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IMPACT OF ELEVATED CO_2 AND ITS INTERACTION WITH ELEVATED TEMPERATURE ON PRODUCTION AND PHYSIOLOGY OF SHIRAZ



FINAL REPORT TO WINE AUSTRALIA

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Impact of elevated CO_2 and its interaction with elevated temperature on production and physiology of shiraz

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Abstract

Using Shiraz vines enclosed in open top chambers, the impacts of warmer CO_2-enriched air on vine phenology, leaf function, vine water use and grape and wine composition were assessed over four seasons. Over and above the seasonal influences, which were strong, warmer air advanced maturity and resulted in lower quality grapes and wine. Elevated CO_2 caused a small improvement in yield. Elevated CO_2 alone and in combination with warmer air increased photosynthesis but not water use by vines. As the climate warms, it is likely that water use in wine grape vineyards will remain static or diminish marginally.

Executive Summary

The advancement of harvest dates over the last three decades has focused attention on what a future climate might mean for wine grape production in Australia, given the predicted rise in global atmospheric temperatures over the coming decades. Despite it being the root cause of warmer air and despite it being the substrate for photosynthesis, the direct effects of elevated atmospheric carbon dioxide concentrations have received far less industry attention. The effects of elevated [CO_2] may be different from, and may modulate, the effects of a warming climate on vine growth, grape production and grape and wine quality. The relationship between the effects of elevated CO_2 and the effects of warmer air have the potential to be very significant for wine grape production in the warm irrigated regions in the intermediate term, and more widely across Australia in the longer term.

Building on an earlier study of the effect of warmer air on the phenology of mature vines in the field using open top chambers (OTCs), a system to enrich the air with CO_2 around mature Shiraz vines in OTCs was designed and built. A factorial combination of ambient/elevated CO_2 and ambient/elevated temperature were imposed for four seasons, and the effects on vine phenology, physiology, grape composition and wine quality assessed.

Most of the variation in phenological development occurred after budburst. The biggest driver of phenology after budburst was season, but air temperature and the concentration of CO_2 also contributed. The effect of the latter was sporadic and less important compared to the influence of air temperature. The supply of air warmed by 2°C relative to the ambient air temperature did result in significant changes in the temperature indices commonly used to relate grapevine phenology to climate across seasons and regions. But here, these indices were seen to have limited explanatory power where a temperature differential was applied across the same climatic backdrop as occurred in this project. In other words, although the steady rise in average air temperatures may result in a general advancement of phenology, it is the unique series of high and low temperatures at different times and for different lengths of time that is unique to each season that will be the ultimate driver of phenology. The widening of the spread in timing to various phenological milestones from a common starting point as each season progressed clearly implicates temperature, but in ways that the current application of climate data to viticulture cannot explain.

Leaf function measurements showed that assessing single components of climate (*e.g.* air temperature) in isolation from other components (*e.g.* CO_2 concentration) gave an incomplete picture of likely leaf function in the projected future climate. Photosynthesis responded positively to elevated CO_2, whereas +2°C air temperature alone had no impact. When the two factors were combined, photosynthesis was greater than that observed in response to elevated CO_2 alone. Thus, projections of the likely impacts of climate change based on assessing the impact of one climatic variable without the other may be misleading.

Annual and perennial growth of grapevines were largely unaffected over the four seasons by either the concentration of CO_2 or air temperature. Nonetheless, elevated CO_2 was associated with an additional two kg of fruit per vine per season overall, but that effect was dwarfed by a factor of five by the seasonal effect. Leaf function measurements combined with whole vine water use measurements suggested that in a warmer climate with higher CO_2 concentrations, whole vine water use may not be any different from the present. Combined with the observation that higher temperature did not carry a yield penalty, and that higher atmospheric CO_2 may even result in a slight improvement in productivity, the volume of water the vines use to produce each tonne of grapes will most likely remain the same or diminish marginally as the climate changes to resemble the climate imposed experimentally here.

The single biggest influence on grape composition at maturity was season, but, over and above the seasonal factors, warmer air resulted in grapes with less tannins, anthocyanins and flavonols. Warmer temperatures also advanced maturity, and the effects of temperature on final grape composition may be due to a combination of less time to accumulate sufficient oenologically-significant compounds and greater thermal degradation of those compounds. For the most part, the effects of the climatic variables on grape composition at harvest translated through to the final wine. Wine stability during storage out to 24 months was not influenced by the grapes' growing conditions. The Australian wine industry may need to consider the wider use of altering the microclimate in the grapevine canopy in order to counter these effects.

The project team gratefully acknowledges the financial support, through Wine Australia, of Australian wine grape producers and the Australian Government, and the co-investment by the Victorian Government and CSIRO. The project team also acknowledges the financial support of the same parties for project DPI 09/01, which enabled the development and testing of the heated OTC concept that was the platform on which the CO_2 enrichment could be added.

Background

The Intergovernmental Panel on Climate Change has repeatedly drawn attention to the expected rise in global atmospheric temperatures over the coming decades (Carter *et al.*, 2007). Models used to simulate atmospheric energy fluxes based on the current trajectory of atmospheric carbon dioxide (CO_2) concentrations and the likely effects of world-wide policy settings, point to a high likelihood of an average 2°C rise in air temperatures by the middle of this century (Carter *et al.*, 2007). Analysis of vintage records suggest that harvest dates have advanced over the last 30 years as a result of warming (Petrie and Sadras, 2008), and this appears to be true even when other viticultural and environmental factors are taken into account (Webb *et al.*, 2011). In an earlier Victorian Government/GWRDC research project (Sommer *et al.*, 2012) an across-the-board temperature increase of 2°C imposed on both red and white wine grape varieties was seen to advance grapevine phenology by up to two weeks, increase the rate of ripening and delay leaf fall.

The primary cause of the predicted elevated atmospheric temperature ("eTemp") is elevated atmospheric CO_2 ("eCO_2"), and this driver of climate change was not a feature of that project. In other words, the impact of eTemp was assessed in the absence of any potentially confounding influence of eCO_2. Because atmospheric CO_2 is the substrate for photosynthesis, eCO_2 has the potential to affect any plant species, including grapevine. Indeed, one of the few eCO_2 experiments conducted on grapevines supports this: Sangiovese vines in the field exposed to free-air eCO_2 demonstrated greater shoot and berry growth (Bindi *et al.*, 2001b). But, the interactive effects of atmospheric temperature and CO_2 might be more important. If the effects of eCO_2 are additive with that of eTemp, impacts on vine growth, grape production or grape and wine quality will be compounded. The extent to which they are compounded may be very significant for wine grape production in the warm irrigated regions in the intermediate term, and more widely across Australia in the longer term.

The earlier project (DPI 09/01) (Sommer *et al.*, 2012) demonstrated that an across-the-board 2°C higher average air temperature around vines could be imposed within open-top chambers (OTCs). In the project reported herein, a free-air CO_2-enrichment system was designed to complement the air warming capability to impose eCO_2 and/or eTemp on Shiraz vines enclosed by OTCs in the field. The use of OTCs avoided the engineering and logistical complexity and cost associated with imposing eCO_2 using the typical free air CO_2 enrichment ("FACE") approach in the field, and in a biometrically robust manner. Elevated CO_2 and/or eTemp was imposed for four seasons allowing the effects eCO_2 alone, eTemp alone and their interactive effects on phenological development, berry development, canopy function, berry and final wine composition to be assessed.

Project Aims

The aims of the project were:

1. To develop and document the impact of elevated temperature and CO_2 on growth and biomass production of Shiraz vines in the field. Specifically, the impact of each factor and their combination on vine phenology and biomass production including commercial grape yield was measured.
2. To quantify the impact of elevated temperature and CO_2 on vine physiology and carbohydrate storage. The focus was on measuring the impact of each factor and their combination on leaf gas exchange (net photosynthesis and stomatal conductance) and vine water relations.
3. To quantify the impact of elevated CO_2 and temperature on grape and wine quality parameters and wine sensory characteristics by an expert panel.
4. To further develop and document a field-based system for studying the effect of elevated temperature and CO_2 on grapevines.

Materials and Methods

Study Site

The experimental site was located at the Victorian Department of Economic Development, Jobs, Transport and Resources (DEDJTR) – Irymple, Victoria, Australia (34°14′07″S, 142°10′16″E; 50 m above sea level). Irymple is located in the far northwest of Victoria, 6 km south of Mildura and 550 km northwest of Melbourne.

The region has a semi-arid climate with hot summers and mild winters. Annual long-term rainfall totals are approximately 290 mm, with winter and spring having the most rainy days[1]. Average temperature maxima and minima were 15 – 32°C and 4 – 17°C, in summer and winter, respectively[2]. Destructive frosts are not uncommon. The region experiences hot summers, and records since mid-1940 indicate the region experiences five days per summer of 40°C and warmer. Those same observations suggest that in the 60 years since records have been kept, the number of days 40°C and warmer has doubled[1].

The soil was a sandy loam (0 – 60 cm) overlaying sandy clay loam (60 – 150 cm).

The Shiraz vines (*Vitis vinifera* L.) on 140 Ruggeri (*V. berlandieri* × *V. rupestris*) rootstock were planted in 1998 and trained to a two-cordon vertical shoot positioned trellis system with the two cordon wires at 1.3 and 1.7 m, respectively, above the soil surface. The intra-row vine spacing was 1.8 m and the vine rows were 3 m apart. The entire planting was drip irrigated at 7.2 L h^{-1} $vine^{-1}$ equating to 1.33 mm h^{-1}. Prior to the installation of any experimental infrastructure, all vines in the planting were assessed to ensure that atypical vines weren't included in the experiment.

Open Top Chamber Design

The OTCs' dimensions (5.4 × 4.8 × 2.4 m; L × W × H) were determined by the need to enclose three mature vines, the need to conduct normal viticultural management practices around those vines, the desire to minimise any impact of the OTC materials on the natural ultraviolet radiation received by the vines and the average maximum height of canopies on the Shiraz vines trained. Each OTC consisted of a frame made from 20 × 20 mm rectangular hollow section steel clad with corrugated Standard SunTuf Clear Greca plastic sheeting (Palram, Cavan, SA, Australia). Each OTC included an access doorway made of the same materials.

Heating

The system to elevate the OTC air temperature incorporated the capacity to produce large volumes of heated air, and deliver that air at low velocity whilst minimizing the loss of warmed air through the open top. The warming was generated by a central heating unit (Affordable Air-conditioning, Frewville, SA, 5063, Australia); each heating unit consisted of triple 2.4-kW finned three-phase heating elements (Stokes Appliance Parts, Ringwood, Victoria, Australia) with an axial single-phase fan (MTS354, Fantech Pty Ltd, Somerton Park, SA, Australia) with a maximum output of 0.77 m^3 s^{-1} running continuously. The temperature of the heated air pumped into half of the OTCs was controlled by a Satchwell climate control

[1] Bureau of Meteorology Climate of Mildura. Retrieved from https://web.archive.org/web/20100914145333/http://www.bom.gov.au:80/vic/mildura/climate.shtml on 2 February 2017.

[2] Bureau of Meteorology Monthly climate statistics. Retrieved from http://www.bom.gov.au/climate/averages/tables/cw_076031.shtml on 2 February 2017.

unit (MN450, TAC Headquarters, Malmo, Sweden) programmed to manage the heating elements to warm the air *en route* to the OTC by approximately 2°C above the vineyard ambient air temperature. The control unit utilized an air temperature sensor 3.75 m outside the OTC to measure ambient conditions whilst a second sensor measured the air temperature in the centre of the OTC, at a height of 1.5 m above the soil surface. The heating unit was connected to a ducting system located directly below the canopy, which directed the warmed air toward the soil surface to maximize the pathway to the top of the chamber and minimize air temperature stratification within the OTC.

Pure CO_2 injection system

The CO_2 injection system was developed through testing of a range of scenarios described by Mollah *et al*. (2014). The system described below was the most suitable of those tested.

The main components of the pure CO_2 injection system were:

- a 30,000 L tank to store liquid CO_2;
- an ambient vaporiser to convert liquid CO_2 into gas;
- bulk gas supply line to the trial site;
- 24 V direct current (DC) power supply to each OTC;
- solenoid valve to each OTC to turn gas on/off;
- a CO_2 sensor and controller at the centre of each OTC;
- a central data logging station for all OTCs.

A schematic representation of the system design appears in Figure 1.

Pure CO_2 was injected at supply pipe pressure (<10 kPa) between sunrise and sunset through 0.30 mm diameter, laser-drilled holes (20 holes m^{-1}) from delivery manifolds on each side of the vine row just below the canopy. The delivery manifolds were positioned 1.3 m above the ground at the level of the bottom cordon, and were parallel and 1.3 m apart in the horizontal plane. The delivery manifolds were oriented to direct the CO_2 jets downwards at an angle of 45°; the intention being to quickly mix the CO_2 with the air inside the OTC and the CO_2-enriched air be transported in and around the grapevines by air movement with or without the fan running.

A CO_2 sensor with a relay facility (SEN51101S01, ETM Pacific Pty Ltd, North Sydney, NSW, Australia) was positioned 1.7 m above the ground near the top of the middle vine's canopy. The sensor signaled a solenoid valve to switch the CO_2 supply on or off to maintain the target [CO_2] of 650 μmol mol^{-1}. Both the CO_2 sensor and the solenoid valve were powered by 24 V DC. The CO_2 sensor was housed in a solar radiation shield (Onset Computer Corporation, Bourne, MA, USA), while the solenoid valve was housed inside a weather proof enclosure box. The CO_2 sensor also measured air temperature.

Data from the housed CO_2/temperature sensors in each OTC were sent via a 2.4 GHz wireless transmitter and a 3G router (ETM Pacific Pty Ltd, North Sydney, NSW, Australia) to an automated remote data logger (IMSE™ Webmaster Pro, Abelko Innovation, Sweden).

Data were collected wirelessly every minute from eight OTCs and four non-OTC ambient controls and logged into files with date and time stamping. The maximum and minimum temperatures were collected from the chamber controls and the heating only OTCs, but no [CO_2] measurements were made in those chambers.

The system was commissioned in September, 2013.

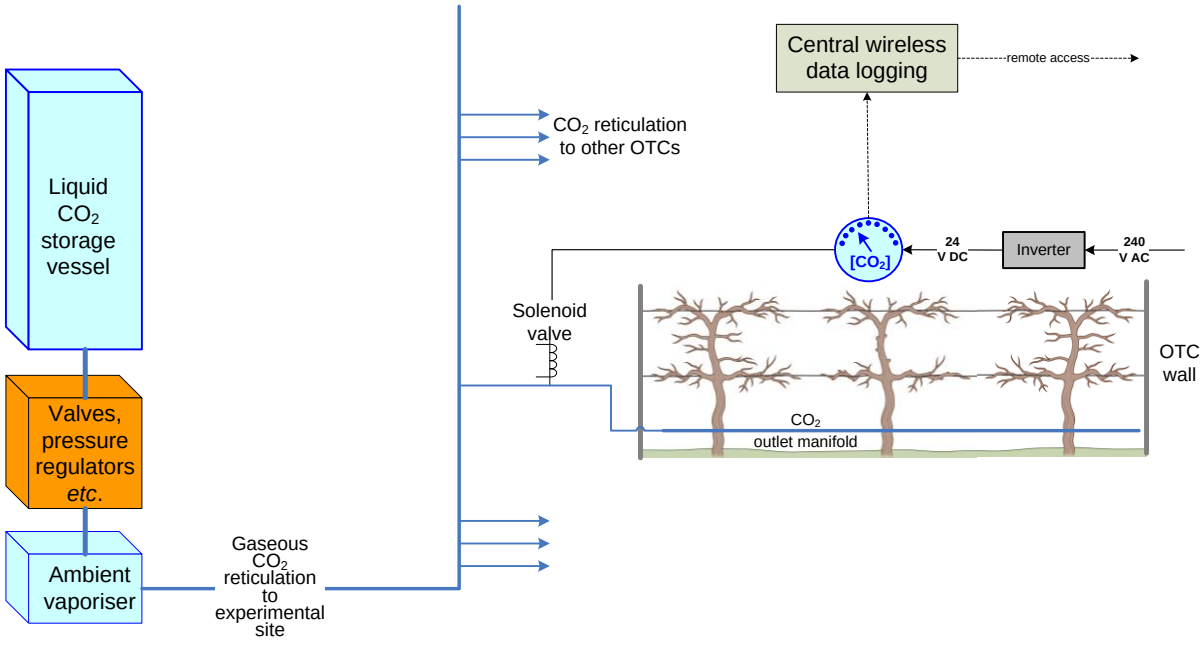


Figure 1 Major components of the pure CO_2 injection system

Vine representations adapted from an image down loaded from http://farhek.com/pt/40v81t/branch/ on March 16, 2018

Treatments

There were four treatments applied to vines in OTCs and there was a non-chambered control; each treatment was replicated four times. The trial therefore consisted of 20 plots which were distributed in a randomised design within the field. Each plot contained a panel of three vines.

The treatments were:

- Control, non-chambered three-vine panels;
- OTC Control, panel of three vines enclosed by an open topped chamber, with a fan unit and ducting and considered to be the "chamber control";
- Elevated CO_2 (eCO_2), as per the OTC Control, plus CO_2 injection;
- Elevated temperature (eTemp), as per the OTC Control with the addition of heating elements to the fan unit;
- Elevated CO_2 and temperature (eCO_2+eTemp), as per the OTC Control plus CO_2 injection and heating elements.

The target $[CO_2]$ for the eCO_2 and eCO_2+eTemp OTCs was set at 650 µmol mol^{-1}, the predicted atmospheric $[CO_2]$ by 2060 – 2070. Carbon dioxide was only supplied during daylight hours when the vines were in leaf, with timing adjusted weekly. The daily supply of CO_2 ran for the duration of four seasons; namely the 2013/14, 2014/15, 2015/16 and 2016/17 southern hemisphere growing seasons (September – April). As grapevines are a deciduous woody perennial plant and are thought to require some chilling for even budburst (Mullins *et*

al., 1992) the temperature treatments were maintained year-round to assess any impacts on this phenological milestone.

Measurements of temperature and relative humidity in the canopy of the middle vine in each OTC, as well as soil temperature at 30 cm, were logged at 30-minute intervals in each of the 20 plots using HOBO Micro Station Data loggers (One Temp Pty Ltd, Marlston SA, Australia) and in the final season Tiny Tag Plus 2 loggers (Gemini Data loggers, Chichester, West Sussex, UK) were used. The sensors were enclosed in mini-Stevenson screens.

Vine water use was monitored at 15-minute intervals from September 2013 to June 2015 using a single SFM sap flow logging system (ICT International, Armidale NSW, Australia) in the central vine of all 20 replicates, providing two full growing seasons of data.

Site map with treatment set-up

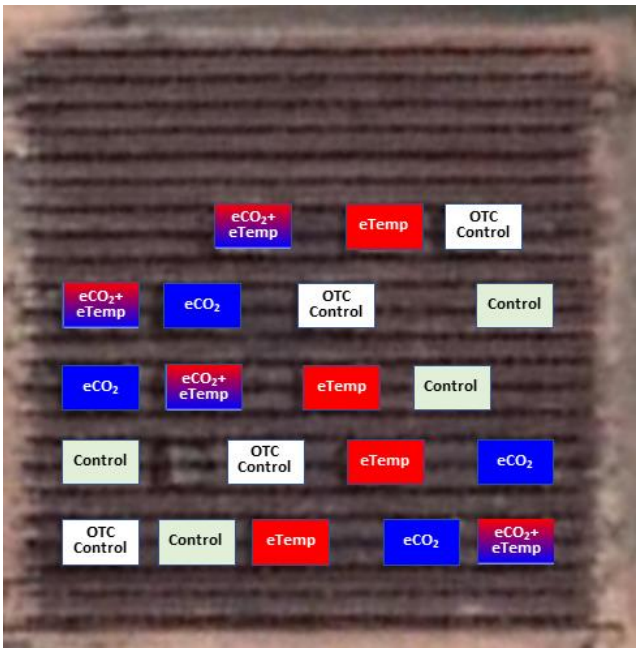
The relative physical distribution of the OTCs and non-chambered Control plots is presented in Figure 2. The treatment locations were selected randomly with the stated proviso that atypical vines were avoided.

Figure 2 Relative locations of non-chambered plots and OTCs† in the Shiraz planting at Irymple overlaid on a satellite image* of the site

- *Control: non-chambered 3-vine plots*
- *OTC Control: fan-only and ambient air*
- *eCO_2: fan, elevated CO_2, ambient temperature*
- *eTemp: fan, heated air, ambient CO_2*
- *eCO_2+eTemp: fan, elevated CO_2, heated air*

†Plots and OTCs not to scale.

**Image downloaded from GoogleEarth*



Spatial variation of OTC vine canopy CO_2

The spatial variation of [CO_2] was assessed using [CO_2] measured by an EGM-4 CO_2 meter (PP Systems International, Massachusetts, USA) within the canopy of each vine. Ten measurement locations were pre-determined for each of three vines inside an OTC to obtain a representative spatial distribution within the vine canopy (Figure 3). On the 5th and 6th February 2014, data were collected from four OTCs; *viz*. an OTC Control, an eCO_2 OTC, an eTemp OTC and an eCO_2+eTemp OTC. A handheld EGM-4 CO_2 meter was used to record [CO_2] manually. The sampling head of the meter was mounted on a purpose-built frame so that the sampling was at least 1.5 m in front and upwind of the operator to reduce the likelihood of contamination by the operator's breath. Fifteen measurements were taken at each of the locations per vine. Thus, there were a total of 45 readings at each location for

each OTC when combined across the three vines in each OTC.

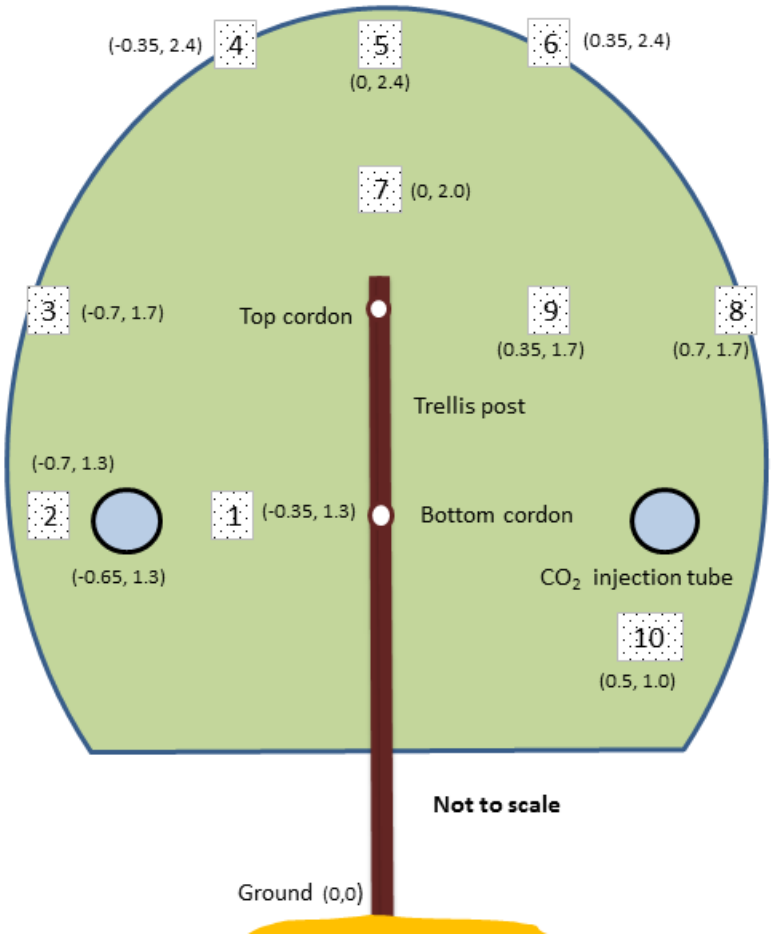


Figure 3 Schematic diagram showing locations (x,y) of [CO_2] measurements in a transect through a vine canopy inside an OTC

Where, in metres, x = horizontal distance relative to the trellis post, and y = vertical distance above the ground.

Location key:

- *1 = inside canopy at the level of bottom cordon wire;*
- *2 = at the edge of the canopy at the level of bottom cordon wire;*
- *3 & 8 = at the edge of the canopy at the level of top cordon;*
- *4 & 6 = at the edge of the canopy top;*
- *5 = at the centre of the canopy top;*
- *7 = at the centre of the canopy above the trellis post;*
- *9 = inside canopy at the level of top cordon wire; and*
- *10 = below CO_2 injection tube.*

Vine growth and development measurements

To minimise any environmental, vine habit and observation/measurement/sampling biases, all observations, measurements, and sampling were conducted in a randomised systematic grid pattern on both sides of the trunk and both sides of the trellis to give an accurate

representation of the development of the vines within the replicate. Sixty percent of observations/measurements were taken from the upper cordon and forty percent from the lower cordon. These proportions were based on the distribution of spurs on vines.

Phenology

The major phenological stages were determined by scoring shoots on 30 spurs within the canopy of the three vines in each of the 20 plots using the Eichhorn-Lorenz scale (Eichhorn and Lorenz, 1977) as modified by Coombe (1995): budburst (EL stage 4), flowering (EL 23) and veraison (EL 36) were assessed by examining buds, inflorescences or bunches, respectively, leading up to, during and post those major phenological milestones. At budburst, each bud was assessed to give a percentage of budburst. At flowering, each inflorescence was observed and a percentage of cap fade/shrivel/fall was allocated to each of the 30 spurs. At veraison, an electronic image of a single bunch from each of the 30 spurs was photographed and later scored against the VITICARE ON FARM TRIALS - MANUAL 3.3 "Botrytis Assessment Key" (page 22)[3]. Rather than being used for assessing botrytis infection levels, the key was used to estimate the number of coloured berries within the bunch, and was recorded as a percentage. Veraison (EL 36) was defined as occurring when berries achieved an average of 50% colour. Harvest (EL 38) was defined as when the berries reached commercial maturity (24°Brix or 13.3 Baumé).

Fruitfulness

In the first two seasons, the bunch to shoot ratio was determined by dividing the number of bunches on the middle vine of each OTC and each 3-vine non-chambered control plot at harvest by the number of mature canes immediately prior to pruning.

In the last two seasons, Merbein bunch counts (Antcliff *et al.*, 1972) were carried out. Prior to budburst, separate counts from the lower and upper cordon were recorded to obtain a total spur count for the central vine in each replicate. At flowering, shoots on 30 randomly selected spurs were assessed for each replicate and scored according to Antcliff *et al.* (1972). To ensure accurate representation, 15 spurs were selected from each side of the trellis, nine from the upper and six from the lower cordon. The bunch to shoot ratio was calculated from the number of bunches on the shoots on each of the spurs.

Maturity monitoring

To establish harvest date, berry maturity was monitored by systematically collecting 60 berries from each replicate at weekly intervals from five weeks post-veraison onwards. Thirty berries were collected from each of Vines 1 and 3 (the central vine, Vine 2, was excluded), 15 berries from each side of the trellis for each vine, with 10 collected from the upper cordon and five from the lower cordon, giving a total of 60 berries.

Total soluble solids

The fresh berry samples were crushed, and the free running juice separated from the solid matter by centrifugation. Total refractable soluble solids (TSS) in the free running juice were measured using a temperature-corrected digital refractometer (Palette PR-101, Atago Co. Ltd, Tokyo, Japan). Berry juice TSS was expressed as °Brix, and is equivalent to g sucrose equivalents g^{-1} of berry juice expressed as %. °Brix were converted to Baumé by dividing by 1.8.

[3] https://www.awri.com.au/wp-content/uploads/2014/09/on-farm-trials-manual-3-3.pdf

Fruit harvest and yield

When fruit for each treatment reached commercial maturity the central vine of each replicate was harvested. The number of bunches per vine was recorded and the fruit transported to the laboratory and weighed. Twenty bunches were then subsampled from each replicate and berries removed for processing and analysis. The remaining fruit from each replicate was then harvested and stored at 1°C for 24 h for winemaking.

Pruning weights

At the end of each growing season the pruning weight of the central vine (Vine 2) of all replicates was measured. Pruned canes from the central vine of each replicate were collected and weighed. A sub-sample (approximately 500 g) was taken, weighed and dried in an oven at 55°C. The oven-dried canes were then re-weighed, and the moisture content calculated. The moisture percentage was in turn used to estimate the dry weight of all pruned canes.

Leaf gas exchange

Leaf gas exchange was measured using an LI 6400 Portable Photosynthesis Analyzer (Li-Cor, Biosciences, Lincoln, Nebraska, USA) on four occasions per season on dates approximately coincident with flowering, canopy closure and veraison, with a final measurement after harvest. Measurements were made ± approximately 2 hours of solar noon in all 20 plots. Each measurement was made on a single fully expanded, sun-exposed leaf per replicate and consisted of a determination of assimilation under saturating light (A_{sat}) at the chamber/atmospheric [CO_2] over the course of a minute, following an equilibration period. The leaves used to measure photosynthesis were harvested at the end of the day, with leaf area and fresh dry weights determined. The dried leaves were then ground and used to determine the concentration of non-structural carbohydrates (NSC) according to Edwards *et al.*, (2011).

Assimilation (A), stomatal conductance (g_s), transpiration (E) and associated parameters were determined from the leaf gas exchange measurements. For each measurement, a predicted median air temperature for the measurement period was estimated from the official BoM weather forecast for the Mallee for that day[4]. The block temperature of the LiCor 6400 leaf chamber was set at this temperature for measurements in the non-chamber control, OTC Control and eCO_2 treatments, whereas for the eTemp and eCO_2+eTemp chambers, the block temperature was set 2°C higher. Similarly, for measurements in the non-chamber control, OTC Control and eTemp treatments, reference air [CO_2] was set at 410 μmol mol^{-1}, resulting in a typical chamber [CO_2] of 390 μmol mol^{-1}, being the approximate ambient atmospheric [CO_2] during the experimental period. Relative humidity in the measurement chamber was not controlled, but was limited to less than 75% irrespective of external conditions, typically being approximately 30 – 40%. The measurement chamber was always illuminated using red-blue LED illumination at a photosynthetic photon flux density (PPFD) of 2000 μmol m^{-2} s^{-1}.

