

P_{UV}	n.s	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{Water}	n.s	<0.001	<0.001	<0.001	<0.001	<0.001	n.s
$P_{UV*water}$	n.s	0.029	<0.001	<0.001	<0.001	0.002	0.031



P_{UV}	n.s	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{Water}	n.s	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$P_{UV*water}$	n.s	<0.001	<0.001	<0.001	<0.001	<0.001	n.s

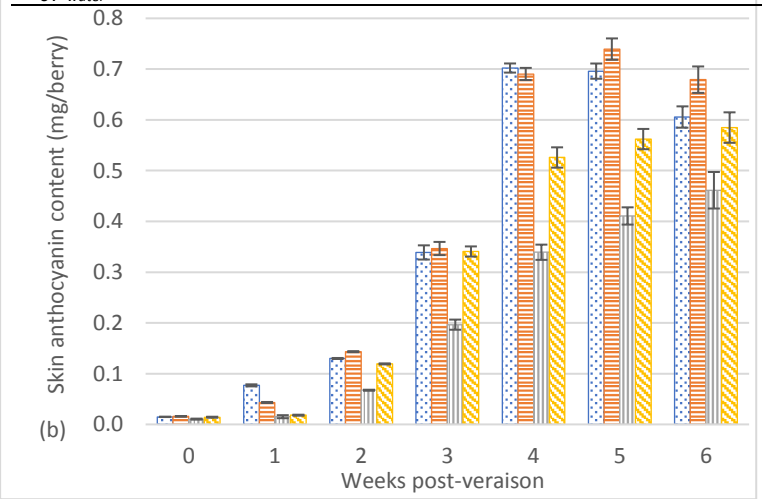
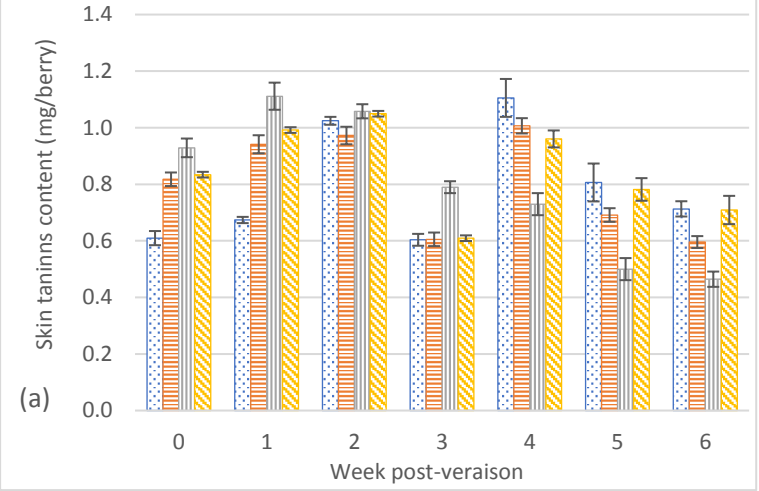


Figure 6.4 (a) and (b): Effects of UV-B and water deficit on skin total phenolic substances and skin anthocyanins in Pinot noir berries during ripening in 2016-2017 glasshouse trials.

Data showed the mean ± standard error of four replicates. *P*-values for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD at the 5% level; P_{UV}, UV effects averaged across water treatments; P_{water}, water effects averaged across UV treatments; $P_{UV*water}$, water effects depend on UV treatments and UV effects depend on water treatments; n.s, no significant difference. +W, well-watered, -W, water deficit; +UV, UV-B radiation, -UV, normal light.

P_{UV}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{Water}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$P_{UV*water}$	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001



P_{UV}	<0.001	0.002	n.s	<0.001	<0.001	<0.001	n.s
P_{Water}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.035
$P_{UV*water}$	<0.001	<0.001	<0.001	<0.001	<0.001	n.s	n.s

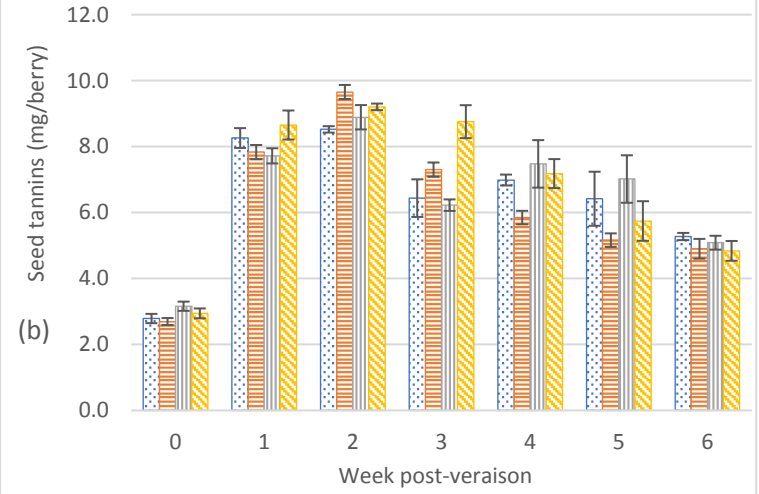


Figure 6.5 (a) and (b): Effects of UV-B and water deficit on skin tannins and seed tannins in Pinot noir berries during ripening in 2016-2017 glasshouse trials.

Volatile compounds

The volatile composition of the fruit was measured from all treatments after the second year of experiments (Table 6.6). In 2015-2016, the effects of UV-B or water deficit were qualified by interactions on hexanal, (E)-2-hexenal, (Z)-3-hexenol, linalool, citronellol and geraniol. Under water deficit effect with UV-B (+UV-W), the concentration of hexanal decreased by 21.3%, compared to -UV-W. Vines in the UV-B treatments had lower (E)-2-hexenal concentrations with water deficit compared to the well-watered vines. Monoterpenes (linalool, citronellol and geraniol) and (Z)-3-hexenol showed similar responses induced by UV-B and water deficit. Under water deficit, their concentrations were increased by UV-B, while they were decreased by UV-B under well-watered.

In 2016-2017, the combination of UV-B and water deficit induced significant changes in hexanol, (E)-2-hexenol and nerol. Vines under water deficit had higher concentrations of hexanol and (E)-2-hexenol in the UV-B treatment than in the no UV-B treatment. The concentration of hexanol and (E)-2-hexenol in +UV-W had values of 1029 µg/L and 790 µg/L, respectively, compared to +UV+W at 752 µg/L and 530 µg/L, respectively. The nerol concentration in -UV+W was 3.4 µg/L, which was the highest of any of the treatments.

Compared with 2016-2017, the concentration of C_6 aldehydes and free monoterpenes were higher in 2015-2016, while C_6 alcohols were lower in 2015-2016. In C_6 aldehydes, the hexanal concentration was over 300 µg/L in the treatments in 2015-2016 but less than 200 µg/L in 2016-2017, particularly 50.6 µg/L in +UV-W. In C_6 alcohols, hexanol concentration ranged from 270 µg/L to 340 µg/L, in 2015-2016, while it was over 640 µg/L, in 2016-2017. In hexanol, +UV-W caused a decrease of 18.2% in 2015-2016 and an increase of 50.5% in 2016-2017, compared to the control. (E)-2-hexenol concentration was below 200 µg/L in 2015-2016 but over 500 µg/L in 2016-2017.

Table 6.6 Effects of UV-B and water deficit on volatile compounds in Pinot noir juice at harvest in 2015-2016 and 2016-2017 glasshouse trials.

	Volatile compounds (μg/L)	+UV+W	+UV-W	-UV+W	-UV-W	P_{UV}	P_{water}	$P_{UV*water}$
2015-2016	C_6 aldehydes							
	Hexanal**	334.2	303.1	317.1	367.7	n.s	n.s	0.050
	(E)-2-hexenal*	177.3	128.7	157.3	140.4	n.s	0.001	0.027
	C_6 alcohols							
	Hexanol**	276.3	272.8	322.5	334.3	<0.001	n.s	n.s
	(E)-3-hexenol**	6.8	6.2	7.2	6.2	n.s	n.s	n.s
	(Z)-3-hexenol*	23.1	32.9	31.2	20.2	0.020	n.s	<0.001
	(E)-2-hexenol**	133.3	151.1	172.6	180.6	0.039	n.s	n.s
	Free monoterpenes							
	Linalool**	1.7	1.7	2.0	1.5	<0.033	<0.001	<0.001
	α-terpineol*	1.5	1.6	2.3	1.4	n.s	n.s	n.s
	Citronellol	1.0	1.1	1.4	0.9	n.s	0.001	0.001
	Nerol	2.5	2.8	3.2	3.0	0.015	n.s	n.s
	Geraniol**	14.1	15.4	18.0	12.6	n.s	0.048	0.005
2016-2017	C_6 aldehydes							
	Hexanal**	223.1	50.6	193.5	169.0	n.s	0.044	n.s
	(E)-2-hexenal*	119.6	79.9	128.8	96.7	n.s	0.016	n.s
	C_6 alcohols							
	Hexanol**	751.8	1028.6	683.3	646.2	0.005	n.s	0.029
	(E)-3-hexenol**	11.9	12.7	11.2	11.8	n.s	n.s	n.s
	(Z)-3-hexenol*	29.3	26.6	30.0	29.6	n.s	n.s	n.s
	(E)-2-hexenol**	529.5	790.3	571.3	562.2	n.s	0.031	0.023
	Free monoterpenes							
	Linaloo**	1.5	1.5	1.5	1.5	n.s	n.s	n.s
	α-terpineol*	1.0	1.1	1.2	1.2	n.s	n.s	n.s
	Citronellol	1.0	1.2	1.1	1.1	n.s	0.037	n.s
	Nerol	3.0	3.0	2.7	3.4	n.s	n.s	0.026
	Geraniol**	13.5	13.3	13.3	14.3	n.s	n.s	n.s

** and * indicates a significant difference between 2015-2016 and 2016-2017, $P<0.01$ and $P<0.05$.

Data showed the mean ± standard error of three replicates from harvest in 2015-2016 and 2016-2017. *P*-values for statistical significance comparing the different treatments according to Two-factor ANOVA and LSD test at 5% level; P_{UV}, UV effects averaged across water treatments; P_{water}, water effects averaged across UV treatments; $P_{UV*water}$, water effects depend on UV treatments and UV effects depend on water treatments; n.s, no significant difference. +W, well-watered, -W, water deficit; +UV, UV-B radiation, -UV, normal light.

6.2.2 Vineyard trials

Effects of UV-B interaction with water deficit on the physiology

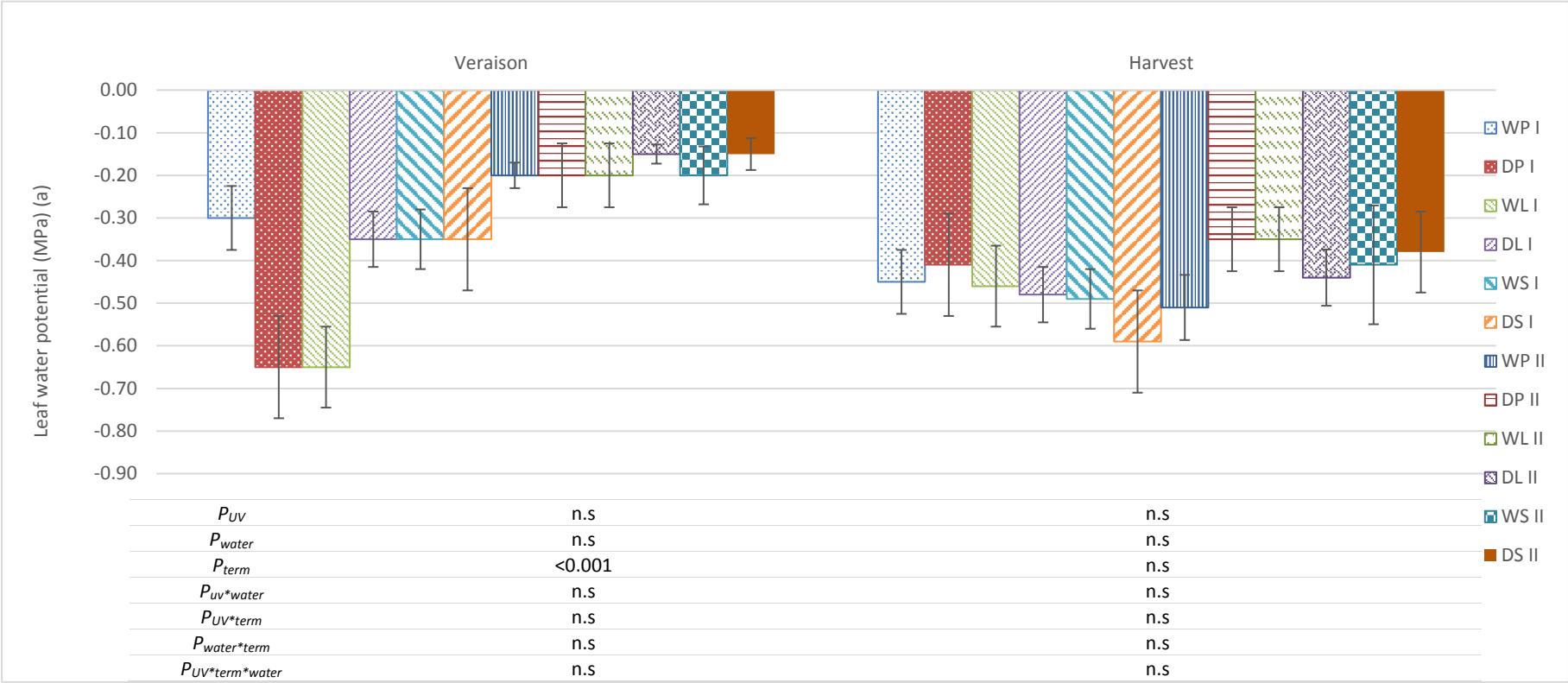
The effects of UV-B exposure/exclusion interactions with water treatments on vine physiology were shown in Table 6.7 and Fig. 6.6/6.7. Pruning weights and TA were changed by UV treatments, and °Brix by the timing of treatment application. UV-B exposure (WL/DL) had the highest value of pruning weights in comparison with shading (WS/DS) and UV-B exclusion (WP/DP). Higher °Brix was shown in the late set of treatments, except for UV-B exclusion with well-watered treatment (WP). TA in WP/DP and WL/DL were around 10 g/L, while in WS/DS it was about 11 g/L of TA. DP and DL affected vine physiology of Pinot noir in the vineyard. In the late set of treatments, the ^{13}C ratio of juice under water deficit was decreased in UV-B exposure (DLII), compared to the shading (DSII) and UV-B exclusion (DPII), while the well-watered vines had the highest $\delta^{13}C‰$ of juice in UV-B exposure (WLII) in comparison with shading (WSII) and UV-B exclusion (WPII) treatments.

