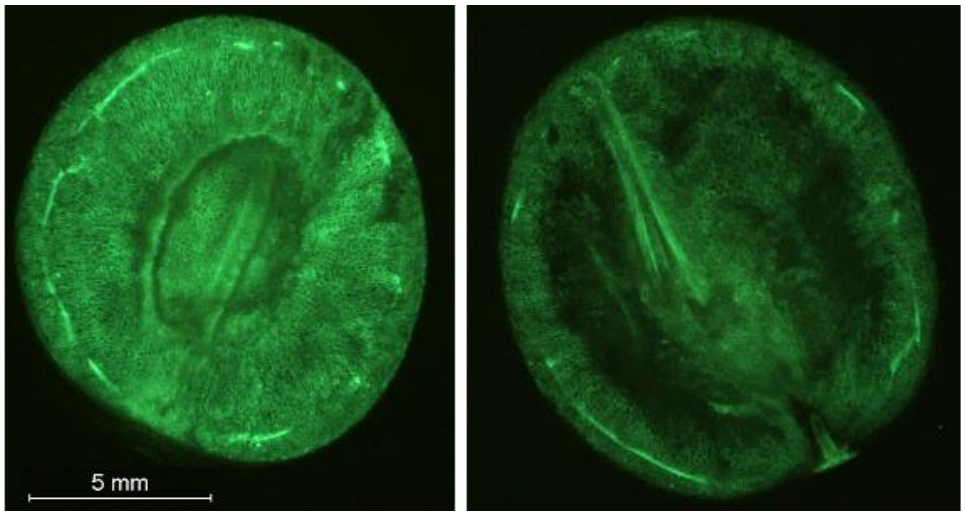
Australian Government

Wine Australia

THE UNIVERSITY of ADELAIDE

Is berry shrivel in Cabernet Sauvignon influenced by climate and does this potentially affect characteristics of the resulting wine?



FINAL REPORT TO WINE AUSTRALIA

Project Number: **UA 1702**

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Research Organisation: **The University of Adelaide**

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1 Abstract

Berry shrivel is a phenomenon of weight loss in berries during the late ripening stage attributed to dehydration and loss of berry cell vitality that results in losses of yield, quality and profitability. However, virtually nothing is known about shrivel in Cabernet Sauvignon (CAS) and, importantly, the effects of shrivelled fruit on grape/wine composition and quality. During the 2017-18 season, the influence of leaf removal, vine water status and row orientation (E-W vs N-S) was assessed on late season berry shrivel in CAS in the Coonawarra viticulture region. Leaf removal resulted in higher canopy and bunch zone light interception and temperature while reduced irrigation levels resulted in lower soil and vine water status, and lower berry weight and yield. Reduced irrigation also negatively affected berry cell vitality resulting in a higher incidence of bunches with shrivelled berries. E-W oriented rows had a higher incidence of shrivelled fruit as well as lower berry weight and yield compared to N-S rows; this was attributed to the higher vine water stress in the E-W rows. The E-W rows generally had higher soluble solids in the fruit. Sensory analysis of the wines revealed that inclusion of shrivelled fruit contributed to higher dark and overripe berry characters with some oxidised notes; these negative descriptors were exacerbated with vine water stress.

2 Executive summary

Climate change in South Australian viticultural regions has resulted in higher growing season temperatures, leading to compressed vintages, critical water scarcities, particularly late in the growing season, and altered fruit composition and wine quality that is redefining the regional wine styles of certain cultivars. Manifesting from these altered environmental conditions is an increased incidence of berry shrivel, a phenomenon associated with cell death in the mesocarp.

In this study, we investigated the dual effects of elevated bunch zone temperature (via bunch zone leaf removal) and vine water stress (via 50% deficit irrigation) on the incidence of berry shrivel in Cabernet Sauvignon (CAS) in Coonawarra. Furthermore, the effect of varying proportions of shrivelled fruit in the must was assessed viz. wine chemical composition and sensory quality, using an expert panel of tasters.

Leaf removal around the bunch zone was effective in increasing canopy porosity and bunch temperatures by ~ 2 °C. Leaf removal did not impact either soil or vine water status. There was no effect of elevated temperature on berry cell death. Yields as well as the proportion of shrivelled bunches on each vine were similar, indicating that basal leaf removal and higher bunch temperatures did not have an impact on berry shrivel. There was no impact of leaf removal on basic fruit composition parameters.

Vine water stress had a significant impact on the incidence of berry shrivel. Water-stressed vines had similar canopy microclimate, leaf and bunch temperatures, porosity and LAI values to non-water stressed vines. Water stress resulted in higher cell death in the berries, with a significant effect of row orientation. Yield components were not severely impacted by water stress but significantly smaller berries were observed in the high water stress treatments, particularly in vines with leaf removal. Basic fruit composition was similar between water stress treatments except for anthocyanins, which were higher in the water stressed vines.

Row orientation affected canopy microclimate: higher ambient temperatures and VPDs were observed in the N-S block during the warmest days. This was consistent with the leaf and bunch surface temperatures measured in that block with differences of up to 5 °C observed. N-S rows had higher canopy porosity, soil moisture and vine water status, i.e. less water stress, compared to the E-W rows. E-W rows also resulted in higher levels of berry cell death at harvest, particularly when water stress and leaf removal treatments were applied; this was not observed in the N-S block. Higher cell death could have resulted from the higher levels of vine water stress in the E-W block. Although neither yield nor berry weight differed between the two blocks, the E-W block had significantly higher amount of shrivelled fruit compared to the N-S block. Must soluble solids, pH, colour and YAN were significantly higher in the E-W block. Wine composition had a similar trend to must composition; the E-W block had higher pH, colour and hue, malvidin glucoside, caffeic acid, quercetin, as well as higher ethanol and volatile acidity. Certain volatile compounds were higher in the N-S block including IBMP. The proportion of shrivelled fruit used for winemaking affected wine composition. Higher proportions of shrivelled fruit resulted in wines with higher anthocyanin and quercetin concentrations but also higher IBMP.

Wine sensory analysis using a modified DA procedure with an expert panel of tasters revealed that addition of shrivelled fruit to the must increased the perception of dark, jammy and oxidised characters, while clean fruit had greater red berry and green character. Vine water stress, higher in the E-W rows compared to the N-S rows, exacerbated the incidence of shrivel and likely also the proportion of berries on each bunch that were shrivelled, consequently increasing the jammy and cooked fruit characters observed in the wines.

3 Background

Berry shrinkage and weight loss is a phenomenon that has been observed in several grape cultivars late during berry development, typically post-véraison. The effect of this is a decrease in yield and potentially quality, and has been reported to be a significant problem in Australia (Fuentes et al. 2010). Yield losses of up to 30% have been reported in Shiraz (McCarthy 1997, Rogiers et al. 2006). Shrinkage and shrivel have been associated with a loss of cell vitality of

mesocarp cells in the berry that begins at different times for different cultivars (Bonada et al. 2013b, Tilbrook and Tyerman 2009). It is a physiological disorder that manifests as visible shrivelling of the pericarp and a loss of membrane competence of the mesocarp cells as well as a loss of the brush of the berry (Bondada and Keller 2012). Programmed cell death has been implicated in this phenomenon; a California study on CAS found a high correlation between berry shrivel and cell death (Krasnow et al. 2008). Shiraz berry shrivel has been observed as early as 90 days after anthesis when mesocarp cell membranes become leaky and osmotically incompetent, and a reduction in hydraulic conductance with the vine occurs (Tilbrook and Tyerman 2008).

Specific environmental conditions including high temperatures and vine water stress pre-véraison have been observed to result in losses in cell vitality or 'cell death' (Bonada et al. 2013a, Bonada et al. 2013b). This results in compromised membrane integrity or lysing of mesocarp cells resulting in a mixing within the berry of various chemical constituents that have been observed to impact sensory attributes of the berry (Bonada et al. 2013a, Caravia et al. 2015). However, to date, no analysis of the effects of berry shrivel has been done on wine composition and quality.
Researchers have shown that specific vineyard treatments such as the application of overhead shade cloth to decrease the amount of incident radiation and decrease bunch temperatures can be effective in reducing the incidence of berry shrivel (Caravia et al. 2016). With temperatures elevated between 1.5-2.3 °C relative to ambient, Chardonnay showed virtually no shrivel whereas Shiraz had 20% more shrivel (Bonada et al. 2013a). Besides heat stress, shrivel in Shiraz has been suggested to be related to water stress (Bonada et al. 2013a), however no specific evidence is available. Berry shrivel in Shiraz was observed to be higher with a warm pre-véraison period despite a cool and wet ripening period (Caravia et al. 2015). Work is also currently underway to study the effect of evaporative cooling using in-canopy misters on the incidence of berry shrivel (Wine Australia project UA 1502).

Fluorescein diacetate (FDA) is a vital stain that permeates cell membranes and is cleaved by esterases in the cytoplasm to form fluorescein, which fluoresces under blue light (Jones and Senft 1985). FDA is a non-polar and non-fluorescent dye (Tilbrook and Tyerman 2009) and has been reported to be a reliable indicator of cell vitality (Krasnow et al. 2008). Indeed, several studies have now used this technique to assess cell death, as a quantitative indicator of berry shrivel (Bonada et al. 2013b, Caravia et al. 2016, Fuentes et al. 2010, Tilbrook and Tyerman 2009). In this study, FDA was used as a cell vitality stain to quantify the extent of cell death in Cabernet Sauvignon grape berries as affected by various environmental factors.

Quantitative assessment of cell death based on impedance spectroscopy can be done in conjunction with fluorescence microscopy. Electrical impedance (Z) is a measure of the resistance

of a circuit when an alternating current is passed through it at specific frequencies. Measurement of impedance provides richer information about the electrical properties of tissues, particularly their ability to store and dissipate electrical charge, similar to a capacitor. Measurements of impedance typically describe the relationships between resistance (in complex numbers, the 'real' part of Z) and reactance (in complex numbers, the 'imaginary' part of Z) at various frequencies between 1 Hz and 1 MHz; these frequencies display the electrical properties of cell structures (Ando et al. 2014). Lower frequencies allow for electrical current to be conducted through the high-resistance extracellular region, i.e. apoplasmic or cell wall path, while higher frequencies result in current also flowing through the low resistance intracellular regions containing fluid (Ando et al. 2014). To quantify the influence of these different cellular regions on impedance, specific electrical circuit parameters that comprise Z such as extra- (R_e) and intra-cellular (R_i) resistances, and membrane capacitance (C_m), are modelled using an equivalent circuit consisting of an extracellular resistor in parallel with an intracellular resistor, the latter in series with a constant phase element (CPE).

The region of Coonawarra, located in the Limestone Coast of South Australia, is renowned for its terra rossa (TR) soil. TR soils are characterised by their red-brown, thick, clay B horizon soils overlying limestone, the depth to which is variable (Longbottom et al. 2011, Mee et al. 2004). While the distribution of TR throughout Coonawarra is limited, current estimates state the area of TR soil to be 23 km in length and 1.7 km in width (Fitzpatrick 2014). The other dominant soil types of the region are rendzina clays and sandy clay loams. The region has approximately 5,700 ha of grapevines with the predominant red varieties being CAS and Shiraz. The region is characterised as relatively cool climate, however, during the growing season, irrigation is required in varying amounts, particularly where the depth to limestone is shallow, or where soil types vary.

Here, we investigated how specific environmental factors (bunch temperature, incident radiation), cultural practices (basal leaf removal, irrigation) and row orientation contribute to the phenomenon of berry shrivel in CAS. Berry shrivel was assessed using morphometric (digital image analysis) and tissue vitality assessment techniques. Fruit at commercial harvest and wines made from varying proportions of shrivelled berries were assessed for key quality metrics (anthocyanins, flavour compounds) as well as sensory quality. A key objective was to link the incidence of shrivelled berries with grape and wine composition and quality, which had not been done previously.