For each measurement the leaf chamber was allowed to equilibrate to the conditions set and then clipped on a lobe of the chosen leaf. Leaf temperature and g_s were monitored until fully equilibrated, which typically took approximately 2 – 10 minutes. Following this equilibration, assimilation under saturating light (A_{sat}) was determined by taking a sequence of measurements every five seconds over a minute. An average of each parameter was recorded as the final result.

[4] http://www.bom.gov.au/vic/forecasts/mallee.shtml

Following the A_{sat} measurement, an A:Ci curve was recorded. An A:Ci curve describes the relationship between [CO_2] in the stomatal cavity and net CO_2 assimilation. Leaf chamber [CO_2] was adjusted to 50 μmol mol^{-1}, the leaf was allowed to equilibrate for approximately two minutes and then an automated stepped sequence was implemented to take a reading of A and associated parameters at eight different [CO_2] between 50 and 1600 μmol mol^{-1}. At each step the leaf was allowed to equilibrate for between two and four minutes. All other leaf chamber conditions were maintained. The A:Ci curves were used to calculate the maximum rate of carboxylation ($V_{c\ max}$) and the maximum electron transport rate (J_{max}) according to (Farquhar *et al.*, 1980). In addition, $V_{c\ max}$ at 25°C was also calculated as described in (Harrison *et al.*, 2009).

At the end of each set of measurements (approximately 15:00 h), the leaves used for the gas exchange measurements were harvested, and fresh weight and leaf area measured. The leaves were then frozen in liquid N_2, freeze-dried and ground to a fine powder. Leaf N concentration (g N 100^{-1} g leaf dry weight) of the dried ground leaf material was determined by the thermal conductivity of the combustion gases method. Samples collected in the first two seasons[5] were analysed using a weighed two mg sub-sample of the ground material on a LECO TruSpec Micro CHN analyser (LECO Corporation, Saint Joseph, Michigan, USA).

Light interception

Photosynthetically active radiation (PAR) interception measurements were collected over the four seasons using a ceptometer (AccuPAR LP-80, Decagon Devices, Inc., Pullman, WA, USA). This involved a measurement sequence, using segmented measurements across the canopy at 90° to the cordon, taking in the whole canopy. Each measurement site was equidistant from any vertical wooded structures with four cross-sections at each site.

Leaf conductance

Stomatal conductance measurements were carried out at 3-week intervals using a leaf porometer (SC-1, Decagon Devices Inc. 2365, WA, USA) across each of the four seasons. Measurements were taken between 09:30 and 11:30 h on clear sunny days on two sun-exposed leaves in the top third of the canopy of the central vine for each replicate in two cycles (*i.e.* four leaves measured for each replicate.). In the 2013/14 and 2014/15 seasons leaf temperature was also measured using an infrared temperature sensor (FLU63, Fluke Corporation, Eindhoven, Netherlands).

Vine water use

In addition to the measurements of leaf level transpiration rate, whole vine water use was monitored at 15-minute intervals from September 2013 to June 2015 using SFM sap flow sensors (ICT International, Armidale, NSW, Australia). A single sensor was installed in the trunk of the central vine of all 20 plots. Each sensor recorded sapflow at depths of 0.75 and 2.25 cm below the bark of the vine. These measurements were then scaled to whole vine water use using the provided Sapflow Tool software and typical vine sapwood properties and the trunk circumference. Vine water use was calculated at 15-minute, daily and seasonal steps for the 2013/14 and 2014/15 seasons.

[5] At the time of report preparation, hardware issues have precluded determination of leaf N content in samples collected in the final two seasons.

Carbohydrate content and dynamics

The diurnal dynamics of leaf carbohydrates were measured by taking leaf samples throughout a 24-hour period on six occasions across the 2014/15, 2015/16 and 2016/17 seasons. Samples were taken at approximately three-hour intervals from all 20 replicates, with each sample consisting of two leaves taken from the sunlit part of the canopy. In all cases there was an 'overlap' with the times, for example, if the first sample was taken at 07:00 h, then a final sample was taken at 07:00 h the following day. Samples were immediately frozen in liquid nitrogen and processed as above. Two of the sampling dates were at veraison (2015/16 and 2016/17), two during ripening (2015/16 and 2016/17) and two post-harvest (2014/15 and 2016/17).

Cane and trunk wood samples were taken from each replicate following leaf fall at the end of all four seasons. For each cane sample, the fourth node and internode, typically the position of the harvested bunch, was cut from four canes and combined. Permanent wood samples were collected from the trunk 30 cm above the ground. Using a cordless drill at a very low speed setting, a 4 mm bit was drilled to the centre of the trunk and the tailings collected and combined into a 2 mL vial. At no point was the drill bit warm to the touch. Cane and trunk samples were immediately frozen and stored at −80°C until being freeze-dried and ground.

According to Edwards *et al.* (2011), non-structural carbohydrate contents in all the samples were determined in the perennial wood samples (*i.e.* cane and trunk) and in the leaf samples taken following gas exchange measurements. The method provided estimates of water soluble carbohydrates and insoluble starch, which were summed to estimate total non-structural carbohydrate (TNC).

Plant tissue analysis

When treatments were at the flowering stage, petiole samples were collected for analysis to give an indication of vine nutrient status (Robinson *et al.*, 1997). From each plot, 20 leaves and petioles were removed from opposite basal bunches. Samples were collected from both sides of the canopy, three from the upper and two from the lower cordons, from the two outer vines in each plot (*i.e.* Vines 1 and 3). The intact leaves (blades and petioles) were washed in 10 L of water with 5 drops of phosphate-free detergent and 2 drops of 1 M HCL, and rinsed twice in two 10 L tubs of distilled water. The petioles were then separated from the blades, placed in paper bags and dried at 55°C for 72 h. The dried samples were then shipped to Phosyn Analytical (Burleigh Heads, Queensland, Australia) for P, K, Ca, Mg, S, Na, Al, Cu, Fe, Mn and Zn determination by inductively-coupled plasma emission spectrometry following acid digestion of ground oven-dried material, and for N determination by combustion and thermal conductivity.

Fruit assessment

Fruit were collected from each replicate at the pea-sized berry stage, veraison, and harvest. No fruit were sampled from the central vine in each replicate during the period leading up to harvest. At the first two growth stages, 180 berries in total were collected from the two outer vines within each replicate. Six berries/bunch were collected from 30 bunches on both sides of the canopy, eight from the lower cordon and 12 from the upper cordon. At harvest, a subsample of fruit from the central vine was taken for fruit composition measurements. At each sampling point, 100 berries were weighed and the skins were removed, weighed, snap frozen in liquid N_2, ground to a fine powder and stored at −80°C for later analysis. The diameter at the equator of 20 berries was measured using digital calipers. A subsample of

100 berries was used for determining pH, titratable acidity (TA) and TSS of the free running juice.

Winemaking

Shiraz grapes for winemaking were harvested from the entire treatment plot (three vines) to maximise the amount of fruit available. Following harvest, fruit was transported to the experimental winery. Wine was made according to the CSIRO standard winemaking practice (Becker and Kerridge, 1972) as described by Gong *et al.* (2010). Fruit from each treatment was crushed and divided between three fermentation vessels. Wines underwent seven days of maceration during fermentation. Following pressing and fermentation to dryness, the wine underwent a series of steps including racking and cold stabilization prior to bottling. Wines were then stored at 15°C until required for post-bottling analysis and sensory and descriptive analyses.

Titratable acidity and pH

Titratable acidity and pH of the juice were measured by titrating with 0.1N sodium hydroxide to an end point of pH 8.3 using a Radiometer combined electrode, a TIM850 Radiometer, TitraLab titrator and a SAC80 Radiometer sample changer (Radiometer Analytical, Pacific Laboratory Products, Blackburn, Victoria, Australia).

Yeast assimilable nitrogen

Yeast assimilable nitrogen (YAN) was determined by quantification of ammonia and primary-amino nitrogen. Juice samples were analysed for YAN content utilising primary amino acid and ammonia assay test kits (Megazyme, K-PANOPA & K-AMIAR, Deltagen Australia, Kilsyth, Victoria) and absorbances determined using a microtitre plate reader (SpectraMax Plus384, Molecular Devices, Sunnyvale, USA).

Extraction, purification and analysis of tannins from grape skins

Three × 400 mg measurement replicate subsamples from each skin sample were weighed out, and 4 mL of 70% acetone/water (v/v) — containing 0.1% ascorbic acid to prevent tannin oxidation — added to each. The sample/extraction liquid slurries were vortex mixed for one minute, sonicated for 20 minutes and centrifuged for 10 minutes (4,000 × g). Tannin extracts were then purified utilising liquid-liquid separation to remove any non-tannin material as described by Seddon and Downey (2008).

Following the removal of non-tannin material, 100 µL of purified sample were transferred to a 1.5 mL microfuge tube and dried under vacuum. Dried down samples then underwent acid catalysed cleavage in the presence of excess phloroglucinol as described by Kennedy and Jones (2001). Samples were then analysed by reverse-phase high performance liquid chromatography as reported by Hanlin and Downey (2009).

Terminal subunit composition was determined utilising standard curves of catechin, epicatechin, and epicatechin-gallate. Extension subunits were quantified by utilising the catechin standard curve and conversion factors as reported by Kennedy and Jones (2001).

Grape skin anthocyanin and flavonols

Anthocyanin and flavonol composition was determined by high performance liquid chromatography (HPLC). For each ground skin sample collected, three × 400 mg measurement replicate subsamples were weighed out into 10 mL tubes and extracted with 4.0

mL of 50% methanol in water (v/v) (Downey *et al.*, 2007). Each tube was then capped, vortexed and sonicated as above at room temperature (approximately 24°C). Following sonication, each tube was re-vortexed and centrifuged at 4,000 × g for 10 minutes. Subsequently, 1.5 mL of the supernatant was transferred to a 2 mL screw-cap tube and centrifuged at 13,000 × g to remove any remaining cell debris prior to analysis.

Following centrifugation, 200 µL of grape skin extract was transferred to a HPLC autosampler vial for separation and analysis using a Wakosil™ analytical column (150 × 4.6 mm, 3 µm packing). The separation utilised a binary solvent gradient as described by Downey and Rochfort (2008). Anthocyanins were expressed as mg malvidin-3-O-glucoside equivalents g^{-1} skin fresh weight (FW), and flavonols were expressed as mg quercetin-3-O-glucoside equivalents g^{-1} skin FW.

Spectrophotometric analysis of wines

At the completion of the winemaking process, and at three additional time points post bottling, samples were collected for spectrophotometric analysis using the methods outlined by Iland *et al.* (2004) for analysis of wine colour density and wine hue. In addition, tannin, anthocyanin, iron-reactive phenolics, and polymeric pigment concentration was determined utilising methods described by Harbertson *et al.* (2003). Prior to analysis, each wine sample was centrifuged at 4,000 × g to remove any solid material.

Determination of fermentation products

Analysis of wine samples was performed at Metabolomics Australia (Australian Wine Research Institute, Urrbrae, SA, Australia) according to their standard protocols. Wine samples were diluted 1:10. A 1 g sample was transferred into 9 mL of buffer solution (at pH 3.5) in a 20 mL vial containing 2 g of salt. Analysis was performed on an Agilent 7890A gas chromatograph equipped with a Gerstel MPS2 multi-purpose sampler and coupled to an Agilent 5975C VL mass selective detector, fitted with a Agilent DB-624UI column (30 m × 0.25 mm × 1.4 µm). The oven started at 40°C, then increasing to 60°C over a minute, and staying at 60°C for 14 minutes. A series of temperature ramps followed, to 80°C at 10°C min^{-1}, and then, 160°C at 20°C min^{-1}, and finally to 260°C at a rate of 10°C min^{-1} and maintained for 2 minutes. The total run time for the complete analysis was 45.5 minutes.

The sample was heated to 40°C for 5 minutes whilst agitating. The SPME fibre was exposed to the headspace for 15 minutes and then desorbed in the injector for 15 minutes, at 260°C.

Wine sensory

Wine sensory analysis were conducted on the finished wine from each vintage. A combination of both difference testing and descriptive analysis was performed by trained panelists at the Australian Wine Research Institute.

Difference testing utilised a balanced reference triangle test.

Descriptive analysis involved eight or nine trained panelists, training sessions to determine appropriate descriptors and final assessment for attributes associated with appearance, aroma and palate.

Details for both difference testing and descriptive analysis can be found in Appendix 5.

Temperature indices

Chill hours ($CH_{0\text{-}7.2}$) (1) were calculated as described by (Darbyshire *et al.*, 2011).

$$CH_{0-7.2} = \sum_{t=start}^{end} CH \quad \begin{cases} T_t < 0℃, & CH_t = 0 \\ 0℃ \leq T_t \leq 7.2℃, & CH_t = 1 \\ T_t > 7.2℃, & CH_t = 0 \end{cases} \qquad (1)$$

Where T_t is the temperature (T) at time t. The starting date for $CH_{0\text{-}7.2}$ accumulation was April 1, but few chill hours were accumulated until May.

Growing degree days (2) using a base temperature of 4.5 ($GDD_{4.5}$) or 10 (GDD_{10}) were calculated as described by Hall and Jones (2009).

$$GDD_i = max\left[\left(\frac{T_{max} - T_{min}}{2} - b\right), 0\right] \qquad (2)$$

Where T_{max} and T_{min} are the maximum and minimum on each day, and b is the base temperature below which plant growth is not considered significant. The starting date for $GDD_{4.5}$ was also April 1; $GDD_{4.5}$ did not begin to be accumulated until late July/August in each season. The starting date for GDD_{10} was September 1.

Statistical analyses

GenStat (17th Edition) was used for all statistical analyses.

Variance homogeneity and data normality were checked and appropriate transformations applied as suggested by GenStat's "Best λ" option.

Where appropriate, parameters such as phenological dates, pruning weights or assimilation rates were analysed as randomised block design. The comparisons of the chambered and non-chambered vines' parameters (*i.e.* Control vs OTC Control) were conducted using the design statement

Season × chamber,

and within chambers using the design statement

Season × [CO_2] × heat.

If a source of variation term's (*i.e.* season, [CO_2] and heat main effects, and interactions thereof) variance ratio F-probability was ≤ 0.05, the least significant difference was used to identify significant differences between means.

Results and Discussion: Objective 1

To quantify the impact of elevated temperature and CO_2 on vine physiology and carbohydrate storage

Phenology

The general climate during the four seasons

Temperature data collected by the Bureau of Meteorology (BoM) at Mildura Airport 9.5 km to the west of the site are summarised in Table 1.

Table 1 Mean seasonal minimum and maximum temperatures at Mildura Airport for each of the four seasons that the experiment was conducted.

	Winter		Spring		Summer		Autumn	
Season	min	max	min	max	min	max	min	max
	———			°C			———	
2013/14	5.6	17.8	10.3	26.2	17.1	33.7	11.9	24.9
2014/15	4.3	17.4	10.5	27.2	16.9	33.0	9.9	23.3
2015/16	4.6	15.9	10.8	27.6	17.6	33.9	12.4	26.2
2016/17	5.7	16.6	9.2	23.5	16.7	33.0	11.5	25.4

The mean temperature of the warmest month (February, in the case of northwest Victoria) has been used to classify regions into cool, warm and hot production areas (Gladstones, 1992). Mean February temperatures for 2013/14, 2014/15, 2015/16 and 2016/17 were 25.4, 26.8, 25.2 and 24.5°C, respectively. Using this index, the 2014/15 season was the warmest and the 2016/17 season the coolest.

Timing of bud burst, flowering, veraison and harvest of chambered vines versus non-chambered vines

Our understanding of the linkage between cause (temperature) and effect (phenological development) is encapsulated in various temperature indices developed empirically *a posteriori* to describe the phenological behaviour of woody perennial plants. In the case of wine grapes, "chill hours" (CH), "growing degree days" (GDD) and "biologically effective degree days" (BEDD); (Gladstones, 2004, Hall and Jones, 2010) are widely used concepts. The current understanding is set out in a modified version of Figure 4 in (Hall *et al.*, 2016) (Figure 4). Included on that modified figure are the average dates of the major phenological milestones for the non-chambered control vines over the four seasons of the experiment. The model used the GDD concept to describe the heat requirements for budburst (4.5°C base temperature; $GDD_{4.5}$) and subsequent phenological development (10°C base temperature; GDD_{10}). As published, the model doesn't consider chilling requirements, most likely because grapevines' chilling requirements are generally believed to be easily met, but in a generally warming climate that may not be so. The heat requirements for bud burst are often referred to as forcing temperatures, and in cooler climates where chilling is more likely to be easily met, more emphasis is placed on forcing temperatures (*e.g.* (Nendel, 2010))

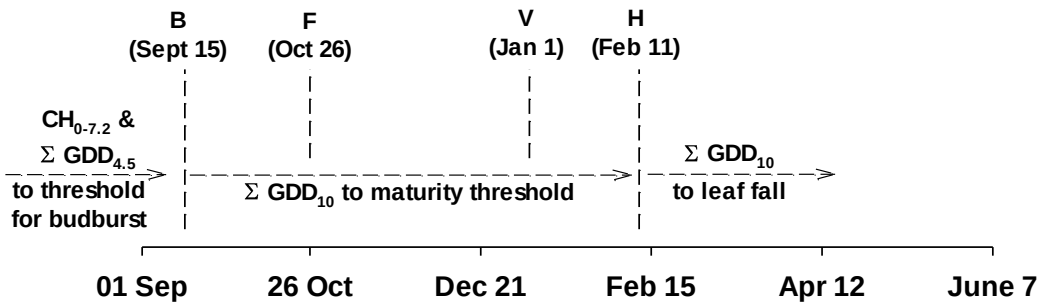


Figure 4 Modified GDD and phenology model with average budburst ("B"), flowering ("F"), veraison "V" and harvest ("H") for the non-chambered Shiraz vines at Irymple

Based on Figure 4 in (Hall et al., 2016), and reproduced with the kind permission of the authors. Dates below each phenological milestone are the average dates across the four seasons that those milestones occurred on the non-chambered control vines.

A summary of the analyses of variance conducted on the timing of the four key phenological milestones by non-chambered control vines and OTC Control vines is presented in Table 2.

Table 2 Summary of analyses of variance of season × chamber effects on the timing of four phenological milestones and the length of the intervals between milestones for Shiraz vines at Irymple

Source of variation	Timing of phenological milestone or interval between milestones						
	bud burst	bud burst to flowering	flowering	flowering to veraison	veraison	veraison to harvest	harvest
	Variance ratios' F probabilities						
Season	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Chamber	0.35	0.81	0.26	0.65	0.28	0.60	0.96
Season × chamber	0.26	0.64	0.27	0.35	0.73	0.19	0.40

Growing Shiraz vines in chambers and feeding those chambers with ambient air had no effect on the timing of the four key phenological milestones — namely, bud burst, flowering, veraison and harvest — relative to non-chambered vines. The intervals between those phenological milestones was similarly unaffected by placing a chamber around the vines. The lack of chamber effect was consistent over the four seasons the trial was conducted.

Mean timings of budburst, flowering, veraison and harvest (at 13.3 Baumé) of the non-chambered Shiraz vines are presented in Table 3. Across the seasons, the spread of timings was of the order of 5 – 6 days for budburst, 22 days for flowering, 23 days for veraison and 41 days, or nearly six weeks, for the attainment of commercial maturity. The last season was the slowest for flowering, veraison and harvest, whilst the first season was the earliest for bud burst, flowering and harvest.

Table 3 Effect of season on timing (days from August 31 in each season) of non-chambered Shiraz vines' phenological milestones at Irymple

Values presented are means (n = 4), and different superscripts within columns indicate significant differences between means at P = 0.05.

	Phenological milestone			
Season	budburst	flowering	veraison	harvest
	Days after August 31			
2013/14	11^{A}	49^{A}	121^{B}	148^{A}
2014/15	17^{B}	54^{B}	124^{C}	167^{B}
2015/16	16^{B}	51^{C}	114^{A}	154^{A}
2016/17	16^{B}	71^{D}	144^{D}	189^{C}

There being no effect attributable to simply surrounding the vines with an OTC structure, subsequent analysis of phenological data were restricted to the chambered vines only.

Effect of [CO_2] and air temperature on timing of bud burst, flowering, veraison and harvest of chambered vines

Analyses of variance conducted on the timing (days after August 31) of bud burst, flowering, veraison and harvest, and the number of days between each of those stages (summarised in Table 4), indicated that season was by far the most consistent and significant source of variation in the time taken to reach the four major phenological milestones and the number of days between those milestones. Of the two other treatment variables — as main effects — air temperature significantly influenced the timing of flowering, veraison and harvest, whereas [CO_2] significantly influenced the timing of veraison and harvest.

Significant interactions occurred at the start and at the end of growing season. Timing of bud burst in some seasons was influenced by heat, and that effect spilled over to affect the number of days to flowering. Significant [CO_2] × heat and season × [CO_2] × heat interaction terms with respect to the number of days between veraison and harvest and on the timing of harvest indicated that in some seasons, [CO_2] and heat may have modulated the effects of each other differently.

Table 4 Summary of analyses of variance for season, [CO_2], heat and interactions thereof on the timing of four phenological milestones and the length of the intervals between milestones for Shiraz vines at Irymple

Source of variation	Phenological milestone or interval between milestones						
	bud burst	bud burst to flowering	flowering	flowering to veraison	veraison	veraison to harvest	harvest
	Variance ratios' F probabilities						
Season	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
[CO_2]	0.08	0.08	0.63	0.13	0.05	0.33	<0.001
Heat	0.97	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001
Season × [CO_2]	0.81	0.85	0.27	0.57	0.82	<0.001	<0.001
Season × heat	0.02	0.01	0.60	0.46	0.78	0.30	0.27
[CO_2] × heat	0.78	0.53	0.23	0.36	0.75	<0.001	<0.001
Season × [CO_2] × heat	0.19	0.69	0.45	0.88	0.49	0.04	0.001

Season, [CO_2] and heat main effects on the timing of bud burst, flowering, veraison and harvest, and the number of days between those phenological milestones are presented in Table 5. The timing of bud burst in the heated chambers was not different to the timing of bud burst in the non-heated chambers in two out of the four seasons. Budburst in the heated chambers was three days later in the second season and three days earlier in the last season compared to the non-heated chambers.

Table 5 Season, [CO_2] and heat main effects on days to four major phenological milestones, and the interval between those milestones for Shiraz vines at Irymple.

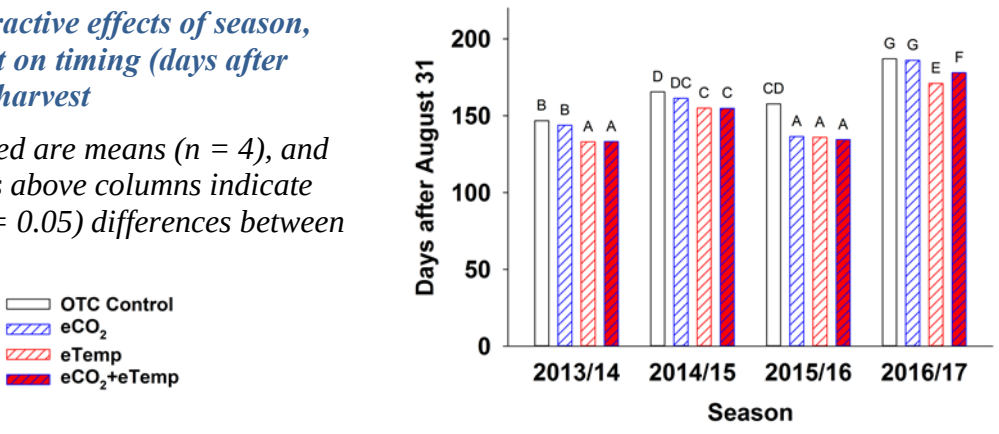
Values presented are means (n = 16, 32 and 32 for season, [CO_2] and heat main effects, respectively). Within season main effects means with the same superscripted letter are not significantly different at P = 0.05, and an asterisk after a mean in either the CO_2 or heat main effects indicates a significant difference between the means at P = 0.05.

Main effect	Treatment	Phenological milestone or interval between milestones						
		bud burst	bud burst to flowering	flowering	flowering to veraison	veraison	veraison to harvest	harvest
		Days from August 31 (phenological milestones) or days between milestones						
Season	2013/14	11^{A}	35^{A}	45^{A}	71^{C}	117^{B}	23^{A}	139^{A}
	2014/15	17^{C}	34^{A}	52^{C}	67^{B}	119^{B}	41^{C}	159^{B}
	2015/16	15^{B}	33^{A}	48^{B}	59^{A}	107^{A}	34^{B}	141^{A}
	2016/17	17^{C}	47^{B}	65^{D}	75^{D}	140^{C}	40^{C}	181^{C}
[CO_2]	Ambient	14	38	53	69	121	35	156
	+CO_2	16	37	52	67	120*	34	154*
Heat	Ambient	15	40	55	70	124	36	161
	+2°C	15	35*	50*	67*	117*	33*	149*

The interactive effects of season, [CO_2] and heat on harvest date are presented in Figure 5.

Figure 5 Interactive effects of season, [CO_2] and heat on timing (days after August 31) of harvest

Values presented are means (n = 4), and different letters above columns indicate significant (P = 0.05) differences between means



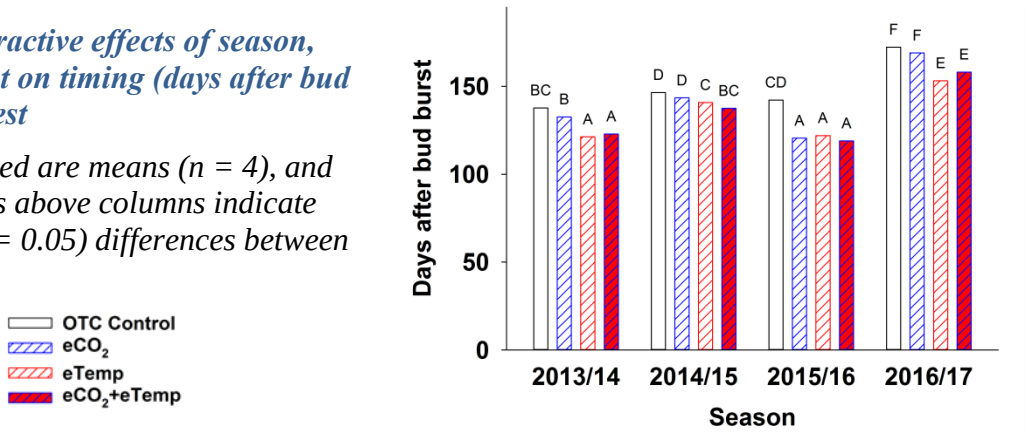
Grapes on vines in heated OTCs always matured earlier than grapes on vines in OTC Control chambers. Grapes on vines in elevated [CO_2] chambers only matured earlier than grapes in OTC Control chambers in one of the four seasons; in 2015/16 grapes in the eCO_2 only chambers matured as early as the grapes in the heated chambers but in 2016/17, elevated CO_2 was seen to modulate the effect of heat on the time to attainment of commercial maturity (*i.e.* 13.3 Baumé or 24°Brix).

To test the robustness of those conclusions, an analysis of variance was conducted on the number of days from bud burst through to commercial maturity. Essentially, the analysis encapsulates the marginal effects of season, [CO_2] and heat on the canopy and berry development that were perhaps too subtle or were undetectable with the level of replication possible. The analysis indicated that all sources of variation — as both main effects and the interactions thereof — exerted significant influences on the number of days from bud burst to maturity. As a main effect, season still accounted for more than 75% of the sums of squares, but the season × [CO_2] × heat interaction term was still significant at P=0.008. More broadly, [CO_2] and heat main effects amounted to four and nine days earlier maturity, respectively, and the [CO_2] × heat term indicated that without elevated CO_2, the effect of heat was of the order of a fortnight, but maturity was only achieved seven days earlier with elevated CO_2. Without warming, elevated CO_2 advanced maturity by eight days, but with warming elevated CO_2 had no effect.

The interactive effects of season × [CO_2] × heat interaction on the timing to harvest from budburst are presented in Figure 6. Broadly the same dominant effect of heat was evident, though the magnitude of the effect was dampened marginally, particularly in the second season. Measuring the length of the season from budburst removed the modulation by elevated CO_2 of the effect of heat on the timing to maturity in the final season (Figure 5). However, the effect of elevated CO_2 alone in the third season was still evident.

Figure 6 Interactive effects of season, [CO_2] and heat on timing (days after bud burst) of harvest

Values presented are means (n = 4), and different letters above columns indicate significant (P = 0.05) differences between means



The timing of budburst also appeared to bear little influence on the time taken to grape maturity. For example, the timing of budburst in the second and fourth seasons was comparable, but harvest was about three weeks later in the fourth compared to the second season. The relative timings of harvest appeared to be more-or-less set by flowering, but with seasonal conditions exerting some influence.

The nature of the effects of season, [CO_2] and heat on the time taken to reach the four major phenological milestones was therefore investigated using the commonly accepted temperature-based indices and bud burst as a pivot point; *i.e.* the accumulation $CH_{0\text{-}7.2}$ and $GDD_{4.5}$ were calculated to the date of budburst for each chamber in each season, and similarly GDD_{10} accumulation was calculated from bud burst. Temperature data were not available to calculate $CH_{0\text{-}7.2}$ and $GDD_{4.5}$ before September 1, 2013. Table 6 contains a summary of analyses of variance of $CH_{0\text{-}7.2}$ and $GDD_{4.5}$ to bud burst, and GDD_{10} from bud burst to flowering, veraison and to the attainment of commercial maturity.