There were significant differences in soil volumetric water content between the well-watered and water deficit treatments (see Chapter 5) before veraison, so the early timing of treatment application decreased the leaf water potential (LWP), compared to the later one at veraison. At harvest, LWP showed little difference in vine water status under the interactive stress treatments at veraison and harvest (Fig. 6.6). In Figure 6.7, leaf SPAD in all treatments substantially decreased during ripening, but there were no significant differences between treatments at harvest. Regardless of water deficit and the timings of treatments, shading cloth treatments (WSI/II and DSI/II) had the biggest value of SPAD than other treatments (WPI/II, DPI/II, WLI/II and DLI/II) from -4-weeks to 4-weeks post-veraison. At 0 and 4-weeks post-veraison, under different fruit exposure, SPAD were increased by water deficit compared to well-watered. Therefore, there was no consistent statistical effect of UV-B treatment over most sampling dates and an apparent weak effect of water deficit, but there was no statistical interaction between UV-B and water deficit treatments.

Table 6.7 Effects of UV-B and water deficit on berry parameters, $\delta^{13}C$‰ of leaf and juice, yield, pruning weight and Ravaz Index in Pinot noir at harvest in 2016-2017 vineyard trials.

Treatment	Leaf $\delta^{13}C$ vs V-PDB ‰	Juice $\delta^{13}C$ vs V-PDB ‰	Vine yield (kg)	Pruning weight (kg)	Ravaz Index	°Brix	TA(g/L)	pH
WP I	-28.90	-27.56	4.37	0.87	5.19	16.6	10.2	3.59
WP II	-29.16	-27.74	3.43	0.62	5.55	16.4	10.5	3.62
DP I	-29.11	-27.47	3.10	0.86	5.29	16.3	10.3	3.63
DP II	-28.30	-26.53	1.80	0.75	2.74	17.9	10.2	3.64
WL I	-28.71	-27.53	3.24	0.92	3.47	16.6	10.7	3.55
WL II	-28.30	-26.53	1.60	1.21	1.98	18.2	10.4	3.76
DL I	-28.58	-27.55	3.06	1.30	2.79	17.2	9.8	3.70
DL II	-28.26	-28.17	3.98	1.12	3.70	18.4	10.1	3.71
WS I	-28.88	-28.47	4.32	0.94	4.81	16.4	11.0	3.57
WS II	-29.13	-28.11	2.97	0.71	4.23	17.1	11.4	3.65
DS I	-28.32	-27.28	4.95	1.04	4.59	16.2	11.0	3.64
DS II	-28.61	-26.93	3.18	0.77	3.85	17.4	10.4	3.68
P_{UV}	n.s	n.s	n.s	**0.**040	n.s	n.s	0.010	n.s
P_{water}	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
P_{term}	n.s	n.s	n.s	n.s	n.s	0.010	n.s	n.s
$P_{uv*water}$	n.s	0.003	n.s	n.s	n.s	n.s	n.s	n.s
$P_{UV*term}$	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
$P_{water*term}$	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
$P_{UV*term*water}$	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

Data showed the mean ± standard error of four replicates. *P*-values for statistical significance comparing the different treatments according to Three-factor ANOVA and LSD test at the 5% level. Main effects of UV (P_{UV}), water deficit (P_{water}), set-up treatments at pre-veraison/veraison (P_{term}), the combination of UV and water deficit ($P_{UV*water}$), the combination of UV and pre-veraison/veraison of set-up treatments ($P_{UV*term}$), the combination of water deficit and pre-veraison/veraison of set-up treatments ($P_{water*term}$) and the combination of UV, water deficit and pre-veraison/veraison of set-up treatments ($P_{UV*term*water}$); n.s, no significant difference. P, PETG screen, L, leaf remove, S, shade cloth; W, well-water, D, water deficit; I, the setup of treatments at pre-veraison, II, the setup of treatments at veraison.



	Veraison	Harvest
P_{UV}	n.s	n.s
P_{water}	n.s	n.s
P_{term}	<0.001	n.s
$P_{uv*water}$	n.s	n.s
$P_{UV*term}$	n.s	n.s
$P_{water*term}$	n.s	n.s
$P_{UV*term*water}$	n.s	n.s

Figure 6.6 Effects of UV-B and water deficit on leaf water potential in Pinot noir at veraison and harvest in 2016-2017 vineyard trials.

Data showed the mean ± standard error of four replicates. *P*-values for statistical significance comparing the different treatments according to Three-factor ANOVA and LSD test at the 5% level. Main effects of UV (P_{UV}), water deficit (P_{water}), set-up treatments at pre-veraison/veraison (P_{term}), the combination of UV and water deficit ($P_{UV*water}$), the combination of UV and pre-veraison/veraison of set-up treatments ($P_{UV*term}$), the combination of water deficit and pre-veraison/veraison of set-up treatments ($P_{water*term}$) and the combination of UV, water deficit and pre-veraison/veraison of set-up treatments ($P_{UV*term*water}$); n.s, no significant difference. P, PETG screen, L, leaf remove, S, shade cloth; W, well-water, D, water deficit; I, the setup of treatments at pre-veraison, II, the setup of treatments at veraison.

P_{UV}	0.040	0.026	0.044	0.003	<0.001	n.s
P_{water}	n.s	n.s	<0.001	n.s	0.015	n.s
P_{term}			n.s	<0.001	n.s	n.s
$P_{uv*water}$	n.s	n.s	n.s	n.s	n.s	n.s
$P_{UV*term}$			n.s	n.s	n.s	n.s
$P_{water*term}$			n.s	n.s	0.048	n.s
$P_{UV*term*water}$			n.s	n.s	n.s	n.s

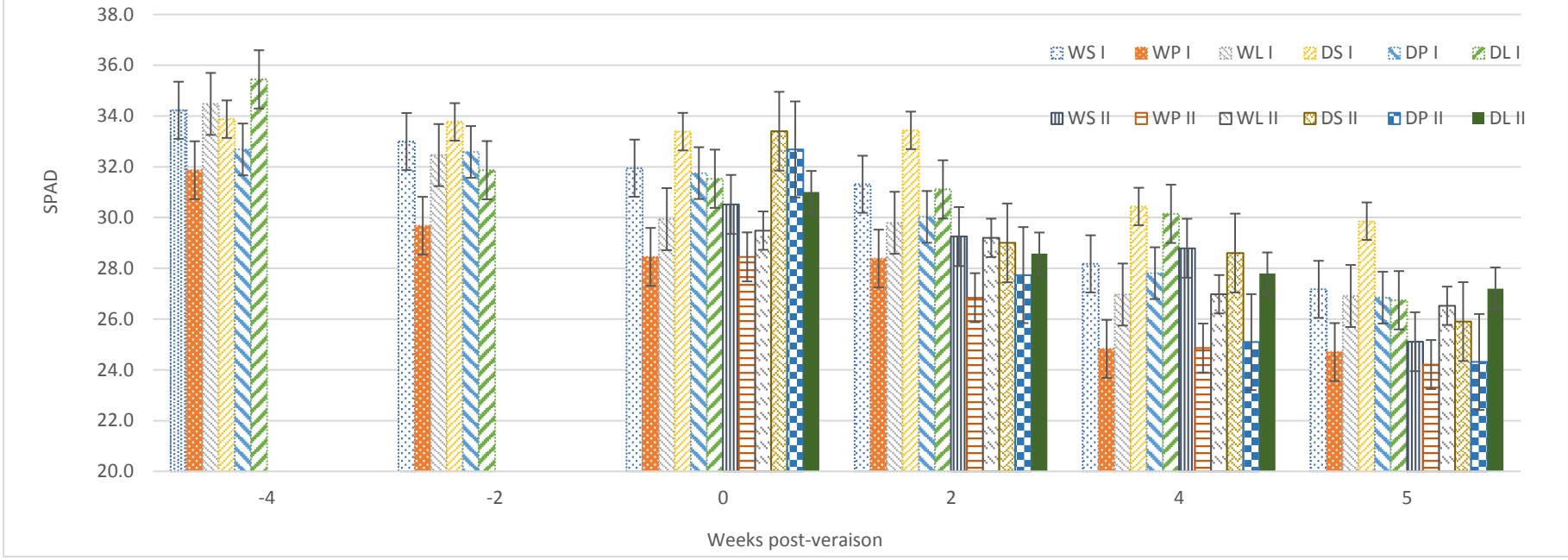


Figure 6.7 Effects of UV-B and water deficit on SPAD level in Pinot noir at veraison and harvest in 2016-2017 vineyard trials.

Effects of UV-B interaction with water deficit on chemical composition

Amino acids

The concentration of amino acids in grape juice were presented in Table 6.8, which showed the effects of UV-B exposure/exclusion interactions with water deficit at two stages of applying treatments. Some amino acids were affected by the sunlight environment, water conditions or treatment timing, but one or more main effects were qualified by an interaction with His, Val, Thr and Lys. Under water deficit, the UV-B exclusion treatments (DPI/II) caused increases in His concentrations, compared to the leaf removal and shading treatments (DLI/II and DSI/II). Also, the increase in His concentration was induced by DPI/II in comparison to WPI/II. Val in DPI/II was 414 μM and 377 μM, respectively, which was lower than DLI/II and DSI/II, while WSI/II caused increases in Val compared to WLI/II and WPI/II. Regardless of the treatment timing, DL and DP had higher Val concentrations than WL and WP. A similar interaction with the sunlight environment and water influenced changes in Thr. UV interaction with water significantly affected Lys. Under water deficit, UV-B exclusion treatments had higher concentrations of Lys than in the shading and leaf removal treatments.

Analysis of the amino acid data expressed on a percentage of the total basis also showed that there were statistically significant effects of the combinations of UV-B and water deficit treatments, such as for Pro, Arg, His, Phe, Val, Asp, Met, Lys and Ser (Table 6.9). Under water deficit, the % of Pro in the UV-B exclusion treatments were 8.5% (DPI)/11.8% (DPII), which were higher than in DSI/II or DL I/II. Also, water deficit treatments dramatically increased the % Pro from 6.1% in WPII to 11.8% in DPII. Arg had the highest percentage in the amino acids, and these values were higher in DS than in DP. Lys, Met and Asp, belonging to aspartate family, accounted for small percentages in the total amino acids. The percentages of Lys were increased by DPI/II (0.3% and 0.4%), compared with DLI/II (0.2% and 0.3%) and WSI/II (0.2% and 0.2%). WPI/ II and DPI/II caused a sharp reduction in Met, dropping to around 0.3%, while the other treatments were over 1%. The percentages of Asp were around 0.5% for all treatments.

Table 6.8 Effects of UV-B and water deficit on amino acids in total amino acids in Pinot noir berries at harvest in 2016-2017 vineyard trials.

Amino Acid (μM)	Treatment												P value						
	WP Ⅰ	WP Ⅱ	DP Ⅰ	DP Ⅱ	WL Ⅰ	WL Ⅱ	DL Ⅰ	DL Ⅱ	WS Ⅰ	WS Ⅱ	DS Ⅰ	DS Ⅱ	P_{water}	P_{UV}	P_{term}	$P_{water*UV}$	$P_{water*term}$	$P_{UV*term}$	$P_{water*UV*term}$
α-ketoglutarate																			
Pro	892	748	1463	1923	820	1332	1095	895	506	516	791	837	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Arg	3066	3349	2849	2711	3357	2854	3267	3331	3314	3256	3121	3098	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Glu	263	262	429	354	270	389	309	286	271	276	294	340	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Gln	2279	2261	4489	3926	2132	3419	2731	1818	2416	2180	2976	2617	n.s	n.s	n.s	n.s	n.s	n.s	n.s
His	164	148	247	249	158	192	153	118	119	117	115	129	n.s	**0.009**	n.s	**0.038**	n.s	n.s	n.s
Shikimate (aromatic)																			
Phe	493	465	606	543	490	582	497	408	440	477	306	455	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Trp	118	114	150	160	99	155	110	85	94	69	49	98	n.s	**0.011**	n.s	n.s	n.s	n.s	n.s
Tyr	57	52	79	65	54	77	59	52	47	52	43	52	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Pyruvate																			
Leu	420	372	509	505	417	577	454	387	383	397	288	373	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Val	313	272	414	377	302	396	829	788	791	776	740	748	**<0.001**	**<0.001**	n.s	**<0.001**	n.s	n.s	n.s
Ala	1521	1612	2358	2216	1809	2749	2249	1860	1881	1895	1973	2292	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Aspartate																			
Asp	234	242	318	248	223	242	223	191	191	218	230	204	n.s	**0.038**	n.s	n.s	n.s	n.s	n.s
Asn	63	65	108	89	66	97	76	58	75	67	86	77	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Thr	1147	1199	1441	1375	1252	1375	1183	1042	1089	1061	1051	1073	n.s	**0.018**	n.s	**0.022**	n.s	n.s	n.s
Ile	344	304	431	414	343	460	365	282	298	309	201	268	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Met	81	40	62	38	13	173	251	231	167	169	186	242	n.s	**0.004**	n.s	n.s	n.s	n.s	n.s
Lys	38	40	56	59	40	50	35	33	27	31	39	40	n.s	**0.027**	n.s	**0.019**	n.s	n.s	n.s
3-phosphoglycerate																			
Ser	775	728	1151	963	874	1113	971	763	798	741	892	841	n.s	n.s	n.s	n.s	n.s	n.s	n.s

Cys	9	12	15	11	10	10	12	11	11	6	5	5	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Gly	34	28	54	45	36	56	46	33	34	30	41	37	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Total	12312	12314	17228	16273	12764	16429	14918	12672	12951	12646	13428	13825	n.s	n.s	n.s	n.s	n.s	n.s	n.s

Data showed the mean ± standard error of four replicates. *P*-values for statistical significance comparing the different treatments according to Three-factor ANOVA and LSD test at the 5% level. Main effects of UV (P_{UV}), water deficit (P_{water}), set-up treatments at pre-veraison/veraison (P_{term}), the combination of UV and water deficit ($P_{UV*water}$), the combination of UV and pre-veraison/veraison of set-up treatments ($P_{UV*term}$), the combination of water deficit and pre-veraison/veraison of set-up treatments ($P_{water*term}$) and the combination of UV, water deficit and pre-veraison/veraison of set-up treatments ($P_{UV*term*water}$); n.s, no significant difference. P, PETG screen, L, leaf remove, S, shade cloth; W, well-water, D, water deficit; I, the setup of treatments at pre-veraison, II, the setup of treatments at veraison.