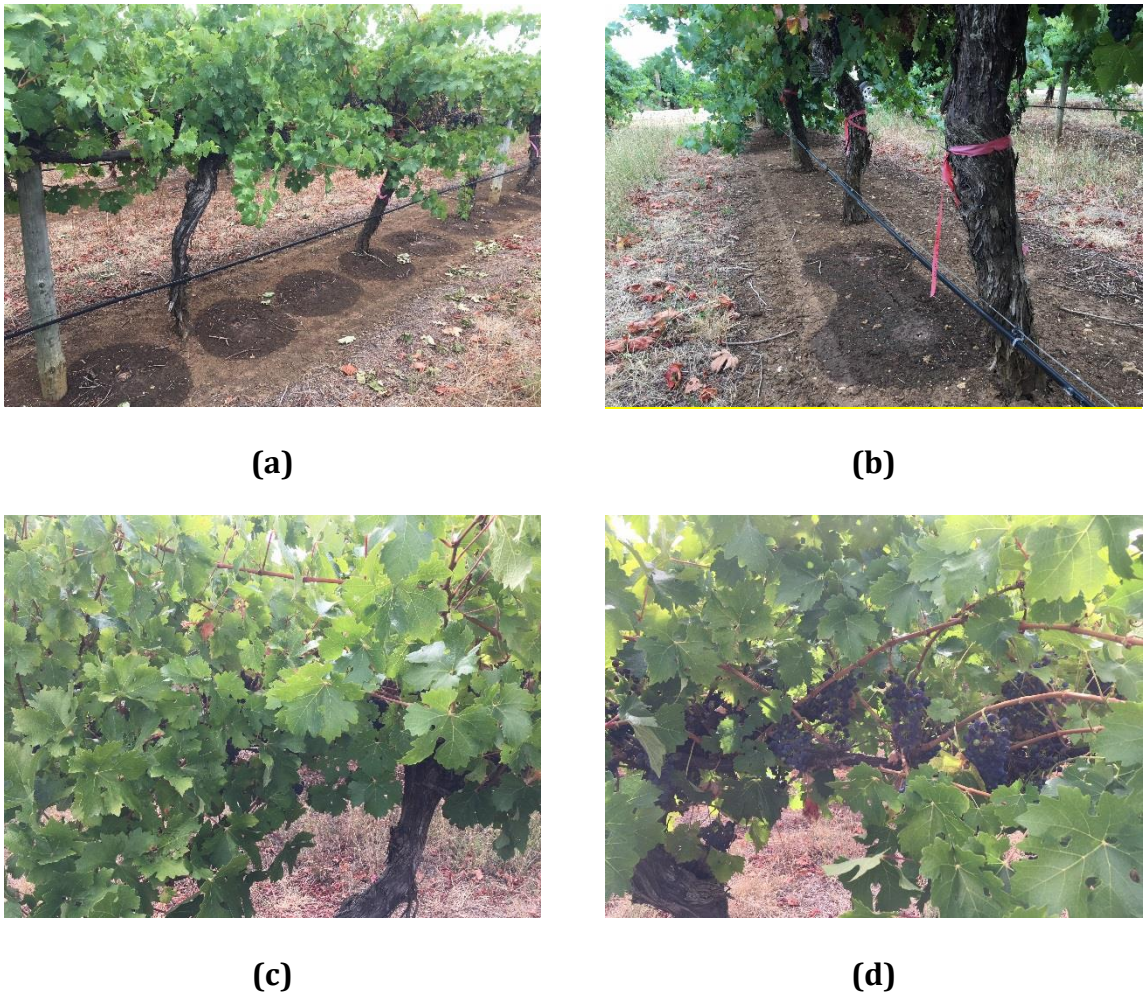
4 Project aims

	Output	Target Date	Activities
a	Final project plan	30/09/2017	Identify vineyard sites (2) for the trial; finalise project design, experimental plan, timeframe of data collection.
b	Instruments and tools working; methods established	31/10/2017	Organise and test all instruments and methods needed for experiments.
c	Treatments established	30/11/2017	Leaf removal treatments set up in two CS vineyards with contrasting water status.
d	Assessment of trial	31/05/2018	Bi-weekly collection of vine water status, light interception, canopy and bunch temperatures, berry samples; analysis of berry composition, berry shrivel.
e	Assessment of wine composition and quality	30/09/2018	Small-scale winemaking with different fractions of shrivelled fruit; wine composition analysis (pH, TA, anthocyanins, polyphenols, tannins, IBMP).
f	Final report	30/09/2018	Collate and analyse data. Submit final project report to Wine Australia.

5 Method

5.1 Vineyard site, plant material and experimental design

The trial was done during the 2017-18 growing season in the Coonawarra region (37.29°S, 140.82°E), part of the Limestone Coast region of SE South Australia. The mean January temperature (MJT) for Coonawarra is 19.3°C, with 1392 growing degree days (October-April; base 10°C). The climate of the area is Mediterranean with winter dominant rainfall and relative summer drought. Typically, annual rainfall for Coonawarra is 569 mm. Two vineyard blocks (henceforth named 'Block P' and 'Block Q') were used in this study. Block P was planted in 1973 to own-rooted *Vitis vinifera* L. cv. Cabernet Sauvignon (Cl. 'Reynella') in north-south oriented rows with a row and vine spacing of 3.38 x 1.83 m. Block Q was planted in 1994 with own-rooted Cabernet Sauvignon (Cl. 'Reynella') in east-west oriented rows with a row and vine spacing of 2.75 x 1.83 m. The vines were cordon-trained and box-pruned with a machine (both blocks), with hand clean-up to two-bud spurs only in Block P. One summer trimming (hedging) was performed shortly prior to véraison when the shoots were horizontal (parallel to the ground). Soil type in both blocks was 'terra rossa', with light to medium red clay, over highly porous limestone typically 1.0-1.5 m below the surface. Block P had a shallower terra rossa profile than Block Q. The vines were drip irrigated at 1.4-1.5 ML/ha in both blocks. Conventional integrated pest management and vineyard floor management practices were applied.



(a) **(b)**

(c) **(d)**

Figure 1: Irrigation treatments showing the wetting pattern on the vine floor **(a)** and deficit irrigation in the vines at top of photo **(b)**; vines without **(c)** and with **(d)** leaf removal in the bunch zone at véraison.

The experimental design in both vineyards was a 2x2 factorial with two irrigation treatments and two leaf removal treatments replicated twice (two sub-blocks) in each vineyard. In each sub-block, irrigation treatments were imposed on five adjacent rows of vines, from which only the middle three rows were used for measurements. Treatments were applied on 12 vines per row with four randomly selected vines per row used for measurements at each timepoint. The two irrigation treatments were: normal irrigation (NI) where 1.4-1.5 ML/ha of water was applied over the season starting on 30 October 2017 on a weekly interval; deficit irrigation (DI) where every second drip emitter was plugged therefore delivering a total of approx. half the NI volume (50% deficit) to the vines or 0.70-0.75 ML/ha over the season (Figure 1 a, b).

Within each irrigation treatment, leaf removal (4-5 leaves) was done to expose the bunches to higher levels of incident radiation and to increase their temperature relative to ambient (Figure 1 c, d). Within the 12 vines per row, four vines were leaf plucked (LR) while the others were not

manipulated (NLR). Due to the high spatial variation in soil moisture in the two blocks, the dataset was sorted based on two vine water status (stem water potential, Ψ_s) levels: mild water stress (Ψ_s > -0.8 MPa) and high water stress (Ψ_s < -1.0 MPa). Leaf removal was done at approx. 50% véraison, on 25 January 2018.

5.2 Environmental parameters

In each treatment, one TinyTag sensor (Model: TGP 4017, Hastings Data Loggers, Port Macquarie, NSW, Australia) was placed in the canopy interior to continuously record ambient temperature every 15 min over the growing season. Seasonal weather data were obtained from the BOM weather station located in Coonawarra, SA. Vapour pressure deficit (VPD) in each canopy and treatment was calculated using the canopy temperature from the TinyTag sensors and relative humidity (RH) from the BOM weather station daily at 1500 h, approx. when the lowest RH of the day was measured to provide the maximum daily VPD.

5.3 Physiological measurements

5.3.1 Canopy light interception, leaf area index, canopy porosity

Canopy light interception in the bunch zone, leaf area index (LAI) and canopy porosity (T) was determined using a portable ceptometer (Model LP-80, Decagon Devices, Pullman, WA, USA). Two measurements per vine were made, one on each side of the trunk and parallel to the cordons. Measurements were made at solar noon between 1100 h and 1400 h.

5.3.2 Soil and vine water status

Soil matric potential was estimated as the pre-dawn leaf water potential (Ψ_{pd}) using a leaf pressure chamber (Model: 1505-EXP, PMS Instruments, OR, USA). Two leaves per vine were measured between 0300-0500 h. Midday stem water potential (Ψ_s) was measured using the same instrument by bagging two leaves per vine with opaque bags for at least one hour prior to measurement.

5.3.3 Leaf and bunch temperatures

Leaf and bunch temperatures were measured simultaneously with light interception measurements using a hand-held infrared thermometer (Model: 62 MAX+, Fluke, Castle Hill, NSW). Two exposed leaves and bunches were measured per vine. In many cases in the NLR treatment, only shaded bunches were available for measurement.

5.4 Berry sampling

Two whole bunches were harvested at each of the following phenological stages for the assessment of berry size, colour, composition and cell vitality: véraison, two weeks post-véraison, two weeks pre-harvest and harvest. Bunches were placed in a cool box with ice packs and transported to the lab where they were stored overnight at 4°C until analysed the next day. Fifty berries from all bunches were sampled for size, colour and composition, while three berries (from all bunches) were sampled for assessment of cell vitality (see below for techniques).

5.5 Berry tissue vitality assessment

Two techniques were used to assess mesocarp tissue cell vitality of the berries at room temperature (~22°C). The first was using the fluorescein diacetate (FDA) staining technique and fluorescence microscopy (Bonada et al. 2013b, Jones and Senft 1985, Krasnow et al. 2008). Using MATLAB software, seeds were first excluded from the image and then analysed using the image analysis code as per Fuentes et al. (2010). In the second technique, electrical impedance spectroscopy was done on the same berries used for the FDA staining method as per the method of Caravia et al. (2015). Impedance spectroscopy provided data on the intracellular (R_i) and extracellular (R_e) resistances of the mesocarp as well as cell membrane capacitance (C_m) (Figure 2).

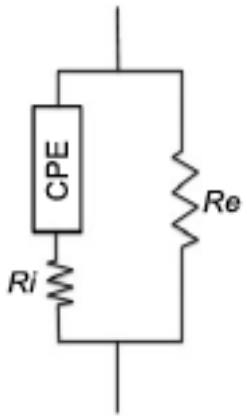


Figure 2: Equivalent electrical circuit for a grape berry (from Caravia et al., 2015).

Model fitting of the impedance data was done as follows. After removing the lowest frequencies (<20 Hz) of the datasets, since they belong to a polarisation effect that does not relate to the cell structure (Ando et al. 2014), each of the electrical impedances measured was fitted to the most appropriate equivalent electric circuit to living tissue (Ando et al. 2014) using EIS Spectrum analyser software (Bondarenko and Ragoisha 2005), to describe impedance as a function of frequency. The equivalent circuit consists of an extracellular resistor (R_e) in parallel with an intracellular resistor (R_i), the latter in series with a constant phase element (Z_{CPE}).

This fitting provides estimates of extracellular resistance (R_e), intracellular resistance (R_i) and constant phase element (CPE) components, T (pre-exponential factor, or coefficient) and p (exponent), that allow for the calculation of cell membrane capacitance (C_m) and real impedance (Z) in the electrical circuit, which in all the cases coincide with measured impedance around f = 36 Hz (Joglar Gonzalez 2018). The following equations were used for the determination of these electrical circuit parameters (Ando et al. 2014):

$$C_m = T^{\frac{1}{p}}(R_e + R_i)^{\frac{1-p}{p}} \quad \text{(Eqn. 1)}$$

$$Z = \frac{(Z_{CPE} + R_i) \cdot R_e}{Z_{CPE} + R_i + R_e} \quad \text{(Eqn. 2)}$$

where,

$$Z_{CPE} = \frac{1}{(j\omega)^p \cdot T} \quad \text{(Eqn. 3)}$$

and,

$$\omega = 2\pi f \quad \text{(Eqn. 4)}$$

where ω is the angular frequency at f = 36 Hz.

5.6 Yield components

The vines were harvested on 21 March 2018. Four vines per treatment were picked based on two levels of water stress (high, low) and leaf removal (no leaf removal, leaf removal) for a total of 16 vines per vineyard. Yield components were measured including yield per vine, yield per metre of cordon (based on measurement of cordon length) and bunch number per vine. Average bunch weight was calculated from these numbers. Fifty berries were later sampled from each lot to calculate average berry weight and average berries per bunch. The fruit from each vine was divided into two groups: shrivelled fruit (bunches with at least three shrivelled berries) and clean or non-shrivelled fruit (no visible signs of shrivelled berries). These lots were weighed separately. The number of bunches in each group was also counted.

5.7 Winemaking

The musts were comprised of 0%, 25% and 50% shrivelled bunches by weight and done in duplicate. The fruit was crushed by hand, adjusted to 26.5 °Brix, and then sampled for later chemical analyses. The musts were inoculated with Lalvin EC-1118 'prise de mousse' yeast (*Saccharomyces cerevisiae bayanus*, Lallemand) at the manufacturer's recommended rate. The ferment size was approx. 4 L. Diammonium phosphate was added at 100 ppm to each must prior

to inoculation with yeast. Skins were punched down twice per day and fermentations were done at 22°C. After approx. two weeks of fermentation, the dry wine was pressed off using a basket press (10 L capacity). Malolactic fermentation was induced by inoculation with lactic acid bacteria and checked at the end of MLF for the absence of malic acid. Sulfur dioxide (30 ppm) was added to the wines, which were then bottled for later analysis.

5.8 Fruit, juice and wine composition

Juice samples were analysed for total soluble solids, pH, titratable acidity, colour and hue, total anthocyanins (pH shift method) and total polyphenols (Folin assay). Wines were analysed similarly except for TSS as they were fermented to dryness. Berry samples were analysed for skin and seed tannins as per Iland (2013).

Residual sugar, malic acid and volatile acidity were measured with the ChemWell® 2910 Autoanalyzer (Awareness Technology Inc., Palm City, FL, USA) using the Sucrose + D-Glucose + D-Fructose kit, the L-Malic Acid and Acetic Acid enzyme kits (Vinessential Laboratories, Orange, NSW), respectively. Alcohol was measured with an Alcolyzer Wine M/ME instrument (Anton Paar GmbH, Graz, Austria).

Total anthocyanins and polyphenols were assessed with the same method used for juice, but in the latter case (for polyphenols), wines were diluted 1:10 with water. Colour intensity and hue were assessed with the same method used for juice, but instead using a 1 mm cuvette due to high turbidity of the wines.