Table 6 Summary of analyses of variance for season, [CO_2], heat and interactions thereof on chill hours ($CH_{0\text{-}7.2}$) and growing degree days (GDD) to bud burst ($GDD_{4.5}$), accumulated GDD_{10} at flowering, veraison and harvest from budburst and GDD_{10} accumulated between each phenological milestone for Shiraz vines at Irymple

**Analyses confined to the 2014/15, 2015/16 and 2016/17 seasons.*

	Phenological milestones & intervals between						
	bud burst*		flowering (from bud burst)	flowering to veraison	veraison	veraison to harvest	harvest
Source of variation	$CH_{0\text{-}7.2}$	$GDD_{4.5}$					
	Variance ratios' F probabilities						
Season	<0.001	0.015	0.004	<0.001	<0.001	<0.001	<0.001
[CO_2]	0.040	0.103	0.670	0.137	0.148	0.877	0.143
Heat	<0.001	<0.001	0.825	<0.001	0.001	0.948	0.004
Season × [CO_2]	0.101	0.113	0.289	0.819	0.931	0.010	0.002
Season × heat	0.484	0.360	0.077	0.094	0.080	0.354	0.386
[CO_2] × heat	0.409	0.133	0.142	0.563	0.962	0.001	<0.001
Season × [CO_2] × heat	0.058	0.081	0.638	0.743	0.591	0.136	0.453

Again, season was the strongest explanatory variable for all indices at all milestones. Heat significantly affected the accumulation of chill hours and $GDD_{4.5}$, but was less important for bud burst-flowering and veraison-harvest GDD_{10} accumulation. The accumulation of chill hours was also affected by [CO_2], but, obviously, that influence was confined to the seasons which temperature data were available to calculate that index. The effects of most interactions were not significant; the exception being season × [CO_2] and [CO_2] × heat effects on GDD_{10} accumulated between veraison and harvest and the GDD_{10} accumulation from budburst through to harvest.

The season, [CO_2] and heat main effects are set out in Table 7. The chilling and warming indices were reduced and increased, respectively, in the heated chambers compared to the non-heated chambers. Elevated [CO_2] was associated with lower $CH_{0\text{-}7.2}$. More pre-bud burst chilling and less warming was apparent in the lead up to the 2015/16 season compared to the season before and the season afterward. Accumulation of GDD_{10} by or between particular phenological milestones was inconsistent across the seasons, and did not follow a pattern consistent with nominal classification of 2014/15 being the warmest season and 2016/17 the coolest based on BoM data.

Table 7 Season, [CO_2], heat main effects on chill hours ($CH_{0\text{-}7.2}$) and growing degree days (GDD) to bud burst ($GDD_{4.5}$), accumulated GDD_{10} at flowering, veraison and harvest from budburst and GDD_{10} accumulated between each phenological milestone for Shiraz vines at Irymple

Values presented are means (n = 16, 32 and 32 for season, [CO_2] and heat main effects, respectively), different superscripted letters within season main effects indicate a significant (P = 0.05) between seasons, and an asterisk after an eTemp mean indicates a significant difference from the ambient mean.

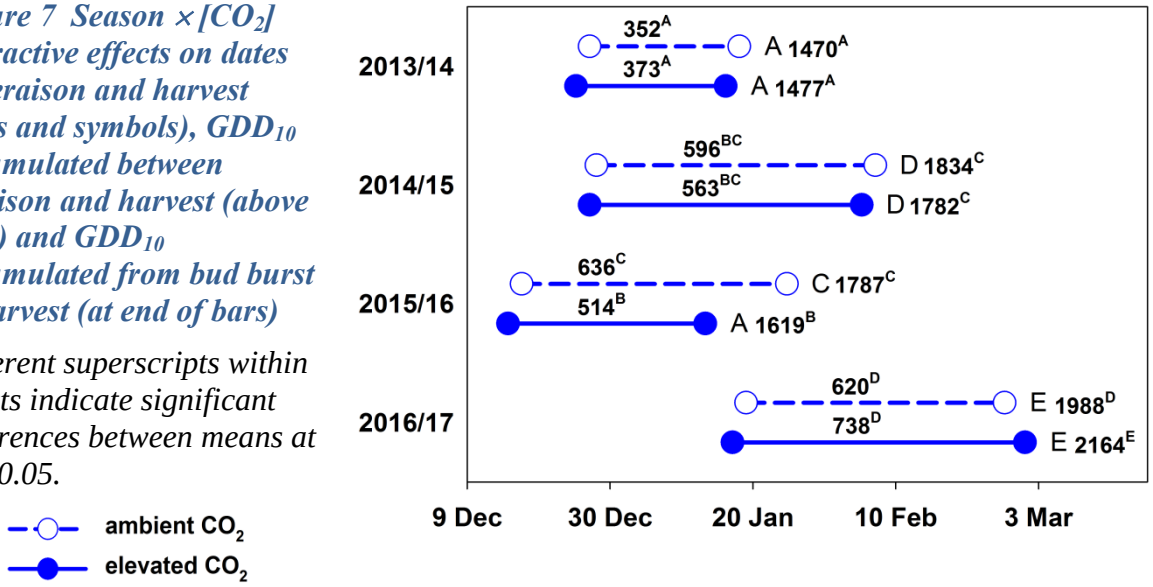
		Phenological milestones & intervals between						
Source of variation		bud burst		bud burst to flowering	flowering	flowering to veraison	veraison	veraison to harvest
		$CH_{0\text{-}7.2}$	$GDD_{4.5}$	GDD_{10}				
Season	2013/14	-	-	280^{A}	831^{A}	1111^{A}	363^{A}	1474^{A}
	2014/15	464^{A}	1037^{A}	317^{C}	912^{B}	1229^{B}	579^{B}	1808^{C}
	2015/16	545^{B}	859^{B}	308^{BC}	820^{A}	1128^{A}	575^{B}	1703^{B}
	2016/17	431^{A}	979^{AB}	294^{AB}	1056^{C}	1350^{C}	679^{C}	2029^{D}
[CO_2]	ambient	501	918	301	918	1219	551	1770
	eCO_2	460*	999	298	892	1190	547	1737
Heat	ambient	642	839	299	872	1170	550	1720
	eTemp	318***	1078***	300	938***	1239**	548	1787***

Significant season × [CO_2] interaction terms were apparent with respect to harvest date, GDD_{10} accumulated between veraison and harvest, and total GDD_{10} accumulated from bud burst to harvest. Those interactions were not significant sources of variation in the veraison date, GDD_{10} accumulated by veraison or the GDD_{10} accumulated between flowering and veraison.

The significant season × [CO_2] interactions referred to in Table 4 and Table 6 are presented in Figure 7. Neither harvest date, nor GDD_{10} accumulation by that date were affected by [CO_2] in the first two seasons. In the third season eCO_2 was seen to accelerate the attainment of commercial maturity by about 11 days, and accumulation of GDD_{10} by that date was reduced as a result. In contrast, eCO_2 delayed maturation by three days in the final season, and more GDD_{10} were accumulated concomitantly.

Figure 7 Season × [CO_2] interactive effects on dates of veraison and harvest (bars and symbols), GDD_{10} accumulated between veraison and harvest (above bars) and GDD_{10} accumulated from bud burst to harvest (at end of bars)

Different superscripts within effects indicate significant differences between means at P = 0.05.



Across the course of the experiment, the number of days from bud burst to attainment of commercial maturity varied from 113 to 179 days; a range of 8 weeks. The date of bud burst on the other hand, only varied by approximately 20 days. As mentioned, season was a strong driver of the length of time between bud burst and harvest, and clearly most of that variation was driven by weather conditions from budburst onwards. However, as applied here, GDD_{10}, was not overly useful in explaining the seasonal effect. Possibly, the index's deficiency in this regard relates to its calculation; GDD_{10} accumulated by a particular event (*e.g.* harvest) from another event (*e.g.* budburst). The relationship may be more granular in the sense that the temperature regime that the vines are experiencing at certain times may delay or advance the developmental processes underpinning major phenological milestones (*e.g.* harvest).

This possibility was explored by calculating GDD_{10} accumulated in 10-days intervals from budburst through to 170 days after budburst. Further, to help identify critical parts of development, data for individual OTCs were used to assess the strength of the relationship between the number of days between phenological stages (budburst, flowering, veraison and harvest) and overall season length (budburst – harvest). The resulting Pearson product moment correlation coefficients are presented in Table 8. Significant correlations were found between the number of days from bud burst to harvest and terms that involved flowering. This leads to the conclusion that although temperature may play a role in the timing of flowering, and hence advance maturity or push maturity further into the future, temperature conditions post-flowering have a strong influence as well. This conclusion is supported by the spread in timing of flowering (31 days), veraison (49 days) and harvest (66 days), but bud burst was only spread by 21 days.

Table 8 Pearson product moment correlation coefficients for days between bud burst, flowering, veraison and harvest for Shiraz vines in OTCs

Bolded coefficients are significant at P = 0.05 (critical value ($t_{0.05[62]}$) = 0.346 for 64 observations)

Bud burst - flowering	-					
Flowering - veraison	**0.583**	-				
Veraison - harvest	0.342	-0.136	-			
Bud burst - veraison	**0.885**	**0.894**	0.110	-		
Flowering - harvest	**0.673**	**0.541**	**0.759**	**0.681**	-	
Budburst – harvest	**0.869**	**0.606**	**0.651**	**0.826**	**0.950**	-
	Bud burst - flowering	Flowering - veraison	Veraison - harvest	Bud burst - veraison	Flowering - harvest	Bud burst – harvest

Fruitfulness, yield and annual biomass production

The overall bunch to shoot ratio was 2.2 bunches per shoot. The only significant source of variation was season, and across the seasons the bunch to shot ratio varied from 2 to 2.5, but season only accounted for 17% of the sums of squares.

A summary of the analyses of variance conducted on the components of yield and berry physical characteristics is presented in Table 9. With the notable exception of bunch weight, season was an important influence on components of yield and on berry physical characteristics. The concentration of CO_2 in the chambers significantly affected the number of bunches per vine and the weight of fruit per vine. There were only three significant interaction terms; season × heat with respect to bunches per vine, and season × [CO_2] with respect to kg fruit vine^{-1} and season × [CO_2] × heat with respect to mg skin berry^{-1}.

Across the four seasons of the experiment, about 17 kg of fruit per vine per season were produced; a level of productivity comparable to other Shiraz vines in the region trained and managed in the same manner. The seasonal means presented in Table 10 indicate that although the bunch number per vine was lower in 2014/15 compared to the 2016/17, yields per vine were similar due to more berries per bunch in 2014/15.

Table 9 Summary of analyses of variance for season, [CO_2], heat and interactions thereof on components of grape yield, berry characteristics and grape yield of Shiraz vines at Irymple

Source of variation	Yield component						
	bunches vine^{-1}	berries bunch^{-1}	g berry^{-1}	berry mm ∅	mg skin berry^{-1}	g bunch^{-1}	kg vine^{-1}
	Variance ratios' F probabilities						
Season	<0.001	<0.001	<0.001	<0.001	0.01	0.79	<0.0
[CO_2]	0.01	0.06	0.93	0.22	0.38	0.23	0.01
Heat	0.12	0.32	0.94	0.28	0.61	0.57	0.24
Season × [CO_2]	0.06	0.39	0.68	0.53	0.04	0.65	0.03
Season × heat	0.01	0.06	0.68	0.21	0.96	0.31	0.47
[CO_2] × heat	0.78	0.99	0.79	0.28	0.77	0.39	0.54
Season × [CO_2] × heat	0.95	0.56	0.40	0.67	0.01	0.46	0.81

The [CO_2] main effect on yield amounted to an additional 2.7 kg of fruit per vine for vines growing in the elevated CO_2 chambers. The additional yield could be attributed to 20 extra bunches per vine in response to elevated CO_2.

Table 10 Season main effects on yield and components of yield

Values presented are means (n = 16), and different superscripted letters within columns indicate a significant difference between means at P = 0.05.

Season	Yield component						
	bunches vine^{-1}	berries bunch^{-1}	g berry^{-1}	berry mm ∅	mg skin berry^{-1}	g bunch^{-1}	kg vine^{-1}
2013/14	157^{A}	73^{A}	1.05^{A}	11.3^{A}	174^{AB}	77	12.1^{A}
2014/15	249^{B}	70^{C}	1.12^{A}	10.4^{B}	159^{A}	78	19.2^{BC}
2015/16	228^{B}	58^{B}	1.25^{B}	11.7^{A}	184^{B}	72	16.5^{B}
2016/17	286^{C}	58^{C}	1.30^{B}	11.5^{A}	221^{C}	76	21.8^{C}

The season × [CO_2] interactions for yield indicated that the effect of elevated CO_2 was confined to the final season; vines growing in the eCO_2 and eCO_2+eTemp chambers produced 7.9 kg more fruit per vine than vines growing in the other chambers (Figure 8). The individual chambers' yield component data were too noisy to identify the basis of the extra yield, but season × [CO_2] means suggest — but do not prove — that more bunches per vine and larger berries may have contributed.

Using the dry weight of winter pruning wood as a proxy for annual shoot growth, there was no evidence over the four seasons of the experiment that either [CO_2] or air temperature had any impact on shoot growth Table 11. Season was a strong source of variation for the number of canes pruned off each season and their individual dry weight, but no seasonal effect was evident in the weight of pruning wood per vine because over seasons, cane numbers were inversely related to their individual dry weight. Across the seasons, about 1.8 kg of wood per vine was pruned off each season.

Figure 8 Season x [CO_2] effects on grape yield

Bars are means (n = 16), and different letters above means indicate significant differences between means P = 0.05.

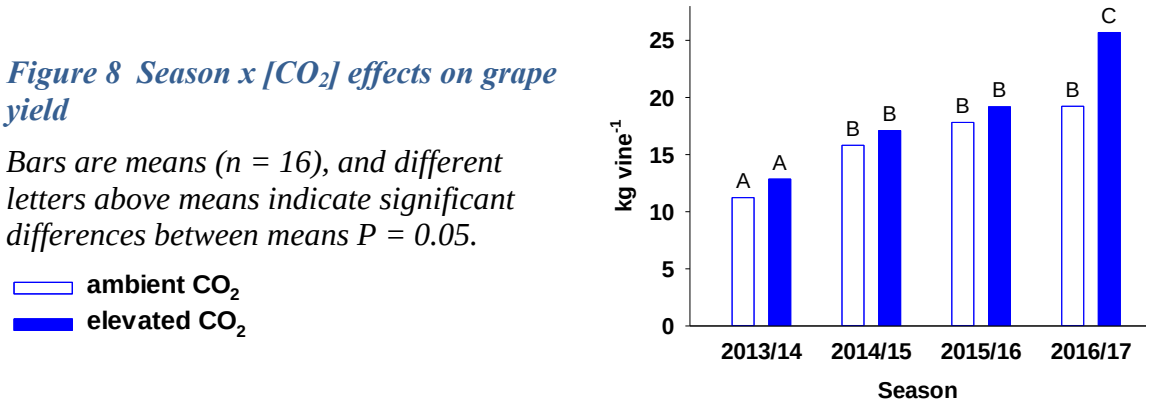


Table 11 Summary of analyses of variance for season, [CO_2], heat and interactions thereof on pruning wood and trunk diameter

**Trunk diameters recorded in the first three seasons only.*

Source of variation	Response			
	canes vine^{-1}	g dw cane^{-1}	g pruning wood vine^{-1}	trunk mm ∅*
	Variance ratios' F probabilities			
Season	<0.001	<0.001	0.18	0.02
[CO_2]	0.08	0.85	0.07	0.01
Heat	0.43	0.56	0.34	0.02
Season × [CO_2]	0.20	0.39	0.18	.85
Season × heat	0.46	0.65	0.77	0.40
[CO_2] × heat	0.99	0.33	0.08	0.50
Season × [CO_2] × heat	0.88	0.38	0.55	0.87

Using trunk diameter as a proxy for perennial wood growth, the analysis of variance indicated significant effects of season, [CO_2] and heat. The effects over the time frame of the experiment were small. The season affect amount to an increment of two mm from 60 mm in 2013/14 to 62 in 2015/16, and the trunk diameters of both eCO_2 and eTemp vines were two mm smaller than the trunk diameters of the ambient [CO_2] and ambient temperature vines.

Summary: Objective 1

For the most part, the scale of the seasonal effect far outweighed the scale of either heat or $[CO_2]$. The concentration of CO_2 in the air inside the chambers was seen to have sporadic inconsistent effects, generally marginal compared to the effects of heat, and almost inconsequential compared to the effect of season.

Elevated CO_2 was associated with a 2.7 kg season^{-1} vine^{-1} yield increase, but the effect was only evident in the final season, raising the possibility that the effect of higher atmospheric $[CO_2]$ may take some time to manifest. The overall effect of elevated CO_2 was far less than the season-to-season variability in yield which was of the order of just less than 10 kg vine^{-1}.

Again across seasons, elevated $[CO_2]$ advanced maturation by two days but under ambient temperatures, elevated $[CO_2]$ advanced maturity by seven days. Under ambient $[CO_2]$, the supply of warmer air advanced maturity by 15 days but under elevated $[CO_2]$, that response was modulated downward due to the effect of eCO_2 at ambient temperatures.

The effect of 2°C warmer air should be to make a cool season a warm season, and a warm season a hot season. Based on the BoM observations, 2016/17 would be considered the coolest, and 2014/15 the warmest. Based on empirical observations by industry and others that seasons are earlier or later depending on the temperatures experienced during the season, the 2014/15 season should have been the earliest and the 2016/17 season the latest. Even if that model doesn't completely hold up, the effect of 2°C warmer air should have been most evident in the warmest season, and least obvious in the coolest season. In fact, the effect of 2°C warmer air was only of the order of 8 days in the warmest season, and 12 days in the other seasons; with eCO_2 ameliorating that effect marginally in the final season.

Attempts to use the commonly accepted temperature indices to understand the effect of seasonal temperatures in terms of the current understanding of the cold and heat requirements of grapevines to burst buds and grow and mature a crop were not successful. It was simple to show that the vines in the heated chambers accumulated less $CH_{0\text{-}7.2}$ and more $GDD_{4.5}$ and GDD_{10}, but those indices did not align fully with the timing of the start of the season and intervals between the phenological milestones. The temperature indices used, namely $CH_{0\text{-}7.2}$, $GDD_{4.5}$ and GDD_{10}, were not exhaustive of the temperature indices used across perennial horticulture, but have proven useful *a posteriori* to explain observations of grapevine phenological behavior. In a sense, the various temperature indices are an attempt to describe the thermal requirements to initiate, carry out and complete various development stages. The experiment enabled the testing of *a priori* models describing vines' thermal requirements because half the vines were experiencing across the board 2°C warmer air compared to the other half of the vines. However, the lack of success using $CH_{0\text{-}7.2}$, $GDD_{4.5}$ and GDD_{10} perhaps reflects the poor descriptive power of the indices in relation to the thermal requirements of Shiraz vines because the phenological milestones were moved more by season than by warming the air in the OTCs by 2°C.

One obvious deficiency of current models is the underlying assumption that "thermal units" have equal value whenever in a season they occur, and that their effect is irreversible. A recent development that seeks to address part of this criticism is the re-conceptualisation — and quantification — of woody perennial plants' chilling requirements in relation to bud burst and spring growth; an approach that has become known as the "Dynamic Model" (Erez and Fishman, 1998, Fishman *et al.*, 1987a, Fishman *et al.*, 1987b, Pope *et al.*, 2014). This model is gaining acceptance as a general model of perennial plants' requirement for chilling to release buds from endodormancy. The calculations are complex, and the algorithms give more weight to prolonged periods of cold compared to short periods of cold. Whilst this

specific approach may have limited applicability to grapevines, which seem to have very low chill requirements in any case, the re-conceptualisation is likely to lead to consideration of the positive and negative impacts of higher and lower temperatures at different parts of the seasonal growth cycle.

Results and Discussion: Objective 2

To quantify the impact of elevated temperature and CO_2 on vine physiology and carbohydrate storage.

Photosynthetic rates of C_3 plants, such as grapevines, are typically limited by CO_2, despite the exponential rise in atmospheric [CO_2] since pre-industrial times, as atmospheric [CO_2] has yet to reach the level required to saturate the C_3 photosynthetic system (Farquhar *et al.*, 1980). As a result, grapevines grown in a high-CO_2 atmosphere, such as the 650 µmol mol^{-1} used during this project, generally have higher rates of photosynthesis than those grown under current atmospheric conditions (da Silva *et al.*, 2017, Martínez-Lüscher *et al.*, 2015, Moutinho-Pereira *et al.*, 2009). Furthermore, this is the primary, direct effect of elevated atmospheric [CO_2] (eCO_2) on plants.

However, C_3 plant species grown under eCO_2, but measured at current atmospheric [CO_2] (approx. 400 µmol mol^{-1}), often have photosynthetic rates that are lower than those of plants grown in the current atmosphere (Bernacchi *et al.*, 2005, Sage, 1994). This is commonly termed photosynthetic acclimation and is usually due to a reduction in photosynthetic capacity, that is, the investment in the proteins that form the photosynthetic machinery (Wong, 1979, Woodrow, 1994).

Grapevines in many Australian growing regions tend to have relatively high carbohydrate reserves and these tend to determine the early season vigour (Holzapfel *et al.*, 2010). Consequently, it is important to determine how grapevine carbon assimilation and allocation responds to the predicted changes in atmospheric [CO_2] and climate, as these responses will determine the adaptation required by the industry to maintain the desired vigour and production levels of wine grape plantings.

A side effect of higher atmospheric [CO_2] — that has implications for vine water use — is that C_3 plants often exhibit reduced stomatal opening (Ainsworth and Rogers, 2007, Medlyn *et al.*, 2001). Stomatal opening, usually defined as stomatal conductance (g_s), is under fine-grained control by the plant and regulated by a number of factors, one of which is the [CO_2] within the stomatal cavity. Typically, the photosynthetic capacity of the leaf is inadequate to fully utilise the additional CO_2 in an eCO_2 atmosphere, such as the 650 µmol mol^{-1} used here, resulting in higher inter-cellular [CO_2] within the leaf (C_i). The increase in C_i then drives a decrease in g_s. The importance of this response is the potential effect on vine water use, which is a product of atmospheric conditions, canopy size, and g_s. Assuming the other factors remain fixed, a decrease in g_s will reduce vine water use. Again, it will be important for industry adaptation to understand how vine water use will respond to a likely future climate.

Photosynthesis in C_3 plants is also temperature sensitive (Berry and Bjorkman, 1980, Sage and Kubien, 2007), although the impact of temperature is generally less than that of the likely future change in atmospheric [CO_2]. Changes in plant tissue temperature can also affect almost every other biochemical process occurring in those tissues, for example, respiration typically doubles for every 10°C temperature increase. Although our previous OTC project (DPI 09/01) examined the impacts of a 2°C increase in temperature on three wine grape cultivars and found minimal effect on leaf level physiology (Sommer *et al.*, 2012), it is far from clear how temperature and eCO_2 might interact on these processes.

Vine carbon acquisition

The effect of eCO_2, eTemp and their interaction on photosynthesis at the leaf level was examined by undertaking leaf gas exchange measurements at key phenological periods, made

at approximately the same time in each of the four seasons. Each measurement assessed maximum assimilation under the prevailing conditions (*i.e.* air temperature and [CO_2]), assimilation at a common [CO_2] and other fundamental photosynthetic parameters, such as maximum rate of carboxylation.

Assimilation under saturating light (A_{sat}) was used as the basic comparison between the treatments as this was representative of the maximum effective photosynthetic rate under the prevailing conditions and, as sunlight in the Murray Valley typically exceeds that needed to light saturate photosynthesis in grapevines, was also representative of the conditions in which the majority of vine carbon would have been fixed. A comparison of the non-chamber control and the OTC Control did not demonstrate any significant chamber effect, whether analysed across the entire data set (split-split plot ANOVA), within a given season (split-plot ANOVA) or within a given measurement date (t-test), P was typically > 0.3. The same was true for all the other gas exchange parameters measured, so chambering vines had no effect on leaf level gas exchange, and the non-chamber control will not be considered further.

Comparing A_{sat} of eTemp and OTC Control across the entire data set or within seasons (Figure 9) showed that +2°C had no impact on A_{sat}, although there was a single measurement date (veraison 2015) where A_{sat} of eTemp vines was significantly lower than OTC Control vines (P = 0.005). This outcome matched that of the previous project (Sommer *et al.*, 2012) in suggesting that, at the leaf level, +2°C is unlikely to have a direct impact on photosynthetic rates in wine grapes.

In contrast, A_{sat} of leaves from the elevated [CO_2] treatments (*i.e.* eCO_2 and eCO_2+eTemp) were significantly higher than leaves from the OTC Control (P = 0.005, split-split plot ANOVA, Figure 9). Comparisons on individual dates were typically significant also, or at least marginally so (P < 0.1). Differences post-harvest were lower than earlier in the season and not significant when measurement dates were examined individually (t-test, P > 0.05). However, a split-plot ANOVA across the three seasons where post-harvest A_{sat} data were available, suggested that the lack of statistical significance on individual dates was probably due to the low replication (n = 4), because there was a significant effect of eCO_2 across the post-harvest data set as a whole (P = 0.025).

When all of the pre-harvest OTC data was examined together (split-split plot ANOVA), there was a significant [CO_2] × heat interaction term (P = 0.036); leaves from the eCO_2+eTemp vines having a higher A_{sat} than leaves from the eCO_2 treatment (also true of the post-harvest A_{sat} measurements, Figure 9). This observation suggests that prior work investigating the impact of climate warming on grapevines and prior work investigating the impact of elevated CO_2 on grapevines have not effectively represented the impact of the likely future climate, where warming and elevated CO_2 will be combined. This is an important finding of the project and only possible due to the unique experimental system developed for this work.

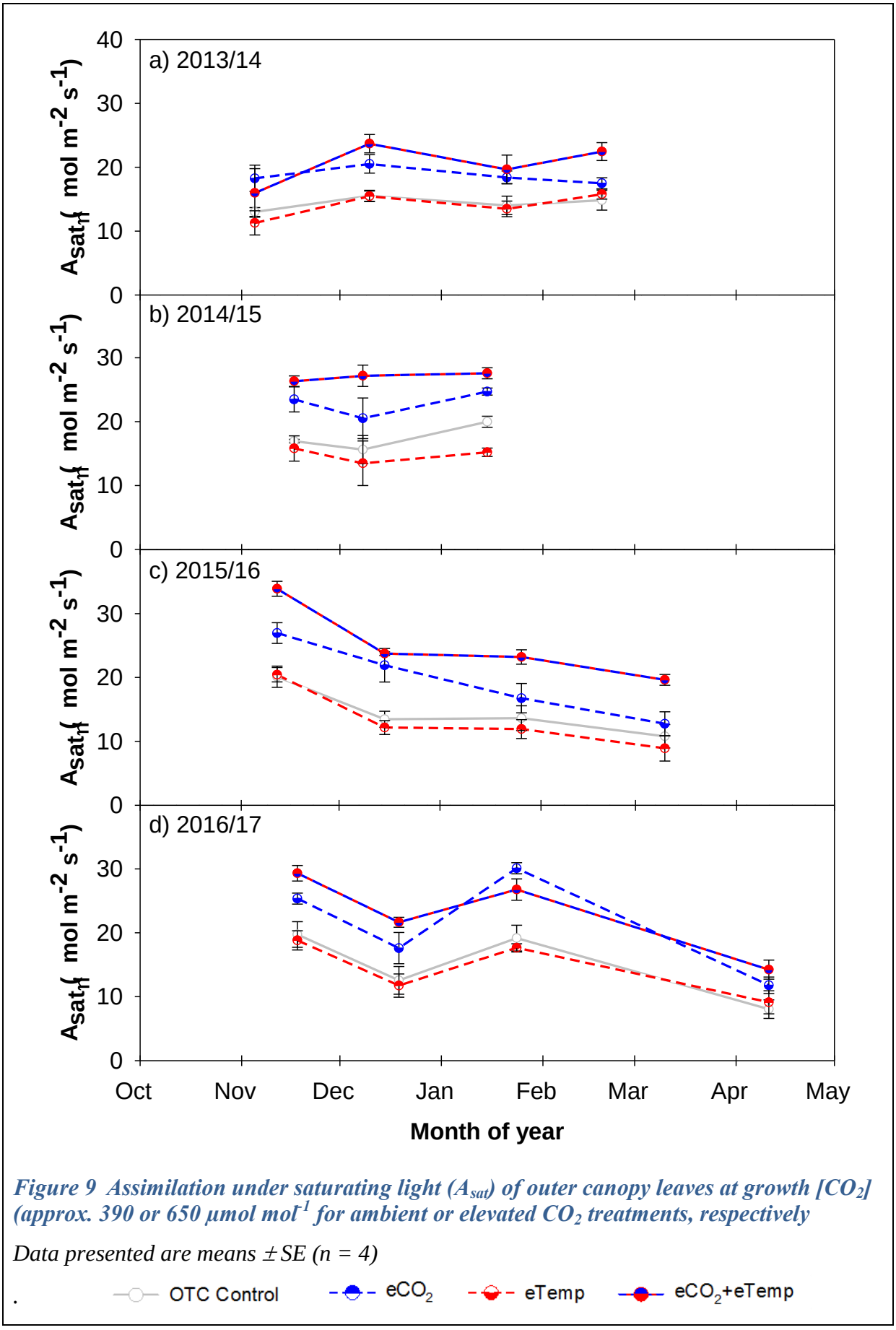


Figure 9 Assimilation under saturating light (A_{sat}) of outer canopy leaves at growth [CO_2] (approx. 390 or 650 µmol mol^{-1} for ambient or elevated CO_2 treatments, respectively

Data presented are means ± SE (n = 4)

.