Table 6.9 Effects of UV-B and water deficit on the percentages of each amino acid in total amino acids in Pinot noir berries at harvest in 2016-2017 vineyard trials.

Amino Acid (μM)	Treatment												*P*						
	WP Ⅰ	WP Ⅱ	DP Ⅰ	DP Ⅱ	WL Ⅰ	WL Ⅱ	DL Ⅰ	DL Ⅱ	WS Ⅰ	WS Ⅱ	DS Ⅰ	DS Ⅱ	P_{water}	P_{UV}	P_{term}	$P_{water*UV}$	$P_{water*term}$	$P_{UV*term}$	$P_{water*UV*term}$
α-ketoglutarate																			
Pro	7.2%	6.1%	8.5%	11.8%	6.4%	8.1%	7.3%	7.1%	3.9%	4.1%	5.9%	6.1%	**0.011**	**0.007**	n.s	**0.045**	n.s	n.s	n.s
Arg	24.9%	27.2%	16.5%	16.7%	26.3%	17.4%	21.9%	26.3%	25.6%	25.8%	23.2%	22.4%	n.s	**0.019**	n.s	n.s	**0.027**	n.s	n.s
Glu	2.1%	2.1%	2.5%	2.2%	2.1%	2.4%	2.1%	2.3%	2.1%	2.2%	2.2%	2.5%	n.s	**0.030**	n.s	n.s	n.s	n.s	n.s
Gln	18.5%	18.4%	26.1%	24.1%	16.7%	20.8%	18.3%	14.4%	18.7%	17.2%	22.2%	18.9%	n.s	n.s	n.s	n.s	n.s	n.s	n.s
His	1.3%	1.2%	1.4%	1.5%	1.2%	1.2%	1.0%	0.9%	0.9%	0.9%	0.9%	0.9%	**<0.001**	n.s	n.s	**0.012**	n.s	n.s	n.s
Shikimate (aromatic)																			
Phe	4.0%	3.8%	3.5%	3.3%	3.8%	3.5%	3.3%	3.2%	3.4%	3.8%	2.3%	3.3%	**0.022**	**0.001**	n.s	**0.014**	n.s	n.s	n.s
Trp	1.0%	0.9%	0.9%	1.0%	0.8%	0.9%	0.7%	0.7%	0.7%	0.5%	0.4%	0.7%	**<0.001**	n.s	n.s	n.s	n.s	n.s	n.s
Tyr	0.5%	0.4%	0.5%	0.4%	0.4%	0.5%	0.4%	0.4%	0.4%	0.4%	0.3%	0.4%	**0.004**	n.s	n.s	n.s	n.s	n.s	n.s
Pyruvate																			
Leu	3.4%	3.0%	3.0%	3.1%	3.3%	3.5%	3.0%	3.1%	3.0%	3.1%	2.1%	2.7%	**0.001**	n.s	**0.027**	n.s	n.s	n.s	n.s
Val	2.5%	2.2%	2.4%	2.3%	2.4%	2.4%	5.6%	6.2%	6.1%	6.1%	5.5%	5.4%	**<0.001**	**<0.001**	n.s	**<0.001**	n.s	n.s	n.s
Ala	12.4%	13.1%	13.7%	13.6%	14.2%	16.7%	15.1%	14.7%	14.5%	15.0%	14.7%	16.6%	**0.002**	**0.046**	**0.020**	n.s	n.s	n.s	n.s
Aspartate																			
Asp	1.9%	2.0%	1.8%	1.5%	1.7%	1.5%	1.5%	1.5%	1.5%	1.7%	1.7%	1.5%	**0.001**	**0.029**	n.s	n.s	n.s	n.s	**0.044**
Asn	0.5%	0.5%	0.6%	0.5%	0.5%	0.6%	0.5%	0.5%	0.6%	0.5%	0.6%	0.6%	**0.044**	n.s	n.s	n.s	n.s	n.s	n.s
Thr	9.3%	9.7%	8.4%	8.5%	9.8%	8.4%	7.9%	8.2%	8.4%	8.4%	7.8%	7.8%	n.s	**0.011**	n.s	n.s	n.s	n.s	n.s
Ile	2.8%	2.5%	2.5%	2.5%	2.7%	2.8%	2.4%	2.2%	2.3%	2.4%	1.5%	1.9%	**0.001**	**0.025**	n.s	n.s	n.s	n.s	n.s
Met	0.7%	0.3%	0.4%	0.2%	0.1%	1.1%	1.7%	1.8%	1.3%	1.3%	1.4%	1.8%	**<0.001**	**<0.001**	n.s	**<0.001**	n.s	n.s	n.s
Lys	0.3%	0.3%	0.3%	0.4%	0.3%	0.3%	0.2%	0.3%	0.2%	0.2%	0.3%	0.3%	**<0.001**	n.s	n.s	**0.014**	n.s	n.s	n.s
3-phosphoglycerate																			

Cys	6.3%	5.9%	6.7%	5.9%	6.8%	6.8%	6.5%	6.0%	6.2%	5.9%	6.6%	6.1%	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Ser	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.0%	n.s	n.s	**0.005**	**0.024**	n.s	n.s	n.s
Gly	0.3%	0.2%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.2%	0.3%	0.3%	n.s	n.s	**0.043**	n.s	n.s	n.s	n.s

Phenolic composition

To determine the effects of UV-B exposure/exclusion interactions with water deficit on Pinot noir berries, samples from pre-veraison/veraison to harvest in 2017 were analysed for their phenolic composition, including skin total phenolics, skin anthocyanins, skin tannins and seed tannins. The contents of skin total phenolics in treatments were from around 0.140 au/berry at -4-weeks post-veraison to about 0.200 au/berry at 5-weeks post-veraison (harvest) and reached their peaks at 4-weeks post-veraison (Fig. 6.8). At harvest, the shading treatments (WSI/II and DSI/II) had the lowest contents of skin total phenolics, which were less than 0.200 au/berry. Compared to WSI/II and DSI/II, the contents of skin total phenolics were significantly increased by WLI/II and DLI/II. DLI/II and WLI/II had higher values, reaching up to 0.208/0.217 au/berry and 0.261/0.292 au/berry, respectively.

The most obvious change was the accumulation of skin anthocyanins from veraison to harvest in all treatments (Fig. 6.9). The contents of skin anthocyanin peaked at 4-weeks post-veraison and, subsequently, declined from there to harvest. At harvest, WLI/II (0.356 mg/berry and 0.466 mg/berry) had the highest contents of skin anthocyanin, while the lowest contents were in DSI/II (0.194 mg/berry and 0.245 mg/berry) and WSI/II (0.213 mg/berry and 0.232 mg/berry). Also, DLI/II had high skin anthocyanin contents, reaching 0.311/0.317 mg/berry. Skin anthocyanins in WPI/II and DPI/II had higher contents than in WSI/II and DSI/II but less than in WLI/II and DLI/II.

As shown In Fig. 6.10, substantial trends were the reduction in skin tannins from -4- to 5-weeks post-veraison (harvest). UV interactions with water deficit significantly affected the contents of skin tannin at harvest. In WPI/II and DPI/II, the skin tannin contents ranged from 0.540 mg/berry to 0.689 mg/berry, respectively, which were more than in the other treatments. The skin tannin contents in WLI/II and DLI/II were less than 0.520 mg/berry, while WSI/II and DSI had more than 0.520 mg/berry, except for 0.165 mg/berry in DSII. There were no statistically significant differences in seed tannins between treatments during berry development (Fig. 6.11), except for at 1-week post-veraison. DLII and DSII had the highest seed tannin contents at 7.003 mg/berry and 7.195 mg/berry than other treatments.

P_{UV}	<0.001	0.010	0.004	0.001	n.s.	n.s	<0.001	0.001
P_{water}	<0.001	0.020	n.s	n.s	0.031	n.s	n.s	0.008
P_{term}				n.s	n.s	n.s	0.046	n.s
$P_{uv*water}$	<0.001	0.002	n.s	n.s	0.015	n.s	n.s	n.s
$P_{UV*term}$				n.s	n.s	n.s	n.s	n.s
$P_{water*term}$				n.s	n.s	n.s	n.s	n.s
$P_{UV*term*water}$				n.s	n.s	n.s	n.s	n.s

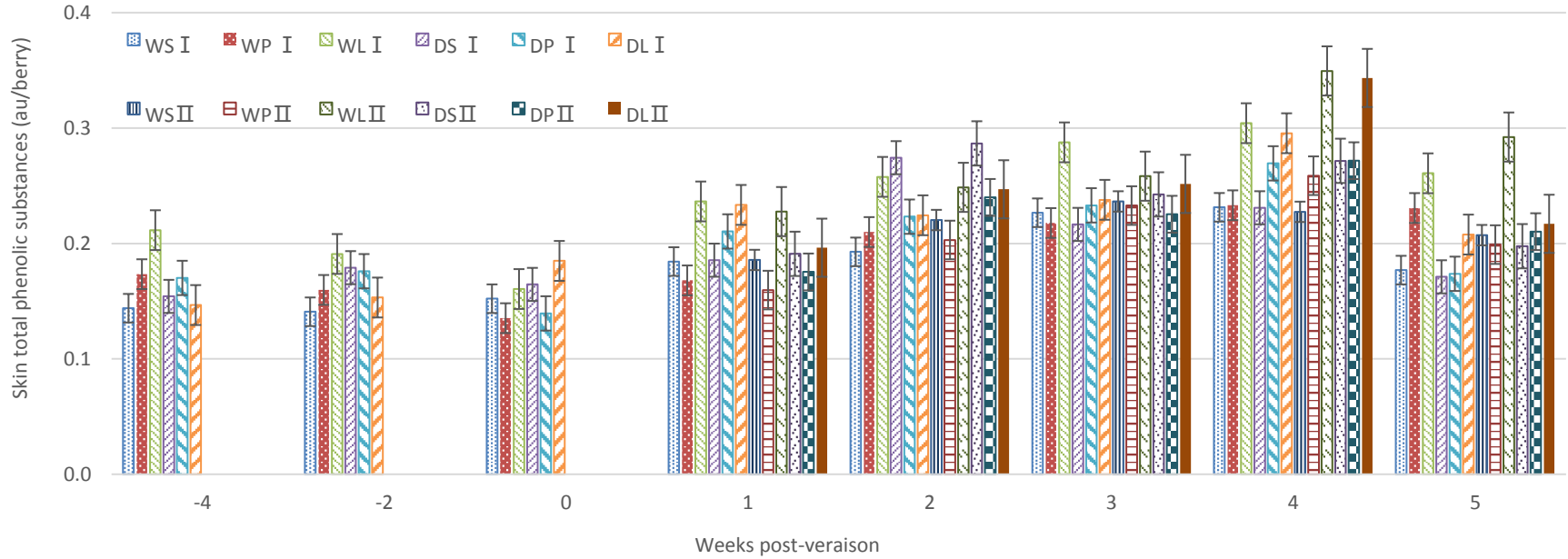


Figure 6.8 Effects of UV-B and water deficit on skin total phenolic substances in Pinot noir berries during ripening in 2016-2017 vineyard trials.

Data showed the mean ± standard error of four replicates. *P*-values for statistical significance comparing the different treatments according to Three-factor ANOVA and LSD test at the 5% level. Main effects of UV (P_{UV}), water deficit (P_{water}), set-up treatments at pre-veraison/veraison (P_{term}), the combination of UV and water deficit ($P_{UV*water}$), the combination of UV and pre-veraison/veraison of set-up treatments ($P_{UV*term}$), the combination of water deficit and pre-veraison/veraison of set-up treatments ($P_{water*term}$) and the combination of UV, water deficit and pre-veraison/veraison of set-up treatments ($P_{UV*term*water}$); n.s, no significant difference. P, PETG screen, L, leaf remove, S, shade cloth; W, well-water, D, water deficit; I, the setup of treatments at pre-veraison, II, the setup of treatments at veraison.

P_{UV}	n.s	n.s	n.s	n.s	0.011	0.028	0.001	<0.001
P_{water}	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
P_{term}				n.s	n.s	n.s	0.006	n.s
$P_{uv*water}$	<0.001	n.s	n.s	n.s	0.032	n.s	n.s	n.s
$P_{UV*term}$				n.s	n.s	n.s	n.s	n.s
$P_{water*term}$				n.s	n.s	n.s	n.s	n.s
$P_{UV*term*water}$				n.s	n.s	n.s	n.s	n.s

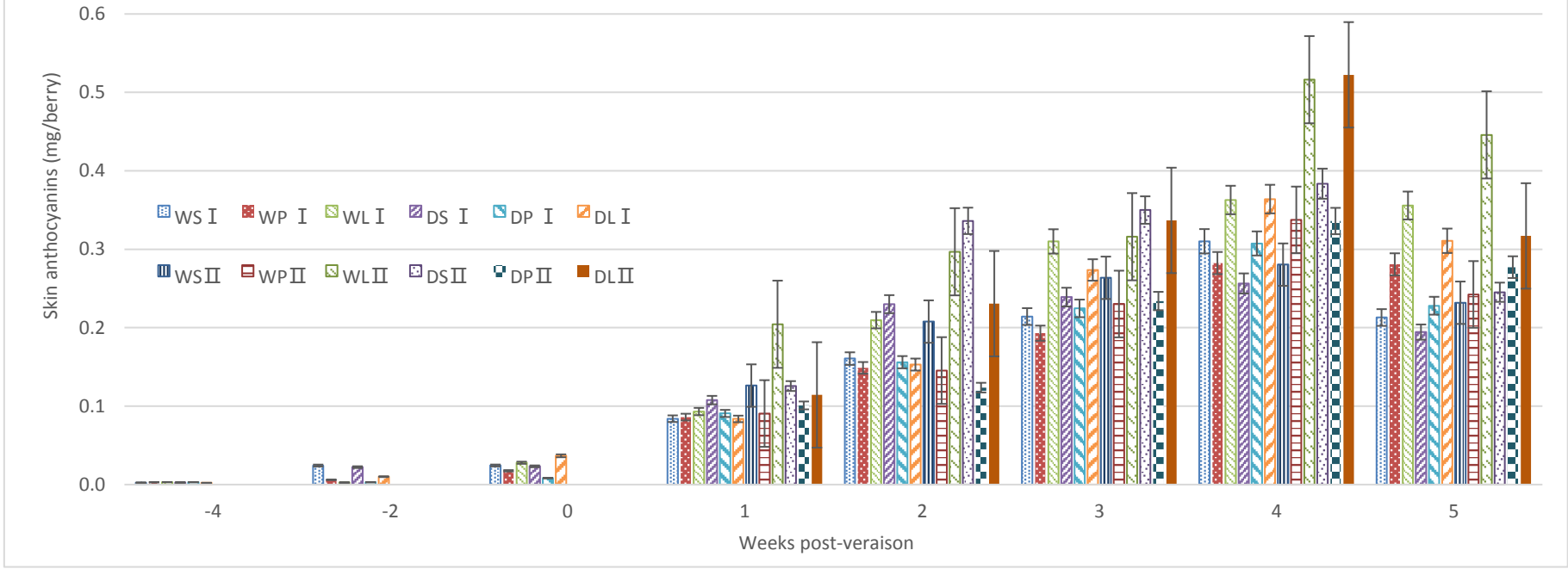


Figure 6.9 Effects of UV-B and water deficit on skin anthocyanins in Pinot noir berries during ripening in 2016-2017 vineyard trials.