5.8.1 Individual anthocyanins and polyphenols in wine

Specific anthocyanins and polyphenols were measured using HPLC, by profiling. All reagents and solvents used in the laboratory were of analytical grade or higher. Formic acid (FA) and standards were purchased from Sigma-Aldrich (St. Louis, MO). The acetonitrile (ACN) of HPLC grade was purchased from RCI Labscan (Thailand) and the MilliQ water was obtained from a MilliQ water purification system (Milli-Q plus, Adelab, SA). Both analyses were performed using Agilent 1100 Series HPLC and ChemStation software (Agilent Technologies, Palo Alto, CA). The separation was carried out using an InertSustain C18 reverse-phase column (250 mm x 4.6 mm, 5 µm; GL Science Inc., Japan) with an InertSustain C18 guard column (33 x 4.6 mm, 5 µm; GL Science Inc., Japan). The temperature of the column was maintained at 30 °C and a flow rate of 1 mL/min was used. All wine samples were filtered through 0.45 µm polytetrafluorethylene (PTFE) syringe filters (CHROMAFIL® O-45/15 MS, Germany) then 70 µL of filtered wine was injected into the HPLC. The binary mobile phase used was buffer A (water/5% FA) and buffer B (ACN/5% FA). The mobile phase gradient was: 5-9 % B (0-10 min), 9-20 % B (10-43 min), 20-60 % (43-73 min), 60-

90 % (73-75 min), 90 % B (75-80 min), 90-5 % B (80-81 min), 5 % B (81-85 min). Individual anthocyanins and polyphenols were detected at 280 nm, 320 nm, 365 nm and 525 nm using a Diode Array Detector (DAD). Identification of the compounds was carried out by comparing their retention times with the standards. Finally, the concentration of each compound was calculated based on the peak area and calibration curves using standards for delphinidin-3-*O*-glucoside (D3OG), cyanidin-3-*O*-glucoside (C3OG), petunidin-3-*O*-glucoside (Pet3OG), peonidin-3-*O*-glucoside (Peo3OG), and malvidin-3-*O*-glucoside (M3OG) obtained from Polyphenols Laboratories (Sandnes, Norway).

5.8.2 Wine volatile compounds

Methoxypyrazines and volatile compounds produced during fermentation were measured by Solid-phase microextraction (SPME) followed by GC-MS. Aliquots of the wines were analysed in a 1:10 dilution with water to a final volume of 10 mL into 20 mL SPME vials in which three grams of NaCl had been added prior to sample addition. Extraction and chromatographic conditions were as described by Keyzers and Boss (2010), but the samples were spiked with five internal standards before SPME-GC-MS analysis: d13-hexanol (9.2 µg for 1:2 dilution, 0.92 µg for 1:100 dilution; C/D/N Isotopes, Pointe-Claire, Canada); d11-hexanoic acid (9.3 µg for 1:2 dilution, 0.93 µg for 1:100 dilution; C/D/N Isotopes); d16-octanal (0.821 µg for 1:2 dilution, 82.1 ng for 1:100 dilution; C/D/N Isotopes); methyl nonanoate (61.2 ng for 1:2 dilution, 6.12 ng for 1:100 dilution; Sigma-Aldrich, Sydney, Australia); and d3-linalool (17.3 ng for 1:2 dilution and 1.7 ng for 1:100 dilution; C/D/N Isotopes).

SPME-GC-MS was carried out using an Agilent 6890 gas chromatograph equipped with a Gerstel MP2 autosampler and using an Agilent Technologies 5973N mass spectrometer for peak detection and compound identification. The identity of the detected volatiles was confirmed by comparing mass spectra with those of authentic standards and spectral libraries such as the laboratory-generated library (328 compounds) as well as the United States National Institute of Standards and Technology-11 (NIST-11) and the Wiley Registry 9th ed. mass spectral library. Compounds were considered positively identified after matching of both mass spectra and linear retention indices (LRI) with those of authentic samples, where the LRI was calculated from a compound's retention time relative to the retention of a series of n-alkanes (C8–C26). The autosampler was operated in SPME mode utilising a divinylbenzene-carboxen-polydimethylsiloxane fibre (2 cm, 23-gauge, 50/30 µm DVB-CAR-PDMS, Supelco, Bellefonte, PA) for extraction. Volatile compounds were extracted using agitation (250 rpm) at 40 °C for 30 min. Chromatography was performed using a ZB-Wax column (length 30 m, 0.25 mm i.d., film thickness 0.25 µm) using helium as a carrier gas at 1.2 mL/min (constant flow). Volatiles were desorbed from the fibre in the GC inlet (220 °C) for 1 min and separated using the following

temperature program: 40 °C for 1.5 min, increasing at 7 °C/min to 245 °C, and held isothermally at 245 °C for 4.5 min. The temperature of the transfer line connecting the GC and MS was held at 250 °C. Positive-ion electron impact spectra (70 eV) were recorded in scan mode (range, m/z 35−350; scan rate, 4.45 scans/s).

5.9 Wine sensory analysis

A sensory analysis of the wines was conducted based on Modified Descriptive Analysis (MDA) using an expert panel of winemakers approx. three months post-bottling. The wines were presented in duplicate and in random in a single session. A total of 12 wines was assessed (2 vineyards x 3 wines per vineyard x 2 replicates) by 11 panellists. A set of aroma, flavour and mouthfeel descriptors typical for Coonawarra Cabernet Sauvignon were provided to each panellist, as follows:

Red berry fruit	Dark berry fruit	Overripe fruit
Eucalyptus	Fresh green	Earthy green
Green tannins	Palate weight	Tannin intensity
Colour density	Oxidised	Acidity

These attributes were scored on an intensity scale of 0 (low/no) to 9 (high/maximum), the intensity scores representing relative intensities within the flight of wines presented to the panellists.

5.10 Statistical analysis

Statistical analyses were performed by either two-way or three-way ANOVA using a generalised linear model. Means comparisons were performed using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$. All statistics were done using the R statistical software package (ver. 3.3.1, The R Foundation for Statistical Computing) and graphed using 'Prism' software (v. 7, GraphPad Software, La Jolla, CA, USA). Results reported as statistically significant refer to the probability of committing a Type I error at the given significance level (*P*-value) for the means comparisons.

For the wine sensory analysis data, a two-way ANOVA was conducted using the restricted maximum likelihood (REML) method with taster (panellist) used as a random variable and treatment (wine) used as a fixed variable. Principal Component Analysis (PCA) was conducted using the mean values for each significant attribute at $P \leq 0.10$.

6 Results and discussion

6.1 Environmental parameters

Figure 3: Seasonal (Oct 1 – Mar 31) GDDs **(a)** and ripening period (Jan 1 – Mar 31) GDDs **(b)** for the measurement season, 2017-18 (red line), compared to the average for the past 12 years (black line). Data sourced from BOM Coonawarra weather station (Station ID: 026091, 140.82°E, 37.29°S). 'V' indicates approx. 50% véraison (25/01/2018), and 'H' indicates harvest date (21/03/2018).

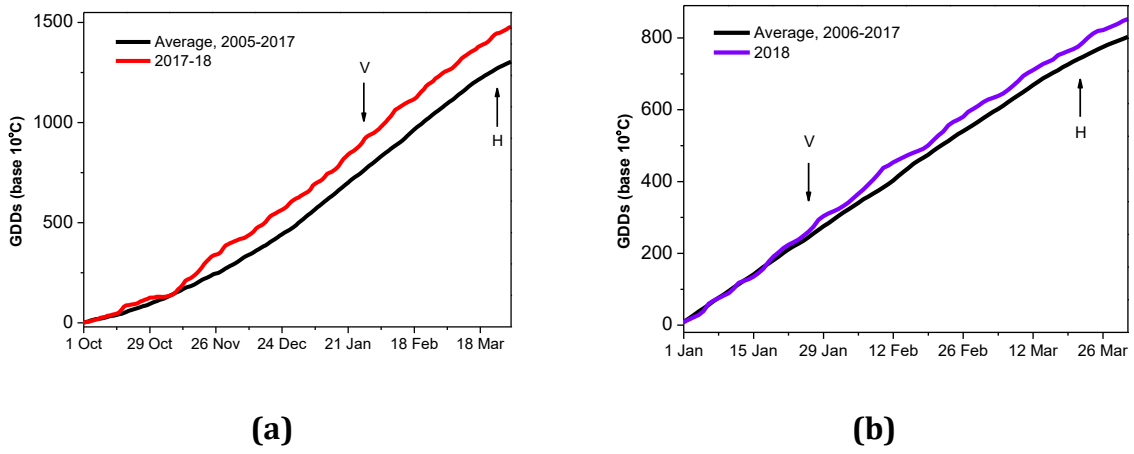
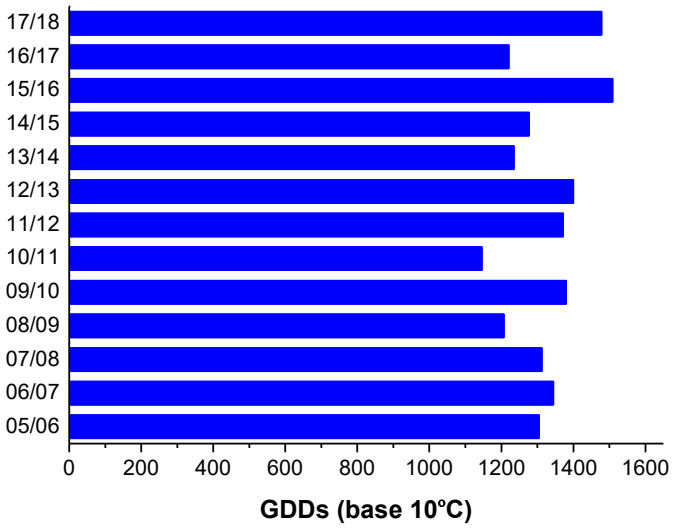


Figure 4: GDD data of past 10 years, 2005-2018. Data sourced from BOM Coonawarra weather station (Station ID: 026091, 140.82°E, 37.29°S).



Seasonal weather conditions as measured by the BOM weather station in Coonawarra indicated that 2017-18 was slightly warmer than the medium-term average of the past 12 years in the region (Figure 3a) and one of the warmest seasons in recent history in Coonawarra, similar to 2015-16 (Figure 4). Between 1 October 2017 and 31 March 2018, approx. 1,479 heat units or growing degree days (GDDs) were accumulated compared to the historical average of 1,309

GDDs, a difference of 168 degree days. Looking at only the berry ripening period between 1 January 2018 and 31 March 2018, the total heat accumulation was approx. 1,479 GDDs compared to the 12-year historical average of 1,305 GDDs, an increase of nearly 174 heat units in the 2017-18 season (Figure 3b). This indicates that much of the heat was received in the latter half of the season, shortly prior to véraison and later. In particular, a heatwave in mid-January (between 17 and 21 January) resulted in considerable heat accumulation around South Australia and would have advanced véraison in many vineyards.

Table 1: Environmental conditions on the four measurements days from véraison to harvest, 2017-2018 in Coonawarra. Data sources from BOM weather station in Coonawarra (Station ID: 026091, 140.82°E, 37.29°S).

Phenological Stage (Date)	**Max. Daily Temp. (°C)**	**Min. Daily Temp (°C)**	**Max. VPD (kPa)**	**Daily Solar Radiation (MJ/m^2)**	**Daily ET_0 (mm)**
Véraison (31/01/2018)	21.1	8.9	1.63	20.2	4.9
Post-véraison (18/02/2018)	22.5	11.6	0.98	7.5	2.5
Pre-harvest (07/03/2018)	32.3	12.0	3.63	22.6	6.0
Harvest (21/03/2018)	26.7	8.5	2.21	18.2	5.3

Detailed environmental conditions during the four measurements timepoints are shown in Table 1: Environmental conditions on the four measurements days from véraison to harvest, 2017-2018 in Coonawarra. Data sources from BOM weather station in Coonawarra (Station ID: 026091, 140.82°E, 37.29°S). Daily maximum temperatures ranged from 21 °C around véraison to 32 °C shortly before harvest. Nights were generally cool during this time with minimum temperatures around 8-12 °C. Atmospheric demand for water vapour (or dryness) as indicated by the vapour pressure deficit (VPD) ranged between 1.0 kPa and 3.6 kPa. The low end of this range was on a relatively cloudy day as indicated by the low solar radiation, 7.5 MJ/m^2. Resulting from this was also low reference evapotranspiration (ET_0) of only 2.5 mm (25 kL/ha).

Microclimatic conditions within the canopies of each treatment and timepoint are presented in Table 2 for the two vineyard sites. Block 'P' has the north-south (N-S) oriented rows, while Block

'Q' has the east-west (E-W) oriented rows. Higher daily maximum temperatures were generally recorded in the N-S oriented vineyard (Block 'P'), across all timepoints that resulted in higher VPDs. The véraison timepoint was the coolest of the three measurement dates, with moderate VPD values between 2.2 and 2.4 kPa in both blocks. Differences between blocks were more apparent during the post-véraison period; maximum canopy temperatures and VPDs were higher in the N-S block by as much as 2 °C and 0.5 kPa, respectively, compared to the E-W block.

The effect of water stress, high or mild, on canopy microclimate was not apparent, but the effect of leaf removal was marked. Leaf removal resulted in higher temperatures, by as much as 2 °C at the véraison timepoint. This validates that the leaf removal treatments were having the desired effect of increasing canopy temperatures. Vines with leaf plucking also had higher VPD levels, by 0.2-0.4 kPa, compared with non-leaf removal vines. In blocks with higher water stress, the leaf removal treatment effect on canopy temperature and VPD was magnified; differences by as much as 0.4 °C and 0.3 kPa were respectively observed due to the more exposed canopy.

Table 2: Daily microclimate averages (in-canopy environmental conditions) for individual experimental treatments and sites (Block P=N-S row orientation; Block Q=E-W row orientation) during the 2017-18 season in Coonawarra.