The significant increase in A_{sat} under eCO_2 (irrespective of temperature) indicates that any acclimation (complete acclimation defined as maintaining homeostasis) to growth under eCO_2, such as adjustment of nitrogen investment into photosynthetic proteins, was, at best, limited. However, there was some evidence of acclimation, with assimilation measured at a common air [CO_2] being significantly lower in eCO_2 vines than OTC Control vines during the 2013/14 season (split-plot ANOVA, P = 0.026) and, individually, on the veraison 2015 measurement date (P = 0.042), although, when the entire data set was examined (split-split plot ANOVA), no effect of eCO_2 was significant (Figure 10 a & b). There was also no significant effect of eTemp, but despite these results there was a significant [CO_2] × heat interaction term. Furthermore, although there was no significant difference in assimilation measured at a common [CO_2] between the OTC Control and any of the other treatments, the eCO_2+eTemp treatment was significantly higher than the eCO_2 treatment (P = 0.040). These mixed results, depending on data set and statistics applied, suggest there may be a small degree of acclimation in the eCO_2 treatment, but importantly, clearly demonstrate no acclimation at all in the combined eTemp+eCO_2 treatment.

The maximum rate of carboxylation ($V_{c\ max}$) and maximum electron transport rate (J_{max}) across the whole data set supports this conclusion, with no significant effects of eCO_2, eTemp or the combined treatment on either parameter (Figure 10 c & d). Even when adjusted for differences in temperature between treatments and measurement date, there were no significant treatment effects on $V_{c\ max}$ (Figure 10 c, grey bars).

Acclimation to eCO_2 in C_3 plants is often driven by changes in N allocation to the leaves and the photosynthetic proteins (Edwards *et al.*, 2006, Temme *et al.*, 2017, Wong, 1979). Changes in the activation state of RuBisCO may also occur (Moore *et al.*, 1999), but it was beyond the scope of this project to examine the latter aspect. Leaf N concentration (mg g^{-1} dry mass) was monitored during the 2013/14 season and assessed early in the 2014/15 season (Figure 11). Prior to harvest in the 2013/14 season there was a significant reduction in leaf N under eCO_2 (P = 0.007), but no effect of, or interaction with, eTemp (Figure 11 d). Leaf N decreased significantly during the season and by post-harvest was less than two thirds the concentration at anthesis. There was also no treatment effect on leaf N by this point. Numerically, leaf N at anthesis in the 2014/15 season followed the same pattern (Figure 11a), but was not statistically significant. It is likely that additional sample analysis would demonstrate an effect in the further seasons. Irrespective, the impact of eCO_2 on leaf N was only large at anthesis in 2013 and appeared to be due to changes in leaf structure or composition, as specific leaf area (SLA; $m^2\ kg^{-1}$) was lower under eCO_2, whether in combination with eTemp or not (Figure 11 b & Figure 12). Adjusting leaf N for changes in SLA, by expressing N on a leaf area basis, negated any treatment impacts (Figure 11 c). Assuming that leaf N was broadly representative of the N investment in, and amount of, photosynthetic proteins (Evans, 1989, Harrison *et al.*, 2009) this suggests that the photosynthetic capacity per unit area of leaf (the light absorbing surface), was unaffected by [CO_2] or heat. This further supports the conclusion that there was no biologically significant acclimation to the eCO_2 treatment under the growth conditions (adequate nutrient supply and water availability).

As with leaf N, SLA also declined during the growing season (Figure 12), due to greater biomass per unit area of leaf, suggesting that the apparent changes in leaf N over time were also due to differences in SLA, rather than a mobilisation of N and export from the leaf. Changes in leaf N immediately prior to leaf-fall were not examined, so any potential impact of eCO_2 on litter quality was not measured.

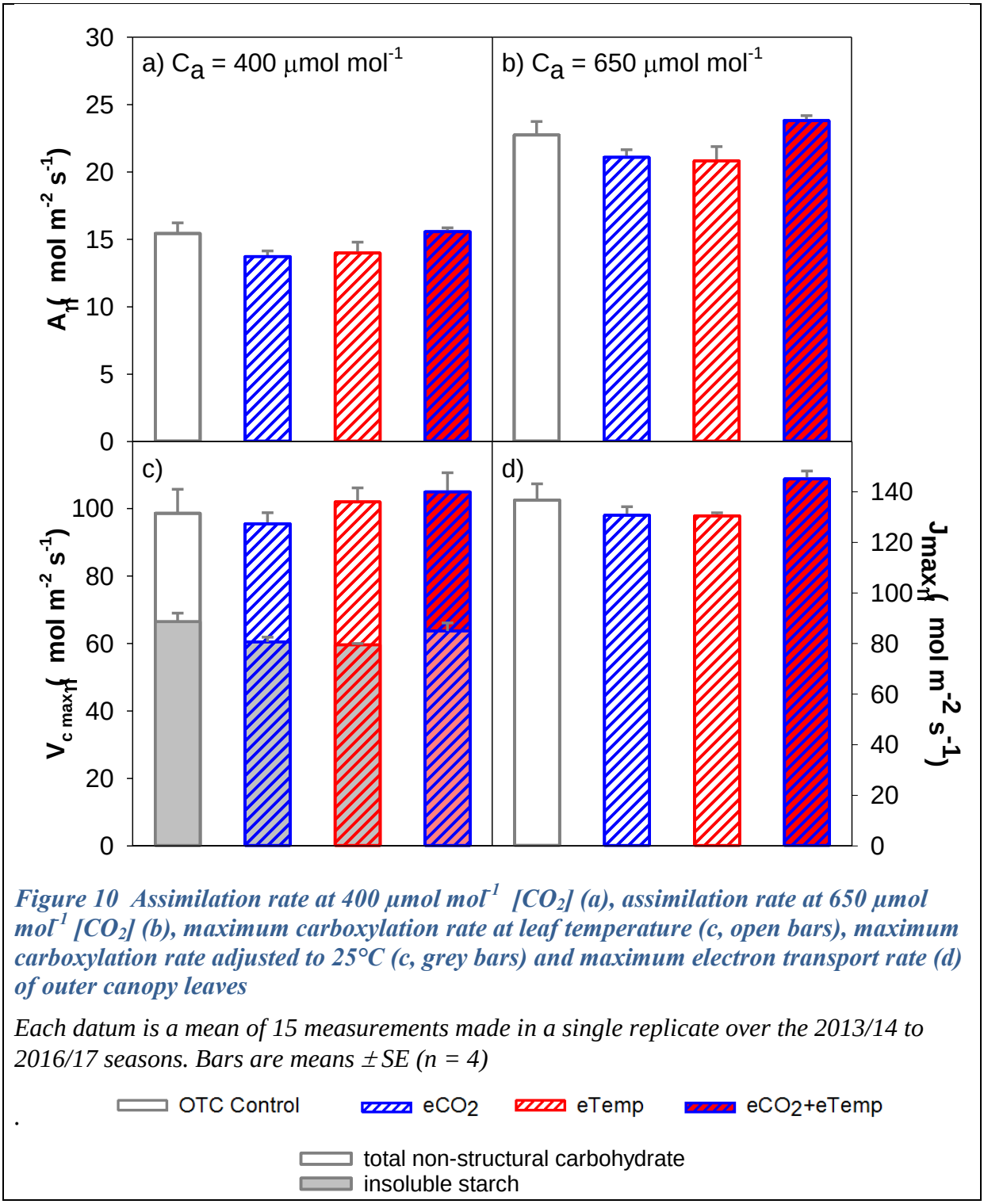


Figure 10 Assimilation rate at 400 µmol mol^{-1} [CO_2] (a), assimilation rate at 650 µmol mol^{-1} [CO_2] (b), maximum carboxylation rate at leaf temperature (c, open bars), maximum carboxylation rate adjusted to 25°C (c, grey bars) and maximum electron transport rate (d) of outer canopy leaves

Each datum is a mean of 15 measurements made in a single replicate over the 2013/14 to 2016/17 seasons. Bars are means ± SE (n = 4)

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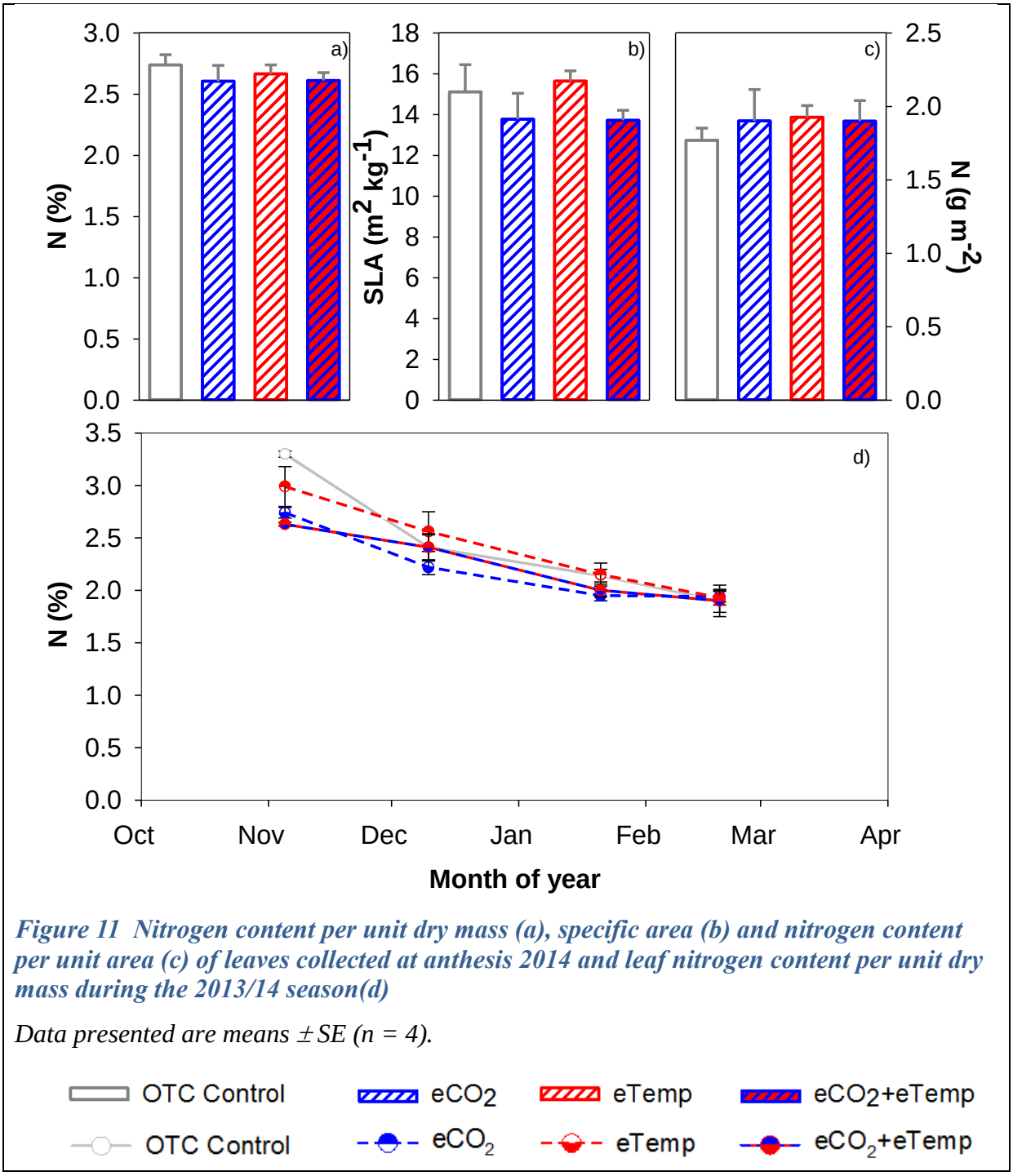


Figure 11 Nitrogen content per unit dry mass (a), specific area (b) and nitrogen content per unit area (c) of leaves collected at anthesis 2014 and leaf nitrogen content per unit dry mass during the 2013/14 season(d)

Data presented are means ± SE (n = 4).

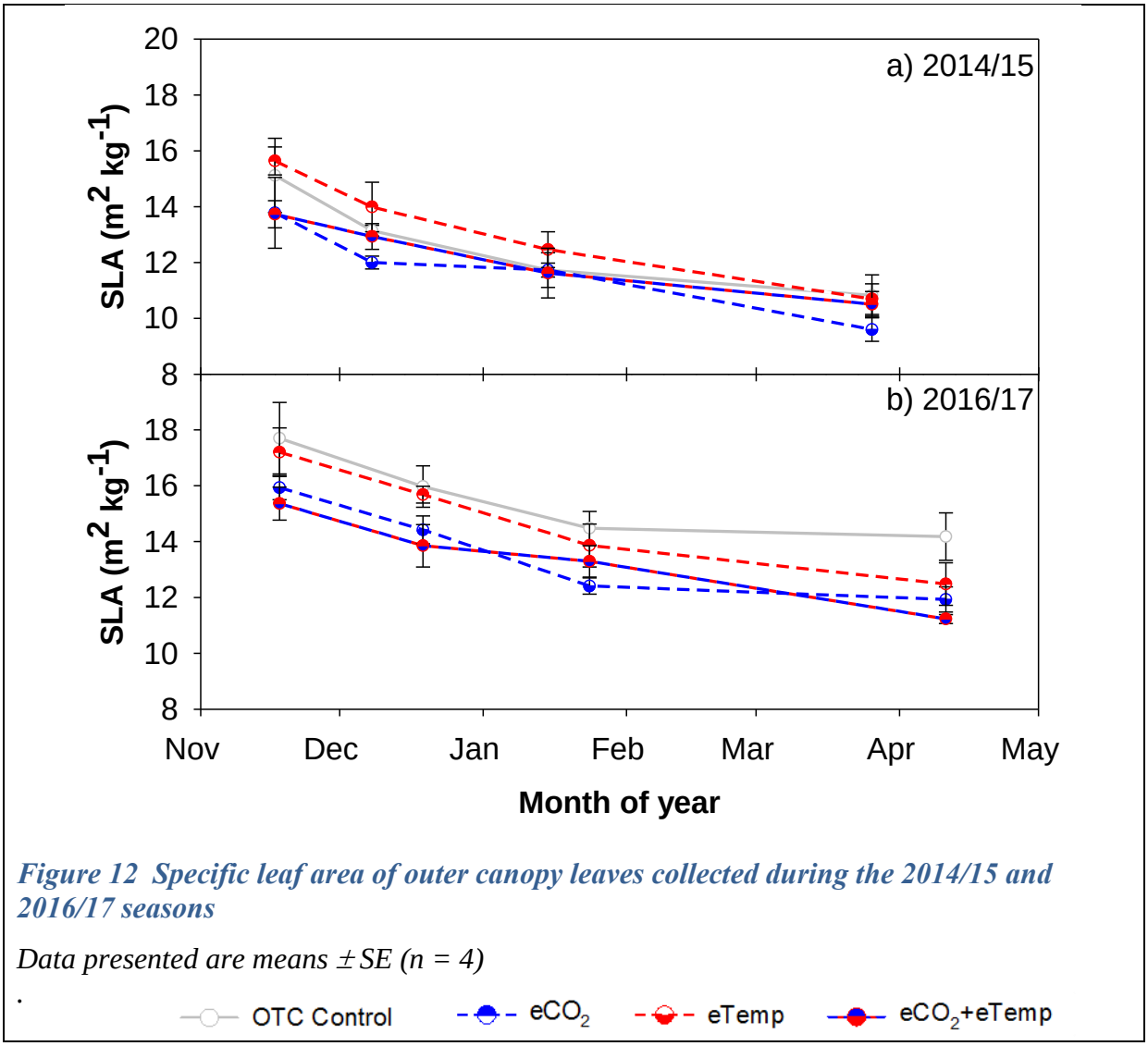


Figure 12 Specific leaf area of outer canopy leaves collected during the 2014/15 and 2016/17 seasons

Data presented are means ± SE (n = 4)

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The observed responses of A_{sat} to eCO_2 and eCO_2+eTemp imply additional production of total non-structural carbohydrates (TNC) in those vines. Indeed, higher TNC may also have played a role in the changes observed in SLA with eCO_2. The leaves used for the gas exchange and N measurements were also analysed for TNC concentration (defined as the sum of water soluble carbohydrates [WSC] and insoluble starch), allowing assimilation rates to be linked to TNC with some degree of certainty that the results are not biased by sampling differences.

As might be anticipated from the A_{sat} data, across the entire data set, leaf TNC concentration was higher under the eCO_2 treatments ($P < 0.001$; Figure 13). This was due to changes in insoluble starch concentration ($P < 0.001$), rather than WSC (not significant). There was also a small reduction in TNC ($P = 0.036$) and starch ($P = 0.023$) in the eTemp treatments. Furthermore, there was no interaction between [CO_2] and heat, implying that, despite the consistent difference in A_{sat} between eCO_2 and eCO_2+eTemp treatments, not only was TNC of eCO_2+eTemp not higher than eCO_2, it was actually lower.

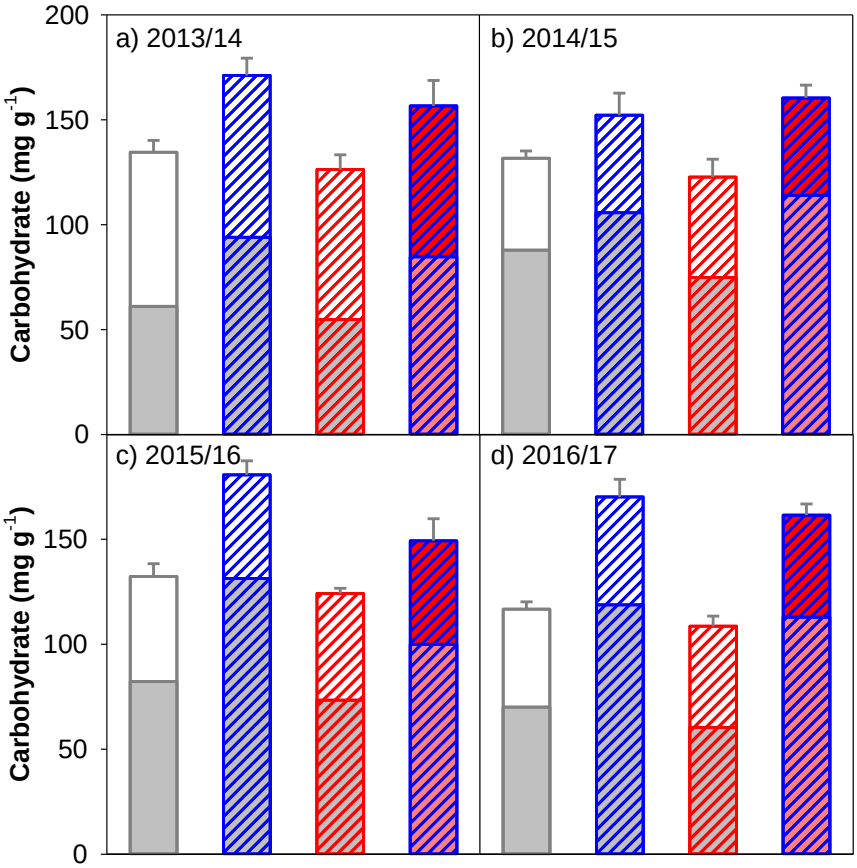


Figure 13 Total non-structural carbohydrate concentration (open bars) and insoluble starch concentration (grey bars) of leaves used for gas exchange measurements during the 2013/14 to 2016/17 seasons.

Each datum is a mean of 3-4 leaves measurements out in a single replicate over the 2013/14 to 2016/17 seasons. Bars are means ± SE (n = 4).

There are two potential explanations for the effect of eTemp on TNC and the discrepancy between the effect of eCO_2+eTemp on A_{sat} and on TNC. Firstly, a change in night-time respiration rate (A_{sat} is net CO_2 uptake, so day-time respiration is already accounted for) and, secondly, a change in export or utilisation of TNC. Changes in these processes are not necessarily mutually exclusive.

It was not possible to examine TNC export, but diurnal changes in leaf TNC were measured on several occasions through three of the four seasons to provide some indication of what might be driving the eTemp effects. A difference in night-time respiration, for example, may be seen as a greater drop in TNC during the hours of darkness. A summary of these data is provided in Figure 14 as line fits through the entire data set, plus 90% confidence intervals for the OTC Control. There was no clear evidence of greater loss of TNC in the eTemp treatments at night, with the shapes of the curve fits being similar in all treatments. However, the data did corroborate the results from the gas exchange leaves, with higher TNC under

eCO_2 and lower TNC under eTemp and no interaction.

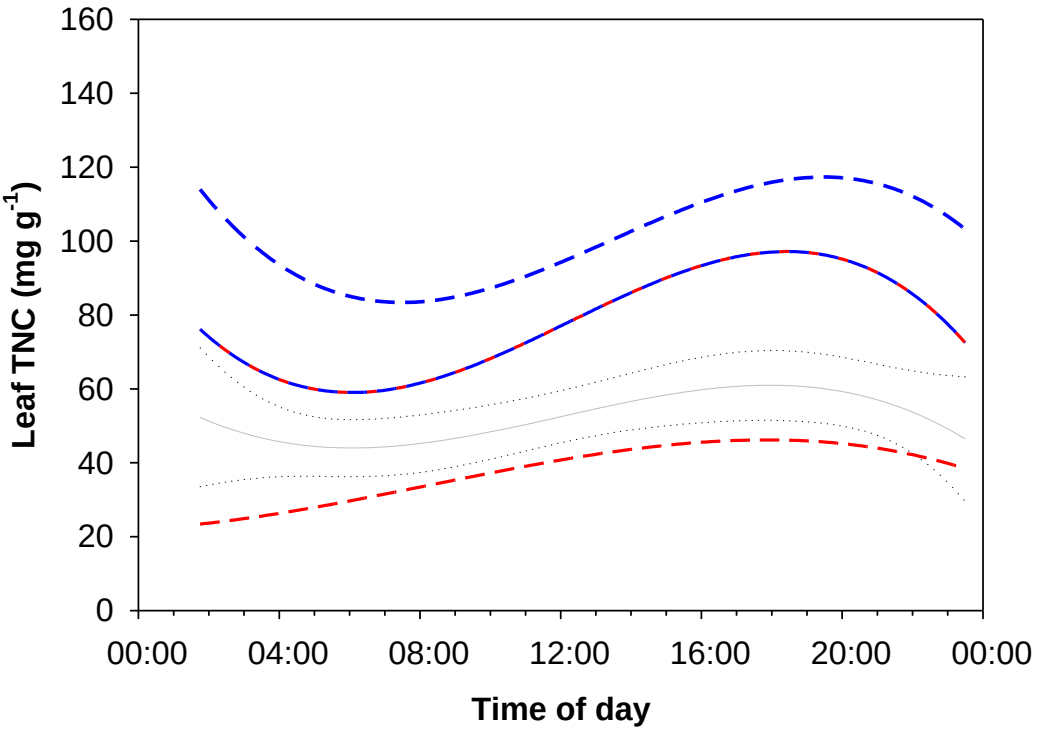


Figure 14 Diurnal changes in total non-structural carbohydrate concentration (TNC) of outer canopy leaves

Each line is a 3rd order polynomial regression of data from approximately 200 leaves collected at 8 - 9 time points on each of six days during the 2014/15 to 2016/17 seasons. The dotted grey lines represent the 90% confidence interval for the fit through data from OTC Control

Although the shape of the curves was similar for all treatments, the absolute amplitude was not. The difference between the maximum and minimum TNC concentration was higher under eCO_2 than the OTC Control or eTemp treatments ($P < 0.001$, Figure 15), but there was no effect of eTemp, and elevated temperature did not modulate the response to elevated [CO_2]. This suggests that not only was C assimilation higher under eCO_2, but the utilisation of that C was also higher. The range was affected by time of season, being reduced post-harvest in line with the lower A_{sat} observed.

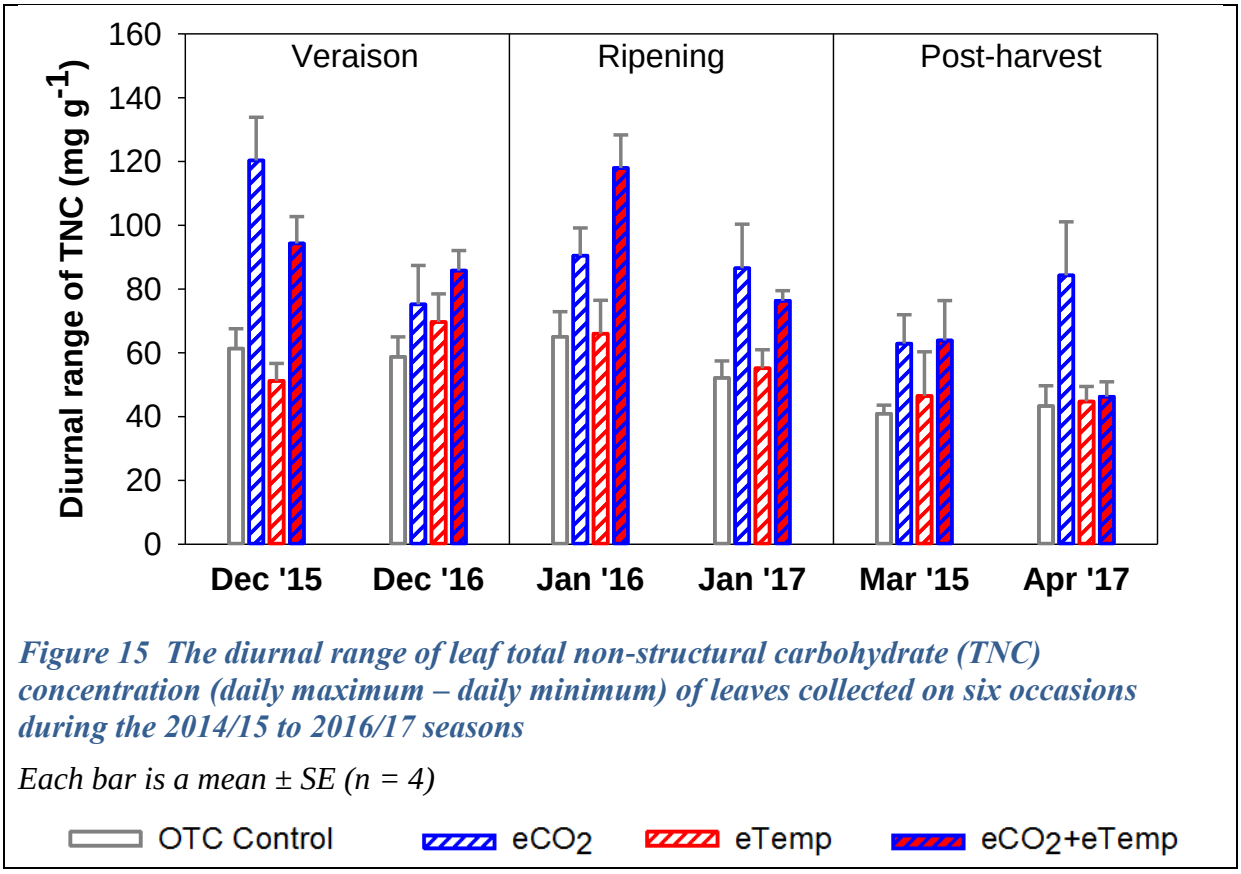


Figure 15 The diurnal range of leaf total non-structural carbohydrate (TNC) concentration (daily maximum – daily minimum) of leaves collected on six occasions during the 2014/15 to 2016/17 seasons

Each bar is a mean ± SE (n = 4)

The difference in TNC range over 24 hours between the OTC Control and eCO_2 treatments was approximately 5% of the dry mass of the leaf. The difference in SLA between the OTC Control and eCO_2 treatments equated to approximately a 15% increase in dry mass. Consequently, it is unlikely that much of this additional TNC is being incorporated into the leaf.

As noted above, although the diurnal data doesn't appear to support it, it was possible that changes in night-time respiration rate were playing a role in the observed differences in leaf TNC and TNC dynamics. Accurately estimation of night-time respiration is technically difficult, especially when comparing across temperatures, as it is difficult to make the measurement without influencing it. Nevertheless, a methodology was developed (see materials and methods) to test this and measurements were made in a sub-set of the chambers at two time-points in January 2017.

Leaf temperature was significantly different between elevated temperature treatments and ambient temperature treatments (Figure 16 a). Leaf temperature was also significantly lower pre-dawn than, at nightfall, although the absolute difference was only 3°C, due to the specific conditions during the night of measurement. When analysing all the data in a single model, there was no significant effect of the eTemp treatments on dark respiration ($P = 0.929$). In contrast there was an effect of measurement time (nightfall vs pre-dawn, $P = 0.020$) and, perhaps surprisingly, a marginal effect of eCO_2 ($P = 0.059$).