P_{UV}	0.021	n.s	n.s	n.s	n.s	n.s	n.s	<0.001
P_{water}	n.s	n.s	n.s	n.s	<0.001	0.015	n.s	0.001
P_{term}				0.002	n.s	n.s	n.s	n.s
$P_{uv*water}$	<0.001	0.007	n.s	0.015	n.s	n.s	n.s	0.023
$P_{UV*term}$				n.s	n.s	n.s	n.s	n.s
$P_{water*term}$				n.s	n.s	n.s	n.s	n.s
$P_{UV*term*water}$				n.s	n.s	n.s	n.s	<0.001

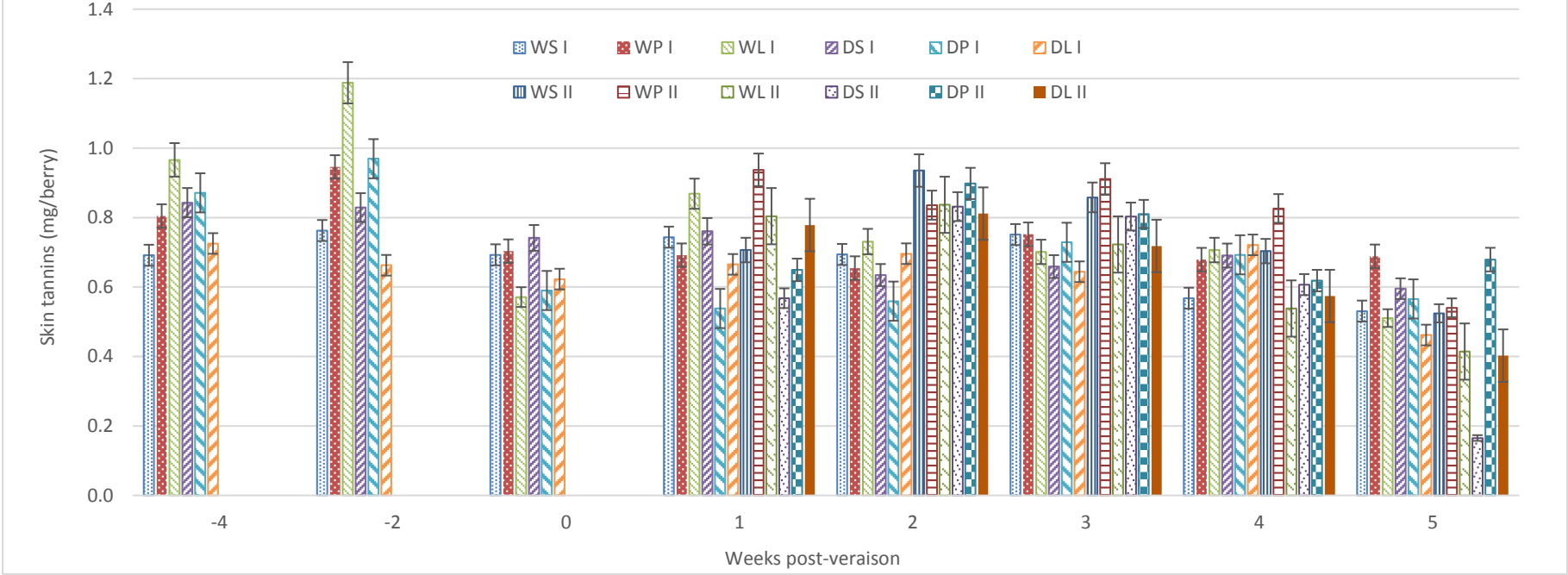


Figure 6.10 Effects of UV-B and water deficit on skin tannins in Pinot noir berries during ripening in 2016-2017 vineyard trials.

P_{UV}	<0.001	n.s	n.s	0.002	n.s	n.s	n.s	n.s
P_{water}	<0.001	n.s	n.s	n.s	n.s	n.s	n.s	n.s
P_{term}				n.s	n.s	n.s	n.s	n.s
$P_{uv*water}$	<0.001	n.s	n.s	n.s	n.s	n.s	n.s	n.s
$P_{UV*term}$				n.s	n.s	n.s	n.s	n.s
$P_{water*term}$				n.s	n.s	n.s	n.s	n.s
$P_{UV*term*water}$				0.027	n.s	n.s	n.s	n.s

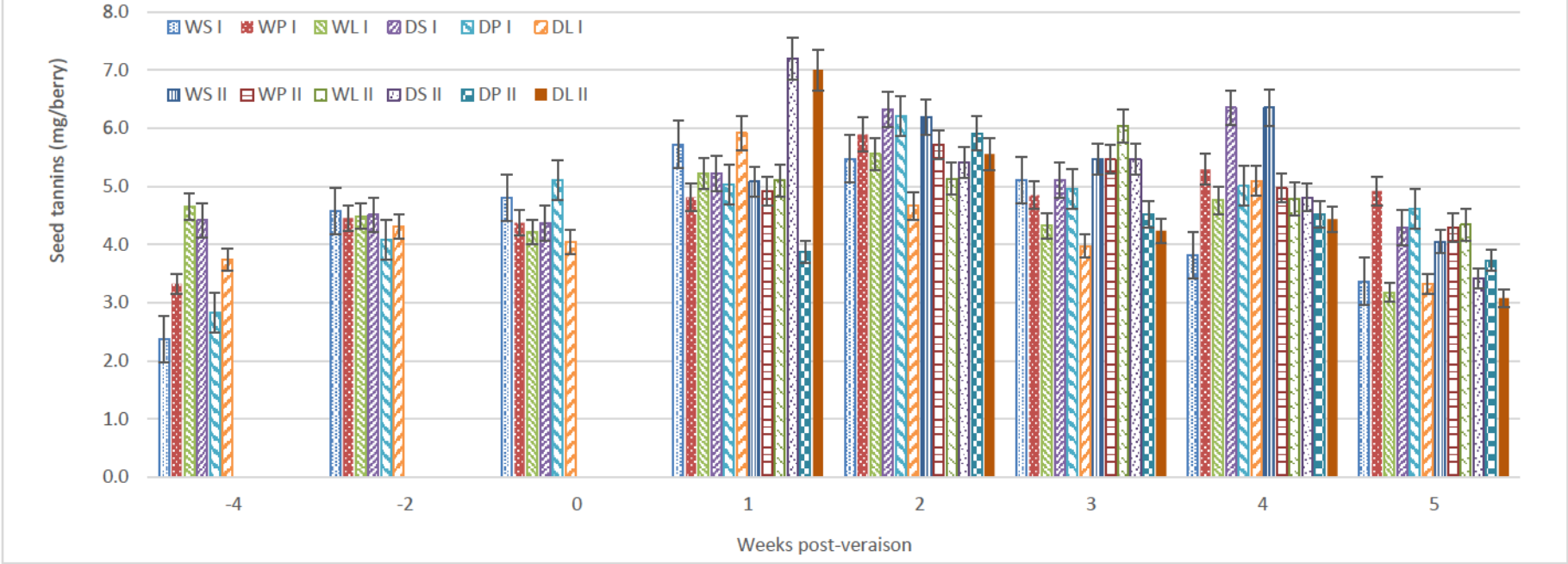


Figure 6.11 Effects of UV-B and water deficit on seed tannins in Pinot noir berries during ripening in 2016-2017 vineyard trials.

Volatile composition

The volatile composition of Pinot noir juice was analysed at harvest under combined UV and water treatments (Table 6.8). There were no statistically significant effects of the interaction on C_6 aldehydes and C_6 alcohols, which were the most abundant volatile compounds at harvest. (E)-3-hexenol was significantly increased by the late set of treatments, except for WLI/II (17.4 µg/L and 15.7 µg/L). Monoterpenes accounted for a small part of the volatile composition, but only α-terpineol was affected by an interaction between treatments. Under water deficit, UV-B exclusion (1.2 µg/L in DPI/II) caused a drop in α-terpineol compared to the shading treatment (1.3/1.4 µg/L in DSI/II). Also, under well-watered conditions, UV-B exclusion (1.3/1.1 µg/L in WPI/II) and UV-B exposure (1.2/1.3 µg/L in WLI/II) caused a drop in α-terpineol compared to the shading treatment (1.4 µg/L in WSI/II). Water deficit induced a reduction in citronellol concentration, such as DP vs. WP (0.7 µg/L vs. 1.0 µg/L) and DL vs. WL (0.8 µg/L vs. 1.2 µg/L). In nerol, the late set of treatments had higher concentrations than the early set of treatments. Also, UV-B exposure caused increased nerol concentration compared to the shading and UV-B exclusion treatments.

Table 6.10 Effects of UV-B and water on volatile compounds in Pinot noir juice at harvest in 2016-2017 vineyard trials.

Volatile compound (μg/L)	C_6 aldehydes		C_6 alcohols				Free monoterpenes				
	Hexanal	(E)-2-hexenal	Hexanol	(E)-3-hexenol	(Z)-3-hexenol	(E)-2-hexenol	Linalool	α-terpineol	Citronellol	Nerol	Geraniol
WP Ⅰ	29.2	28.3	1032.2	12.9	105.0	76.5	1.8	1.3	0.8	4.6	12.4
WP Ⅱ	31.4	33.9	1062.2	14.9	95.2	156.1	1.8	1.1	1.0	4.8	13.8
DP Ⅰ	39.6	41.2	966.6	15.3	108.2	163.4	1.7	1.2	0.8	4.7	12.3
DP Ⅱ	33.0	27.9	1042.4	16.7	67.6	75.8	1.7	1.2	0.7	5.4	10.7
WL Ⅰ	37.6	37.4	1198.1	17.4	112.9	49.8	1.7	1.2	0.9	5.2	11.1
WL Ⅱ	43.7	51.9	1064.8	15.7	50.8	11.7	1.8	1.3	1.2	7.8	13.3
DL Ⅰ	38.3	36.4	1188.1	18.9	64.5	32.9	1.7	1.2	0.6	5.2	10.1
DL Ⅱ	38.6	51.5	1113.7	19.6	53.2	109.1	1.6	1.3	0.8	6.1	10.3
WS Ⅰ	31.1	56.6	1083.3	16.7	88.4	206.3	1.8	1.4	1.0	4.8	12.1
WS Ⅱ	35.0	35.1	1118.2	17.4	77.0	86.8	1.8	1.4	1.0	5.1	12.7
DS Ⅰ	30.4	35.7	936.8	15.3	119.8	214.3	1.8	1.3	1.0	4.7	13.3
DS Ⅱ	44.9	43.2	1101.4	19.7	48.0	89.1	1.8	1.4	0.9	5.7	11.5
P_{water}	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	**0.024**	n.s	n.s
P_{UV}	n.s	n.s	n.s	n.s	n.s	n.s	n.s	**<0.001**	n.s	**0.046**	n.s
P_{term}	n.s	n.s	n.s	**0.009**	n.s	n.s	n.s	n.s	n.s	**0.025**	n.s
$P_{water*UV}$	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
$P_{water*term}$	n.s	n.s	n.s	n.s	n.s	n.s	n.s	**0.043**	n.s	n.s	n.s
$P_{UV*term}$	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
$P_{water*UV*term}$	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

Data showed the mean ± standard error of four replicates. *P*-values for statistical significance comparing the different treatments according to Three-factor ANOVA and LSD test at the 5% level. Main effects of UV (P_{UV}), water deficit (P_{water}), set-up treatments at pre-veraison/veraison (P_{term}), the combination of UV and water deficit ($P_{UV*water}$), the combination of UV and pre-veraison/veraison of set-up treatments ($P_{UV*term}$), the combination of water deficit and pre-veraison/veraison of set-up treatments ($P_{water*term}$) and the combination of UV, water deficit and pre-veraison/veraison of set-up treatments ($P_{UV*term*water}$); n.s, no significant difference. P, PETG screen, L, leaf remove, S, shade cloth; W, well-water, D, water deficit; I, the setup of treatments at pre-veraison, II, the setup of treatments at veraison.

6.3 Discussion

From the literature, water deficit can dramatically influence UV-B-induced responses, but the responses from water deficit and UV-B depended on the plant species. Interactions between UV-B exposure and water deficit in plants have been investigated for about 30 years, but few have been undertaken about the effects in Pinot noir. In this chapter, we investigated the effects of UV-B interaction with water deficit on the vine physiology and chemical composition of fruit.

6.3.1 The alteration of vine physiological indices induced by UV-B interaction with water deficit

In the glasshouse, the combination of UV-B and water deficit caused decreases in leaf water potential (LWP) (Table 6.1). Compared to either individual stress, the combination of UV-B and water deficit did not increase the magnitude of the responses in the glasshouse. Therefore, the combination of UV-B and water deficit decreased LWP induced by water deficit alone (-1.31MPa in +UV-W and -1.38MPa in -UV-W). In the vineyard, UV-B interaction with water deficit did not alter LWP at harvest (Fig. 6.4b) as a result of the rainfall. However, at veraison, LWP in the early timing of treatments was lower than the late treatments in the vineyard. This may result from the increases in the weights of the shoots and clusters and pulling the basal part of shoots apart from other shoots from fruit-set to veraison. Therefore, providing more light into the canopy centre and more air circulation into the canopy could lead to increase the vine transpiration, compared to the late timing of treatments (having full canopies from fruit-set to veraison) (Gu et al., 2004; Tardaguila et al., 2010; Williams, 2012).