Row Orientation	**N-S (Block P)**				**E-W (Block Q)**			
Water Stress	**High**		**Mild**		**High**		**Mild**	
Leaf Removal	**LR**	**NLR**	**LR**	**NLR**	**LR**	**NLR**	**LR**	**NLR**
Véraison								
Max Temp (°C)	27.4	25.5	27.0	25.5	26.9	26.0	26.5	26.0
Min Temp (°C)	12.6	12.5	12.6	12.5	12.3	12.3	12.3	12.3
Max VPD (kPa)	2.4	2.2	2.4	2.2	2.4	2.3	2.2	2.3
Post-véraison								
Max Temp (°C)	38.9	37.4	39.0	37.4	36.6	37.1	37.3	37.1
Min Temp (°C)	15.5	15.6	15.5	15.6	15.2	15.3	15.3	15.3
Max VPD (kPa)	5.0	4.6	5.0	4.6	4.4	4.6	4.6	4.6
Pre-harvest								
Max Temp (°C)	36.5	35.9	36.3	35.9	36.7	35.9	35.5	35.9
Min Temp (°C)	12.0	12.1	12.1	12.1	11.5	11.6	11.5	11.6
Max VPD (kPa)	4.6	4.4	4.5	4.4	4.6	4.4	4.3	4.4

6.2 Physiological measurements

6.2.1 Canopy light interception

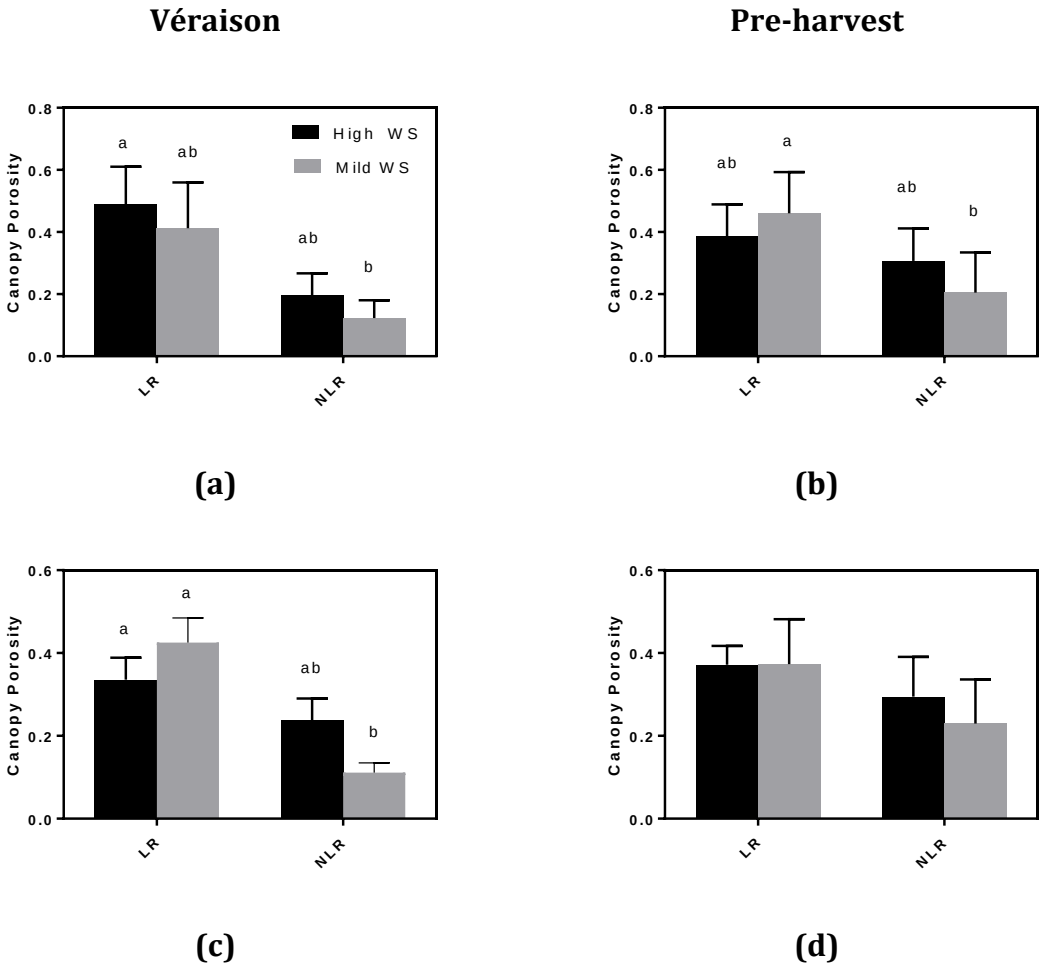


Figure 5: Canopy porosity (fraction of 1.0) for the N-S row orientation (a, b), and E-W row-orientation (c, d). Dark/black bars represent high vine water stress levels while grey bars indicate mild vine water stress levels over the period between véraison and pre-harvest. Different letters indicate significant differences between means of all treatments based on Tukey's HSD test ($P \leq 0.05$). Data for the post-véraison timepoint not shown.

The leaf removal treatment was applied at mid-véraison (25 January 2018) in both vineyards, while deficit irrigation commenced in early January. Canopy light interception in the bunch zone as indicated by canopy porosity indicated that the leaf removal treatment increased porosity by more than two-fold to over 40% in both blocks (Figure 5). Differences between treatments were greater at véraison than at pre-harvest due to the growth of lateral (secondary) shoots that were stimulated by leaf removal, and consequent 're-filling' of the canopy gaps. Row orientation did not affect light interception in the bunch zone, however, the E-W oriented rows had lower

porosity values compared to the N-S rows. By pre-harvest, these differences were reduced. Water stress did not affect canopy porosity in either block, and there was no interaction with leaf removal.

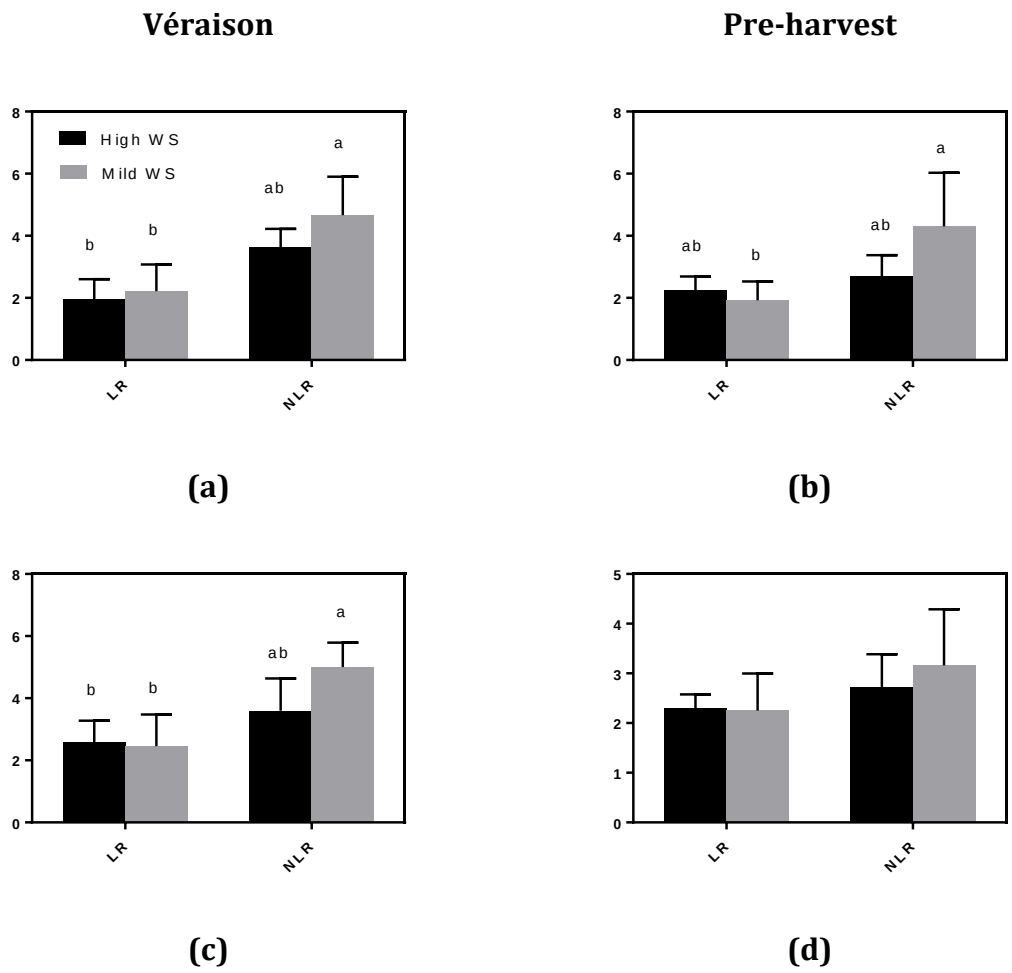


Figure 6: Canopy Leaf Area Index (LAI, unitless) for the N-S row orientation (a, b), and E-W row-orientation (c, d). Dark/black bars represent high vine water stress levels while grey bars indicate mild/low vine water stress levels over the period between véraison and pre-harvest. Different letters indicate significant differences between means of all treatments based on Tukey's HSD test ($P \leq 0.05$). Data for the post-véraison timepoint not shown.

Characterisation of leaf area index (LAI), which is the total leaf area of a canopy per unit ground area, using ceptometry revealed results consistent with canopy porosity measurements. Around véraison, leaf removal had a significant effect on lowering LAI, as expected, by as much as half (Figure 6 a, c). In the N-S block, there were also significant differences in LAI between the same treatments although less than at véraison. The effect of water stress was insignificant on LAI values despite the higher water stressed vines having slightly lower LAI values. Finally, row orientation did not have an effect on LAI.

6.2.2 Soil and vine water status

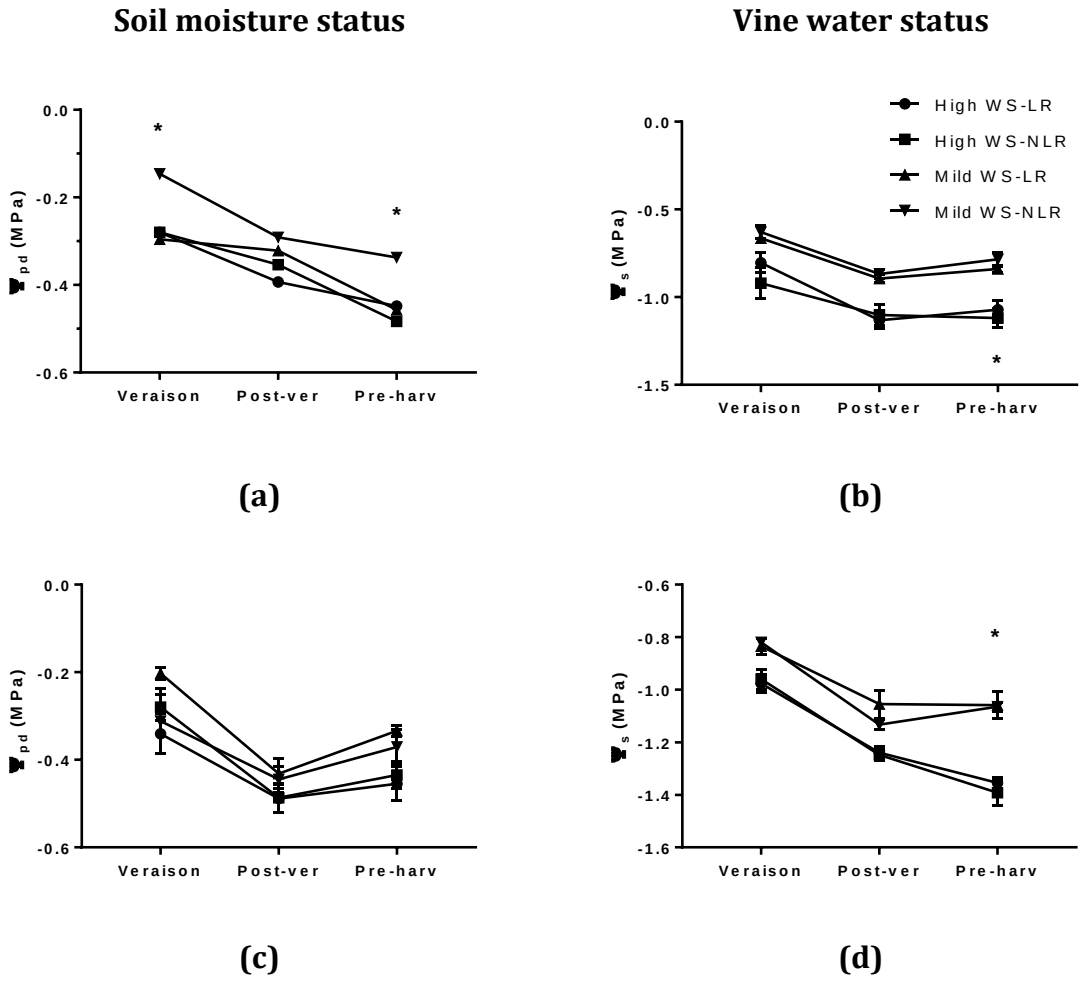


Figure 7: Soil and vine water status over the ripening period for the N-S row orientation (a, b), and E-W row-orientation (c, d). Soil moisture is estimated as the pre-dawn leaf water potential (Ψ_{pd}) while vine water status is represented as the midday stem water potential (Ψ_s). Dark/black bars represent high vine water stress levels while grey bars indicate mild/low vine water stress levels. Asterisks indicate significant differences between means of all treatments based on Tukey's HSD test ($P \leq 0.05$).

Imposition of deficit irrigation, where 50% of the drip emitters were plugged, resulted in a gradual dry down of the soil in both vineyards as shown in Figure 7 (a, c) of the predawn leaf water potential (Ψ_{pd}). Ψ_{pd} is an estimate of soil moisture in the wettest portion of the rootzone. Ψ_{pd} values decreased from -0.1 MPa down to -0.5 MPa between the véraison to pre-harvest period in the N-S block, and -0.2 to 0.45 MPa in the E-W block. Differences were significant at the véraison and pre-harvest timepoints in the N-S block, particularly between the low/mild water stress and the other treatments (Figure 7a). In the E-W oriented block, greater soil moisture deficits were

generated at the post-véraison timepoint compared to the N-S block; this was consistent with the objectives of the grower.