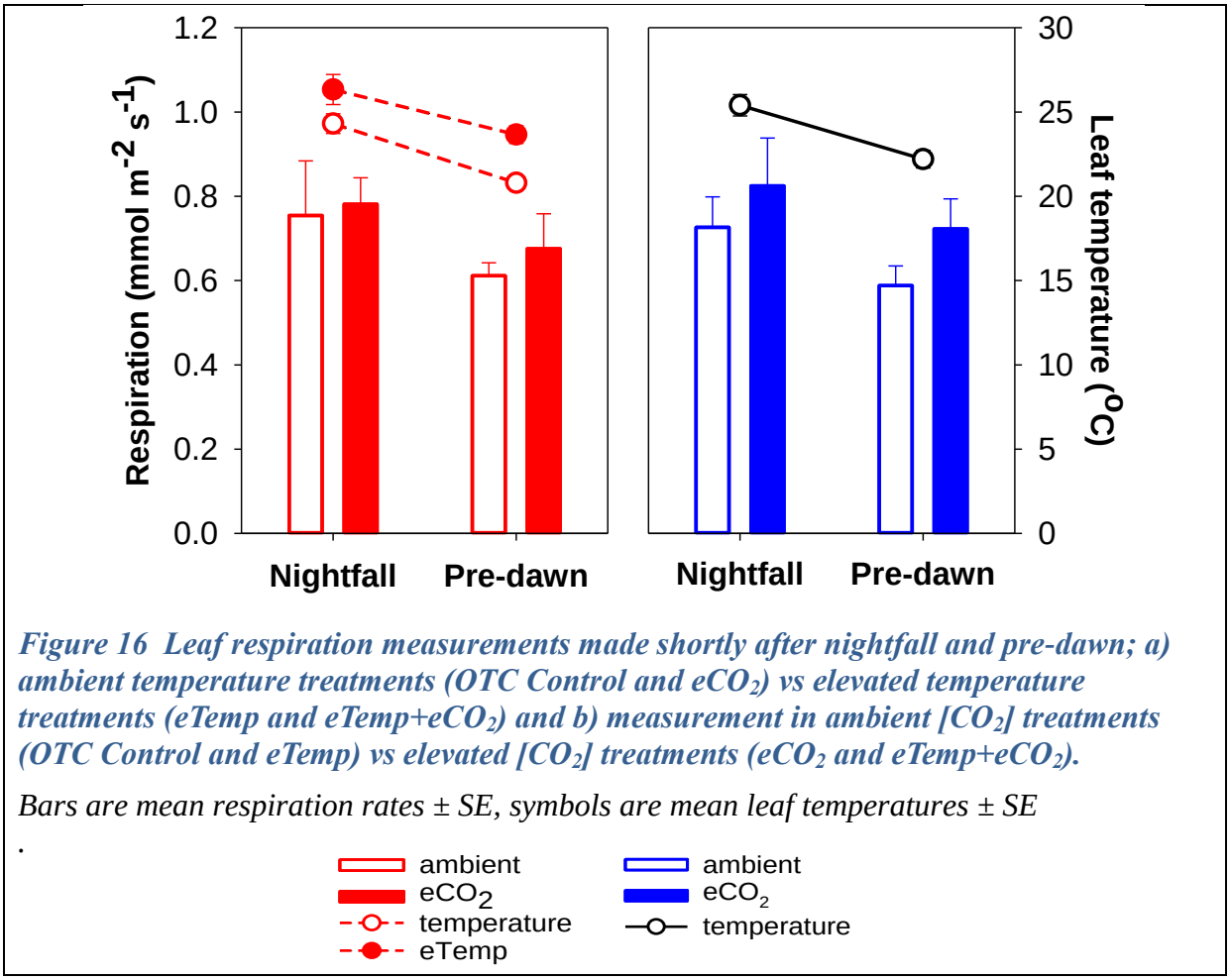


Figure 16 Leaf respiration measurements made shortly after nightfall and pre-dawn; a) ambient temperature treatments (OTC Control and eCO_2) vs elevated temperature treatments (eTemp and eTemp+eCO_2) and b) measurement in ambient [CO_2] treatments (OTC Control and eTemp) vs elevated [CO_2] treatments (eCO_2 and eTemp+eCO_2).

Bars are mean respiration rates ± SE, symbols are mean leaf temperatures ± SE

.

Either as a result of falling temperatures or reducing sink for respiratory products (Bruhn *et al.*, 2008), a reduction in leaf respiration during the night is commonly observed and would be expected.

The temperature response of respiration (calculated as Q_{10}, the increase in respiration for a temperature rise of 10°C) was also measured. Based on those Q_{10} measurements and the measured difference in leaf temperature between the ambient and eTemp treatments, the elevated temperature treatments could have been expected to be 12% higher than the ambient temperature treatments at nightfall and 35% higher pre-dawn. The actual differences were only 4 and 10%. Combined with the lack of a statistically significant effect, it appears that night-time leaf respiration had largely or completely acclimated to the eTemp treatment. This, in turn, suggests that the difference observed in leaf TNC with the eTemp treatment may be due to changes in C export and utilisation, therefore leaf TNC levels may not be representative of the whole vine.

The marginally significant response of leaf respiration in the elevated [CO_2] treatments (eCO_2 and eCO_2+eTemp), despite no difference in temperature (*i.e.* between eCO_2 and OTC Control, or eCO_2+eTemp and eTemp) may be indicative of a higher demand for respiratory products, perhaps driven by the greater C availability in these vines. However, conclusions need to be treated with caution due to the limited leaf respiration dataset.

One sink for the additional carbon fixed and, apparently, exported from the canopies of the vines grown under elevated [CO_2] is the vine's storage reserves. Carbohydrates are stored in

the canes, trunk and roots systems of vines, although under normal circumstances, most of the carbohydrate in the canes is removed during pruning. The most important pool for spring growth is probably the root system (Holzapfel *et al.*, 2010), but this was, in effect, inaccessible during this project due to the limited number of vines available for study and the need to leave those vines undisturbed for future seasons. The potential pool sizes of the canes (as estimated by pruning weight), and trunk (as estimated by circumference), were not affected by any of the treatments, thus any change in TNC concentration of these tissues would represent a change in the actual carbohydrate pool. However, neither cane nor trunk TNC concentration were significantly altered by any treatment over the four seasons of the project work (Figure 17). The lack of effect, despite the demonstrated increase in C availability within the vine, may have been because the storage reserve sink as a whole did not alter, due to the pre-eminence of the root TNC pool for growth, or simply because any changes were not large enough to be detected over the background TNC in these tissues.

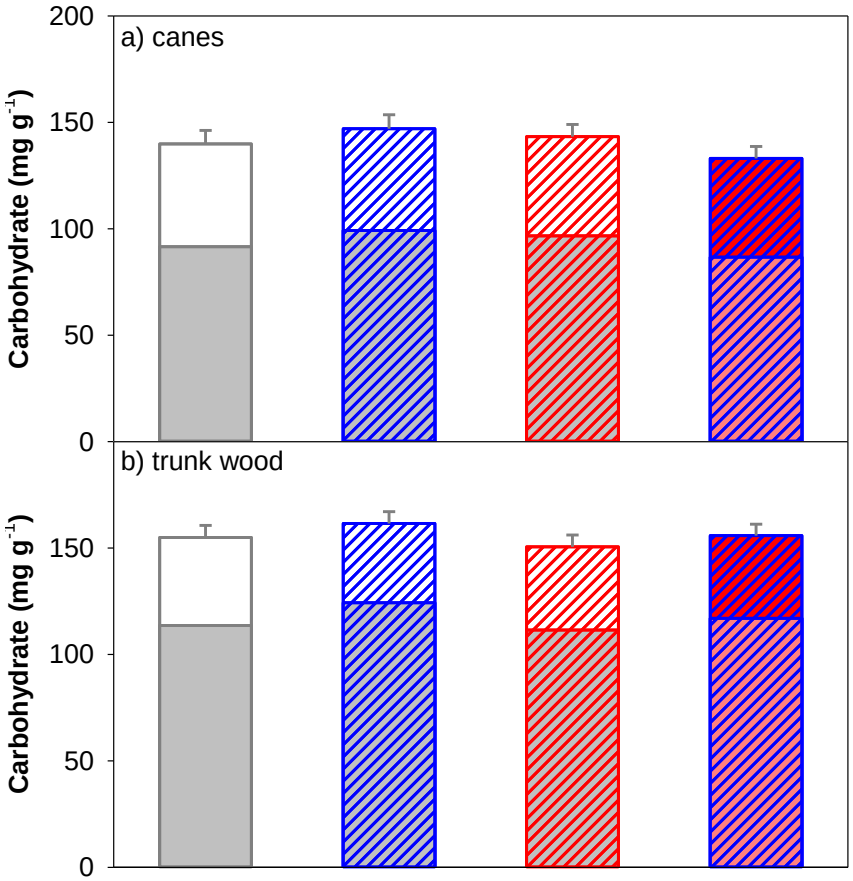


Figure 17 Carbohydrate reserves of a) canes and b) trunk wood between 2013 and 2017.

Each datum is a mean of one measurement per season per replicate for four seasons
Reserves are defined as total non-structural carbohydrates (open bars) and insoluble starch (grey fill bars) and are means ± SE (n = 4).

Vine water use and water use efficiency

As an irrigated crop, utilising a highly controlled and limited water supply, any impact of climate change on viticultural water use efficiency could have large ramifications for industry profitability. All of the components, of climate change, including warming and elevated atmospheric [CO_2], have the potential to influence water use efficiency.

Gas exchange measurements have been used throughout the project to examine treatment effects on g_s and leaf level water use. In addition, whole vine water use was examined using sapflow sensors in two seasons. Both sets of data allow various forms of water use efficiency to be calculated and assessed.

There were no consistent significant treatment effects of either eCO_2 or eTemp, or consistent interactions between season or phenological period and treatment on g_s as measured by gas exchange (Figure 18). However, g_s is particularly sensitive to small environmental changes,

so absolute values of g_s varied strongly between measurements and the gas exchange measurement system itself alters the leaf environment to some extent. Consequently, g_s was also examined using a different instrument (a porometer) which is much faster and has fewer impacts on the leaf environment. These data are summarised in Figure 19**,** normalised to the OTC Control (at each sample date) and averaged across each season. There appeared to be a small effect of eCO_2 (<10% reduction relative to the OTC Control) and this was significant when eCO_2 was compared to OTC Control across all four seasons (P = 0.047).

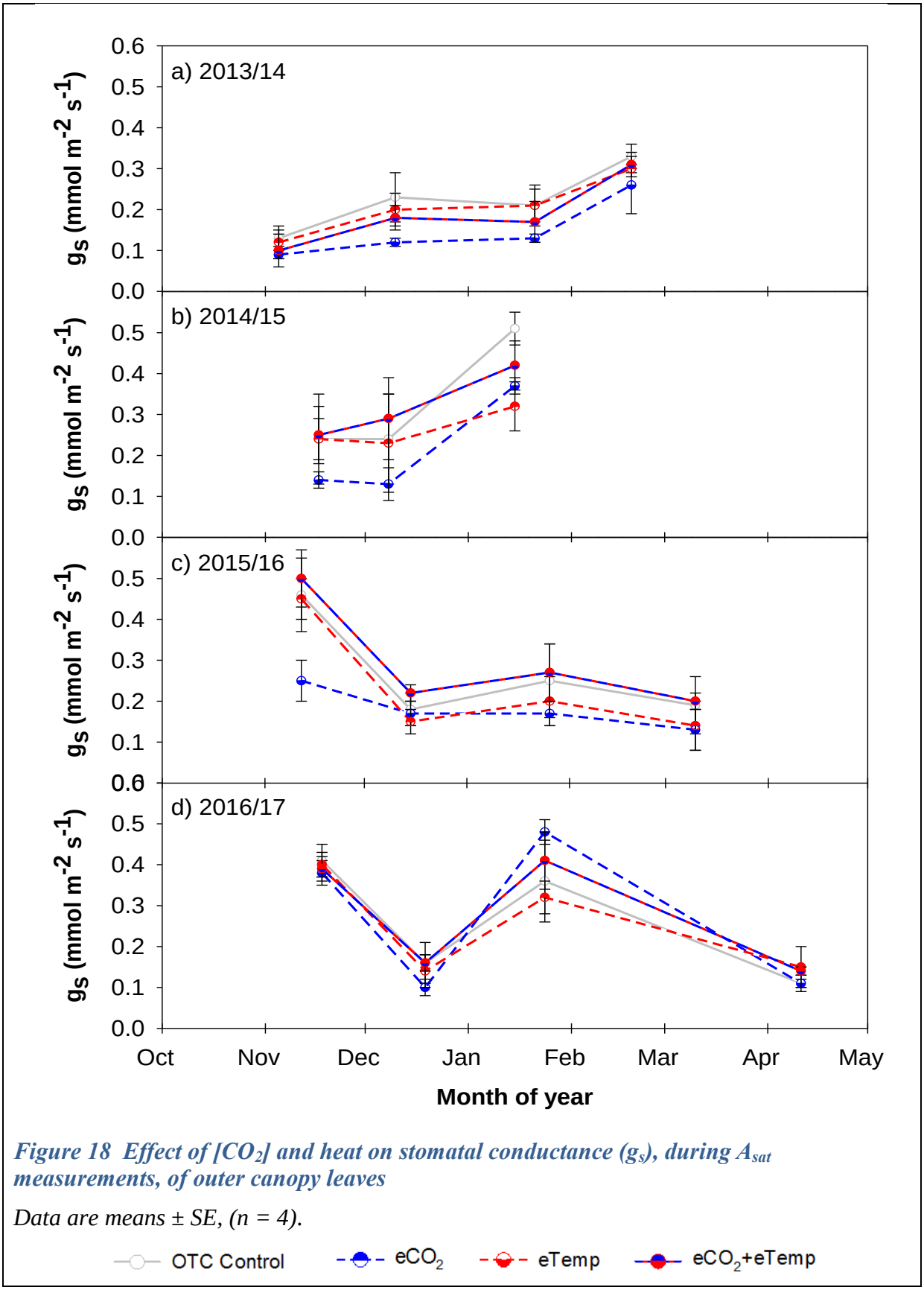


Figure 18 Effect of [CO_2] and heat on stomatal conductance (g_s), during A_{sat} measurements, of outer canopy leaves

Data are means ± SE, (n = 4).

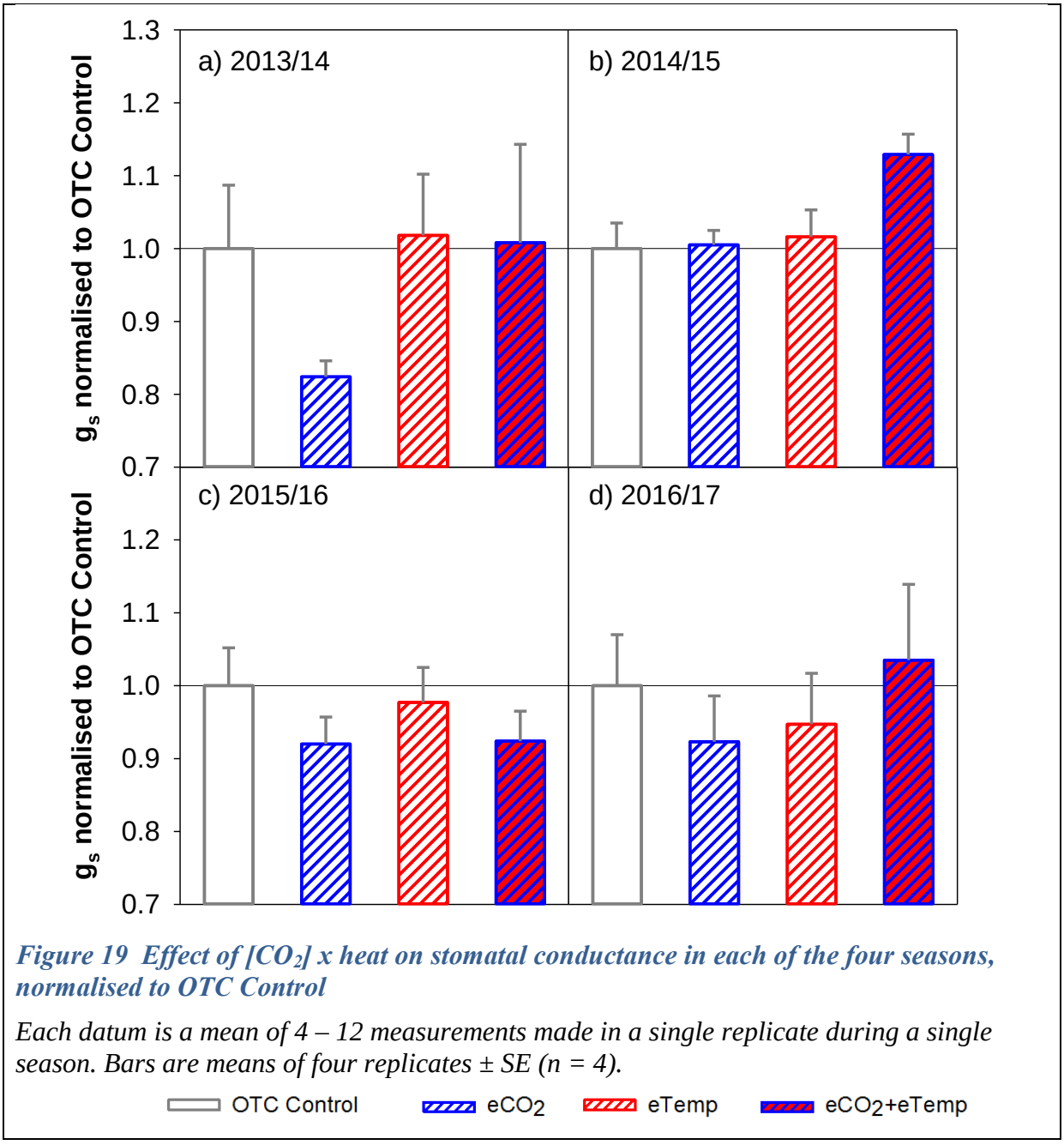


Figure 19 Effect of [CO_2] x heat on stomatal conductance in each of the four seasons, normalised to OTC Control

Each datum is a mean of 4 – 12 measurements made in a single replicate during a single season. Bars are means of four replicates ± SE (n = 4).

Irrespective of any marginal reduction in g_s under eCO_2, there was no change in g_s of the eCO_2+eTemp vines. Consequently, it is unlikely that there will be any reduction in water use, even at the leaf level, of vines grown under a future climate.

Leaf level water use, E, was also measured during the gas exchange measurements, but not during the porometer measurements (Figure 20). There was a very marginal effect of the warming treatments (P = 0.098) that was only slightly stronger if the first season was excluded (P = 0.080), but there was little evidence of a major treatment effect.

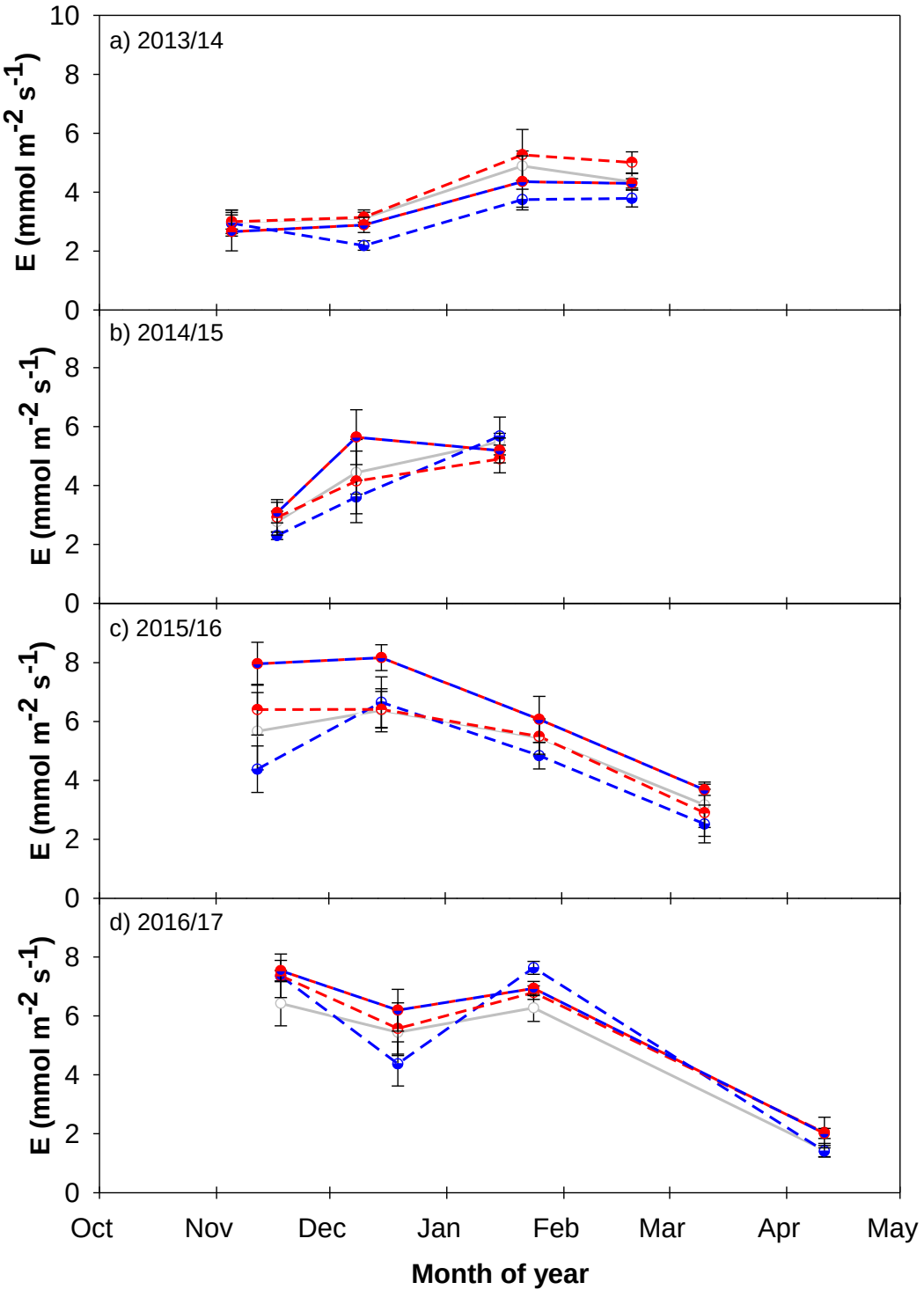


Figure 20 Effect of [CO_2] x heat on leaf level transpiration (E), during A_{sat} measurements, of outer canopy leaves in each of the four seasons.

Data presented are means ± SE, (n = 4).

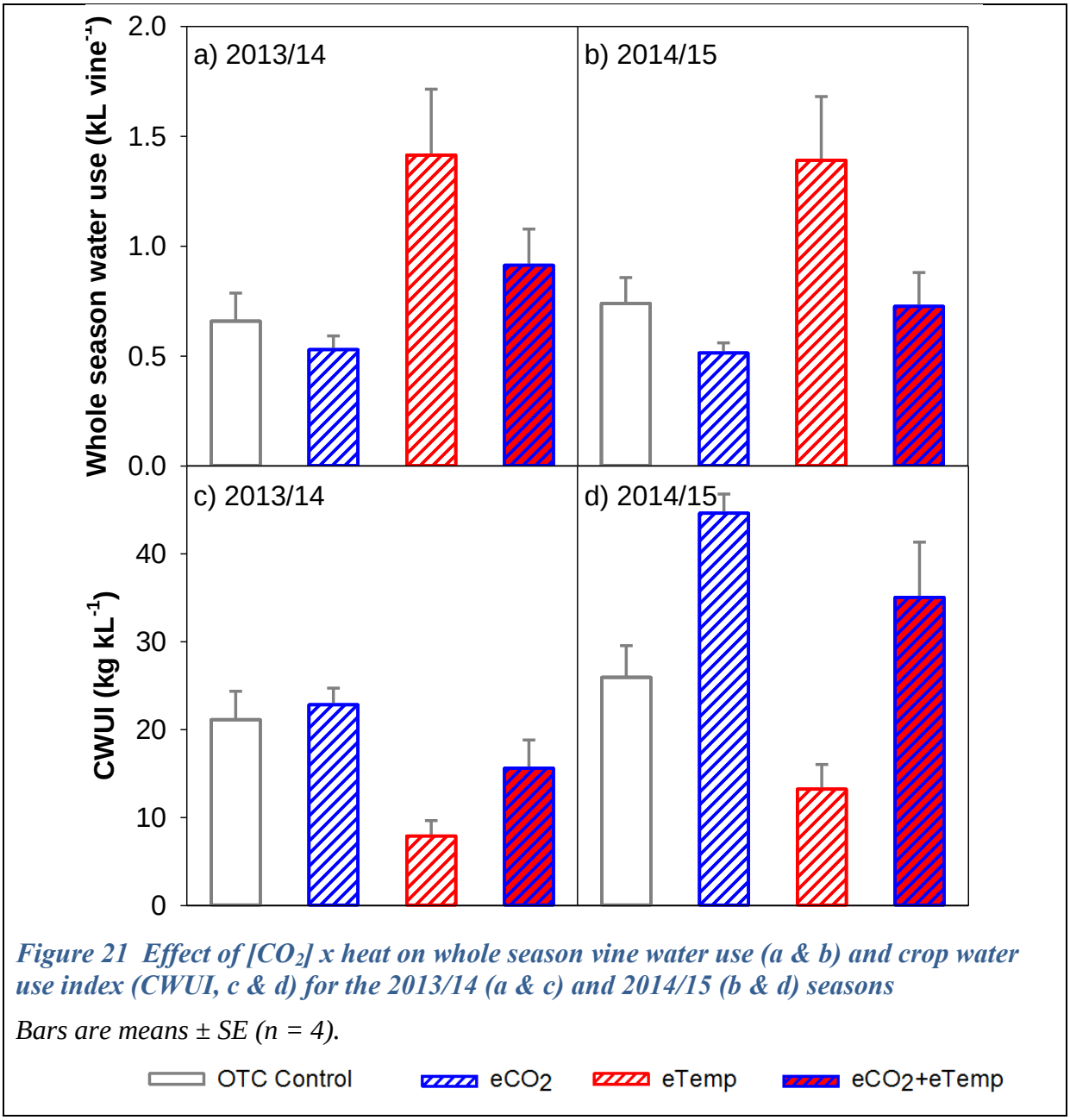


Figure 21 Effect of [CO_2] x heat on whole season vine water use (a & b) and crop water use index (CWUI, c & d) for the 2013/14 (a & c) and 2014/15 (b & d) seasons

Bars are means ± SE (n = 4).

This is consistent with the lack of an impact on g_s, taking into account the increase in VPD as a result of the warming treatments. The marginal mean for eCO_2+eTemp was 5.6 mmol m^{-2} s^{-1}, compared with 4.9 for the OTC Control vines, 4.6 for the eCO_2 vines and 5.1 for the eTemp vines.

The leaf level estimates of water use do not take account of the roles of canopy size, canopy structure or of leaf longevity in determining whole vine water use. Sapflow sensors were used during the 2013/14 and 2014/15 seasons to estimate the whole vine and whole season water use and any treatment effects. All vines were adequately irrigated, so no water limitation was expected, even if vines of one treatment were utilising more water than those of another.

The sapflow data were consistent across the two seasons (Figure 21 a & b) with a large effect of eTemp (P = 0.016) and a marginal effect of eCO_2 (P = 0.055). The lack of seasonal effect may have been due to the strong correlation between daily water use and reference crop

evapotranspiration (ET_o, $r^2 = 0.69$) and the almost identical seasonal sum of ET_o, 1101 and 1102 mm for 2013/14 and 2014/15, respectively. The eTemp effect observed was far larger than that seen at the leaf level, which may have been due to a combination of various factors, including differences in canopy duration (earlier budburst, altered leaf fall), canopy structure (water use is strongly driven by degree of sun exposure) and diurnal responses not captured by the gas exchange measurements. In addition, the absolute values recorded by the sensors used are highly dependent on positioning within the trunk and as n = 4 in each case, it is possible the response was over-estimated due to the limited number of probes used.

Although a reduction in seasonal sapflow was observed under eCO_2 and there was no interaction with warming, the eCO_2+eTemp treatment still had seasonal water use equal to or greater than the OTC Control vines. Consequently, even though an effect of eCO_2 on whole vine water use was observed, where an effect at the leaf level was not, there was no evidence that a likely future climate would reduce vineyard water use.

However, grapevines are an irrigated crop, so, from a viticultural perspective, water use efficiency is potentially more important than water use per se. The data reported above provide two ways of determining water use efficiency (WUE). Firstly, WUE at the leaf level (A_{sat}/E), termed instantaneous WUE (iWUE), and representing the photosynthetic carbon gain per unit of water use, and secondly, WUE of the crop produced (yield/total sapflow), termed crop water use index (CWUI).

At the leaf level iWUE was always high in the eCO_2 vines, due to the high rates of A_{sat} (Figure 22). This suggests that even if production was not higher under eCO_2, it should be possible to adjust vine management to take advantage of the improved iWUE in order to improve CWUI; for instance by reducing canopy size.