In the glasshouse, there was no interaction effect of UV-B and water deficit on SPAD levels (Fig.6.1b), but the statistical analyses showed a UV effect averaged across water treatments (+UV+W and +UV-W vs. -UV+W and -UV-W), as well as significant differences in water deficit. The effects of UV or water deficit individually on SPAD has been explained in

the previous chapters. In the literature, it was assumed that the combined stresses can lead to enhanced light transmittance through leaves, resulting in a decrease in SPAD when compared to the individual treatments. However, the final results showed that the combination of UV-B and water deficit did not change the response compared to either UV-B or water deficit. Furthermore, there were no interaction effects of UV, water deficit and the timing of treatments on SPAD in the vineyard (Fig. 6.4a). The significant differences between UV treatments were shown from -4 to 4-weeks post-veraison. There were no significant differences caused by different fruit exposure, averaged across water and the timings of treatments. According to the results in Chapter 4, the individual different fruit exposure did not have significant effects on SPAD from pre-veraison to harvest, so effects of UV in the figure could be induced by water deficit from pre-veraison to veraison, particularly veraison, and by the timings of treatments from 2- and 4-weeks post-veraison.

As with water, carbon dioxide (CO_2) is another important compound for the synthesis of carbohydrate through photosynthesis. So, measurements of the abundant stable isotope carbon atoms in plant tissues is a way to evaluate the effects of UV-B and water deficit on grapevines (Taiz et al., 2015). We found UV-B in combination with water deficit caused increased juice $\delta^{13}C‰$ and no significant changes in leaf $\delta^{13}C‰$ in both the glasshouse (Table 6.1) and the vineyard (Table 6.7). In grapevine tissues, the $^{13}C/^{12}C$ isotope ratio is determined by the gradient of CO_2 concentrations between the atmospheric and intercellular spaces in the leaf, which is influenced by environmental stresses (Farquhar et al., 1982; Gaudillère et al., 2002). UV-B radiation decreases the activity of Rubisco (Choi and Roh, 2003). Stomatal closure leads to less diffusion and then decreases CO_2 uptake in grapevine leaves induced by water deficit. Thus, intercellular $^{13}CO_2$ is more likely to be used as the substrate of Rubisco in the carboxylation reaction (Farquhar et al., 1989). Grapevines grown under water stress, such as Merlot, Cabernet Sauvignon, Cabernet franc and Tempranillo, tend to have more positive carbon isotope ratios ($\delta^{13}C‰$) (Gaudillère et al., 2002; Santesteban et al., 2012). So, the results stated that a water deficit can enhance

UV-B-induced responses in vine carbon isotope assimilation. More severe stress and more restricted stomata openings lead to photosynthates with a greater proportion of ^{13}C (des Gachons et al., 2005). From source to sink, most sucrose (photosynthates) containing ^{13}C is translocated from leaves to grapes and converted to fructose and glucose, resulting in increasing ^{13}C in fructose and glucose (Centritto et al., 2009; Chaves et al., 2003). Additionally, less photosynthates incorporating ^{13}C in leaves are used to maintain function, such as respiration (des Gachons et al., 2005). Thus, the combination of UV-B and water deficit increased the $\delta^{13}C‰$ of juice but not in leaf $\delta^{13}C‰$.

In 2016-2017, °Brix was higher in the later timing of treatment application compared to the earlier one in the vineyard trials (Table 6.7). Red winegrapes should achieve a value higher than 18°Brix for commercial harvest of table wines (Keller, 2015), but due to the growing conditions, all treatments had low (<18)°Brix. After veraison, heavy rainfall with low light intensities may have prevented photosynthesis producing carbohydrate in the leaves, resulting in less accumulation of sugar in berries (Jackson and Lombard, 1993). The effects of leaf removal around the fruiting zone on sugar accumulation are quite variable depending on timing and severity. Removing source leaves caused dynamic changes in photosynthesis leading to changes in the source-sink balance (Palliotti et al., 2011; Poni et al., 2006). The removal basal leaves (around the fruiting zone) at veraison had a little effect on the sugar accumulation, because medial and apical leaves were mature at veraison which had higher photosynthetic performance than basal leaves during ripening (Herrera et al., 2015). Therefore, in the previous study, leaf removal at the earlier time (before veraison) may lead to less photosynthate that can be translocated into berries, compared to the late treatments (Bledsoe et al., 1988). Thus, the condition significantly caused this to be an effect on the berry maturity in 2016-2017 vineyard trials.

6.3.2 Effects of UV-B interaction with water deficit on amino acids in berries

There is little research about the effects of UV-B interaction with water deficit on amino acids in grapes. In the literature review (2.6.3), the study hypotheses that UV-B interaction with water deficit will change concentrations of amino acids. In 2015-2016, there were decreases in amino acids under both UV-B and water deficit in the glasshouse (Table 6.2). This could be because berries suffered from *Botrytis*. At harvest, clusters under the separated or combined water deficit and UV-B had a more serious infection from *Botrytis* than the control. It may be that the *Botrytis* infection, in combination with water deficit and UV-B, resulted in the reduction in the amino acids to synthesise pathogen-related proteins (Azarkan et al., 2004; Dhekney et al., 2011; Linthorst and Van Loon, 1991).

In the 2016-2017 glasshouse trials, UV-B radiation in combination with water deficit increased the concentration of total amino acids and some individual amino acids (Table 6.3). There was no significant effect on the concentration of total amino acids, but there were increases in His, Val, Thr and Lys in the vineyard (Table 6.8). There was a higher concentration of free Arg than Pro in grapes at harvest in both the glasshouse and the vineyard, because Pinot noir is an Arg accumulating cultivar (Berdeja et al., 2014). The increases in amino acids in 2016-2017 in the glasshouse appeared to be from water deficit increasing the concentration of total free amino acids due to increases in some individual amino acids of berries, particularly Pro, Arg, Ala and Thr (Bertamini et al., 2006). Pro and Arg are major components of total amino acids in grapes and can function for an osmotic adjustment and act as antioxidants (Grimplet et al., 2007). Pro biosynthesis is a reductive pathway controlled by the activation of Δ1-PYRROLINE-5-CARBOXYLATE SYNTHETASE (*P5CS*) and Δ1-PYRROLINE-5-CARBOXYLATE REDUCTASE (*P5CR*) genes and requires NADPH. A water deficit induces the accumulation of NADPH due to the inhibition of the Calvin cycle, so the accumulation of Pro under water deficit generates $NADP^+$ and maintains a low NADPH:$NADP^+$ ratio for the Calvin cycle (Allan et al., 2008; Jiménez et al., 2013). UV-B may

enhance the increases in Pro and Arg under water deficit. UV-B, in combination with a water deficit, could induce more degradation of proteins to produce more amino acids for osmotic adjustment (Hollósy, 2002; Martínez-Esteso et al., 2011). Therefore, the combination of UV-B and water deficit can increase the concentration of total amino acids, particularly Arg and Pro, in the secondary year trials of the glasshouse.

However, in the vineyard, there was no water deficit after veraison resulting in little change in total amino acids under UV-B interaction with water deficit at harvest. The percentages of Pro were very low, while %Gln was high in the vineyard. This may be related to the low level of grape maturity, which was only around 17°Brix at harvest in 2016-2017. Pro accumulation through the whole ripening period was converted from Gln via aminotransferases in berries (van Heeswijck et al., 2001). Thus, some Gln may not have been converted into Pro at this early state of berry maturation, leading to the high and low percentages of Gln and Pro, respectively, in total amino acids at harvest in 2016-2017.

6.3.3 The phenolic composition in berries in response to the combination of UV-B and water deficit

The composition of phenolics accumulated was analysed in Pinot noir berries at different stages during ripening. The contents of skin anthocyanins and total phenolics showed developmental regulation in Pinot noir berries during berry development in the treatments (Fig. 6.2, 6.3 and 6.5).

At the late pre-harvest stage, there were significantly higher amounts of skin anthocyanins and total phenolics in grape berries compared to the fruit collected around veraison (Fig. 6.2a/b and 6.3a/b). The berries collected at veraison had significantly higher levels of skin tannins than at harvest (Fig. 6.2c and 6.3c). These results are consistent with previous findings that skin anthocyanins and total phenolics are mainly produced from veraison to harvest (Martínez-Lüscher et al., 2014b; Roby et al., 2004). Also, compared with the control, UV-B interaction with water deficit increased the contents of skin anthocyanin and

skin tannin in the glasshouse trials. Similar results were found by Martínez-Lüscher et al. (2014a) in Tempranillo berries. UV-B or water deficit can induce the accumulation of ROS, and anthocyanins in grape skins can play a role as antioxidants to scavenge ROS (Berli et al., 2011). The accumulation of skin anthocyanins is also induced by the up-regulation of *FLS1, UFGT* and *F3H* through UV-B, and up-regulation of *F3H* and *OMT2* through water deficit (Berli et al., 2011; Cook et al., 2015; Martínez-Lüscher et al., 2014a). Furthermore, as described previously, studies have shown that skin anthocyanins and skin total phenolics were increased by UV-B exposure, while shaded fruit had lower levels. Our results in this study further determined that UV-B was the major component of radiation that induced anthocyanins in grape berries (see above 5.3.3), and this is supported by previous UV-B exclusion experiments (Núñez-Olivera et al., 2006).

In the vineyard, different canopy temperatures could induce changes in skin anthocyanins. Higher temperatures of about 30-35℃ can stimulate the degradation of skin anthocyanins, for example (Downey et al., 2006; Teixeira et al., 2013). Gregan et al. (2012), who used the same screening system and vineyard as used in this research, reported that daily temperatures around the fruiting area was slightly raised with leaf removal and PETG covers (0.2/0.6℃, respectively). The increases in temperature were found at solar noon and there were no differences in temperature during the morning and evening. In the study, the highest skin anthocyanins were found in the UV-B exposure treatment and the lowest in the shading treatment at harvest. Given the magnitude of air temperature change around the fruit in this research, it seems likely that temperature did not significantly affect the skin anthocyanins.

In the 2015-2016 glasshouse trials, UV-B interaction with water deficit induced the highest accumulation of skin tannins compared to the control and UV-B or water deficit alone treatments at harvest, but skin tannin contents in +UV-W were higher than -UV+W and lower than +UV+W and -UV-W in 2016-2017 trials of the glasshouse. Also, in the vineyard, skin tannin contents frequently changed in all treatments during ripening, but there were no consistent significant differences of skin tannins in treatments. The fluctuation of skin

tannins during ripening may relate to the polymeric flavan-3-ols. Polymerization is dramatically influenced by environmental factors, such as UV-B and water deficit, and changed at different stages of berry development (Cortell and Kennedy, 2006; Downey et al., 2003; Kennedy et al., 2002). Water deficit or UV can increase a greater of degree of polymerization in skin tannins (Cortell and Kennedy, 2006; Ojeda et al., 2002). Also, skin tannins are synthesised via a branch in the anthocyanin-forming pathway by LAR that is affected by UV and water deficit (Bindon et al., 2011; Del-Castillo-Alonso et al., 2016). The accumulation of flavonoids in skins acts as UV protectants and free-radical scavengers to protect berries from the damage of UV-B and water deficit (Downey et al., 2006).

However, there were no consistent results of the changes in skin tannins between the combined and separated treatments in two years of glasshouse trials. In 2015-2016, skin tannin contents in the combined treatment were more than in either UV or water deficit treatments, but the opposite results were shown in 2016-2017. These results may reflect that pathogens may affect the skin tannins accumulation during ripening. *Botrytis* infection vines in the 2015-2016 glasshouse trial may have caused the changes in skin tannins. The fungal mycelium grew on the fruit surface and within the skin tissue leading to direct contact between the fungal extracellular enzymes and skin tannins. The fungal extracellular enzymes were specific to the inhibitory activity of tannins, particularly the stilbene oxidase (Ky et al., 2012). So, the different levels of infection interaction with UV and water deficit probably induced the inconsistent changes in skin tannins contents.

6.3.4 The effects of UV-B interaction with water deficit on volatile composition in berry juice

To investigate the effects of UV-B radiation interacting with water deficit on volatile compounds in Pinot noir juice, samples were taken at harvest from both the glasshouse (Table 6.6) and the vineyard (Table 6.10) trials. There was no consistent pattern of changes to the volatile composition at harvest in any of the trials. C_6 compounds were not different in either year of the glasshouse and vineyard trials, except for hexanol and (Z)-3-hexenol in

+UV-W. In the berry skin of mesocarps, C_6 compounds (C_6-aldehydes and C_6-alcohols) are formed by enzymatic oxidation of unsaturated lipids during ripening. Some alterations of C_6 compounds in berries may be explained by UV-B causing an increase in the abundance of transcripts of several lipoxygenases (LOX) in grapevines to produce a cascade of damaging ROS, resulting in the increased catabolism of fatty acids to C_6 compounds (Gil et al., 2013; Mendez-Costabel et al., 2014). Water deficit causes an increase in the transcript abundance of LOX and HPL, resulting in the increase in the catabolism of fatty acids. However, it also increases the transcript abundance of ADH and AAT, which can finally convert the hydroperoxides to volatile esters (Cramer et al., 2007a; Deluc et al., 2009). Therefore, UV-B interaction with water deficit potentially enhanced the abundance of transcripts of LOX, HPL and ADH to convert fatty acids into C_6 alcohols (hexanol and (Z)-3-hexenol), compared to either UV-B or water deficit alone.

However, in 2015-2016, vines under the combination of water stress and UV-B treatment were infected by *Botrytis* more seriously than the control vines. *Botrytis* is primarily characterized by degradation of terpenoids (Bell and Henschke, 2005; Ribéreau-Gayon, 1988; Williams et al., 1987). It has been shown that *Botrytis* can metabolise major flavour compounds in the fruit to unpleasant 'phenol' or 'iodine' characters (Ribéreau-Gayon, 1988; Williams et al., 1987). In healthy plants, C_6 aldehydes are usually low, but *Botrytis*-infection can induce their rapid formation (Matsui, 2006), which may have been the case with our results, where there were more C_6 aldehydes in 2015-2016 than 2016-2017. C_6 aldehydes can be bactericidal and fungicidal compounds in infected fruit (Myung et al., 2007). Additionally, *Botrytis* can stimulate *hydroperoxide lyase* (*HPL*) expression to enhance C_6 aldehydes formation in grapes. It was reported that HPL catalysed 13-hydroperoxides of linolenic acids and linoleic to generate (Z)-3-hexenal and hexanal (García et al., 2003; Kalua and Boss, 2010; Kishimoto et al., 2008).