Soil moisture deficits translated in all cases to vine water stress; the low/mild water stress treatments had higher midday stem water potentials (Ψ_s) compared to the high water stress treatments, particularly at pre-harvest. The N-S block had lower vine water stress compared to the E-W block at all timepoints; N-S rows had Ψ_s values ranging from -0.6 MPa to -1.0 MPa, indicating low to moderate water stress, while the E-W vines had Ψ_s values ranging from -0.8 MPa to nearly -1.4 MPa, indicating that relatively high water stress was reached.

6.2.3 Leaf and bunch temperatures

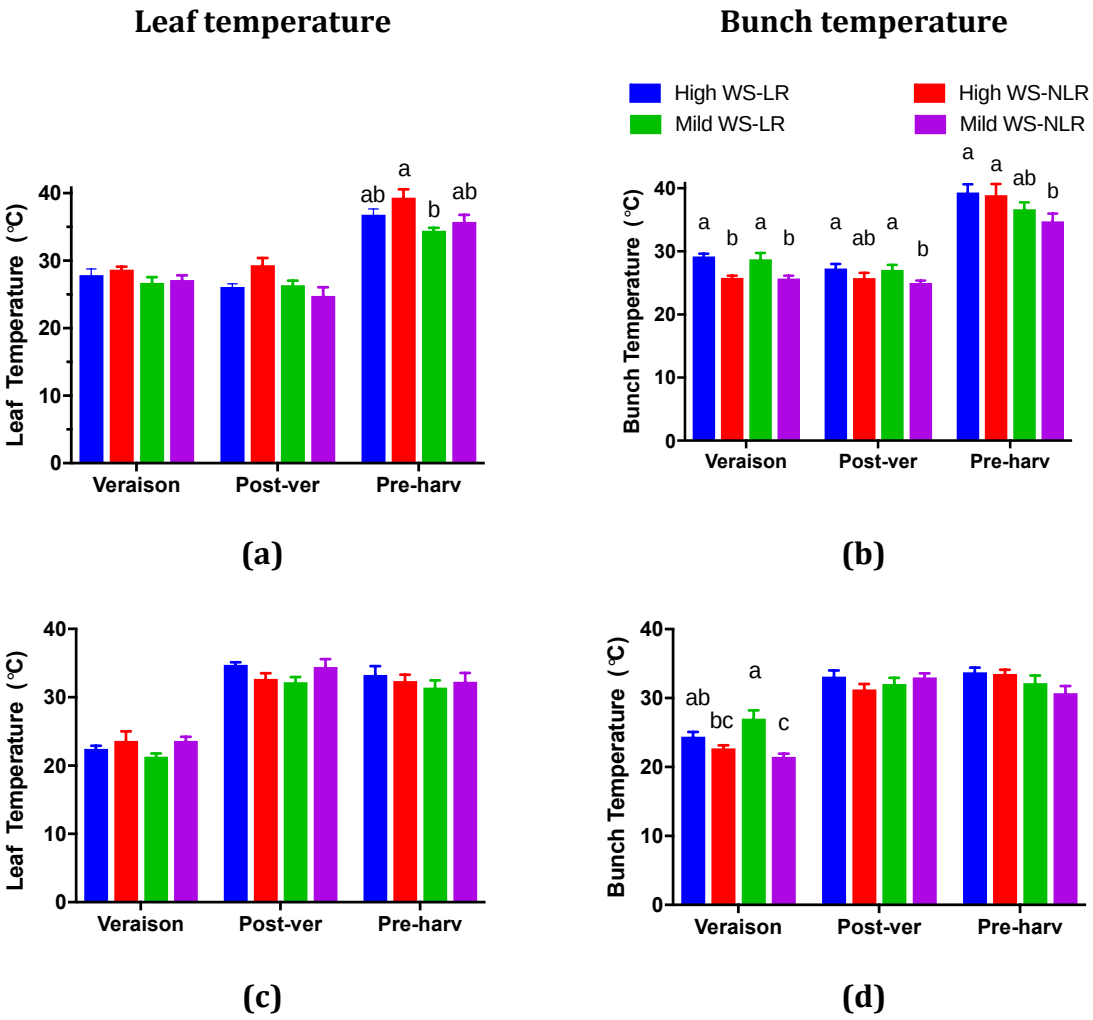


Figure 8: Leaf and bunch surface temperatures as measured with a hand-held IR thermometer for the N-S row orientation (a, b), and E-W row-orientation (c, d). Different letters indicate significant differences between means of all treatments within a vineyard based on Tukey's HSD test ($P \leq 0.05$).

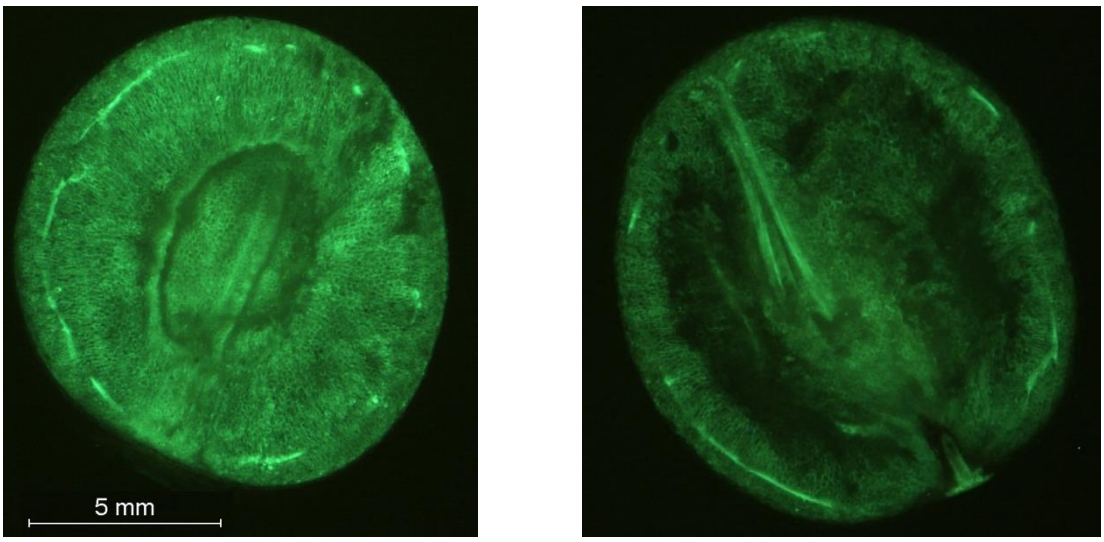
The surface temperature of leaves and bunches was measured using a hand-held spot IR thermometer. In general, it was observed that leaves were cooler than bunches around véraison, but bunches became gradually warmer following véraison, and were warmer than leaves at the post-véraison timepoint.

No differences were observed in leaf temperature between the various leaf removal and water stress treatments at véraison and post-véraison (Figure 8 a, b). However, by pre-harvest, the N-S oriented vineyard had higher leaf temperatures in the non-leaf removed vines, independent of water status. The leaf removal treatment with mild water status had the lowest temperature of all treatments possibly due to higher transpiration rates (on a whole vine basis) resulting in evaporative cooling, as well as fewer leaves resulting in more convective cooling of the canopy. In the post-véraison and pre-harvest timepoints, all canopies were considerably warmer in both vineyards, which is consistent with the environmental conditions at those timepoints (Table 1), but also likely due to lower water availability (Figure 7) and reduced transpirational cooling.

Bunch temperatures were significantly altered due to both leaf removal and water deficits. In the N-S vineyard, vines with leaf removal had higher bunch temperatures at all timepoints, in some cases, significantly (Figure 8 c, d). Differences in bunch temperature were as much as 2 °C in those more porous canopies with higher light infiltration into the bunch zone. In all treatments, bunch temperature was considerably higher at the pre-harvest timepoint, which was likely related to the higher ambient temperature at that time compared to the previous timepoints. In the E-W oriented block, bunch temperature was higher in the leaf removal treatments but only at the véraison timepoint. Water stress did not impact bunch temperature in this block although differences between vine water status did not appear until pre-harvest (Figure 7d).

Row orientation seemed to have a marked effect on leaf and bunch temperatures. In general, it was observed that the E-W rows had cooler canopies and fruit (the post-véraison differences were statistically non-significant). This suggests that E-W oriented rows are a better option than N-S oriented rows in warmer climates. The primary reason for this difference is related to the cosine law; during the warmest part of the day, mid-afternoon, the angle of the sun relative to the canopy is acute and hence contributes less to radiative heating (sensible heat). In contrast, the incident angle of solar radiation in N-S rows is closer to perpendicular (less acute), contributing to greater heating.

6.3 Assessment of berry cell vitality



(a) **(b)**

Figure 9: Fluorescein diacetate (FDA) stained grape berries images using fluorescence microscopy (with UV light). **(a)** healthy berry; **(b)** shrivelled berry with cell death. Photos on the right show the extent of cell death; green regions represent healthy tissue, dark regions represent dead cells. Scale bar = 5 mm.

The extent of berry shrivel was quantified using two independent techniques simultaneously on the same berry. The first of these techniques is based on fluorescence microscopy using a vital stain, FDA. The stain permeates cell membranes and gets cleaved by cytoplasmic esterases that produce fluorescein that fluoresces under blue light. The presence of fluorescein is a reliable indicator of cell membrane integrity as well as the presence of active esterases in the cytoplasm, both of which are indicators of vital cells (Krasnow et al. 2008). We adopted this staining technique to assess the vitality of grape berry cells as affected by the heat and water stress treatments. Fluorescence microscopy indicated that shrivelled berries had a greater proportion of non-vital (i.e. dead) cells in the mesocarp (Figure 9b) compared to non-shrivelled or healthy berries (Figure 9a). Cell death appeared to be higher in the regions around the seed, i.e. in the locule.

Our assessment of the vitality of cells in berries obtained from different treatments and vineyards did not show large differences in cell death across various timepoints (Table 3). Water stress appeared to result in higher cell death (lower cell vitality) where no leaf removal was done suggesting that increased canopy size and demand for water would lead to some degree of berry shrivelling, possibly from backflow of water from the berry to the leaves via xylem. At harvest, higher cell death appeared to be seen in leaf removed and high water stress vines in the E-W block

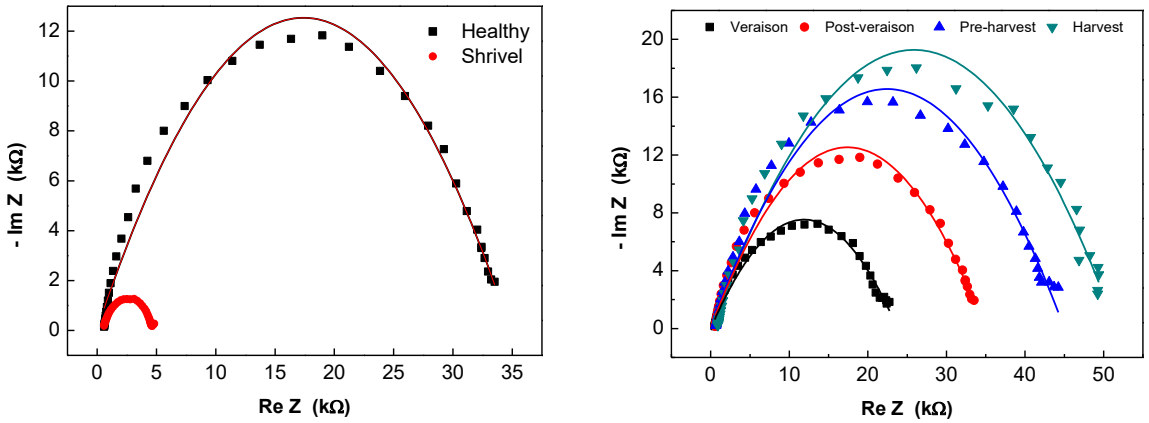
but not in the N-S block; this could be the result of higher vine water stress in that block compared to the N-S block.

Electrical impedance spectroscopy was used to quantify the relative extent of cell death by measuring various equivalent circuit parameters such as cell membrane resistances and capacitances. Measurement of impedance of berries obtained from our trial indicated that little to no difference in impedance parameters was present at any timepoint or vineyard (Table 3). In one case, the total impedance (Z) measured at 36 Hz was significantly higher in the high water stress vines without leaf removal compared to the mild water stress vines with leaf removal. The progressive increase in R_e and C_m during berry ripening has been previously observed (Caravia et al. 2015). Our data indicate that this increase stops 2-3 weeks prior to harvest and then starts decreasing, indicative of a breakdown of membrane integrity. A comparison of healthy berries and shrivelled berries indicated that Z values of healthy berries ranged from 22-34 kΩ while for shrivelled berries, Z ranged from 8-11 kΩ (data not presented). Healthy berries also show higher reactance values for various resistance values (Figure 10a) and this relationship, given by the Cole-Cole (or Nyquist) plot, changes during berry ripening (Figure 10b).

Table 3: Cell vitality assessment by FDA staining (% living cells) and electrical circuit parameters (R_e, R_i, C_m and Z) for Cabernet Sauvignon berries based on impedance spectroscopy analysis from various treatments and trial sites in Coonawarra at several maturity stages of berry development in the 2017-18 season. Different letters indicate significant differences between means of all treatments within a vineyard based on Tukey's HSD test ($P \leq 0.05$). 'Z' (total impedance) values reported at 36 Hz. n=6.