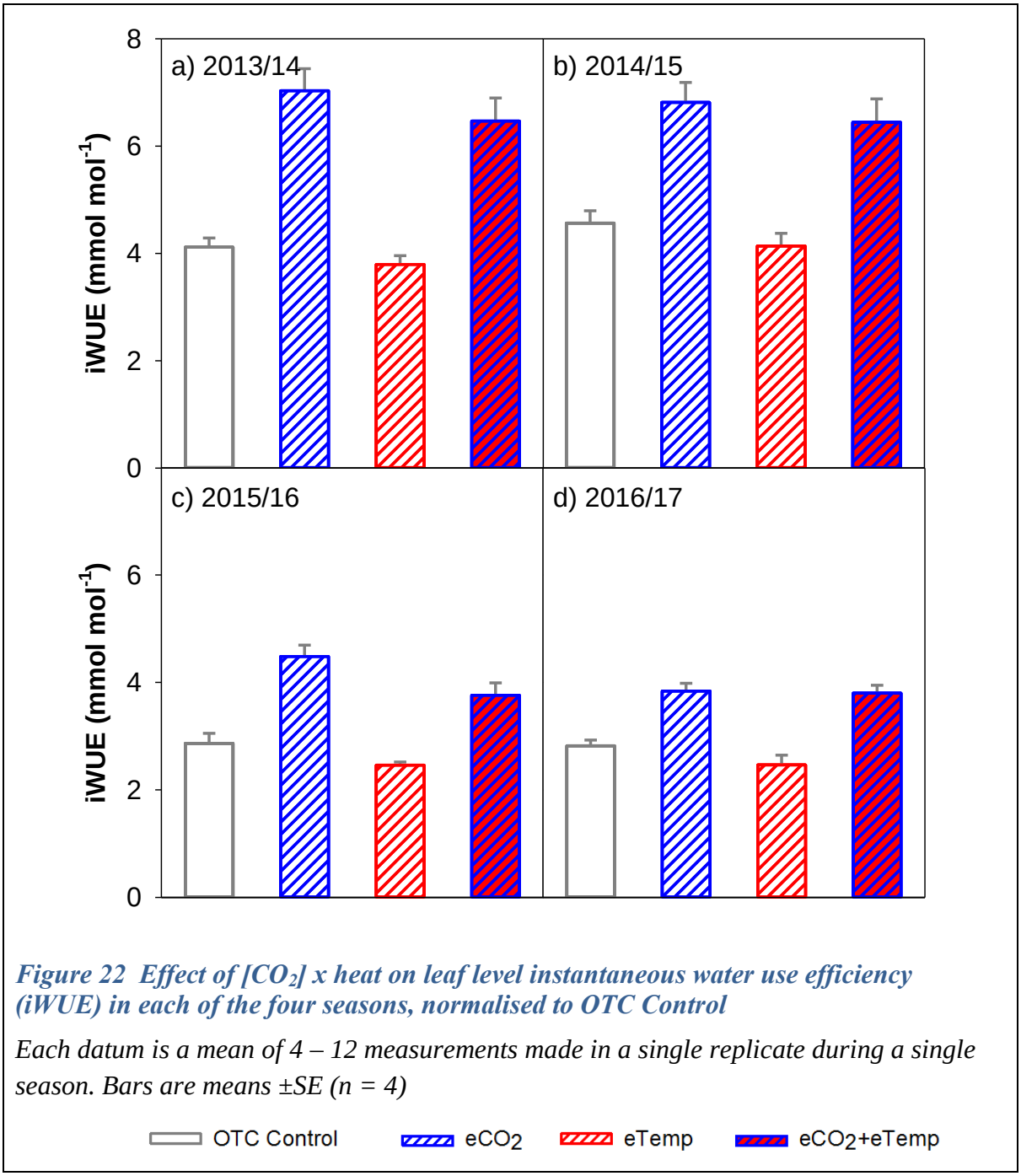


Figure 22 Effect of [CO_2] x heat on leaf level instantaneous water use efficiency (iWUE) in each of the four seasons, normalised to OTC Control

Each datum is a mean of 4 – 12 measurements made in a single replicate during a single season. Bars are means ±SE (n = 4)

Actual CWUI during the 2013/14 and 2014/15 seasons was improved by eCO_2 (P = 0.001), but reduced by eTemp (P = 0.004), with no interaction. However, there was a significant seasonal interaction with the eCO_2 effect, suggesting that CWUI in the future will be highly dependent on any season × eCO_2 interaction on yield.

Summary: Objective 2

The most surprising aspect of the physiology work was the lack of any acclimation of vine photosynthesis. Coupled with the positive interaction between warming and elevated CO_2, this suggests that under a future climate, vines will be able to sustain very high rates of carbon gain, for a similar water and nutrient use as under current conditions. What is less

clear is whether this will be a benefit or a hindrance to wine grape production. The photosynthetic results, together with the carbohydrate results, suggest that vine reserves are likely to increase year on year, which is likely to increase vine vigour over a number of years, requiring additional management costs. However, this may allow a leaner vine, able to produce the same volume of fruit with a smaller canopy. It is possible that this may, in turn, allow the observed improvements in water use efficiency to be exploited, despite the lack of any reduction in actual vine water use observed during this project.

Results and Discussion: Objective 3

To quantify the impact of elevated CO_2 and temperature on grape and wine quality parameters and wine sensory characteristics by an expert panel

Influence of open-topped chamber design on grape quality parameters

The OTCs allowed the imposition of elevated CO_2 and/or warmer air to vines in a vineyard. In order to assess the impact of enclosing vines in OTCs on grape quality parameters, non-chambered and OTC Control fruit were sampled and assessed at three phenological stages in each of the four seasons. Across the seasons, chambering vines had no effect on berry size and composition at commercial maturity (Table 12). As a result, hereafter only the chambered vines and grapes are discussed.

Table 12 Effect of chambering Shiraz vines main effects on grape physical characteristics and composition at harvest

Values presented are means (n = 16).

Parameter	Non-chambered control	OTC Control
TSS (°Brix)	25.2	25.1
pH	4.08	4.01
TA	4.06	4.11
YAN (mg L^{-1})	91	81
total tannin (mg CE[†] g^{-1})	7.61	8.59
total anthocyanins (mg M3G[‡] g^{-1})	5.84	5.99
total flavonols (mg Q3G[§] g^{-1})	1.27	1.25

[†]mg catechin equivalents; [‡mg] malvidin-3-glucose equivalents; [§]mg quercetin-3-glucose equivalents
TA: titratable acidity; YAN: yeast assimilable nitrogen

Effect of [CO_2] and air temperature on juice pH and TA, and YAN

Some viticultural management techniques such as irrigation, pruning, canopy management and crop nutrition are known to influence berry physical properties (Downey *et al.*, 2006). Season, [CO_2] and heat main effects, and individual treatment effects on grape juice pH and TA are presented in Figure 23 and Figure 24, respectively. Overall, juice pH increased during berry ripening whilst TA decreased. Season had very little effect on juice pH at harvest, which remained consistent between seasons, ranging in pH from 3.94 in 2015 to pH 4.04 in 2014.

There were small differences observed in juice pH in grapes from the heated treatments (eTemp and eCO_2+eTemp) at pea size berries and veraison, specifically, slightly higher pH

and slightly lower juice pH, respectively. However, as berries matured and reached harvest maturity there was no difference observed for juice pH in response to elevated temperature and/or elevated [CO_2].

Unlike observations for juice pH, there was considerable variation in the TA between seasons and treatments. The average seasonal TA at harvest ranged from 3.63 g L^{-1} in 2016, to 6.89 g L^{-1} in 2014 (Figure 24). Whilst there was significant variation in measured TA between seasons, elevated [CO_2] resulted in no change to the TA of grape juice. Warmer air (eTemp and eCO_2+eTemp) resulted in an increase in the TA of grape juice post veraison, with an average of 1.3 g L^{-1} higher TA observed in fruit exposed to elevated temperature. The eTemp treatment had an average increase in TA of 1.1 g L^{-1}, the addition of elevated carbon dioxide into the mix (*i.e.* the eCO_2+eTemp treatment) resulted in an average increase in acidity of 1.4 g L^{-1}.

Nitrogen content is critical for successful fermentation and is often measured prior to the winemaking process to determine if and how many nitrogen additives may be required in the winery. YAN is a combination of the nitrogen in free amino acids, and ammonium and ammonia, is available to yeast during the winemaking process. For a low risk wine fermentation, the minimum YAN requirement for a red wine is 150 mg L^{-1} (Blateyron *et al.*, 2003). YAN concentration can vary with variety, region, and viticultural management such as soil nitrogen content and irrigation practices.

Similar to observations for other fruit parameters, there was considerable variation in the measured YAN between seasons **(**Figure 25). Fruit exposed to elevated atmospheric [CO_2] generally had lower measured YAN than fruit exposed to ambient [CO_2]. Across all four treatments (OTC Control, eTemp, eCO_2 and eTemp+eCO_2), YAN ranged in concentration from 68 to 81 mg L^{-1} with the lowest measured YAN detected in the juice of those treatments exposed to higher [CO_2]. Whilst none of the treatments met the minimum specification of YAN for a successful fermentation, the fruit from the elevated CO_2 treatments (eCO_2 and eCO_2+eTemp) would appear to require the greatest intervention in the winery to ensure sufficient nutrient availability and successful fermentation.

Figure 23 Shiraz juice pH at three developmental stages: (top) pea-size berries; (middle) veraison, and (bottom) harvest over four growing seasons.

Values presented are means (n = 16, 32 and 32, for season, [CO_2] and heat main effects, respectively, and 16 for [CO_2] and heat interactions). Different letters above columns within comparisons, or an asterisk above a column indicate significant (P = 0.05) differences between means.

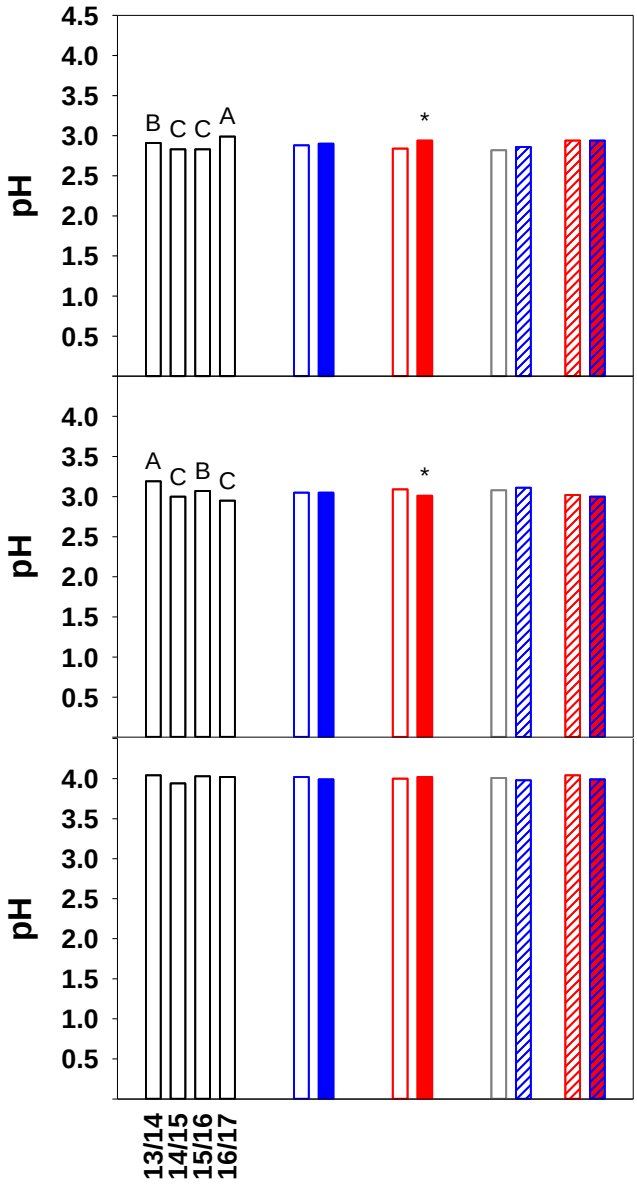
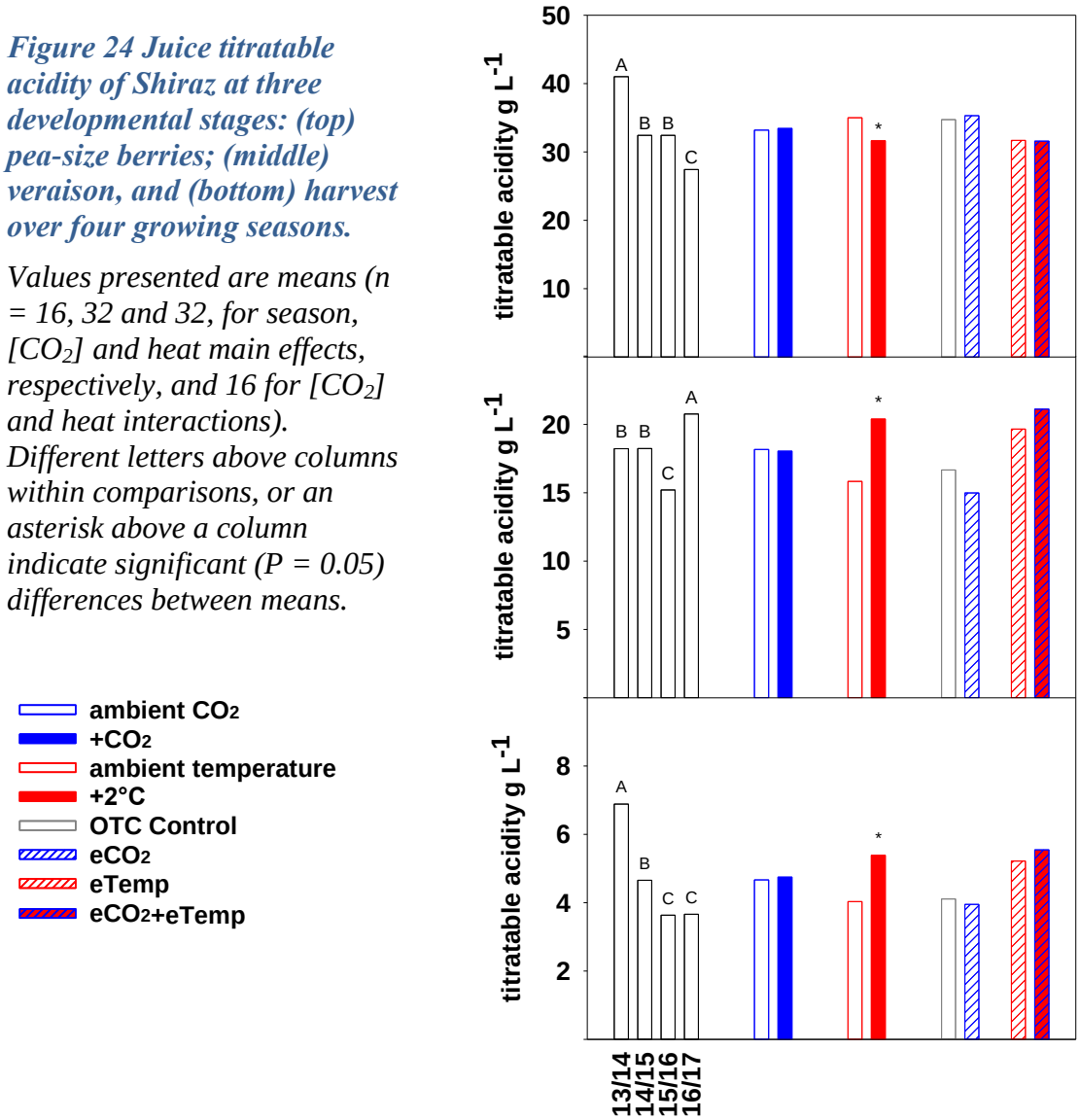


Figure 24 Juice titratable acidity of Shiraz at three developmental stages: (top) pea-size berries; (middle) veraison, and (bottom) harvest over four growing seasons.

Values presented are means (n = 16, 32 and 32, for season, [CO_2] and heat main effects, respectively, and 16 for [CO_2] and heat interactions). Different letters above columns within comparisons, or an asterisk above a column indicate significant (P = 0.05) differences between means.



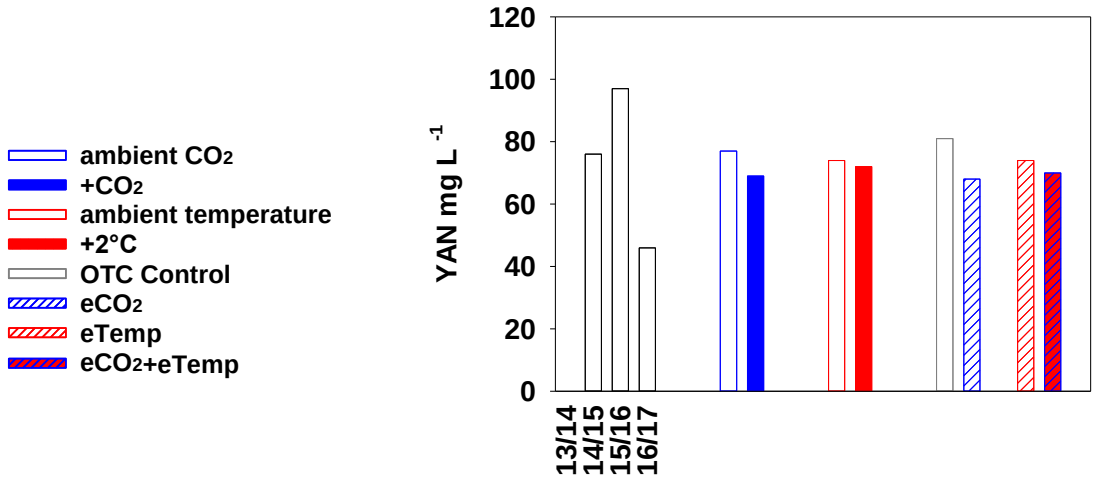


Figure 25 Yeast assimilable nitrogen (YAN) of Shiraz juice at harvest over three seasons.

Values presented are means (n = 16, 24 and 24, for season, [CO_2] and heat main effects, respectively, and 12 for [CO_2] and heat interactions). Different letters above columns within comparisons, or an asterisk above a column indicate significant (P = 0.05) differences between means.

Effect of [CO_2] and air temperature on grape skin tannin, anthocyanin and flavonol concentration and composition

Tannin concentration was assessed in grape skins over four seasons at three phenological time points, namely pea-sized berries, veraison, and harvest (Figure 26)**.** For all seasons, tannin concentration varied between time points. Tannins were detected at the highest concentrations when berries were at pea-size stage, decreased at veraison, and measured lowest at harvest. This pattern of accumulation is the same as that observed by (Hanlin and Downey, 2009). At the pea-size berry and veraison developmental time points, there was very little difference in skin tannin concentration between treatments with concentration ranging from 12.19 to 12.76 mg g^{-1} of skin and 9.33 to 10.07 mg g^{-1} skin respectively. As berries ripened the differences between treatments became more pronounced, with tannin concentration being significantly lower in the skins of berries exposed to elevated temperature conditions **(**Figure 26). Overall the main effect of [CO_2] had minimal or no impact on total skin tannin concentration. An elevation in temperature by 2°C (eTemp and eCO_2+eTemp) resulted in significantly lower skin tannin concentrations in comparison to fruit exposed to ambient temperatures. On average, at harvest, the heated treatments had a decrease in skin tannin concentration of approximately 2 mg g^{-1} fresh weight of skin.

HPLC analysis of Shiraz skin tannin enabled condensed tannin polymer subunit composition to be determined under different atmospheric conditions (Figure 27). Two terminal subunits were identified, catechin being the most abundant, accounting for approximately 64% of the terminal subunits and epicatechin accounting for the remaining 36%. Extension subunits were comprised of 37% epigallocatechin, 4% catechin, 52% epicatechin, and 7% epicatechin-gallate. These relative proportions of terminal and extensions subunits are consistent with other studies of Shiraz berries. Despite being known to be present in Shiraz grape skin

(Downey *et al.*, 2003, Downey *et al.*, 2012), in this instance, the terminal subunit epicatechin-gallate was not detected.

Terminal subunit composition remained consistent, with very little difference (up to 2%) observed in the proportional composition of catechin versus epicatechin between seasons and treatments. Extension subunit composition appeared to be more prone to variation as a result of underlying season, with individual subunit composition between seasons differing by up to 14% for epicatechin, and up to 3% for catechin subunits. Elevated [CO_2] had very little impact on the relative proportion of extension subunits. Conversely, fruit exposed to elevated temperature conditions (heat effect) had a lower proportion of epigallocatechin (4.1%) and higher proportion of epicatechin (5.7%) extension subunits.

Figure 26 Tannin concentration of Shiraz skin at three developmental stages: (top) pea-size berries; (middle) veraison, and (bottom) harvest over four growing seasons expressed as mg catechin equivalents g^{-1} fresh weight of skin.

Values presented are means (n = 16, 32 and 32, for season, [CO_2] and heat main effects, respectively, and 16 for [CO_2] and heat interactions). Different letters above columns within comparisons, or an asterisk above a column indicate significant (P = 0.05) differences between means.

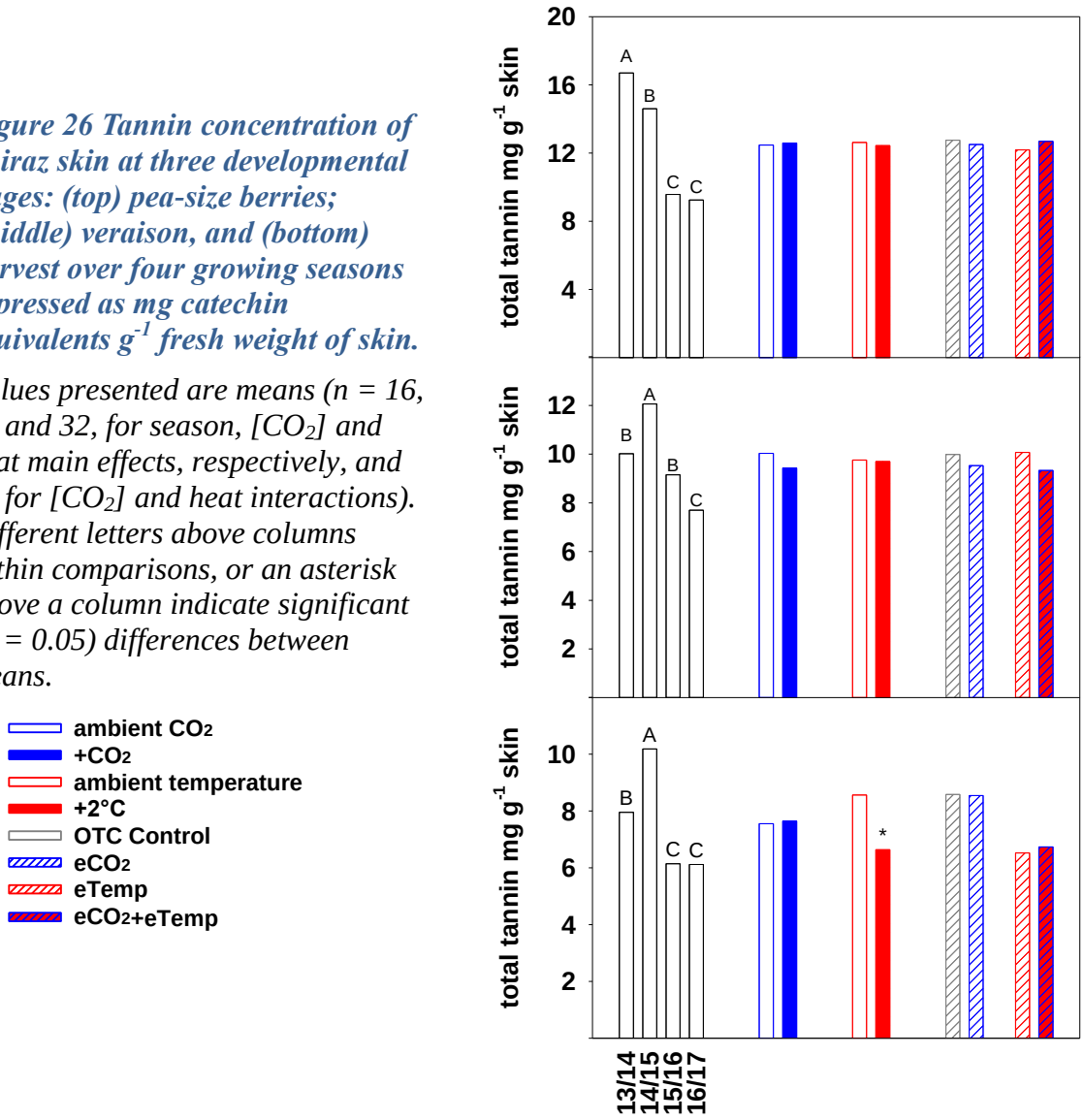
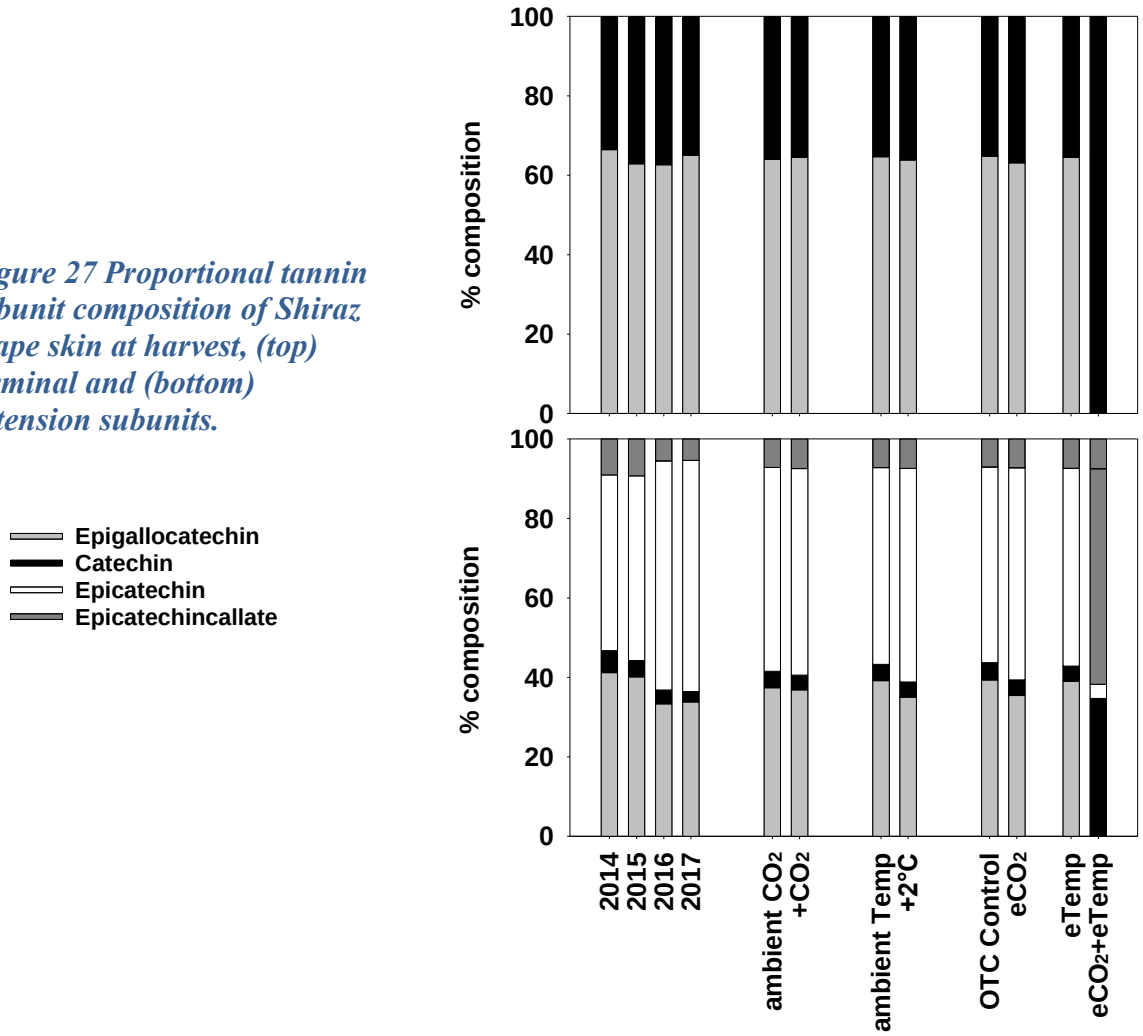


Figure 27 Proportional tannin subunit composition of Shiraz grape skin at harvest, (top) terminal and (bottom) extension subunits.

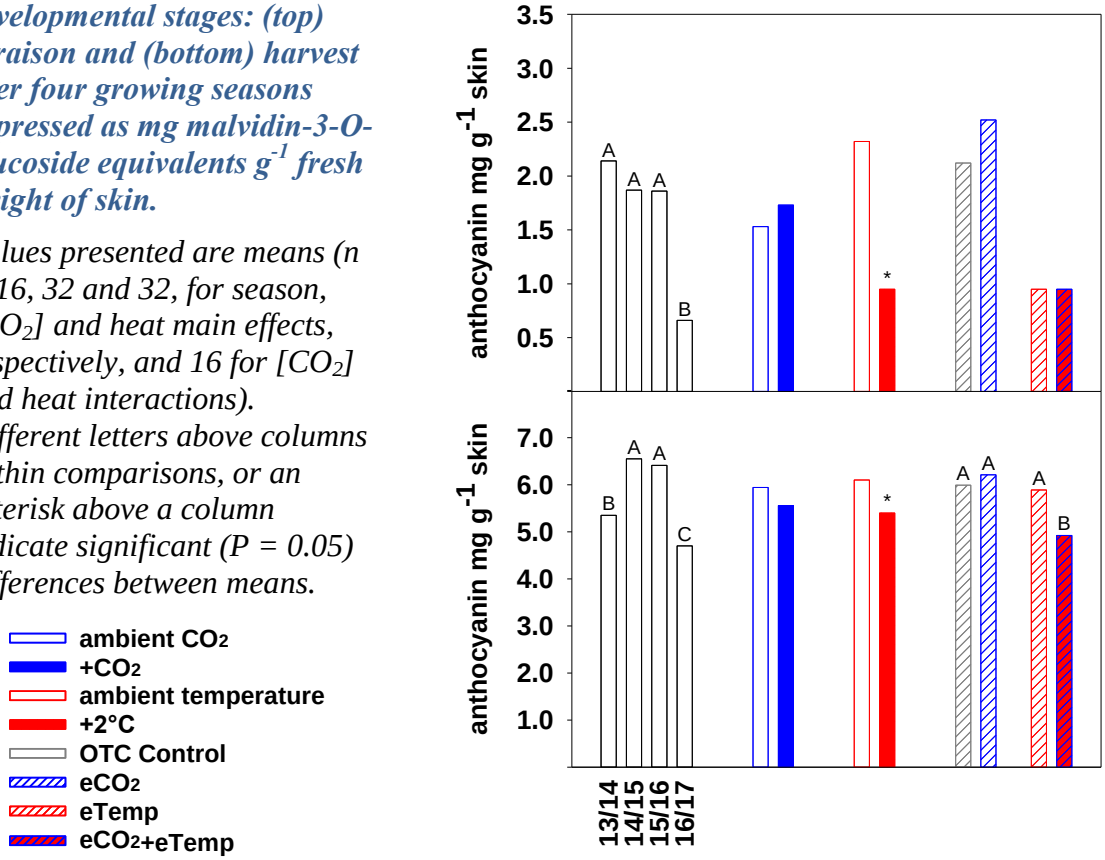


Anthocyanins accumulate in the skin of grape berries during the third phase of berry development, the ripening phase, with veraison being the commencement of this final phase of development. Anthocyanin concentration was determined in the skin of Shiraz grapes at veraison and at harvest. The effect of season, [CO_2] and heat on skin anthocyanin concentration at veraison and harvest is shown in Figure 28. At veraison, anthocyanin concentration was significantly lower in the skins of grapes exposed to elevated temperature conditions (main heat effect), with almost a 50% reduction in anthocyanin concentrations in the skins. At harvest this difference was not as pronounced. In general, fruit exposed to an elevation in temperature of 2°C had significantly lower anthocyanin concentration at harvest. Lower anthocyanin concentration was also observed in fruit exposed to elevated [CO_2] ([CO_2] effect) however, it is apparent that the level of anthocyanin, in this instance, is driven by the interactive effects of an elevation in atmospheric [CO_2] and temperature, with the

eTemp+eCO_2 treatment overall having an 18% reduction in anthocyanins at harvest and little difference observed between the control, eCO_2 and the eTemp treatments. This suggests that it is the interactive effects of both temperature and CO_2 driving lower accumulation of anthocyanins.