6.4 Conclusion

In this chapter, the physiological indices and chemical compositions from the glasshouse and vineyard experiments in Pinot noir were examined under a combination of UV-B and water deficit. The combined treatments caused decreases in leaf water potential and SPAD, and an increase in juice $\delta^{13}C‰$ at harvest. These changes were shown in the vines in response to the combination of water deficit and UV-B. The concentration of amino acids and volatile compounds in berries were determined at harvest. Amino acids were significantly increased by the combined treatment, particularly Pro, Arg, Ala and Thr. There were slight increases in volatile compounds. Quantitative changes in anthocyanins and tannins were observed in response to berry development. The total phenolics and anthocyanins in skins showed increasing patterns through ripening, while the skin tannins decreased from veraison to harvest. Total phenolics, anthocyanins and tannins in berry skins showed significant responses to UV-B and water deficit, regardless of their developmental stage. UV-B interaction with water deficit can enhance the accumulation of total phenolics, anthocyanins and tannins in grape skins, compared to the control, while the combined treatment did not have the consistent changes in skin phenolic compounds in comparison with either UV-B or water deficit treatments across the two years of glasshouse and vineyard trials. The results in the glasshouse showed more significant and different changes in vine physiology and chemical compounds than in the vineyard.

Chapter 7

General discussion and conclusions

7.1 General discussion

Vine physiology and fruit chemical composition are affected by interactions between the vines and their environment. UV-B (280—315 nm) as an environmental factor can induce a series of responses in grapevines, including changes in physiology, together with the accumulation of specific metabolic compounds (Berdeja et al., 2014; Teixeira et al., 2013). In the vineyard, the accumulation of phenolic compounds in berries can be induced by increased light/radiation exposure, in particular, UV-B (Del-Castillo-Alonso et al., 2016; Gil et al., 2013). Following exposure to supplemental UV-B in the glasshouse, both anthocyanins and flavonoids showed UV-B-induced responses (Martínez-Lüscher et al., 2014b). The changes in the vine physiology and chemical composition of the fruit also respond to water deficit, such as decreases in leaf water potential and leaf chlorophyll content and increases in total amino acids and phenolic compounds (Berdeja et al., 2014; Bertamini et al., 2006; Martinez-Luscher et al., 2015).

There has been very limited research into the effects of UV-B radiation interaction with water deficit on the vine physiology and fruit chemical composition in Pinot noir. Previous research has investigated changes in phenolic and volatile compounds in Pinot noir fruit and wines as induced by shading and sun exposure (Cortell and Kennedy, 2006; Feng et al., 2017; Price et al., 1995; Song et al., 2015), and water deficit (Berdeja et al., 2014; Griesser et al., 2015). Our research focused on changes in phenolic composition during berry development, amino acids, and the volatile composition in fruit at harvest to water deficit and UV-B in both the glasshouse and vineyard in 2015-2016 and 2016-2017. In the glasshouse, potted vines were exposed to UV radiation or not, while trials in the vineyard removed leaves around the fruiting zone, covered it with a plastic screen (PETG, eliminating UV-B) or shade cloth (SC). Regulated deficit irrigation was used in both the

glasshouse and vineyard to alter the water supply. The hope is that this research can provide important information about the influence of the canopy environment on Pinot noir fruit composition to the New Zealand wine industry.

However, no sustained and comprehensive evidence for an interaction with UV-B and water deficit was found in both glasshouse and vineyard trials. In the 2015-2016 glasshouse trials, *Botrytis*-infection occurred, leading to unhealthy appearing clusters. In 2015-2016 vintage, the rainfall was high from January to March, so there was no difference between water treatments in the vineyard. The following vintage was also a challenge season with abundant rainfall after veraison, resulting in no difference between water treatments after that period and low °Brix of berries at harvest.

7.1.1 Implications of leaf removal in the vineyard and UV-B in the glasshouse

Vine physiology

Leaf removal is an important canopy management approach in the vineyard. Removing some or all leaves around the fruiting zone enhances the exposure of fruit to sunlight. In our study, fruit was subject to different environments through 100% leaf removal around the fruiting zone (LR), leaf removal in combination with covering with a plastic screen (PETG, UV-B exclusion) and leaf removal followed by the application of shade cloth (SC, 70% light exclusion). There were no statistically significant differences in SPAD induced by the treatments in the vineyard (Fig. 4.4a), resulting from a high ratio of PAR to UV-B. Compared with WS and WP, which protected the vines from 70% of incident radiation and UV-B, respectively, WL had the least negative juice $\delta^{13}C‰$ (Table 4.5). This result indicated that UV-B induced stomatal closure resulted in the use of $^{13}CO_2$ in photosynthesis. Similar results were shown by Alonso et al. (2015); Núñez-Olivera et al. (2006) and Santesteban et al. (2015). At veraison, leaf water potential in the early timing of treatments was lower than the late treatments in the vineyard, but there were no differences in LR, PETG and SC treatments (Fig. 6.4b). This may indicate that leaf removal at the early timing (pea-size)

causes more decrease in vine water status, compared to leaf removal at the late timing (veraison). It is because plucking the basal part of shoots at pea-set provides more light into the canopy center, leading to increase the vine transpiration, (Gu et al., 2004; Tardaguila et al., 2010; Williams, 2012).

At harvest, we found no significant differences in juice °Brix, pH, TA, yields or pruning weights (Table 4.5), which may be related to leaf area. Leaf removal significantly decreased the main leaf area but stimulated the lateral regrowth, resulting in compensating for the abscission of basal leaves and finally maintaining the total leaf area per shoot (Koblet et al., 1994; Williams, 2012). So, there was also no effect of leaf removal around the fruiting zone on the amount of light interception by the canopies (Poni et al., 2006; Tardaguila et al., 2010). Furthermore, leaf removal at pre-veraison/veraison should not affect the number of berries, because fruit set has the critical period from bloom to 2-3 weeks post-bloom (Koblet et al., 1994). Consistent with Bavaresco et al. (2008) and Downey et al. (2006) , we also found that leaf removal around the fruiting zone at pre-veraison/veraison did not decrease berry maturity or yields.

In comparison with the vineyard trials, the environment was very different in the glasshouse. UVI-6 for 8h and a reduction of approximately 66% PAR was maintained in the glasshouse. The glasshouse had a low ratio of PAR to UV-B, while there were natural UV-B and PAR conditions (a high ratio of PAR to UV-B) in the vineyard. The levels of UV-B damage in plants depend on the quantity of PAR (Jordan, 2011). High PAR levels can reduce UV-B-induced damage levels in plants, while low PAR levels enhance the damage in plants under UV-B (Jordan, 2002; Jordan, 2011). In the glasshouse, UV-B caused decreases in SPAD during ripening (Fig. 4.1b) at harvest, compared to the control (Table 4.1), finding that were consistent with Dehariya et al. (2012) and Martínez-Lüscher et al. (2013). The decreases in SPAD indicate a reduction in leaf greenness under UV-B, resulting from the chloroplasts moving to the periclinal cell walls with an increase in light transmittance (Martínez and Guiamet, 2004).

Amino acids

Amino acids in berries are a main component of yeast-assimilable-nitrogen (YAN) and act as precursors for some aroma compounds, meaning they contribute to the efficiency of fermentation and the characteristic aromas of wines (Bell and Henschke, 2005; Loulakakis and Roubelakis-Angelakis, 2001). In the 2015-2016 and 2016-2017 vineyard trials, the concentrations and percentages of amino acids were not dramatically altered by the treatments (Table 4.6-4.8). These results were consistent with Gregan et al., (2002), Keller and Torres-Martinez (2002) and Martínez-Lüscher et al. (2014b). However, the shading treatments produced lower concentrations of Pro than PETG or LR treatments. LR, which included UV-B as a component of radiation, significantly increased Pro by 12% in 2016 and 31% in 2017 compared with PETG. This result supports the idea that the accumulation of Pro was promoted by the increased Δ1-PYRROLINE-5-CARBOXYLATE SYNTHETASE1 (P5CS1) activation (Aleksza et al., 2017). P5CS1 induction depends on light (Ábrahám et al., 2003) and responds to ROS signals (Ben Rejeb et al., 2015). UV-B stimulates the accumulation of ROS as signal molecules (Apel and Hirt, 2004). Therefore, PETG could increase Pro induced by light, and both of light and UV-B stimulate the Pro accumulation in LR. The lowest concentration of Pro in SC may be affected by proline dehydrogenase (PDH), which can be activated in darkness for proline catabolism (Szabados and Savouré, 2010).

In the glasshouse, there were no consistent changes in amino acid concentrations in either 2015-2016 or 2016-2017 (Table 4.2). The changes in amino acids under UV-B might have been induced by the *Botrytis* infection in 2015-2016. Although visually *Botrytis*-infected clusters were removed, PR protein can be produced in fruit that is not expressing symptoms consume amino acids in the vines. UV-B also can induce synthesis of PR protein in the vines (Liu et al., 2018). However, total amino acids were increased by UV-B in 2016-2017. It is possible that UV-B changes the levels of protein activation and enzyme activity (Jordan, 2017), leading to the degradation of protein, but no known gene relates to amino

acid biosynthesis or turnover in response to UV-B in grapes (Gregan *et al*., unpublished observations).

Phenolic composition

UV exposure through leaf removal had a positive influence on the chemical composition of fruit and, possibly, could have an impact on wine quality (Jackson and Lombard, 1993; Price et al., 1995). This research focused on the changes in phenolic composition during ripening. The skin total phenolics, skin anthocyanins and skin tannins under UV exposure (LR) increased by 31%, 50% and 5%, respectively, compared to the shade cloth (SC) (Fig. 4.5 and 4.6). UV-B radiation was the component of sunlight responsible for these increases, as berries exposed through leaf removal, but protected from UV-B radiation by PETG, showed no statistically significant changes in skin anthocyanins, skin total phenolics or skin tannins. These results follow anthocyanins and tannins profile responses in previous research (Martinez-Luscher et al., 2015; Martínez-Lüscher et al., 2014b). UV exclusion (PETG) had similar results on skin anthocyanin contents as the shade cloth (SC), which was that the skin anthocyanin content was increased by UV-B exposure. UV-B may have up-regulated the expression of UFGT, leading to an increase in skin anthocyanins (Martínez-Lüscher et al., 2014a). Additionally, °Brix levels are related to the accumulation of skin anthocyanins. It could be explained that biosynthesis of anthocyanins appeared to highly depend on berry sugar levels, of which sugar levels are not only carbohydrate sources, but also involved in the stimulation of gene activities (Bobeica et al., 2015; Dai et al., 2014; Zheng et al., 2009). Sucrose can up-regulate *F3H* expression, coinciding with the enhancement of anthocyanin levels (Dai et al., 2014; Solfanelli et al., 2006). Furthermore, skin anthocyanin contents in Tempranillo are influenced by PAR and temperature between UV-transmitting and UV-blocking treatments (Del-Castillo-Alonso et al., 2016). However, the changes in skin anthocyanins contents were consistent during ripening across two seasons. Skin anthocyanin contents had no significant increases under PETG but dramatically increases under LR, so the influence of PAR and temperature cannot be completely ruled out in our study.

The increases in skin total phenolics were induced by LR in comparison with PETG and SC, which can be attributed to the increases in skin anthocyanins and skin total phenolics. In the glasshouse, UV-B caused increases in skin anthocyanins and skin total phenolics during ripening, compared to the control (Fig.4.2/4.3), which were consistent with the vineyard results and previous findings (Del-Castillo-Alonso et al., 2016; González et al., 2015; Sun et al., 2017). Flavonols are the component of skin total phenolics and can act as UV screening to protect grapes from UV-B damage (Gregan et al., 2012). *FLS* was activated by UV-B radiation in grapes, resulting in the accumulation of flavonols, as reported in previous research (Liu et al., 2015; Martínez-Lüscher et al., 2014a). This was because the *VvMYB1* transcription factor was a flavonol biosynthesis-specific regulator in grapevine interception by the UV-B photoreceptor, UVR8. VvMYBF1 has a high specificity for FLS to stimulate flavonol biosynthesis (Liu et al., 2015; Yin et al., 2015). Therefore, the increases in skin total phenolics are not only the increase in skin anthocyanins but also the accumulation of flavonols.

The accumulation of phenolics in berry skins under UV-B is to provide protection by absorption of UV-B and as antioxidants (Alonso et al., 2016; Jordan, 2018). In other aspects, the accumulation of phenolic compounds in berry skins was involved in a specific UV-B signal transduction pathway (Binkert and Ulm, 2017; Jenkins, 2014b). UVR8, a specific UV-B photoreceptor in the low fluence UV-B signal transduction pathway, interacts with transcription factors to mediate the photomorphogenic UV-B response (Brown et al., 2009; Brown and Jenkins, 2008). The action spectrum for UVR8 acts at 280 nm (Brown et al., 2009), but the action spectrum for UVR8 monomerisation and *HY5* transcript accumulation shows peaks at 290 nm and 300 nm (Díaz-Ramos et al., 2018). This new result indicated that longer UV-B wavelengths were also involved in the phenolic secondary metabolites. Thus, although there was a supplemental UV-B environment in the glasshouse and natural UV environment in the vineyard, the majority of UV-B induced responses were similar increases in phenolic composition in the glasshouse and vineyard.

Volatile compouds

Pinot noir fruit from harvest was used to measure volatile compounds, including C_6 aldehydes, C_6 alcohols and free monoterpenes. Compared with SC, berries exposed to radiation with a UV-B component or exclusion of UV-B had no significant changes in C_6 volatile composition (C_6 aldehydes and C_6 alcohols) at harvest in the glasshouse and the vineyard (Table 4.4 and 4.9), which was consistent with Feng et al. (2015) and Gil et al. (2013). The free monoterpenes in berries were decreased by UV-B exposure in the glasshouse and the vineyard. Recent findings suggest that the production of terpenoids in competition with the accumulation of phenolics uses carbon substrates (Dudareva et al., 2013; Xie et al., 2008). Thus, it can be inferred that phenolics can be dramatically increased by UV-B, resulting in fewer carbon substrates to support the synthesis of terpenoids.

7.1.2 Restricted irrigation in the glasshouse and vineyard

Vine physiology

Water is one of the most important inputs for supporting grapevine growth and function. In the east of the South Island, summer temperatures are warm with low rainfall and long dry periods, resulting in soil and atmospheric water deficits in vineyards. Therefore, water application is a valuable tool to manage berry development for grape production in many regions (Cramer et al., 2007b; Jackson and Lombard, 1993). If supplementary water is in excess, it can reduce colour and sugar contents in grapes and produce an imbalance in the acidity in wines. However, an appropriate water supplement can stabilize yields and maintain or even improve grape quality (Chaves et al., 2007; Conde et al., 2007). Previous research has shown that a moderate water deficit can reduce vine vigour and increase red grape composition and wine quality (Chaves et al., 2007; Intrigliolo and Castel, 2011; Jackson and Lombard, 1993).