Row Orientation	**N-S**				**E-W**			
Water Stress	**High**		**Mild**		**High**		**Mild**	
Leaf Removal	**LR**	**NLR**	**LR**	**NLR**	**LR**	**NLR**	**LR**	**NLR**
Véraison								
Living cells (%)	41 ± 14	47 ± 11	71 ± 9	51 ± 17	29 ± 10b	66 ± 8a	29 ± 7b	65 ± 13a
R_e (kΩ)	15.5 ± 5.4	18.2 ± 4.4	19.1 ± 3.4	14.1 ± 3.9	13.2 ± 3.8	20.2 ± 2.0	12.5 ± 3.3	14.2 ± 2.7
R_i (Ω)	0.9 ± 0.3	0.7 ± 0.2	0.4 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.0	0.7 ± 0.1	0.4 ± 0.0
C_m (nF)	2.4 ± 0.8	3.3 ± 0.7	3.8 ± 0.4	3.0 ± 0.7	1.9 ± 0.5	4.3 ± 0.3	2.4 ± 0.5	3.9 ± 0.7
Z (kΩ)	15.5 ± 5.4	17.9 ± 4.3	19.1 ± 3.4	14.1 ± 3.9	13.3 ± 3.8ab	20.1 ± 1.9a	12.3 ± 3.3b	14.1 ± 2.6ab
Post-véraison								
Living cells (%)	52 ± 6	60 ± 12	45 ± 14	60 ± 9	56 ± 8	51 ± 16	64 ± 11	51 ± 10
R_e (kΩ)	25.9 ± 4.5	27.9 ± 6.5	11.3 ± 5.5	27.0 ± 6.9	39.3 ± 3.6	25.4 ± 6.9	26.8 ± 5.7	24.8 ± 5.7
R_i (Ω)	0.6 ± 0.0	0.6 ± 0.0	0.9 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
C_m (nF)	3.6 ± 0.3	2.8 ± 0.5	1.6 ± 0.6	3.2 ± 0.4	3.2 ± 0.2	3.4 ± 0.2	3.4 ± 0.4	3.7 ± 0.8
Z (kΩ)	25.5 ± 4.4	27.7 ± 6.4	11.3 ± 5.6	26.7 ± 6.8	38.6 ± 3.6	25.1 ± 6.8	26.5 ± 5.6	24.6 ± 5.7
Pre-harvest								
Living cells (%)	45 ± 11	73 ± 9	48 ± 12	64 ± 11	57 ± 10	39 ± 13	43 ± 9	39 ± 14
R_e (kΩ)	24.2 ± 9.9	22.3 ± 6.7	18.2 ± 6.9	28.7 ± 7.3	23.9 ± 7.8	23.5 ± 5.4	18.7 ± 8.3	33.7 ± 13.0
R_i (Ω)	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.5 ± 0.0	0.8 ± 0.2	0.6 ± 0.0	0.7 ± 0.0
C_m (nF)	2.9 ± 0.1	2.9 ± 0.2	2.5 ± 0.4	2.9 ± 0.5	2.8 ± 0.3	2.8 ± 0.4	2.3 ± 0.6	2.5 ± 0.2
Z (kΩ)	23.9 ± 9.6	22.4 ± 6.8	18.0 ± 0.7	28.7 ± 7.3	23.8 ± 7.9	23.4 ± 5.4	18.5 ± 8.2	32.7 ± 12.3
Harvest								
Living cells (%)	50 ± 15	43 ± 14	22 ± 4	49 ± 10	34 ± 15	36 ± 20	57 ± 8	53 ± 9
R_e (kΩ)	11.5 ± 4.6	7.6 ± 2.5	9.1 ± 3.1	21.2 ± 14.5	7.8 ± 2.3	7.5 ± 2.9	11.4 ± 4.4	11.7 ± 5.7
R_i (Ω)	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0

C_m (nF)	2.7 ± 0.4	2.7 ± 0.5	2.5 ± 0.2	2.9 ± 0.1	2.8 ± 0.3	2.9 ± 0.4	3.1 ± 0.3	2.9 ± 0.1
Z (kΩ)	11.5 ± 4.6	7.6 ± 2.5	9.1 ± 3.1	5.9 ± 2.8	7.7 ± 2.2	7.5 ± 2.9	11.4 ± 4.4	11.5 ± 5.6



(a) **(b)**

Figure 10: (a) Cole-Cole (Nyquist) plot of healthy (black dots) vs. shrivelled (red dots) berries with polynomial fitting at post-véraison. R^2 values: shrivel berry (1.00), healthy berry (0.98)**; (b**) Cole-Cole plot of a healthy berry at various stages of berry ripening with polynomial fitting. R^2 values: véraison *(*0.97), post-véraison *(*0.97), pre-harvest (0.98), harvest (0.98).

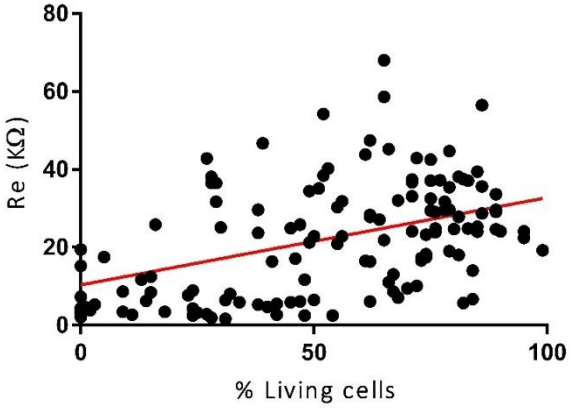


Figure 11: Relationship between cell vitality with FDA staining (% living cells) and extracellular resistance (R_e) as measured with impedance spectroscopy. Each point represents an individual berry that was analysed using both methods. R^2=0.16.

The two methods used in this study, fluorescence microscopy and impedance spectroscopy, to quantify berry shrivel associated with cell death did not appear to be well correlated (Figure 11). This could be due to several factors including the decrease in R_e as a function of soluble solids as well as the variations in berry size affecting the spacing between the impedance electrodes (Caravia et al. 2015).

While these two methodologies have potential as tools to assess cell vitality, clearly more refinement is required before they can be used as reliable measures of berry shrivel and cell death.

6.4 Yield components

Table 4: Yield components of harvested fruit including shrivelled fruit. Yield reported on a cordon length basis due to lack of uniformity of vine size (cordon length). Different letters indicate significant differences of means between all treatments within a vineyard based on Tukey's HSD test ($P \leq 0.05$).

	N-S				
	High Water Stress		**Mild Water Stress**		**Vineyard Effect**
	LR	**NLR**	**LR**	**NLR**	***P*-value**
Average Bunch Weight (g)	47.1 ± 3.3b	55.1 ± 8.3ab	60.4 ± 3.3ab	71.6 ± 5.4a	ns
Average Berries per Bunch	50 ± 6	60 ± 10	54 ± 3	55 ± 6	ns
Average Berry Weight (g)	0.96 ± 0.09	0.93 ± 0.07	1.13 ± 0.09	1.33 ± 0.08	ns
Yield (kg/m)	1.76 ± 0.12	2.12 ± 0.45	1.46 ± 0.21	1.95 ± 0.24	ns
Shrivelled Yield (%)	6 ± 1	10 ± 3	8 ± 1	3 ± 1	0.00
Shrivelled Bunches (%)	7 ± 0	9 ± 2	9 ± 2	3 ± 1	0.00
Total Bunches per m	37.47 ± 0.91	38.72 ± 5.62	24.83 ± 4.43	28.08 ± 5.00	0.02
	E-W				
	High Water Stress		**Mild Water Stress**		**Vineyard Effect**
	LR	**NLR**	**LR**	**NLR**	***P*-value**
Average Bunch Weight (g)	61.5 ± 6.0	71.2 ± 8.3	55.2 ± 1.5	64.3 ± 10.6	ns
Average Berries per Bunch	68 ± 4	73 ± 6	49 ± 2	59 ± 7	ns
Average Berry Weight (g)	0.91 ± 0.06	0.97 ± 0.05	1.12 ± 0.02	1.08 ± 0.05	ns
Yield (kg/m)	1.4 ± 0.06	1.67 ± 0.22	1.57 ± 0.20	1.52 ± 0.34	ns
Shrivelled Yield (%)	23 ± 8	23 ± 7	23 ± 4	19 ± 11	0.00
Shrivelled Bunches (%)	24 ± 9	24 ± 5	20 ± 4	18 ± 11	0.00
Total Bunches per m	23.59 ± 2.57	24.32 ± 4.54	28.3 ± 3.15	23.76 ± 4.80	0.02

At harvest, yield components were collected or calculated as shown in Table 4. There was considerable bunch stem necrosis observed in the vineyard in both blocks. This fruit was not included in the harvest nor was it included in the determination of proportion of shrivelled fruit. Bunch stem necrosis is quite easy to distinguish from shrivelled berries as the former tends to look raisined and also light purple in colour whereas the shrivelled berries appear darker purple in colour.

Yield was not significantly different between vineyards. However, the E-W rows had a significantly higher percentage of shrivelled fruit, both from a yield and bunch count standpoint, compared to the N-S vineyard, and in all treatments. The N-S vineyard had between 3-10% shrivelled fruit at harvest compared to 19-23% in the E-W vineyard. This shows the influence of row orientation on berry shrivel and the benefit of planting in the E-W orientation, especially in warmer regions. Shrivel did not affect either average bunch weight or average berry weight.

However, water stress did have an impact; grapes from high water stress vines were approx. 20% and 30% lower in berry weight compared to the mild water stress vines, in the E-W and N-S blocks, respectively.

No effect of leaf removal was observed in berry weight, bunch weight, or yield. In the N-S block, under mild water stress, leaf removal appeared to increase the incidence of shrivelled fruit by nearly three-fold. Results of our study indicate that although yield was not directly impacted by either late season water stress (post-véraison) or leaf removal (at véraison), higher light exposure and temperatures in the bunch zone from both leaf removal and water stress could have contributed to the increase in shrivelled fruit observed, particularly in the N-S block.

6.5 Must and wine composition

6.5.1 Basic chemical composition – must

Table 5: Must chemical composition of harvested fruit from the two trial sites. Different letters indicate significant differences between means of all treatments within a vineyard based on Tukey's HSD test ($P \leq 0.05$).

Row Orientation	**N-S**				**E-W**				**Vineyard Effect (*P*-value)**
Water Stress	**High**		**Mild**		**High**		**Mild**		
Leaf Removal	**LR**	**NLR**	**LR**	**NLR**	**LR**	**NLR**	**LR**	**NLR**	
TSS (°Brix)	25.6±0.8	25.9±0.4	24.6±0.1	24.2±5.1	29.4±1.8	27.5±0.8	26.4±0.4	23.3±0.5	0.00
Titratable Acidity (g/L)	3.7±0.3	4.6±0.8	4.8±0.2	6.4±0.7	4.6±0.2	4.7±0.6	4.6±0.4	5.4±0.9	ns
pH	3.48±0.07	3.5±0.09	3.5±0.01	3.42±0.04	3.83±0.1	3.7±0.11	3.53±0.04	3.63±0.07	0.00
Colour (a.U.)	1.82±0.7	1.77±0.17	1.3±0.33	1.16±0.15	3.71±1.27	2.45±0.51	2.03±0.37	1.68±0.19	0.01
Hue (a.U.)	1.34±0.12	1.29±0.09	1.28±0.03	1.58±0.09	1.12±0.07	1.39±0.09	1.43±0.1	1.5±0.14	ns
Total Anthocyanins (mg/L)	5.0±1.0ab	15.4±5.0a	7.7±3.4ab	2.8±0.2b	10.0±2.0	20.7±9.0	12.7±7.7	6.7±2.4	ns
Total Polyphenols (mg/L)	317±67	334±62	260±25	304±29	558±162	342±28	325±79	280±42	ns

Yeast Assimilable Nitrogen (mg/L)	100±5	118±25	76±14	117±55	84±18	72±23	25±14	35±18	0.02

Fruit samples were analysed for basic chemical composition at each timepoint starting at post-véraison with the data from only the harvest sample presented in Table 5. The harvested fruit was crushed and analysis of the must was conducted for chemical composition prior to fermentation. Total soluble solids (TSS) were higher in the E-W rows in the water-stressed vines compared to all the other treatments or N-S block, which were similar to each other. Differences of between 2-4 °Brix were observed in the fruit between the E-W water stressed vines' fruit and N-S vines or non-water stressed vines from either block. Titratable acidity was the same in all treatments and blocks (range 3.7 – 6.4 g/L), as was the pH (range 3.42 – 3.83), colour intensity (range 1.2 – 3.7 a.U.), and hue or tint (range 1.1 – 1.5 a.U.).

Total anthocyanins in the musts were significantly higher in the water stressed vines that did not have leaf removal in the N-S block. Those vines resulted in musts with between 15-19 g/L of anthocyanins compared to mild water stressed vines that produced musts with under 3 g/L of anthocyanins. In all blocks (independent of row orientation), leaf removal resulted in greater anthocyanin concentration in the fruit. Total polyphenols in the musts were similar across treatments although the range was quite large. While there were no differences between the two blocks (row orientation effect), the water stressed vines had slightly higher polyphenol concentrations in both blocks. The range of polyphenols measured in the musts were 260 – 558 mg/L. Finally, yeast assimilable nitrogen content in the musts was found to be significantly higher in the N-S block compared to the E-W block. Within the block, however, the water stress and leaf removal treatments did not impact YAN concentrations.

6.5.2 Basic chemical composition – wine

Table 6: Basic wine chemical composition from the two trial sites. Wines were made using different proportions of shrivelled fruit independently of vineyard treatments. Different letters indicate significant differences between means of all treatments within a vineyard based on Tukey's HSD test ($P \leq 0.05$).