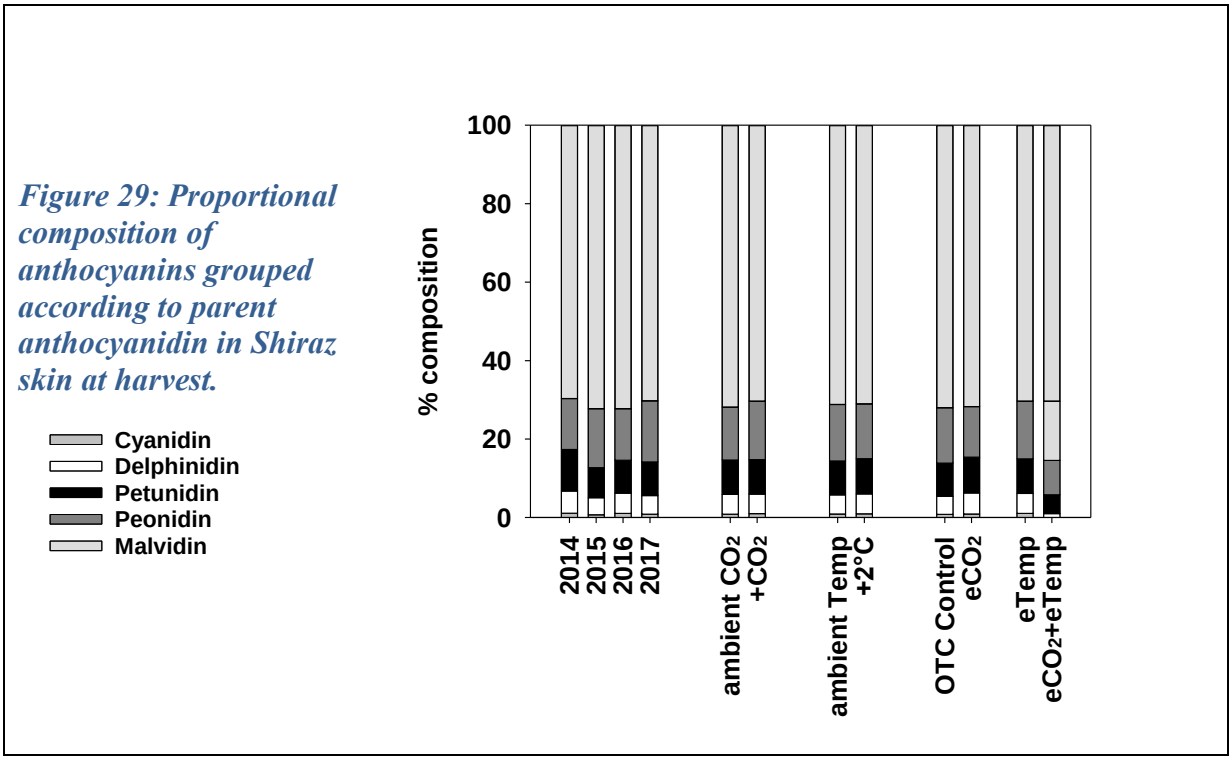
Figure 28 Total anthocyanin in Shiraz grape skin at two developmental stages: (top) veraison and (bottom) harvest over four growing seasons expressed as mg malvidin-3-O-glucoside equivalents g^{-1} fresh weight of skin.

Values presented are means (n = 16, 32 and 32, for season, [CO_2] and heat main effects, respectively, and 16 for [CO_2] and heat interactions). Different letters above columns within comparisons, or an asterisk above a column indicate significant (P = 0.05) differences between means.



The anthocyanins identified in Shiraz are derived from five different anthocyanidins. The overall anthocyanin composition at harvest over four seasons in response to changes in [CO_2] and temperature conditions is shown in Figure 29. Anthocyanin composition remained relatively consistent between seasons and treatments, with less than 3% difference between seasons of the relative proportions of anthocyanins. Malvidin-derived anthocyanins are the most abundant anthocyanin present, accounting for approximately 70 – 72% of anthocyanins, followed by peonidin (13 – 15%), petunidin (8 – 9%), delphinidin (5 – 6%) and finally cyanidin derived anthocyanins accounting for less than 1.0%. Despite some of the changes in total anthocyanin concentration observed, and more specifically the decrease in anthocyanins observed in the eCO_2+eTemp treatment, there was no change in the relative proportion of the different anthocyanins present. This suggests that whilst changes in anthocyanin concentration were observed, particularly for the eCO_2+eTemp treatment, these conditions had no effect of the synthesis of the relative proportions of individual anthocyanins.

Figure 29: Proportional composition of anthocyanins grouped according to parent anthocyanidin in Shiraz skin at harvest.



Flavonol accumulation begins in the early stages of berry development, is responsive to light conditions (Downey *et al.*, 2004) and may act as a UV protectant (Flint *et al.*, 1985). Total flavonol concentration varied between seasons and increased from the pea-sized berry developmental time point through to harvest (Figure 30). Similar to the pattern of accumulation observed for anthocyanin concentration, +2°C resulted in lower flavonol concentration in grape skin. As with [CO_2] observations for anthocyanins, this is driven by the significant reduction in flavonol concentration observed in the fruit exposed to both elevated temperature and carbon dioxide conditions (eCO_2+eTemp). Flavonols were significantly lower in the eCO_2+eTemp treatment compared to the control. This would indicate that, similar to the observation for anthocyanin concentration, it is the interactive effect of elevated temperature and [CO_2] that has the most profound effect.

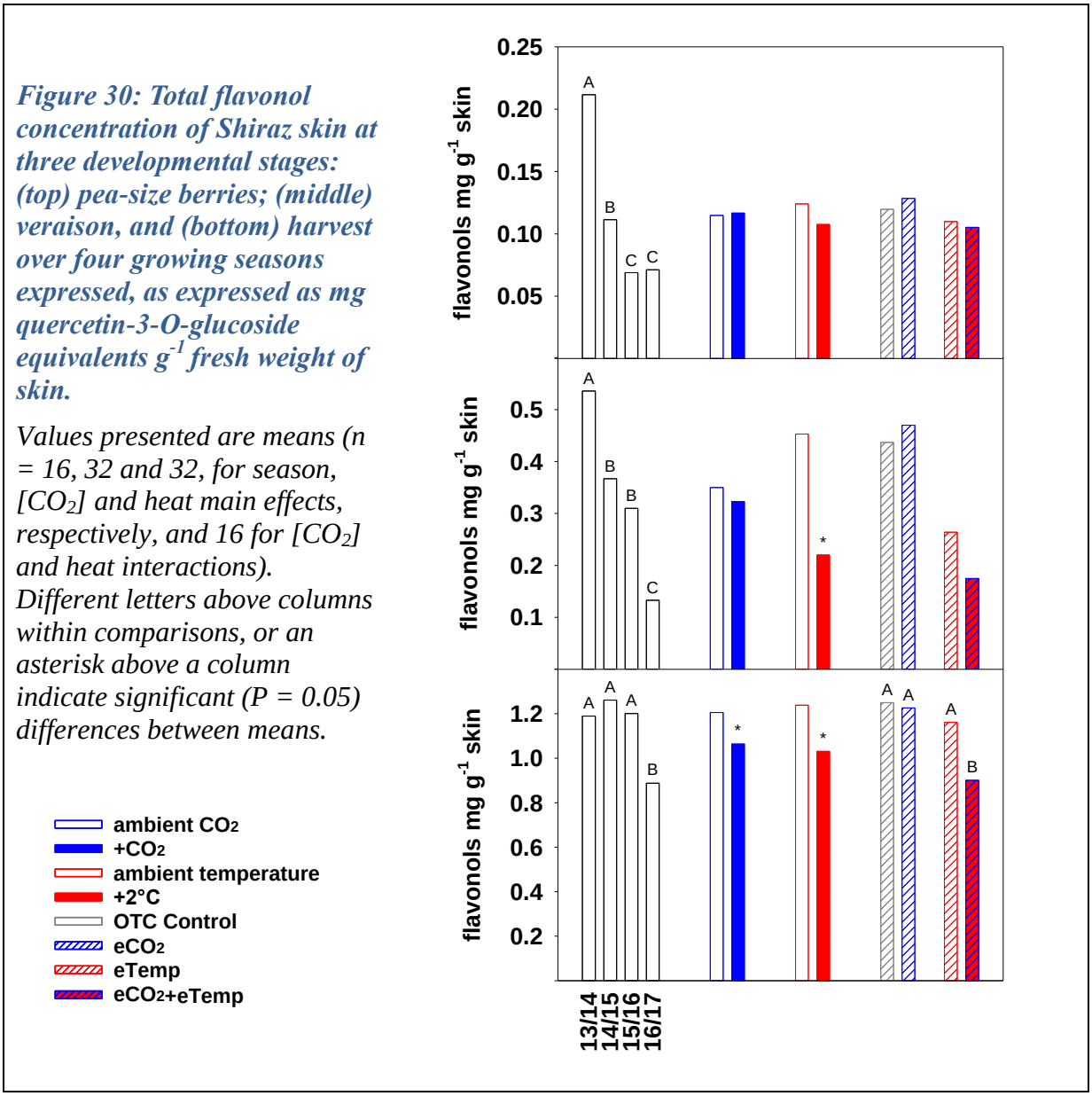


Figure 30: Total flavonol concentration of Shiraz skin at three developmental stages: (top) pea-size berries; (middle) veraison, and (bottom) harvest over four growing seasons expressed, as expressed as mg quercetin-3-O-glucoside equivalents g^{-1} fresh weight of skin.

Values presented are means (n = 16, 32 and 32, for season, $[CO_2]$ and heat main effects, respectively, and 16 for $[CO_2]$ and heat interactions). Different letters above columns within comparisons, or an asterisk above a column indicate significant (P = 0.05) differences between means.

Effect of $[CO_2]$ and air temperature on quality parameters of finished wine

Phenolics in red wine, such as anthocyanins and tannins, are extracted from the skins and seeds of berries during the maceration phase of the winemaking process. Anthocyanins contribute to the colour of red wines whilst tannins play a significant role in the mouthfeel properties of a wine. Generally, the relationship between grape anthocyanin concentration and wine colour is strong, with grape colour measures providing a reasonable basis for predicting final wine colour. The relationship between grape tannin and wine tannin concentration is often more complex, with grape tannin concentration not always a good indicator of final wine tannin concentration (Kilmister *et al.*, 2015, Kilmister *et al.*, 2014).

The anthocyanin concentration in Shiraz wines at bottling are presented in Figure 31. As observed for grape anthocyanin concentration, wine anthocyanin concentration varied considerably between seasons. For example, anthocyanin concentration was 30% higher in the 2014/15 wines compared to the 2013/14 wines. Overall, wine made from fruit exposed to

elevated [CO_2], irrespective of other conditions, had significantly lower anthocyanin concentration than wines made from fruit exposed to ambient [CO_2]. Similarly, wines made from fruit exposed to an elevation in temperature (heat main effect), had lower anthocyanins than wines made from fruit exposed to ambient temperature conditions. The [CO_2] × heat interaction suggests that the bulk of the reduction in wine anthocyanin in wines made from fruit exposed to both elevated temperature and CO_2 is due to air temperature. At bottling, wines made from the eCO_2+eTemp OTCs had an average of 81mg L^{-1} less anthocyanins than wines made OTC Control wines, equating to an 18% reduction. Whilst wines made from fruit exposed to elevated temperature alone (eTemp) were also observed to have lower anthocyanins, that effect was greater in eCO_2+eTemp wines.

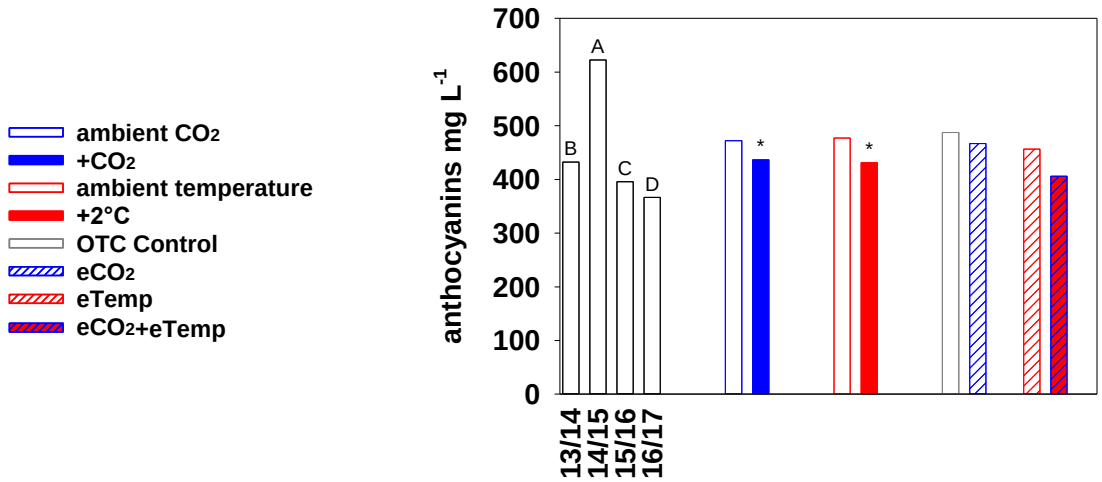


Figure 31: Total anthocyanin in finished Shiraz wine at bottling over four vintages, expressed as mg malvidin-3-O-glucoside equivalents L^{-1}.

Values presented are means (n = 16, 32 and 32, for season, [CO_2] and heat main effects, respectively, and 16 for [CO_2] and heat interactions). Different letters above columns within comparisons, or an asterisk above a column indicate significant (P = 0.05) differences between means.

Wine colour density (WCD) is an indicator of the colour intensity or redness of a wine. Wine colour density is generally high in young wines; decreasing as wines age and yellow/brown pigments develop. The effect of [CO_2] and heat on finished Shiraz wine WCD over four seasons is presented in Figure 32. As observed with wine anthocyanin concentration at bottling, WCD varied considerably between seasons. The main effect of increased atmospheric [CO_2] was a reduction in WCD. Likewise, +2°C in the vineyard resulted in a reduction in the wine colour density of wines at bottling. These observations for WCD, like that for grape and wine anthocyanin, are driven predominantly by interactive effects of temperature and [CO_2]; eCO_2+eTemp WCD (8.1) was lower than OTC Control (9.7), eTemp (9.1), and eCO_2 (9.3). The WCD for the wines from each treatment reflect the observations for wine anthocyanin concentration.

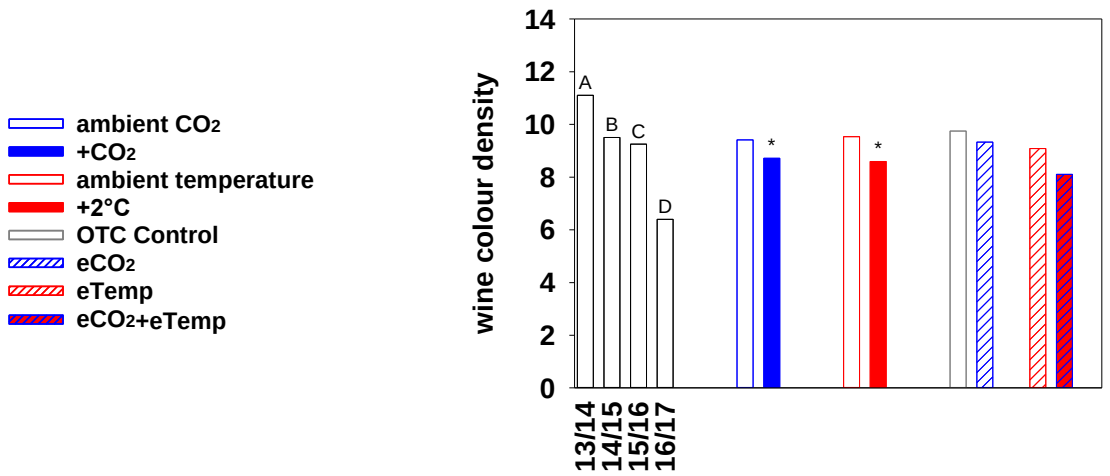


Figure 32 Wine colour density of finished Shiraz wine at bottling over four vintages

Values presented are means (n = 16, 32 and 32, for season, [CO_2] and heat main effects, respectively, and 16 for [CO_2] and heat interactions). Different letters above columns within comparisons, or an asterisk above a column indicate significant (P = 0.05) differences between means.

Where WCD is an indicator of the redness and intensity of a wine, wine hue is an indicator of the yellow-brown pigments of a wine. The effects of [CO_2] and heat on wine hue of finished Shiraz wine over four seasons are presented in Figure 33. The elevated [CO_2] and heat effects amounted to increases of wine hue by 0.018 a.u. and 0.028 a.u., respectively. Wines made from fruit from the eTemp, eCO_2 and eCO_2+eTemp treatments were all observed to have higher wine hue compared to the OTC Control; however, these differences were not significant.

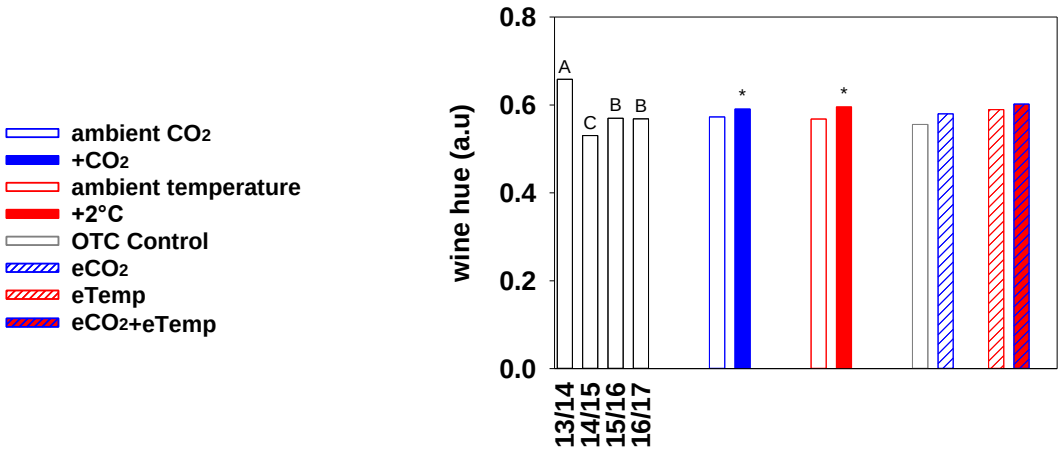


Figure 33: Wine hue of finished Shiraz wine at bottling over four vintages.

Values presented are means (n = 16, 32 and 32, for season, [CO_2] and heat main effects, respectively, and 16 for [CO_2] and heat interactions). Different letters above columns within comparisons, or an asterisk above a column indicate significant (P = 0.05) differences between means.

The effects of [CO_2] and heat on tannin concentration in the finished wines at bottling are presented in Figure 34. Like other grape and wine phenolics, tannin concentration in wine was heavily influenced by the underlying season-to-season variability irrespective of conditions imposed in the vineyard. Overall, [CO_2] and air temperature were not significant sources of variation with respect to the total tannin concentration of finished wine at bottling. However, examination of the interaction means indicates that the imposition of elevated air temperature alone or elevated CO_2 imposed alone was associated with significant reductions in tannin concentration of finished wines at bottling of 61 mg L^{-1} and 47 mg L^{-1}, respectively, compared to the OTC Control wines. But, wines made from the grapes grown on vines subject to both elevated CO_2 and elevated air temperature had tannin levels comparable to OTC Control wines. This is contrary to the effect observed on wine anthocyanins and WCD at bottling.

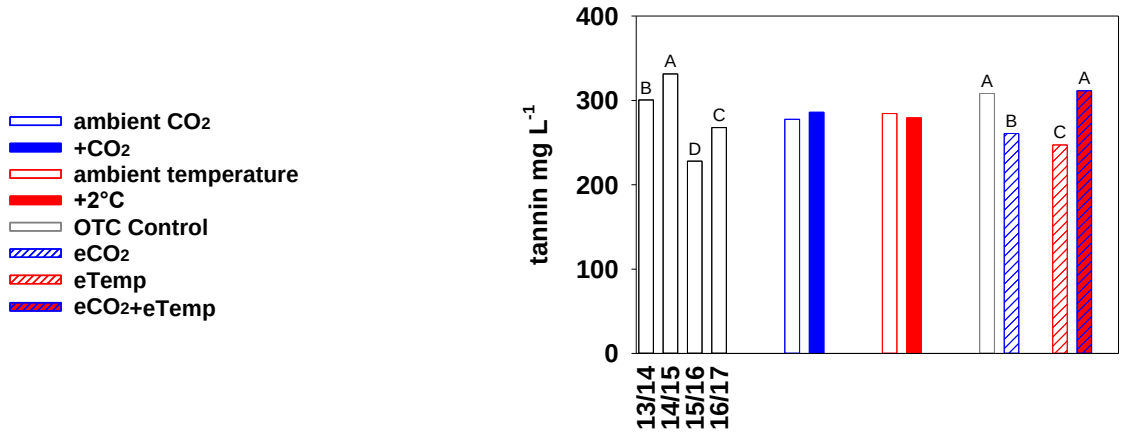


Figure 34: Total tannin in finished Shiraz wine at bottling over four vintages, expressed as mg catechin equivalents L^{-1}.

Values presented are means (n = 16, 32 and 32, for season, [CO_2] and heat main effects, respectively, and 16 for [CO_2] and heat interactions). Different letters above columns within comparisons, or an asterisk above a column indicate significant (P = 0.05) differences between means.

Iron-reactive phenolics are an indicator of the total pool of coloured and non-coloured phenolics extracted during the winemaking process. Iron-reactive phenolics are expressed as catechin equivalents. The effects of [CO_2] and heat on the total phenolic concentration of the finished wines at bottling are presented in Figure 35. As observed with other measures of wine quality, season exerted the strongest influence on wine phenolics; mean iron-reactive phenolics varied by a factor of two from one season to the next. The imposition of elevated CO_2 and/or elevated temperature on vines did not result in any effect on total phenolics across the four seasons.

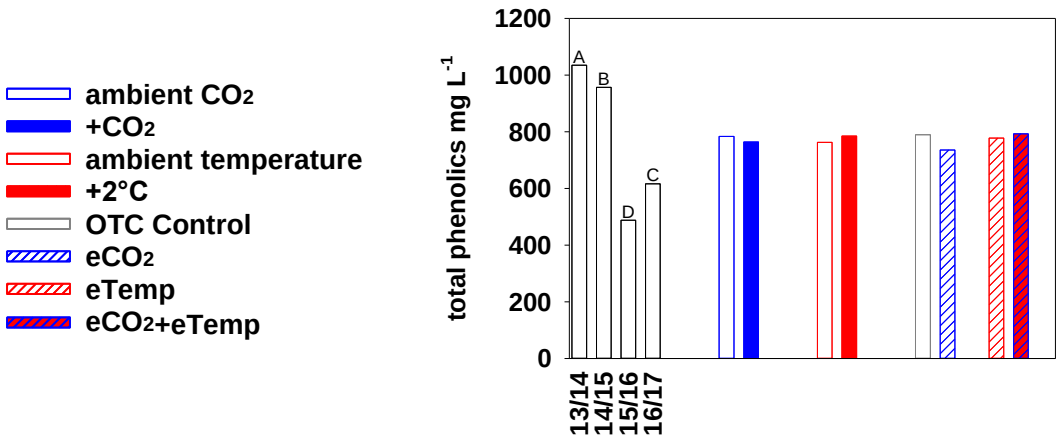


Figure 35: Total iron reactive phenolics in finished Shiraz wine at bottling over four vintages, expressed as mg catechin equivalents L^{-1}.

Values presented are means (n = 16, 32 and 32, for season, [CO_2] and heat main effects, respectively, and 16 for [CO_2] and heat interactions). Different letters above columns within comparisons, or an asterisk above a column indicate significant (P = 0.05) differences between means.

A total of 29 fermentation products were quantified. Analyses of variance for season, [CO_2], heat, and [CO_2] x heat interactions are presented in Table 13. Season was the greatest source of variation for all fermentation products assessed. [CO_2] and heat factors impacted on eight and seven fermentation products, respectively.

Table 13: Summary of analyses of variance for season, [CO_2], heat and interactions thereof on fermentation products quantified in finished wine.

Fermentation product	Source of variation			
	Season	CO_2	Heat	CO_2 × Heat
	Variance ratios' F probabilities			
2-methyl propanoic acid	-	<0.001	0.923	0.336
2-methylbutanol	<0.001	0.032	0.019	0.756
2-methylbutyl acetate	<0.001	0.298	0.742	0.167
2-methylpropanol	<0.001	0.406	0.943	0.288
2-methylpropyl acetate	<0.001	<0.001	0.002	<0.001
2-phenyl ethyl acetate	<0.001	0.464	0.531	0.165
2-phenyl ethyl ethanol	<0.001	0.398	0.504	0.409
2-methyl butanoic acid	<0.001	0.23	0.74	0.185
3-methylbutanol	<0.001	0.733	0.047	0.165
3-methylbutyl acetate	0.009	0.694	0.892	0.149
acetic acid	<0.001	0.161	0.038	0.009
butanoic acid	<0.001	0.42	0.046	0.720
butanol	-	<0.001	<0.001	<0.001
decanoic acid	<0.001	0.003	<0.001	<0.001
ethyl 2-methylbutanoate	<0.001	0.607	0.26	0.366
ethyl 2-methylpropanoate	<0.001	0.627	0.086	0.618
ethyl-3-methylbutanoate	<0.001	0.151	0.079	0.988
ethyl acetate	<0.001	<0.001	0.031	0.024
ethyl butanoate	<0.001	0.889	0.213	0.792
ethyl decanoate	<0.001	0.07	0.093	0.243
ethyl hexanoate	<0.001	0.028	0.189	0.051
ethyl octanoate	<0.001	0.041	0.193	0.183
hexanoic acid	<0.001	0.302	0.035	0.026
hexanol	<0.001	<0.001	0.812	<0.001
hexyl acetate	0.027	0.996	0.158	0.354
3-methyl butanoic acid	<0.001	0.829	0.52	0.006
ethyl propanoate	<0.001	0.142	0.817	0.01
octanoic acid	<0.001	0.148	0.034	0.921
propanoic acid	<0.001	0.646	0.072	0.053

Effect of [CO_2] and air temperature on wine quality parameters during storage

Shifts in the intense red and violet hues of young wines to pale red and orange hues are often seen as wines age. During the aging process, monomeric anthocyanins bind with tannin polymers to form more stable pigments with different spectrophotometric properties.

The effects of [CO_2] and/or heat on anthocyanin concentration of Shiraz wines during storage at 15°C for up to 2 years are presented in Figure 36. On average and based on the first two

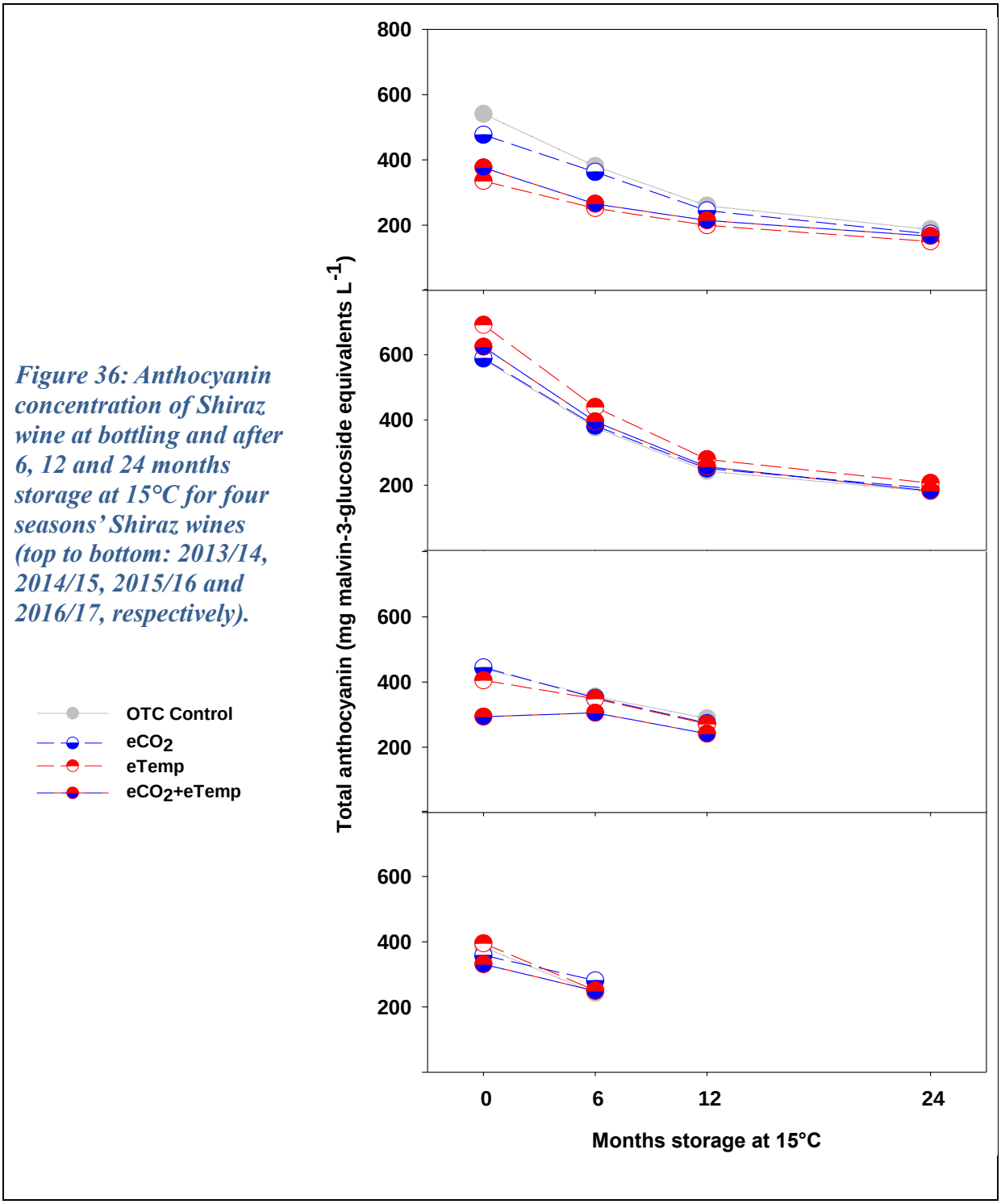
seasons' wines, wine anthocyanin concentration dropped by approximately 65% after 24 months across all four treatments. Despite the differences in anthocyanin concentration of young wines at bottling (for example, by 38% and 34% in the first and third seasons, respectively), wines analysed after 12 months bottle storage had very little or no difference observed between treatments.