Rainfall and irrigation are two major components of vine water status in the vineyard. Although we restricted the irrigation in two vintages, the vineyard suffered significant rainfall throughout the ripening period in 2015-2016 and, in 2016-2017, rainfall after

veraison. So, it was difficult to achieve water deficit in the trials, which led to no, or only early season differences in vine water status in 2016-2017 (Fig.5.4). Restricted irrigation led to decrease the soil volumetric water content from -4-weeks post-veraison to veraison (early season). The water deficit may induce vine physiological responses, but SPAD and leaf water potential did not show significant differences between the treatments and the control before veraison. Also, there were no significant differences in leaf water potential between treatments at harvest, leading to no significant changes in berry parameters (°Brix, pH and TA), yields or pruning weights at harvest in 2016-2017 (Table 5.6).

It is easier to control soil water in the glasshouse. Also, the glasshouse maintains the same temperature and humidity. Therefore, the glasshouse trials provided a more stable environment and clear treatment differences than the field experiments could provide. This was important for the thesis work to ensure that relevant experimental results could be obtained, even though the glasshouse is a model system and not entirely representative of a field situation. In the glasshouse, water deficit caused decreases in SPAD during ripening (Fig. 5.1b) and increases in leaf and juice $\delta^{13}C‰$ (Table 5.1), which were consistent with (Flexas et al., 2000). °Brix and LWP were also increased and decreased by 8% and 32%, respectively, under water deficit in 2016-2017 (Table 5.1). The results indicate that LWP was indicative of vine water status relative to the soil water volume content. Water deficit enhances the loss of leaf greenness and leads to stomatal closure (Gao et al., 2002; Hailemichael et al., 2016).

Amino acids

Total amino acids were slightly affected by the water treatments in the 2016-2017 vineyard trials (Table 5.7), except for Pro. Although there was no strong water deficit after veraison, the increase in Pro still appeared to be related to the water deficit. Similar results reported that in vines that had water withheld for two weeks before veraison and rewatered at veraison increased the concentration of Pro, compared to the control (Matthews and Anderson, 1988). These results supported the idea that Pro accumulation was sensitive to vine water status and was significantly induced by a slight water deficit

(Coombe and Monk, 1979; Matthews and Anderson, 1988). Water deficit stimulated Pro as an osmolyte and antioxidant in grapevines (Szabados and Savouré, 2010).

Water deficit did not significantly affect amino acids in 2015-2016 in the glasshouse, but the accumulation of total amino acids was induced by water deficit in 2016-2017 (Table 5.2), which was consistent with previous studies (Berdeja et al., 2014; Krüger, 2002). These increases were mainly reflected in the induction of two major amino acids: Pro and Ala. Water deficit-induced Pro accumulation can protect berries from excessive osmotic stress and maintain cell turgor pressure at a low water potential (Hochberg et al., 2013; Hong et al., 2000). Also, water deficit stimulates protein breakdown to increase amino acids (Less and Galili, 2008). Therefore, amino acids can be increased by water deficit.

Phenolic compounds

A water deficit may have positive effects on phenolic composition of fruit during ripening in berries, which may contribute to changes in the quality of Pinot noir wines (Berdeja et al., 2014; Roby et al., 2004). However, in 2016-2017 there was no prominent change in phenolics from the water treatments in the vineyard (Fig. 5.5). Compared to the field experiments, the glasshouse had the significant changes in phenolics during ripening (Fig. 5.2/5.3). These changes suggested that an increasing water deficit can induce osmotic adjustments through the active intracellular accumulation of organic solutes (Falginella et al., 2012; Zandalinas et al., 2018). ABA is a drought inducible hormone and can act as a signal to stimulate anthocyanin biosynthesis (Ferrero et al., 2018; González‐Villagra et al., 2018). In addition, water deficit had been reported to cause increases in gene expression of *F3H*, *LDOX*, *UFGT* and *GST*, resulting in the accumulation of skin total phenolics, skin anthocyanins and skin tannins during ripening, consistent with previous findings (Berdeja et al., 2014; Del-Castillo-Alonso et al., 2016).

Volatile compouds

Compared with well-watered, water deficit caused significant decreases in C_6 volatile composition (C_6 aldehydes and C_6 alcohols) but few changes in monoterpenes at harvest in

the glasshouse and the vineyard (Table 4.4 and 4.9), which were consistent with Fang and Qian (2005) and Mendez-Costabel et al. (2014). The concentrations of C_6 compounds decrease with increases in the sugar level in berries. Fatty acids also are catabolised into C_6 compounds. Water deficit causes an increase in the transcript abundance of LOX, HPL, ADH and AAT, resulting in the increase in the catabolism of fatty acids to volatile esters (Cramer et al., 2007a; Deluc et al., 2009). Therefore, water deficit causes the decreases in C_6 compounds, due to be converted to volatile esters. However, C_6 compounds mainly contributed to the 'fresh green', 'grassy' and 'herbaceous' aroma of berries (González-Barreiro et al., 2015), so the reduction in them may decrease undesirable aromas in the final wines.

7.1.3 Combination of leaf removal/UV-B and restricted irrigation in the glasshouse and vineyard

Vine physiology

In this study, one hypothesis is that UV-B interaction with water deficit will change the physiology in grapevines. In the vineyards, effects of the combination of UV-B and water deficit on the vine physiology and chemical composition of the fruit were only seen in 2016-2017, due to rainfall in 2015-2016 (see above). The combination of the different light environments and water deficit did not affect vine physiological parameters and did cause only a slight increase in juice $\delta^{13}C‰$ (Table 6.7). The slight increase in juice $\delta^{13}C‰$ is because, from source to sink, most sucrose (photosynthates) containing ^{13}C is translocated from leaves to grapes and converted to fructose and glucose, resulting in increasing ^{13}C in fructose and glucose (Centritto et al., 2009; Chaves et al., 2003). Additionally, less photosynthates incorporating ^{13}C in leaves are used to maintain function, such as respiration (des Gachons et al., 2005). Thus, the combination of UV-B and water deficit increased the $\delta^{13}C‰$ of juice but not in leaf $\delta^{13}C‰$. Also, UV-B interaction with water deficit did not induce sustained and greater responses in the grapevine physiology in the glasshouse experiments (Fig. 6.1 and Table 6.1). It could that the changes in vine

physiology in response to water deficit may negate UV-B damage (Sullivan and Teramura, 1990).

Amino acids

In this research, there were few effects of the combination of UV-B and water deficit on amino acids in the 2016-2017 vineyard trials (Table 6.8). In total, although there was not a perfect vintage for the vineyard experiments in 2016-2017, the results still showed some tendencies in the vine physiology and fruit chemical composition during berry development. The changes in metabolites were mainly induced by the different light environments, and water deficit may have an influence on UV-B-induced responses. However, none of the expected results for the combined treatments were found, possibly due to the rainfall after veraison in 2016-2017.

In the glasshouse, under the combined stresses, amino acids had the lowest concentrations in 2015-2016 (Table 6.2), but the highest concentrations compared to the other treatments (Table 6.3). In 2015-2016, the grapes had the most serious *Botrytis* infection in the combination of UV-B with water deficit treatment. Although unhealthy grapes had been removed, the intruder can induce reductions in amino acids (La Guerche et al., 2006). Therefore, total amino acids in grapes may be dramatically decreased by a *Botrytis* infection at harvest. In 2016-2017, amino acids were increased by UV-B interactions with a water deficit, except for Cys, which as evidence supported our hypotheses. It was possible that the increases in amino acids were induced by water deficit, and UV-B could potentially enhance the responses to the water deficit. The combined stresses may enhance the accumulation of proline to protect from excessive osmotic stress and protein breakdown to amino acids to maintain cell turgor pressure (Ábrahám et al., 2003; Ashraf et al., 2018).

Phenolic compounds

In a previous study, the accumulation of anthocyanins can significantly increase under UV-B interaction with water deficit in Tempranillo fruit (Martinez-Luscher et al., 2015). In the

vineyard, when berries were shaded (SC) or exposed to UV-B (LR) and protected from UV-B (PETG), combined with water deficit, the most obvious increases in phenolic composition were skin anthocyanins and skin total phenolics during ripening (Fig. 6.5). The interaction of leaf removal with water deficit (DL) increased skin anthocyanins and skin total phenolics by around 40% and 17%, respectively, compared to the shading and well-watered treatments (WS) at harvest. Moreover, DP increased skin anthocyanins by around 13% and skin total phenolics by less than 10%. This stated the exposure can increase the accumulation of anthocyanin and total phenolics in berry skins under water deficit, and the key component of radiation was UV-B to increase the levels of phenolics.

In the glasshouse, the combination of UV-B treatment and water deficit showed a greater accumulation of skin anthocyanins than the control but not than UV-B or water deficit treatments (Fig. 6.2b/6.3b), so the combined treatments did not increase responses compared to the individual stress. This increase may result in UV-B inducing the stimulation of the genes regulating anthocyanin biosynthesis and the activity of the corresponding enzymes (Lee and Skinkis, 2013; Martínez-Lüscher et al., 2014a; Schreiner et al., 2017). UV radiation or water deficit enhances methane release from cell wall pectins via ROS generation (Messenger et al., 2009). The accumulation of skin anthocyanins responded to UV-B or water deficit as ROS scavengers (Del-Castillo-Alonso et al., 2016), but water deficit may not enhance the response induced by UV-B. However, total phenolics and tannins in berry skins presented a greater accumulation in the combined stresses than in the individual stresses in 2015-2016 (Fig. 6.2a/c) but not in 2016-2017 (Fig. 6.3a/c). This may indicate that water deficit and UV-B can increase the polymerisation of proanthocyanidins to tannin (Berli et al., 2011). In 2015-2016, *Botrytis* infection may have caused the oxidation of phenolic compounds, resulting in changing the polymerization of proanthocyanidins. Thus, more accumulation of extracted skin tannins occurred in the combination of UV-B and water deficit than in the individual stresses in 2015-2016. Skin tannins are the most abundant class of total phenolics, so their contents directly affected

the content of skin total phenolics. At harvest, skin total phenolics showed similar effects from UV-B and water deficit as skin tannins over the two years.

Volatile compounds

In the vineyard, there was no statistically significant effect of the combination of UV-B and water deficit on volatile compounds (Table 6.10), while the combined stresses caused increases in hexanol and (E)-2-hexenol in the 2016-2017 of glasshouse trials (Table 6.6). These increases in C_6 alcohols demonstrated that water deficit-induced osmotic adjustments and oxidant stress were enhanced by UV-B. UV-B or water deficit caused an increase in the abundance of transcripts of several lipoxygenases (LOX) in grapevines to produce a cascade of damaging ROS resulting in increased catabolism of fatty acids to C_6 compounds (Deluc et al., 2009; Jordan, 2017). In addition, lipids in skins were catabolised into monoterpenes through the MEP pathway to protect berries from oxidative stress (Garcia-Esparza et al., 2018). Therefore, the UV-B interaction with water deficit may promote the production of C_6 compounds, which supports our hypotheses about the increases in accumulation of volatile compounds in response to the combination of UV-B and water deficit (see above 2.6.3).

As an overview, the goal of this research was to investigate the responses of the vine physiological indices and chemical composition of fruit to UV-B and water deficit singly and in combination. This was carried out on vines at different stages of fruit development. UV-B or water deficit stimulated the accumulations of amino acids, and the combined stresses enhanced the increases in amino acid concentrations at harvest. Skin anthocyanins and skin total phenolics were increased by UV-B or water deficit during ripening, and UV interaction with water deficit caused more increases in skin anthocyanins, but not in skin total phenolics. C_6 alcohols (one group of volatile compounds) showed the highest concentration in the combined treatment compared to individual stresses. This chapter also compared vine responses to water deficit in combination with UV-B radiation in the glasshouse and UV-B exposure/exclusion in the vineyard. The obvious differences of trials between the glasshouse and vineyard were shown in SPAD. There were no differences of

SPAD in the vineyard, but the glasshouse showed significant decreases in SPAD under treatments. Changes in chemical composition were consistent in the glasshouse and vineyard. Our hypotheses were supported by changes in metabolites in fruit, especially in the glasshouse trials. Chemical composition at harvest was considered an important quality-related attribute in fruit and the final wine.

7.2 Conclusions and recommendations

This study's goal was to investigate the responses of grapevines to UV-B radiation and water deficit in terms of vine physiology during berry development and the chemical composition of the fruit at harvest. Key findings are:

1. This research demonstrated that the physiology of *Vitis vinifera* L. var. Pinot noir vines were altered by water deficit or UV-B. The combination of UV-B and water deficit changed vine water status and leaf greenness in the glasshouse, but there was no effect on berry composition (°Brix, pH and TA). However, sunlight exposure with or without UV-B and interaction with water deficit did not affect vine water status and leaf greenness, even with no significant changes in fruit production capacity and °Brix in the vineyard.
2. Amino acid concentrations did not show consistent results in the glasshouse two years trials. Amino acids were decreased by UV-B and water deficit in 2015-2016. However, no change in amino acids in response to UV-B in 2016-2017. The increases in amino acids under water deficit were enhanced by the combination of UV-B and water deficit. In the vineyard, berries exposed to sunlight with or without UV-B resulted in no changes in amino acids over the two years, while their interaction with water deficit significantly changed some amino acids in 2016-2017.

3. This research reports increased contents of skin anthocyanin and skin total phenolics in fruit under an individual stress, but there were no greater accumulations of skin anthocyanin and skin total phenolics under a combined UV-B and water deficit than UV-B or water deficit. When berries were directly exposed to UV-B, more phenolic compounds accumulated in the berry skins. This research also reported that an increase in water deficit could potentially enhance the responses in skin phenolic compounds to UV-B.
4. The research showed increases in the concentration of hexanol and (E)-2-hexenol in berries under combined UV-B and water deficit in 2016-2017 glasshouse trial, but there were no changes in C_6 compounds in the vineyard. Some monoterpenes concentrations were decreased by the combined treatments in the vineyard, but not in the glasshouse.

The information presented in this thesis also indicated how important leaf canopy management and water deficit were for vineyard management. Recommendations for vineyard management are as follows:

1. The combination of leaf removal around the fruiting zone and water deficit at veraison had no negative effects on berry sugar accumulation and yield under the conditions of the trials run for this work.
2. Effective increases in amino acids under UV-B interaction with water deficit may be as the precursors of volatile compounds in grapes, resulting in significant effects on the aroma and flavour characteristics of wine.
3. Leaf removal interacting with a moderate water deficit will be a good way for vineyard management to increase the accumulation of anthocyanins and tannins in berry skins. These increases in phenolic composition may be maintained through the winemaking process to affect wine properties.
4. Leaf removal around the fruiting zone in combination with water deficit at veraison was a practical way to increase the volatile compounds of fruit in the vineyard, which may contribute to changed notes in the final wines.