Row Orientation	**N-S**	**E-W**	**Vineyard Effect**

Shrivelled Fruit (%)	**0**	**25**	**50**	**0**	**25**	**50**	***P*-value**
Ethanol (% v/v)	12.0±0.1	12.4±0.0	12.3±0.1	12.6±0.1 b	13.0±0.0 a	13.1±0.1 a	0.00
Volatile Acidity (g/L)	0.64±0.0	0.64±0.0	0.64±0.0	0.64±0.0 a	0.51±0.0 b	0.52±0.0 ab	0.04
pH	3.88±0.02 a	3.69±0.02 b	3.66±0.03 b	4.00±0.01	3.92±0.00	3.95±0.03	0.00
Titratable Acidity (g/L)	6.0±0.1	6.2±0.1	6.1±0.3	6.0± 0.1	5.7±0.1	5.7±0.1	ns
Colour	0.73±0.01 a	0.62±0.03 b	0.61± 0.01 b	0.76±0.01	0.75±0.01	0.78±0.01	0.00
Hue	10.01±0.31 b	13.76±0.47 a	12.64±0.06 a	11.43±0.86	12.53±0.97	12.52±0.11	0.01
Total Anthocyanins (mg/L)	294±29	294±9	290±12	338±5 b	355±3 ab	369±4 a	ns
Total Polyphenols (mg/L)	1919±20	2026±132	2116±11	2264±65	2472±85	2340±83	ns

One of the primary objectives of the study was to assess the effect of shrivelled fruit on Cabernet Sauvignon wine composition and quality. Following harvest, three different wines were produced from each vineyard, each wine with varying proportions of shrivelled bunches: 0%, 25% and 50%. A bunch was considered shrivelled if three or more berries in that bunch were observed to be shrivelled at harvest. Prior to fermentation, all musts were adjusted to the same TSS values.

Differences were found between the vineyards and between the treatments. The E-W block had higher alcohol, pH and hue, whereas the N-S block had higher volatile acidity, total acidity and colour intensity. Within the N-S block, wines with clean (0% shrivelled) fruit had significantly higher pH and colour intensity, and lowest hue compared to wines made with 25% or 50% shrivelled fruit. There was no effect of shrivelled fruit on wine anthocyanins or polyphenols.

In the E-W block, wines without shrivelled fruit resulted in lowest ethanol and highest VA. Total anthocyanins were significantly higher in the wines made from shrivelled fruit at both levels, 25% and 50%, while polyphenols were not significantly different between treatments or vineyards. Anthocyanins were slightly higher in the N-S block compared to the E-W block, likely due to the

higher light exposure levels (Figure 5) and temperatures (Figure 8) measured in those vines and bunches.

6.5.3 Individual anthocyanins – wine

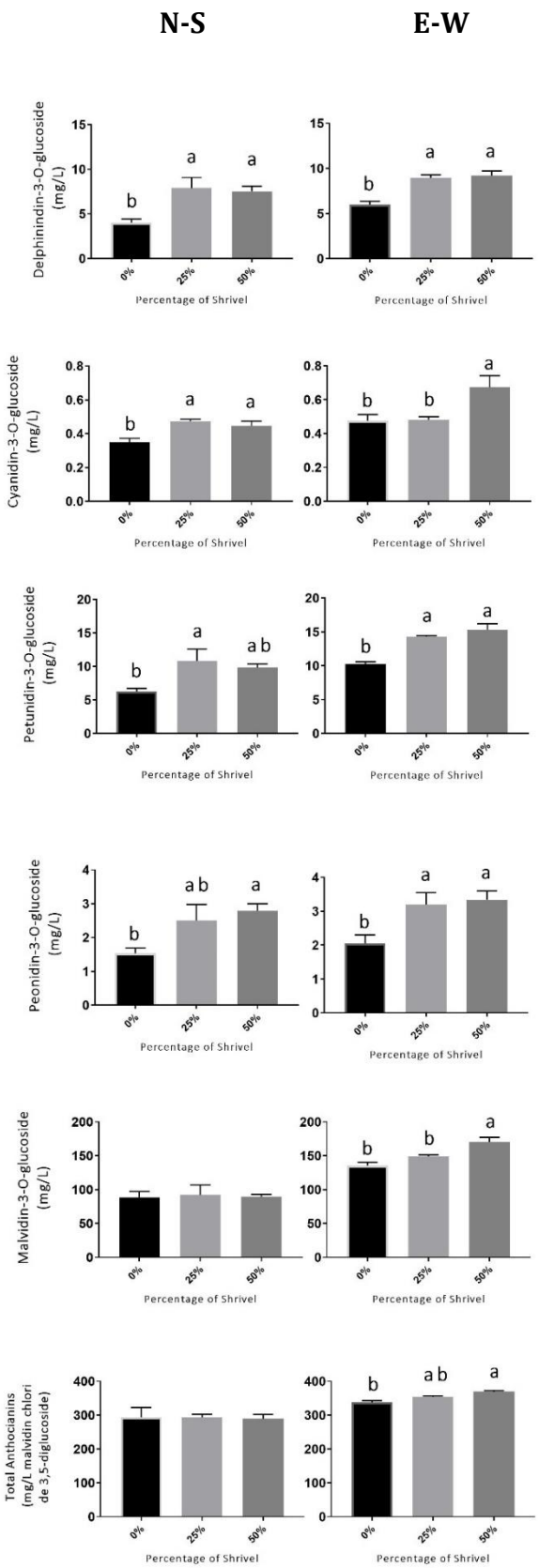


Figure 12: Wine individual anthocyanins based on HPLC analysis. Different letters indicate significant differences between means between all treatments within a vineyard based on Tukey's HSD test ($P \leq 0.05$).

Specific anthocyanins were analysed in the wines using reverse phase HPLC-DAD. The individual anthocyanins quantified were: delphinidin-3-*O*-glucoside (D3OG), cyanidin-3-*O*-glucoside (C3OG), petunidin-3-*O*-glucoside (Pet3OG), peonidin-3-*O*-glucoside (Peo3OG), and malvidin-3-*O*-glucoside (M3OG). There were significant differences between vineyards for all individual anthocyanins, all five being higher in the N-S block compared to the E-W block (Figure 12). M3OG was the predominant anthocyanin in these wines, as expected. Higher anthocyanin levels have been linked directly to higher sun exposure while the increase of malvidin derivates was related to higher temperatures (Tarara et al. 2008).

Comparing the effect of shrivelled fruit on wine individual anthocyanins, it was observed that in the N-S block, all the anthocyanins measured except for M3OG had significant differences between the wines; with the exception of M3OG, the presence of shrivelled fruit in the must resulted in higher anthocyanin concentrations in the wines. However, no differences in any of the anthocyanins were observed between 25% and 50% shrivel wines. Similar results were obtained for E-W vineyard wines; shrivelled fruit resulted in higher anthocyanin concentrations compared to wines made from clean fruit without shrivel. C3OG and M3OG, although not different from each other, were significantly lower in the 0% and 25% shrivel wines compared to the 50% shrivel wines.

Our results suggest that the presence of even small amounts of shrivelled fruit can significantly increase the concentration of anthocyanin glucosides in CAS wine. In particular, shrivelled fruit was seen to increase anthocyanin concentrations by 15-100% depending on the particular anthocyanin compared to wines made from clean or non-shrivelled fruit. It should be noted that our selection of shrivelled bunches that were used to make the shrivelled wines was based on a minimum of only three berries per bunch showing shrivel, which is a relatively small proportion ($\sim$ 5%) of berries on a bunch (average in our trial was $\sim$ 55 berries per bunch).

6.5.4 Individual polyphenols – wine

Table 7: Wine individual polyphenols based on HPLC analysis. All values reported in mg/L. Different letters indicate significant differences between means of all treatments within a vineyard based on Tukey's HSD test ($P \leq 0.05$).

Row Orientation	N-S			E-W			Vineyard Effect
Shrivelled Fruit (%)	**0**	**25**	**50**	**0**	**25**	**50**	***P*-value**
Gallic acid	5.3±0.1	5.6±0.2	5.5±0.1	5.5±0.2	5.6±0.2	5.8±0.1	0.08
Caffeic acid	0.9±0.0	0.8±0.0	0.8±0.0	1.2±0.1	1.1±0.0	1.0±0.1	0.03
Quercetin	3.4±0.1	3.6±0.4	3.7±0.3	4.2±0.2 c	5.4±0.5 b	6.7±0.2 a	0.00
Epicatechin	29.3±0.8	29.3±0.4	32.5±1.6	26.8±0.7	28.1±0.5	28.8±0.7	0.11

Individual polyphenol compounds were analysed in the wine samples using HPLC-DAD; results are presented in Table 7. Phenolic compounds analysed were: caffeic acid (hydroxycinnamic acid), gallic acid (benzoic acid), quercetin (flavonol), and epicatechin (flavan-3-ol; a tannin monomer). Significant differences between vineyards were found for caffeic acid and quercetin; the latter is a powerful UV-protectant for grapes, the synthesis of which is stimulated by high light exposure. Caffeic acid and quercetin were always higher in the N-S block wines than the E-W block wines. Quercetin was the only phenolic compound of this group that had differences between the wines, and only in E-W block wines were the wines significantly different from each other: the 50% shrivel wine had the highest concentration whereas the 0% shrivel wine had the lowest concentration. This indicates the increase in quercetin levels in the berries as a result of shrivelling. No differences were found in the polyphenols in the N-S block in wines made from varying proportions of shrivelled fruit.

In conclusion, the effect of shrivelled fruit on specific wine polyphenols was only to increase the concentration of quercetin that positively correlated with the proportion of shrivelled fruit in the must. The E-W row orientation appeared to positively impact both caffeic acid and quercetin levels in the wine, which has been suggested to be related to canopy light exposure, with higher light exposures increasing the hydrolysis of caftaric acid to caffeic acid (Pagay et al. 2013).

6.5.5 Individual volatiles – wine

Table 8: Organic volatile (aroma) compounds in the CAS wines from the two vineyards. Differences between vineyards given at $P \leq 0.05$.

Family	Compound	Vineyard effect *P*-value

Alcohols	1-hexanol	0.02
	(*Z*)3-hexen-1-ol	ns
	(*E*)3-hexen-1-ol	ns
	2-heptanol	ns
	2-ethyl-1-hexanol	0.01
Benzene derivatives	benzaldehyde	0.01
	benzyl alcohol	ns
	phenylethyl alcohol	0.00
C13 Norisoprenoids	β -damascenone	ns
	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	ns
Esters	methyl hexanoate	ns
	ethyl acetate	ns
	ethyl 2-methylbutanoate	ns
	ethyl decanoate	ns
	ethyl dodecanoate	ns
	ethyl hexanoate	ns
	ethyl tetradecanoate	ns
	isoamyl propanoate	ns
	hexyl acetate	ns
Terpenes	β-citronellol	ns
	geraniol	ns
	linalool	ns
	nerol	ns
	1,8-cineole (eucalyptol)	0.03
Methoxypyrazines	3-isobutyl-2-methoxypyrazine (IBMP)	0.00
Other	acetic acid	ns
	acetaldehyde	ns

Table 8 shows a select list of organic volatile compounds (from over 100 volatiles) analysed by SPME-GC-MS for all the wines from the two vineyard blocks, N-S and E-W. The table shows the effect of row orientation on volatile composition. Differences were found within the vineyards for alcohols, benzene derivates, terpenes and methoxypyrazines (p-values in Table XV). Both 1-hexanol and 2-ethyl-1-hexanol were higher in the N-S block wines, while benzaldehyde and phenylethyl alcohol were higher in E-W block wines. 1,8-cineole (or eucalyptol) was significantly different between vineyards and was found to be ten-fold higher in the E-W block wines compared to the N-S block wines.

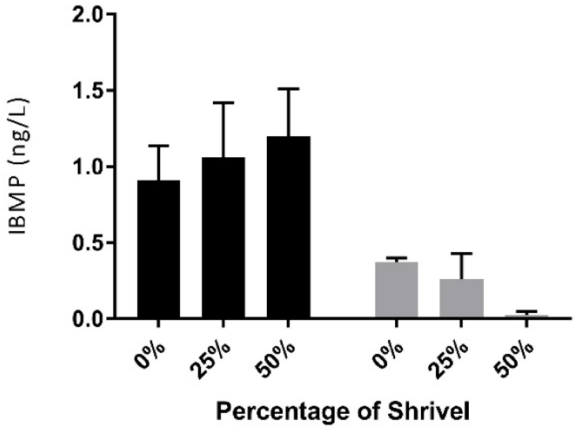


Figure 13: IBMP concentration in the wines made with different proportions of shrivelled fruit from the two vineyards, N-S (black bars) and E-W (grey bars) row orientations. Error bars represent SEM.

IBMP, a methoxypyrazine that contributes green, capsicum and herbaceous aromas that are often undesirable in wine, was significantly higher in N-S wines compared to E-W wines, however, no significant differences were found between wines with different proportions of shrivelled fruit in the must within each block's wines (Figure 13). The higher IBMP levels in the N-S oriented rows was a surprising finding as those vines had higher light exposure levels in the bunch zone as well as higher leaf and bunch temperatures at all timepoints after véraison. Light exposure, via its effect on temperature, has been shown to negatively affect IBMP biosynthesis and/or catabolism in Cabernet Franc (Scheiner et al. 2010), contrary to our findings for CAS in Coonawarra.

In the N-S block, 0% shrivel wines had higher (*E*) 3-hexen-1-ol and (*Z*) 3-hexen-1-ol, which have been negatively associated with flavour and attributed to greenness in the wines (Bindon et al. 2014, Schelezki et al. 2018). However, 0% shrivel wines had the highest pH, possibly due to the lower concentration of TA in shrivelled berries compared to the 25% and 50% shrivelled fruit wines. Acetic acid was also found to be lower in the 0% shrivel wine, which corroborates the literature suggesting that shrivelled berries result in higher VA and benzyl alcohol that relates to viscosity in the wine, which decreases as the proportion of shrivel increases (Schelezki et al. 2018). Wines with 50% shrivel had the highest concentration of isoamyl propanoate, which is related to pepper and woody/tobacco aromas.

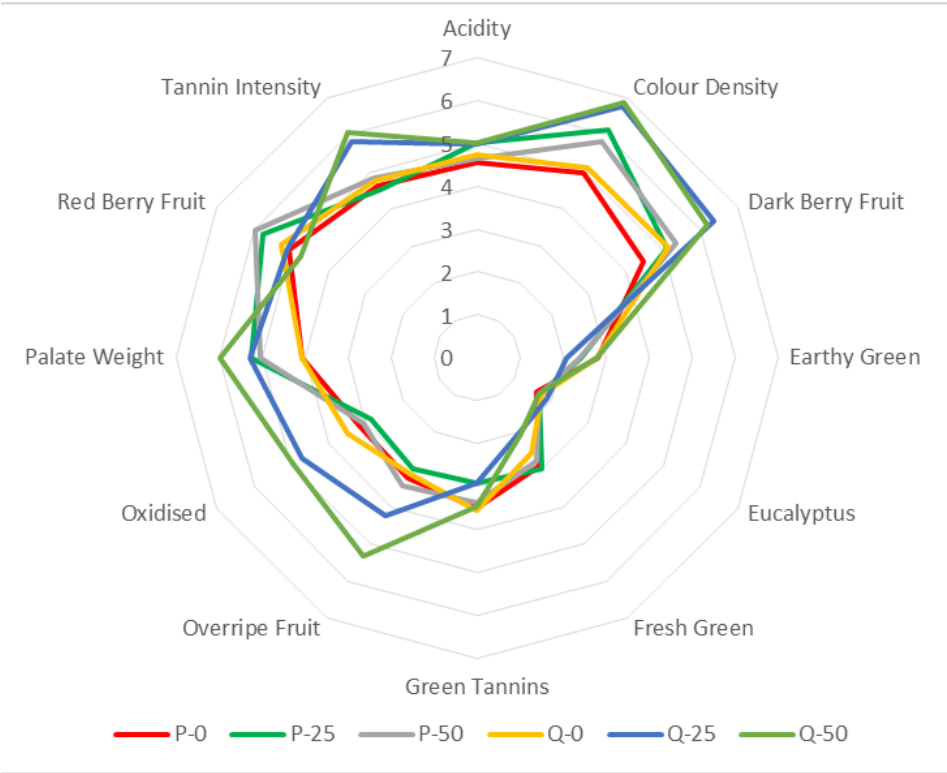
As for some other compounds of relevance, acetic acid was observed to be consistently higher in the shrivelled wines at both levels and in both blocks while acetaldehyde was five-fold or higher in the shrivelled wines from the N-S block (data not shown). This could be related to the breakdown of skins in the shrivelled berry resulting in microbial growth of acetic acid and other undesirable bacteria that are commonly found on grape skins and cause wine spoilage (Kántor et al. 2015). Acetic acid and acetaldehyde are amongst the main spoilage compounds associated with acetic acid bacteria, e.g. *Acetobacter*, *Gluconobacter* (Bartowsky 2009). Further work needs

to be done to investigate the relationship between wine spoilage microbes in the vineyard and the incidence of berry shrivel.

6.6 Wine sensory analysis

A sensory analysis of wines was conducted with an expert panel of tasters that consisted of experienced winemakers and viticulturists from the Coonawarra region on 17 September 2018. Sensory attributes consisted of aroma and flavour compounds, as well as mouthfeel attributes e.g. palate weight and tannin intensity. These specific descriptors are routinely used to evaluate the sensory profiles of Cabernet Sauvignon wines from the Coonawarra region.

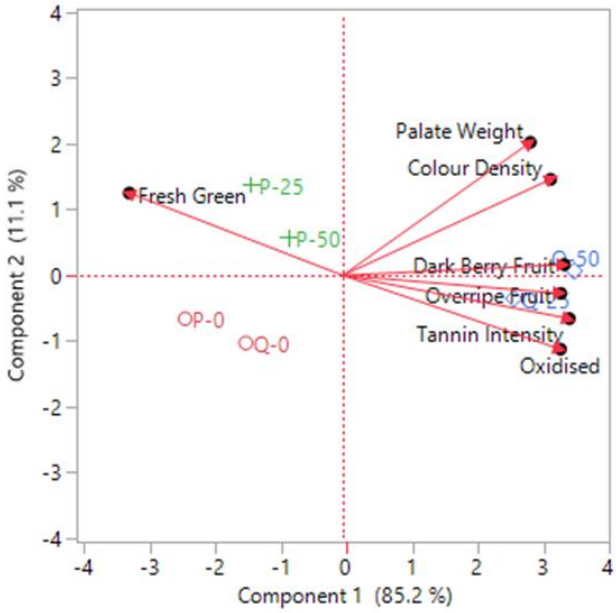
Figure 14: Sensory profiles from the modified descriptive analysis of wines with various proportions (0, 25, 50%) of shrivelled fruit from two vineyards, Punters 'P' (N-S rows) and Quarry 'Q' (E-W rows). (n=11)



The sensory attributes assessed by the panellists were rated on a relative intensity scale (0-9) and their averages shown in Figure 14. Red berry fruit characters were higher in the Punters (N-S) wines with higher levels of shrivelled fruit (25%, 50%) compared to the low/no shrivelled fruit, the latter which were similar to the Quarry (E-W) wines. The Quarry wines tended to have higher intensities of dark berry fruit, tannins, palate weight and colour density compared to the other block and also compared to the same vineyard with no shrivelled fruit. Addition of either 25% or 50% shrivelled fruit to the must resulted in overripe and oxidised characters appearing

in the E-W wines. There are no previous similar studies, making comparisons with the current work difficult. However, it was not unexpected that addition of shrivelled fruit to the must would alter the sensory profile of the resulting wines towards more dark/ripe fruit and oxidised characters since these descriptors are often used for port (Vilela et al. 2015) and sherry (Moyano et al. 2009) style wines.

Figure 15: Principal component analysis bi-plot from the modified descriptive analysis of wines with various proportions (0, 25, 50%) of shrivelled fruit from two vineyards, Punters 'P' (N-S rows) and Quarry 'Q' (E-W rows). (n=11)



A PCA bi-plot with statistically significant relationships between select sensory attributes and wines is shown in Figure 15. 85% of the variation in the dataset was associated with PC1 while 11% of the variation was associated with PC2; together, they accounted for 96% of the total variation in the data set. The attributes dark berry fruit, overripe fruit, tannins, and colour density were on the right side of PC1 and inversely related to fresh green, which was on the left side of PC1. Wines that corresponded to the fresh green attribute included those from Punters (N-S) and Quarry (E-W) with no shrivelled fruit. In contrast, wines that had dominant dark berry, overripe fruit, oxidised character, and tannin intensity tended to be Quarry (E-W) wines with shrivelled fruit. This could be attributed to the higher levels of water stress in the E-W vines compared to the N-S vines as bunch temperatures were higher in the latter row orientation. Further work in this area could determine whether smaller (< 25%) proportions of shrivelled fruit can also impact wine quality similarly.

7 Conclusions

This is the first study of berry shrivel in Cabernet Sauvignon as affected by row orientation and environmental factors of temperature and water availability. Furthermore, this study also investigated the link between berry shrivel and wine composition and quality. Although the trial was done for only one season, some important findings were obtained.

Leaf removal around the bunch zone was effective in increasing canopy porosity, reducing LAI, and increased bunch temperatures, both around the bunch zone (ambient temperatures), as well as bunch surface temperatures (by ~ 2 °C). Leaf removal did not impact either soil moisture (Ψ_{pd}) or vine water status (Ψ_s); values in both cases were around -0.4 MPa and -1.0 MPa, respectively, late in the season, indicating moderate water stress levels. Cell death, as measured by fluorescence microscopy and impedance spectroscopy, appeared to be unrelated to leaf removal and its impact on elevated temperatures, at least to the extent measured in this study. Yields were similar between leaf removal treatments as was the proportion of shrivelled bunches on each vine indicating that this cultural practice does not appear to have an impact on berry shrivel. There was no impact of leaf removal on basic fruit composition parameters.

Water stress as imposed via deficit irrigation appeared to influence vine physiology and berry shrivel. Water stressed vines had similar canopy microclimate, leaf and bunch temperatures, porosity and LAI values to non-water stressed vines. Deficit irrigation decreased soil moisture availability adequately to impose water stress; the most stressed vines had average Ψ_s values around -1.4 MPa indicating considerable stress on the vine. Water stress resulted in higher cell death where no leaf removal was done with a significant block effect (due to row orientation). Yield components were not severely impacted by water stress but significantly smaller berries were observed in the high water stress treatments, particularly in vines with leaf removal. Basic fruit composition was similar between water stress treatments except for anthocyanins, which were higher in the high water stress vines.

Row orientation had a significant impact on canopy microclimate; higher ambient temperatures and VPD levels were measured in the N-S block during the warmest days. This was consistent with the leaf and bunch surface temperatures measured in that block, with differences of as much as 5 °C observed in bunch temperatures. The N-S rows tended to have higher canopy porosity, soil moisture and vine water status i.e. less water stress, compared to the E-W block. Higher levels of cell death in the berries were observed at harvest in the E-W block when water stress and leaf removal treatments were applied; this was not observed in the N-S block. This could have resulted from the higher levels of vine water stress in the E-W block. Although yield or berry weight did not differ between the two blocks, the E-W block had significantly higher amounts of shrivelled fruit at harvest compared to the N-S block; differences up to three-fold were observed in the incidence of shrivelled bunches. Must soluble solids, pH, colour and YAN were significantly higher in the E-W block. Wine composition had a similar trend to must composition; the E-W block had higher pH, colour and hue, malvidin glucoside, caffeic acid, quercetin, as well as higher ethanol and volatile acidity. A few volatile compounds were higher in the N-S block including 1-hexanol,

ethyl hexanol, and IBMP, the latter despite higher exposure levels and temperatures compared to the E-W block.

The proportion of shrivelled fruit used for winemaking affected the wine composition. Higher proportions of shrivelled fruit resulted in wines with higher anthocyanin and quercetin concentrations but also higher IBMP, which is not desirable.

Wine sensory analysis using a modified DA procedure with an expert panel of tasters revealed overall that the two vineyard row orientations had a significant influence on quality. N-S oriented rows tended to produce fruit with more red berry characters regardless of the proportion of shrivelled fruit in the must while E-W rows tended to produce wines that were jammy and oxidised when even 25% shrivelled fruit was present in the must (but were similar to the N-S rows when no shrivelled fruit was included). This could be due to the higher water stress in the E-W vines compared to the N-S vines, especially since the N-S bunches were warmer than the E-W bunches. It should be noted that shrivelled bunches that were included in the must, in certain cases, may have had only three berries that appeared visibly shrivelled, suggesting that not much shrivelled fruit is required to negatively impact wine quality. Therefore, future decisions around harvest should include considerations relating to the presence of shrivel in vineyards and the implication of including this (shrivelled) fruit in the crush.

Future work in this area should consider imposing leaf removal at earlier phenological stages (pre-véraison), as well as investigating the possibility that shrivel (or cell death) begins in the berries prior to the appearance of visible symptoms, which is typically 100 days post-anthesis. Wines could also be made with smaller proportions of shrivelled fruit, e.g. 10%, to investigate effects on composition and quality. Finally, new and improved techniques could be developed for quantitation of cell death in the field to provide practitioners with a rapid, non-destructive tool to assess shrivel prior to harvest.

8 Recommendations

This study provided some valuable information on the effects of canopy management (via leaf removal) and water stress. Our results indicate that water stress has an overriding effect on berry shrivel compared to high temperature. High levels of water stress result in increased incidence of berry shrivel and smaller berries, particularly when combined with leaf removal. This finding has important implications for viticulture; maintaining good vine water status ($\Psi_s \geq$ -1.0 MPa) and avoiding leaf removal on the west (afternoon) side of N-S rows and north side of E-W rows. Where

new blocks are being planted, particularly in warm regions, our results indicate that, from a berry shrivel standpoint, the N-S row orientation is recommended as the risk of shrivel is lower even with moderate water stress levels in the vines. If vineyards are oriented E-W, the risk of obtaining shrivelled fruit can be minimised by avoiding leaf removal and maintain good water status as mentioned above. Greater amounts of shrivelled fruit in the must results in higher anthocyanins but also IBMP and acetic acid which are not desirable, therefore including shrivelled fruit in the crush should be minimised or avoided. Based on the results of the wine sensory analysis, it is recommended that visibly shrivelled bunches be excluded from the crush (e.g. dropped prior to harvesting) since jammy and oxidised characters in the wine are generally undesirable. These negative attributes can be exacerbated with high vine water stress, which was observed to a greater extent in the E-W rows, therefore water stress should be minimised during the post-véraison period.

This study provided, for the first time in Coonawarra Cabernet Sauvignon, insights into the effects of elevated heat and water stress on the incidence of shrivel, as well as the effects of shrivel on wine composition and quality.

Appendix 1: Communication

The information from this trial has been featured or presented at the following sites and locations:

- Article on Wine Australia website (13/10/2017)
  Putting berry shrivel under the microscope
  https://www.wineaustralia.com/au/news/articles/putting-berry-shrivel-under-the-microscope

- Invited seminar presented at the Australian Cabernet Symposium, Penola, SA (05/07/2018)
  The impacts of berry shrivel on Cabernet
  https://www.naracoorteherald.com.au/story/5512230/australian-cabernet-symposium-held-at-penola/

- Two peer-reviewed manuscripts currently being prepared for publication and one in an industry journal *(Wine and Viticulture Journal*)

Appendix 2: Intellectual Property

There is no intellectual property arising from this study.

Appendix 3: References

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