5. Thus, the timing of leaf removal and the severity of water deficit should be considered with caution to change quality parameters in berries. A balance needed to be kept between amino acids and phenolic and volatile compounds to produce high quality wine.

Overall, this research substantially contributed to improve our scientific understanding of UV-B and water deficit responses in an important commercial species. In addition, it provided valuable information for possible changes to vineyard management and highlighted some future research to produce high quality wines with the anticipated specific characteristics.

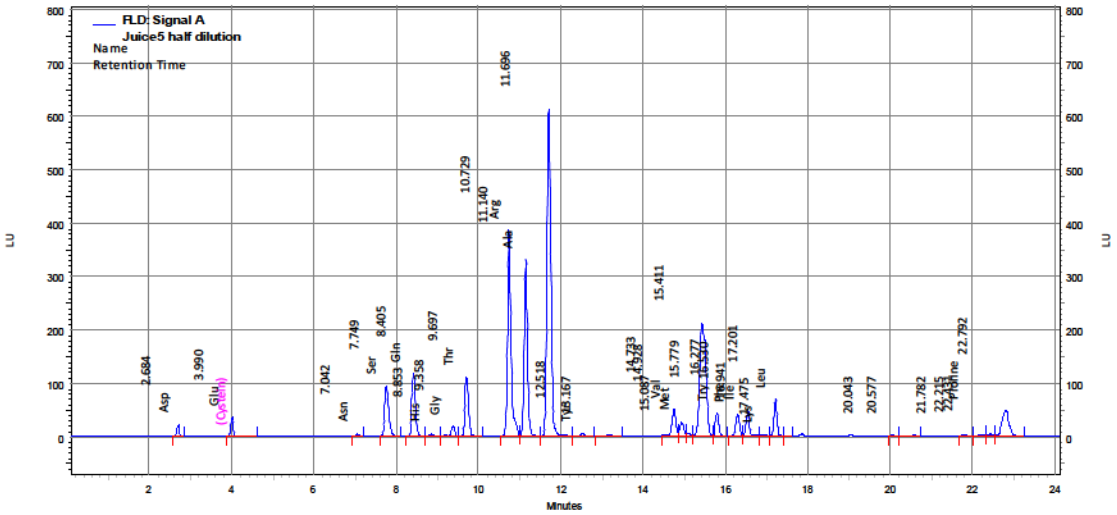
7.3 Future work

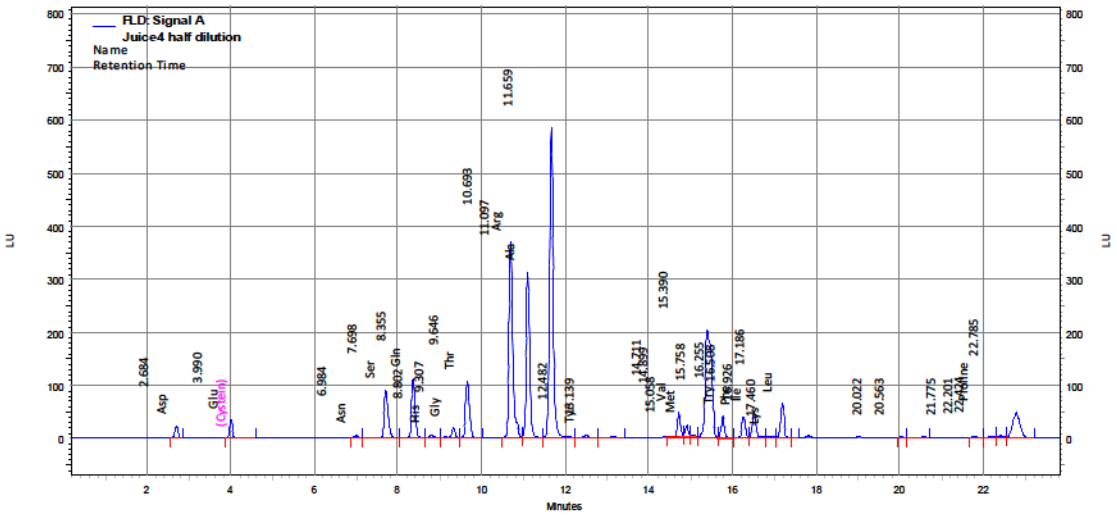
1. In this research, there was no statistical changes in the vine physiological indicators under the combination of UV-B and water deficit, but metabolites (amino acids, phenolic compounds and volatile composition) were changed. Thus, it will be critical what is the mechanism of the combined stresses on the first and secondary metabolite.
2. The research has reported the changes in amino acids under water deficit and its interaction with UV-B. Amino acids may be changed by a water deficit, but not directly affected by UV-B. So, future work should focus on understanding the mechanisms of amino acid biosynthesis in response to UV-B interaction with water deficit in grapes. This will be crucial in further explaining the changes in amino acids of grapes in response to UV-B.
3. Future work may focus on different leaf removal patterns, such as different densities of leaf removal within or above the fruiting zone and decreases in leaf numbers per shoot on the top of shoots. Leaf removal can have significant effects on berry quality parameters. For example, excessive removal of leaves may lead to a reduction in photosynthesis and nitrogen assimilation, resulting in a delay in ripening and a decrease in the concentrations of amino acids.

4. The imposition of several levels of water deficit at different stages, such as using restricted deficit irrigation (RDI) from pre-veraison, veraison and pre-harvest to harvest, may also be interesting to study. The supplementation of water deficit at different stages can change the berry size, the degree of tannin polymerization and anthocyanins (Herrera et al., 2017; Ojeda et al., 2002; Ollé et al., 2011). A moderate water deficit from veraison to harvest should enhance the development of phenolic and aroma compounds and the accumulation of amino acids. The supplement of an excessive water deficit at different stages can increase sugar accumulation and decrease yields (Cáceres-Mella et al., 2017; van Leeuwen and Darriet, 2016). When and how to combine leaf canopy and regulated deficient irrigation is, consequently, critical to the accumulation of these chemical compounds in berries at harvest.
5. Climate change undoubtedly affects global grapevine and wine production. The key issues are changes in temperature, water availability and CO_2, which directly influenced not only vine growth and yield but also the pest and pathogen spectra (Koch and Oehl, 2018). In this research, the effects of water and UV-B have been investigated, but changes in temperature and CO_2 under the frames were not controlled, which should be considered in future research. Therefore, it was essential that the consequences of a changing environment be considered along with multiple environmental parameters.

Appendix A

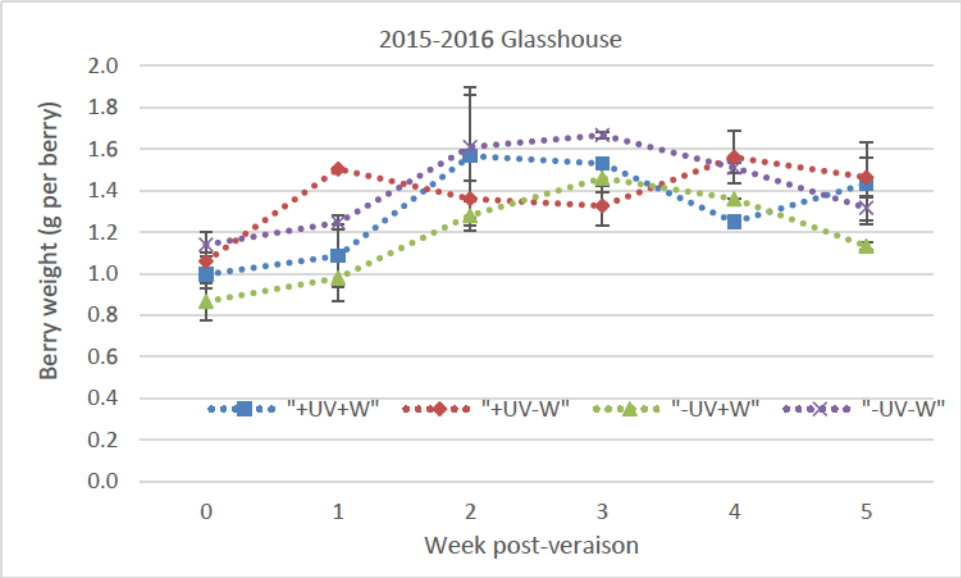
Amino acids chromatograms

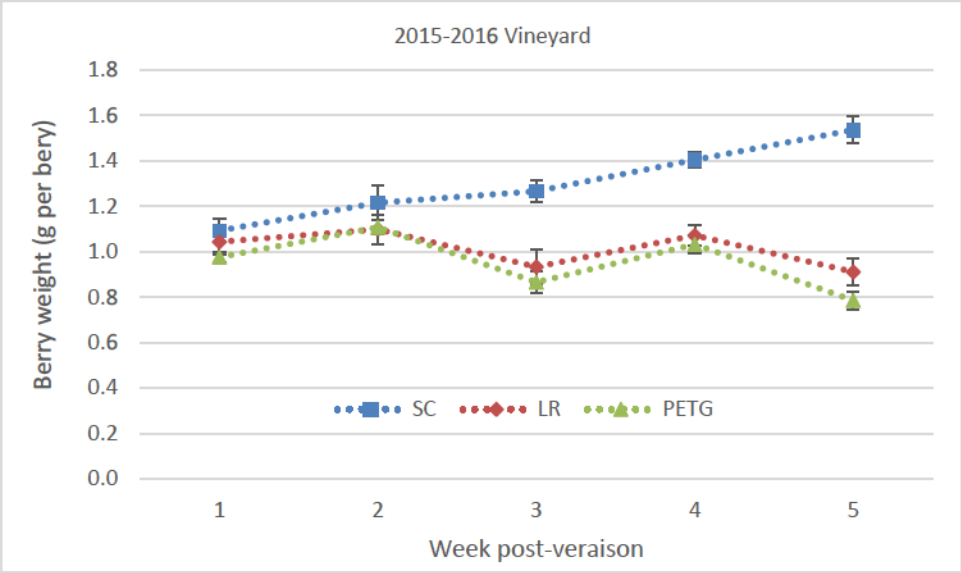


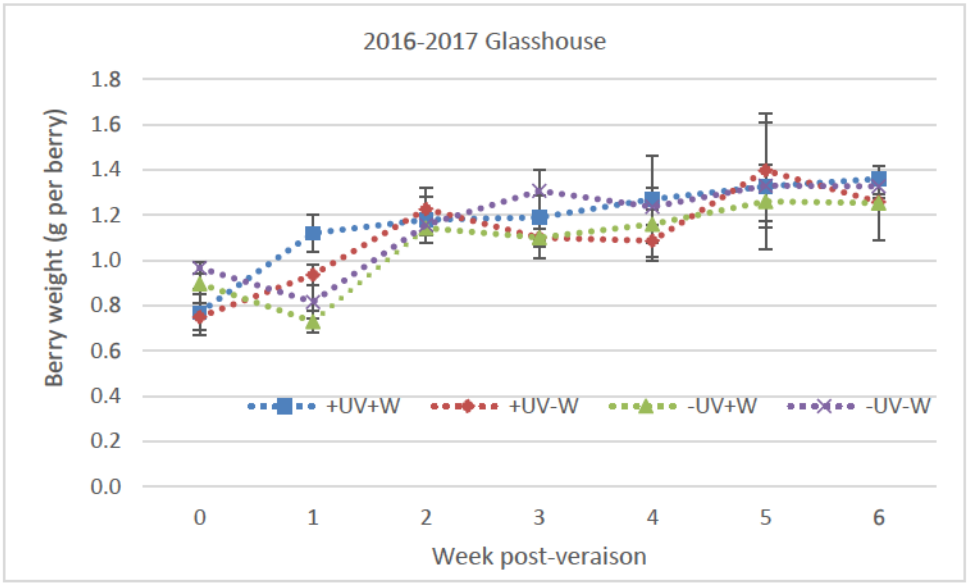


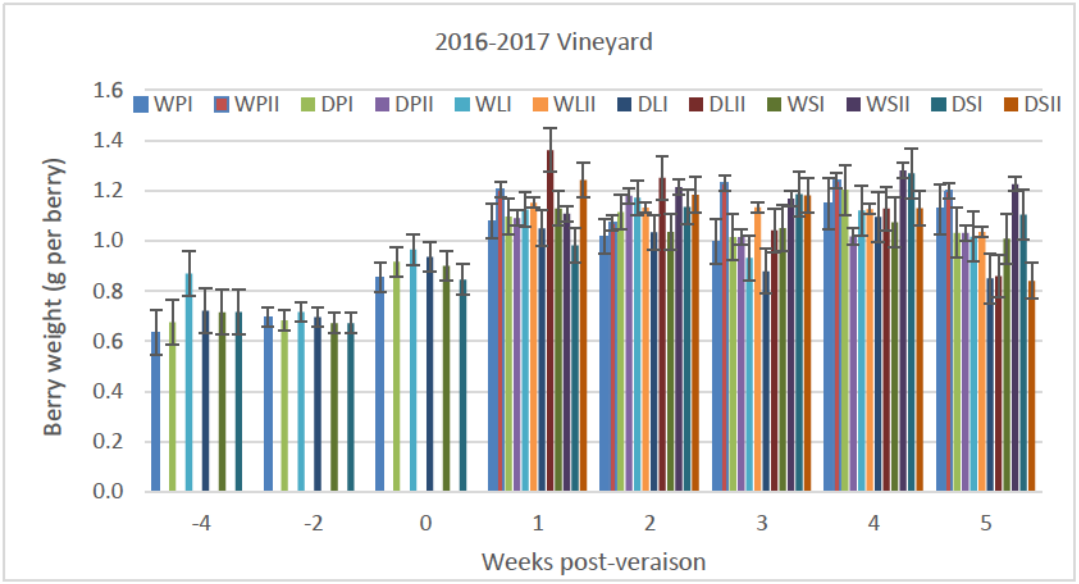
Appendix B

Berry weight






2016-2017 Glasshouse
Berry weight (g per berry)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
+UV+W
+UV-W
-UV+W
-UV-W
0
1
2
3
4
5
6
Week post-veraison


2016-2017 Vineyard
WPI
WPII
DPI
DPII
WLI
WLII
DLI
DLII
WSI
WSII
DSI
DSII
Berry weight (g per berry)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-4
-2
0
1
2
3
4
5
Weeks post-veraison

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