The effects of [CO_2] and heat on WCD and wine hue of Shiraz wines during storage are presented in Figure 37 and Figure 38, respectively. Except for the first season, WCD was lowest in wines made from fruit grown on vines in the eCO_2+eTemp OTCs, and these relativities were maintained during storage. Colour density of the first two seasons' wines remained stable during bottle storage, with the differences observed at bottling remaining consistent over the 24-month period.

As expected, wine hue increased during bottle storage; on average by approximately 29% across all treatments after 24 months of storage. Some seasonal variability was present, but the overall rate of increase was similar across most treatments. The exception was wines from the heated treatments (eTemp and eCO_2+eTemp) in the first season, where the change observed over time was not as large. Wine aging, therefore, as indicated by hue, didn't increase at a proportionately higher rate for wines produced from fruit from any particular treatment.

During wine aging, there is a decrease in anthocyanins and formation of more stable polymeric pigments. The effects of [CO_2] and heat on the levels of short polymeric pigments and total polymeric pigments of Shiraz wines during storage at 15°C for up to 2 years are presented in Figure 39. Across all seasons' wines, total polymeric pigments increased in concentration during the first 12 months of storage. For those wines for which measurements could be conducted at 24 months, the rate of the formation of pigmented polymers was generally higher during the first 12 months of aging, and slowed subsequently. Short polymeric pigments accounted for marginally more than half of total polymeric pigments in these wines, and broadly the same observations could be made regarding concentration trends during storage. The relative differences between treatments in polymeric pigment concentration at bottling remained similar for the duration of storage, with the exception of the 2015 season where the observed differences between the four treatments increased between 6 and 12 months of bottle aging. The results from this study gave no indication that wines made from fruit exposed to one treatment compared to another were more or less likely to form the more stable polymeric pigments during the bottle aging process.

Figure 36: Anthocyanin concentration of Shiraz wine at bottling and after 6, 12 and 24 months storage at 15°C for four seasons' Shiraz wines (top to bottom: 2013/14, 2014/15, 2015/16 and 2016/17, respectively).



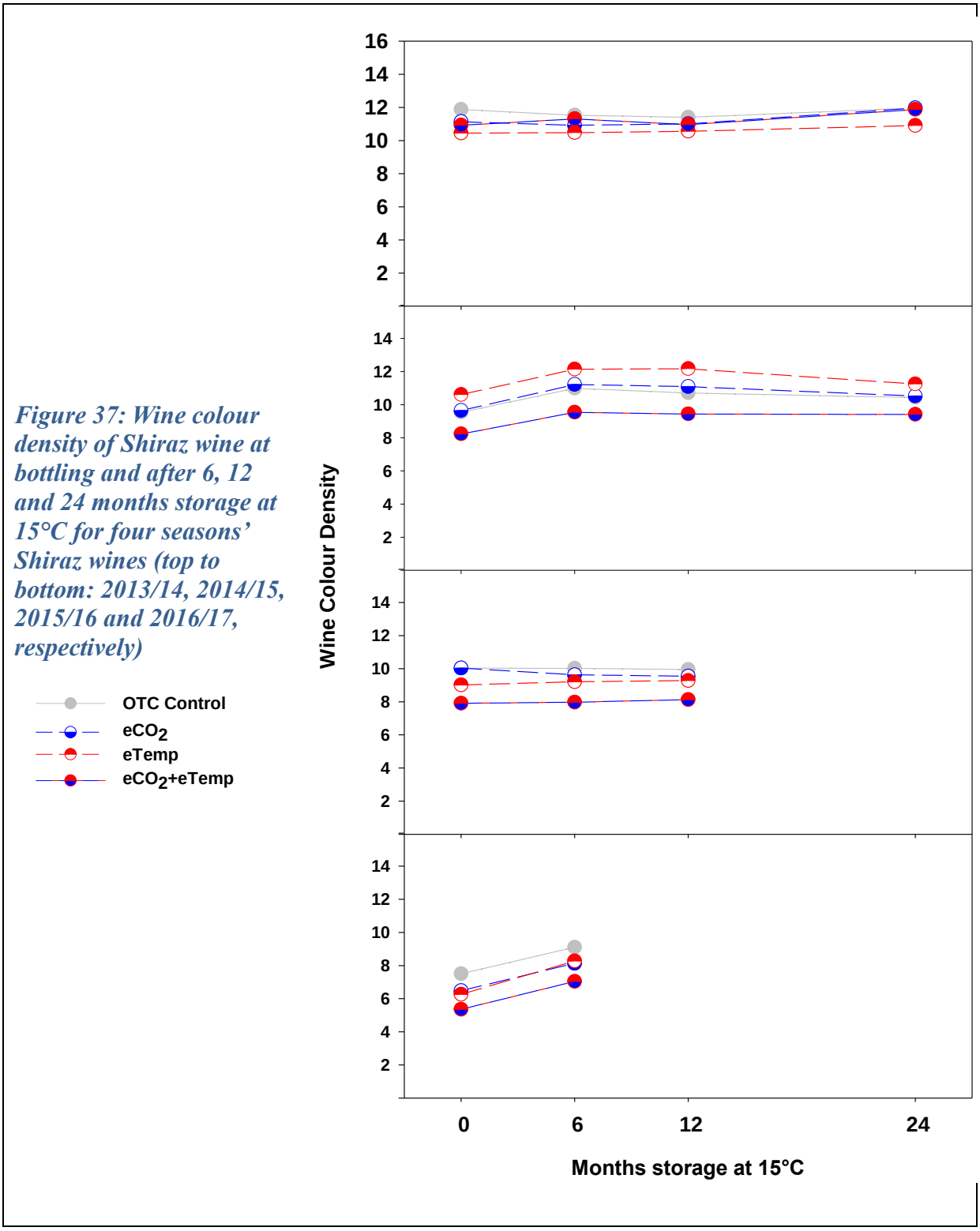
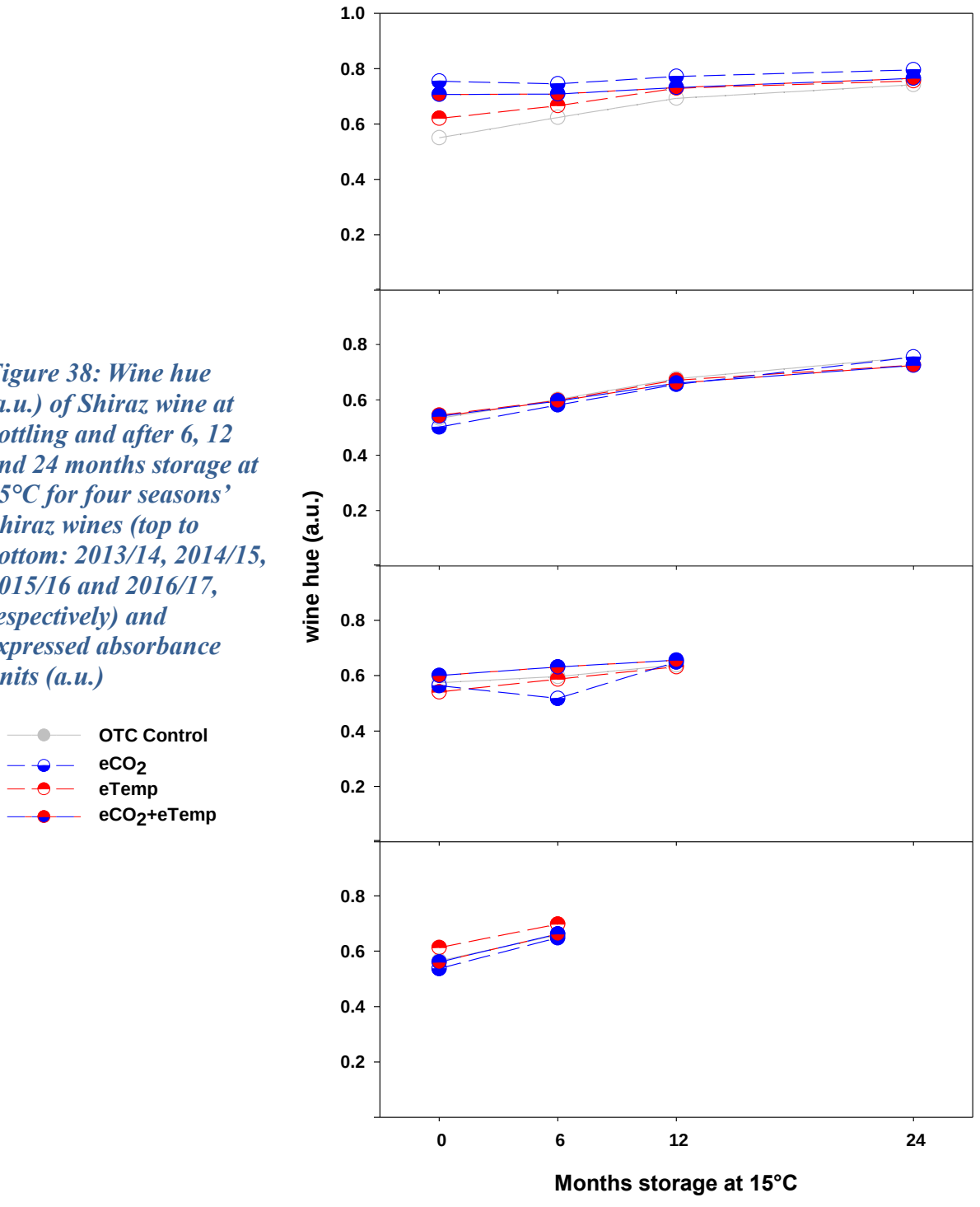


Figure 37: Wine colour density of Shiraz wine at bottling and after 6, 12 and 24 months storage at 15°C for four seasons' Shiraz wines (top to bottom: 2013/14, 2014/15, 2015/16 and 2016/17, respectively)

Figure 38: Wine hue (a.u.) of Shiraz wine at bottling and after 6, 12 and 24 months storage at 15°C for four seasons' Shiraz wines (top to bottom: 2013/14, 2014/15, 2015/16 and 2016/17, respectively) and expressed absorbance units (a.u.)



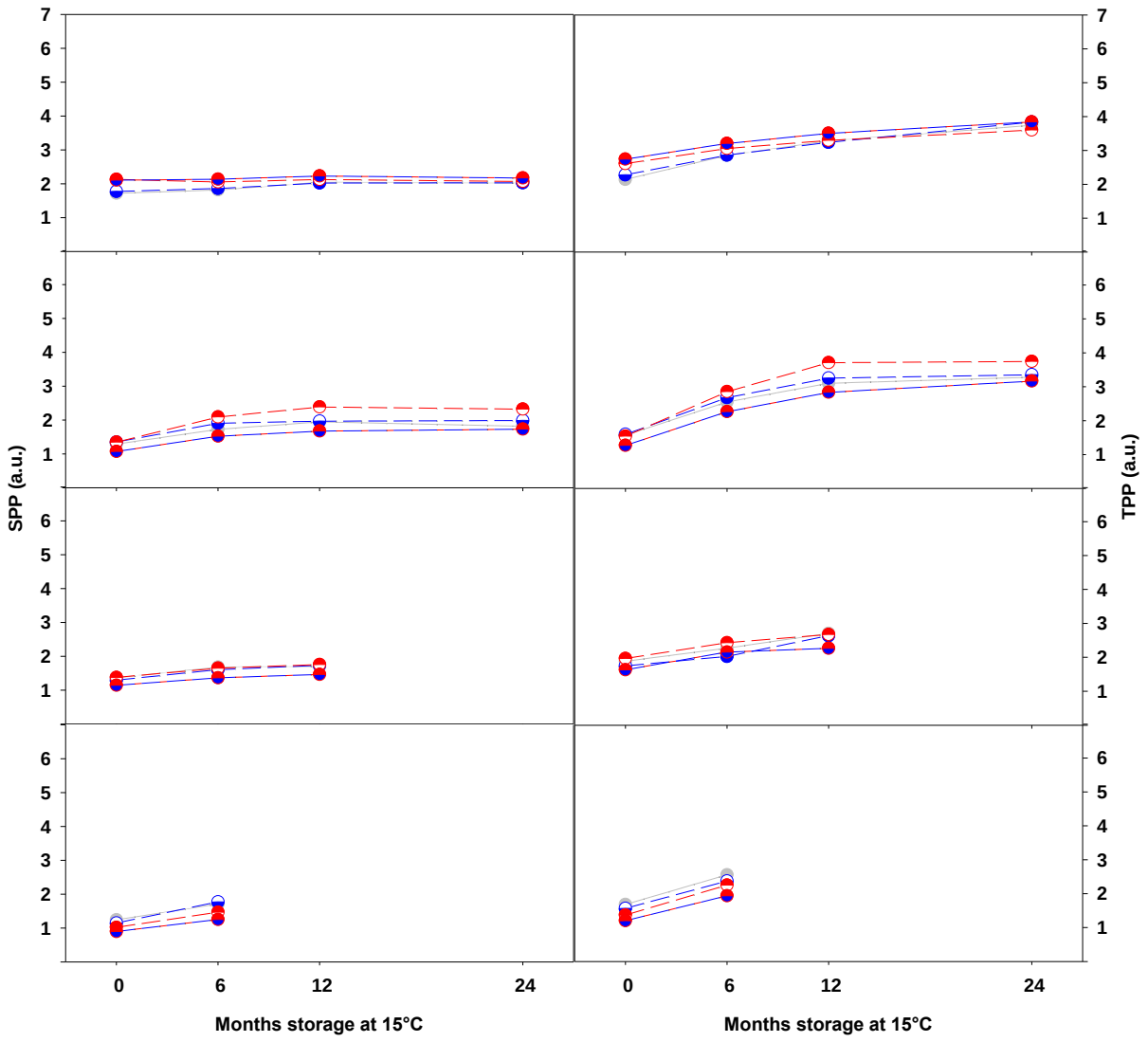


Figure 39: Shiraz wine short (SPP; left panels) and total polymeric pigments (TPP; right panels), expressed in absorbance units (a.u.), of at bottling and after 6, 12, 24 months storage at 15°C over four seasons top to bottom, 2013/14, 2014/15, 2015/16 and 2016/17, respectively.

Tannins contribute to the mouthfeel properties of wine and interact with anthocyanins to form the stable polymeric pigments. The effects of [CO_2] and heat on the tannin concentration of Shiraz wines during storage at 15°C for up to 2 years are presented in Figure 40. The tannin concentrations of the first season's eCO_2 and eTemp wines at bottling varied by a factor of two. The spread was less in the second and fourth season, and minimal in the third. Some minor fluctuation in tannin concentration were observed during aging (for example, the 2013/14 wines), but generally the level of tannins measured at 24 months were similar to

these measured at bottling. The relative differences observed between treatments remained similar, with no wines from any particular treatment exhibiting a disproportionate change in wine tannin concentration during the aging process.

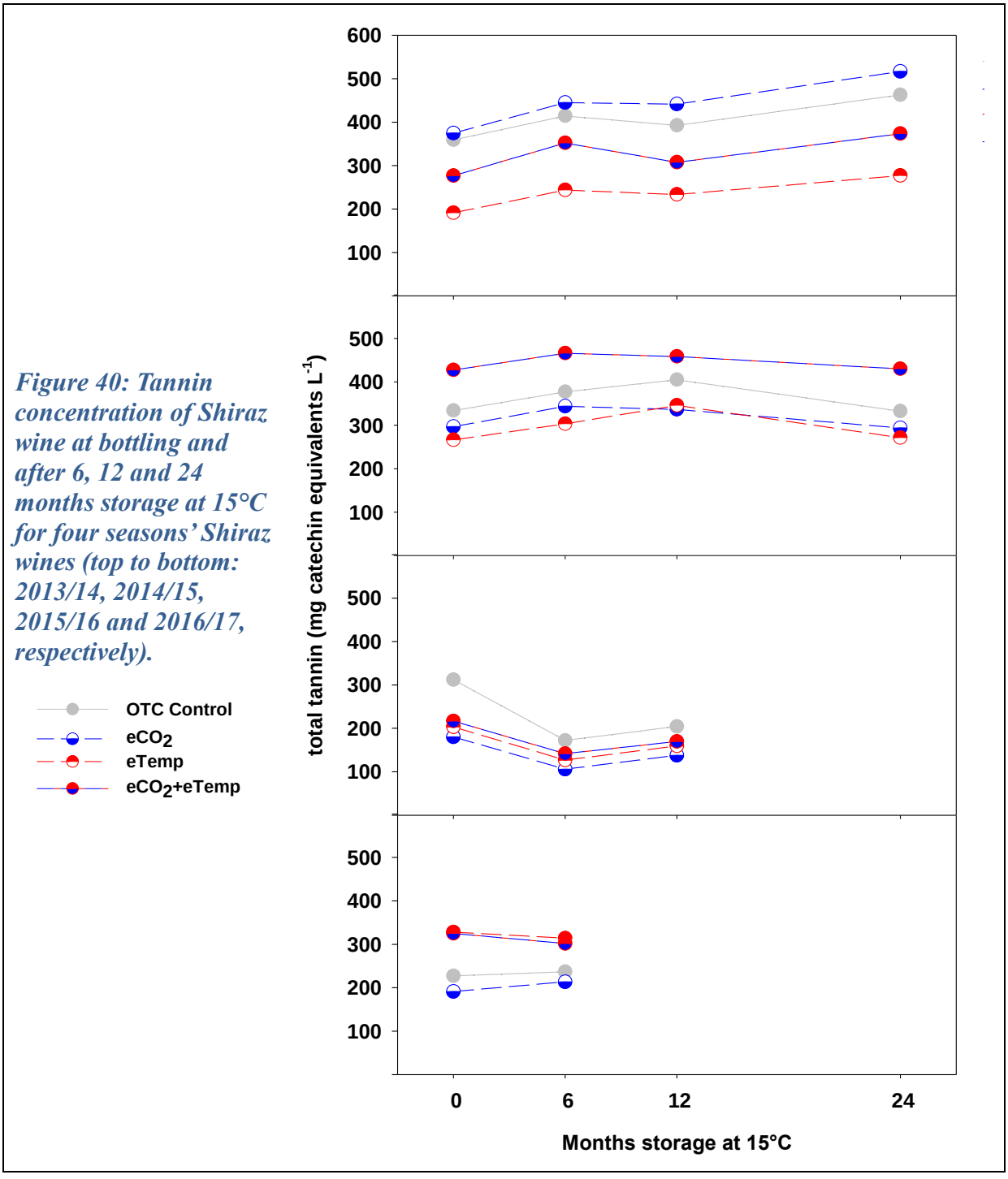


Figure 40: Tannin concentration of Shiraz wine at bottling and after 6, 12 and 24 months storage at 15°C for four seasons' Shiraz wines (top to bottom: 2013/14, 2014/15, 2015/16 and 2016/17, respectively).

Effect of [CO_2] and air temperature on sensory properties of wine

In order to the assess the impact of elevated [CO_2] and temperature conditions in the vineyard on wine sensory properties, finished wines were sent to the Australian Wine Research Institute for sensory assessment by a trained panel. Difference testing, an assessment of a

perceivable difference between two wines, was performed on wines made from fruit exposed to elevated [CO_2] and/or temperature conditions for four vintages and is summarised in Table 14. More comprehensive difference testing reports are included in Appendix 5.

Table 14 Summary of statistical analyses of difference testing for each season's wines made from Shiraz grapes grown in OTCs exposed to elevated [CO_2] and/or elevated temperature conditions

Season		OTC Control	eCO_2	eTemp
	eCO_2	ns		
2013/14	eTemp	***	*	
	eCO_2+eTemp	***	***	Ns
	eCO_2	ns		
2014/15	eTemp	ns	ns	
	eCO_2+eTemp	***	**	Ns
	eCO_2	ns		
2015/16	eTemp	*	ns	
	eCO_2+eTemp	ns	ns	*
	eCO_2	ns		
2016/17	eTemp	ns	ns	
	eCO_2+eTemp	ns	ns	Ns

The perceived sensory differences between treatments varied between seasons. In all seasons, there was no difference detected between wines made from fruit from the eCO_2 treatments compared to the OTC Control wines. In 2013/14 and Season 2014/15 wines from the eCO_2+eTemp were distinguishable from OTC Control wines. The wines from the eTemp treatment were distinguishable from OTC Control wines in two of the four seasons, Season 2013/14 and Season 2015/16. There were no differences observed between any wines in the final season, 2016/17.

Whilst difference testing enables wines to be assessed for distinguishable differences between two wines, the basis of the difference and the direction (*i.e.* better or worse) is unknown. A more detailed sensory evaluation was performed on wines from the final three seasons. Descriptive analysis involves attributes relating to appearance, aroma and palate being assessed by an expert panel.

Of the attributes assessed on the 2014/15 wines, eight were rated as being significantly different between treatments. The wines made from the OTC Control and the eCO_2+eTemp treatments rated low for vegetal and barnyard aromas and highest for the confection, red fruit, floral and overall fruit attributes. Wines from the eTemp treatment were rated high for colour intensity and low for bitterness, which is consistent with the spectral analyses where these wines were observed to have the highest anthocyanins and lowest tannins for the 2015 season.

Sixteen of the attributes assessed on the 2015/16 wines were significantly different between treatments. Generally, wines from the eTemp and OTC Control rated highly for dark fruit, overall fruit and colour intensity. Although there was some variation within treatments, the eCO_2 wines generally rated higher for red fruit and confectionary and the eCO_2+eTemp wines were not rated high for any attribute and lower for colour intensity. This is consistent with the spectral analyses, where wines from the eCO_2+eTemp treatments were observed to have the lowest anthocyanin and WCD for the 2016 season.

Of the attributes assessed in the 2016/17 wines, nine were rated as being significantly different by the panel and six were close to being significantly different. The eTemp wines were rated highly for overall fruit flavour, red fruit flavour and fruit aftertaste, whilst the OTC Control, eCO_2, and eCO_2+eTemp wines were rated high for the more undesirable cooked/tinned vegetable aroma, drain aroma, and sweaty aroma. The wines made from the eCO_2+eTemp treatment were rated lower for colour intensity; a result consistent these wines having the lowest anthocyanin concentration and WCD.

Further details regarding sensory attributes for treatments across the three seasons refer to the reports of descriptive analysis included in Appendix 5.

Summary: Objective 3

As with grapevine characteristics, seasonal climate was the biggest influence on all grape quality parameters, irrespective of any treatment imposed in the vineyard. Season-to-season variability in anthocyanin and tannin concentration were as much as 30% and 40%, respectively.

Other than season, heat had the greatest effect on the grape quality parameters assessed. Elevated temperature, for example, was associated with 1.3 g L^{-1} more titratable acidity at harvest than fruit grown on vines at ambient temperatures. This effect may be indirect because elevated temperature was associated with earlier attainment of commercial maturity than ambient temperature; the effect of warmer air may have been to accelerate sugar transport and accumulation more than the 2°C warmer air encouraged overnight respiratory consumption of organic acids.

Elevated temperature was associated with lower skin tannin concentration at harvest, but not at earlier sampling points (*i.e.* peasize and veraison). Skin tannin concentrations drop from pea size through to harvest due to tannins becoming less extractable, and the effect of the +2°C air may have been to further decrease or alter the capacity of tannin to be extracted during the veraison to harvest period.

There were also qualitative changes apparent in tannin structure attributable to elevated temperature. The extension subunits had lower and higher proportions of epigallocatechin and epicatechin extension subunits, respectively. Structural variation such as polymer length and subunit composition effect tannin extractability. These qualitative changes may be either a cause or an effect of the lower skin tannin; the change in the proportions of epigallocatechin and epicatechin in the extension subunits and/or potential changes to polymer length and tannin structure

Anthocyanin and flavonol concentrations at harvest were also significantly lower in fruit grown on vines exposed to higher temperatures (*i.e.* a heat main effect). This heat effect appeared to be driven predominantly by the 18% lower anthocyanin concentration observed in fruit from the eCO_2+eTemp treatment, with no difference observed between fruit from the OTC Control, eCO_2, and eTemp treatments. Proportional composition of anthocyanins did not change as a result of an increase in [CO_2] or temperature.

The effects of the elevated CO_2 and temperature on flavonol accumulation were similar, both resulting in a reduction in flavonol concentration. Like the observation for anthocyanin, these treatment effects are driven by the significant reduction in flavonol concentration observed for the eCO_2+eTemp treatment, with no difference between the OTC Control, eCO_2 and eTemp treatments.

Tannins, anthocyanins and flavonols are all products of the same biosynthetic pathway, and appear to have been similarly affected by growing the vines and maturing the crop in air warmed by 2°C relative to ambient air.

The effects of the treatments, and season, on grape composition at harvest translated through to the wine quality parameters assessed, and the relative importance of the sources of variation was similar. Season aside, finished wine anthocyanin and wine colour density was the strongest influence for all wine quality parameters assessed at bottling. Other than season, low wine anthocyanin and colour density and high wine hue were associated with 2°C warmer air in the vineyard.

While anthocyanin levels in grapes at harvest and wine at bottling followed similar trends, grape and wine tannin did not. Grape tannin was lowest in the elevated temperature treatments (eTemp and eCO_2+eTemp), with no difference between the OTC Control and the eCO_2 treatment. Conversely, wine tannin was lower in eCO_2 and eTemp wines compared to OTC Control and eCO_2+eTemp wines, which weren't different. The linkage between grape tannin and wine tannin is the complex series of biophysical processes collectively termed vinification. The amount of tannin that ends up in the wine is a function of the amount present in the skins at crushing and the amount of those tannins extracted during vinification. One potential explanation for the disconnect between how much tannin was in the grapes and how much was present in the wine could be an effect of [CO_2] on the grapes that affected how easily tannin was extracted during the maceration process and during subsequent pumping over events during vinification, An obvious candidate here would be the nature of the cell walls of grapes grown on vines exposed to elevated [CO_2] and/or elevated temperature.

Equally important, when considering the impact that the climate in which the grapes are produced has on wine quality, is the potential for carry over effects on long-term wine stability. During wine aging, stable pigments can form as a result of anthocyanins and tannin polymers binding. This was observed in the wines during storage; anthocyanin concentrations declined and polymeric pigment concentration increased for all treatments in all seasons. Wine colour density remained relatively constant following two years of storage at 15°C and wine hue increased. The relativities observed at bottling remained similar following 24 months of storage at 15°C, with no particular treatment having disproportionate increases or decreases of wine quality parameters assessed.

With respect to the sensory evaluation, the eTemp and eCO_2+eTemp wines were more commonly distinguishable from OTC Control wines, and the eCO_2 wines were not distinguishable from OTC Control wines in any season. Wines from each treatment were not particularly prone to having undesirable or desirable wine attributes over multiple vintages. For example, the eCO_2 and eTemp+eCO_2 wines were rated highly for cooked/tinned vegetable aroma, drain aroma, and sweaty aroma in 2015/16, but these attributes did not rate highly in other vintages for wines from the same treatments. In two of the three seasons assessed, the eCO_2+eTemp wines were rated lowest for colour intensity, and this aligned with the wine quality chemical analyses.

Results and Discussion: Objective 4

To further develop and document a field-based system for studying the effect of elevated temperature and CO_2 on grapevines.

System validation

Over the course of the 2013/14 season, the system was constantly monitored to ensure its performance complied with the target performance criteria (*i.e.* 650 µmol mol^{-1} and +2°C relative to ambient). The short-term comparisons described below demonstrate the broader performance of the system over the first season, and were the basis of the project team's confidence that the system was imposing the desired treatments.

OTC [CO_2] temporal variation

The 1-min average [CO_2] from the centres of the OTCs were used to assess the temporal variation of [CO_2] within each OTC. Typical averaged [CO_2] for a single OTC over a week in February 2014, are presented in Figure 41. The averages show a [CO_2] spike when the CO_2 supply is switched on at sunrise, but the average settles down quickly to oscillate between 550 and 770 µmol mol^{-1}, and averaging 661 µmol mol^{-1}. The concentration of CO_2 at the measurement point dropped quickly to ambient levels following the supply being switched off, underscoring the rapid flux of air upward due to the fans pumping ambient air continuously into the OTC. On some days, prior to [CO_2] falling to ambient levels when the supply was turned off, concentrations rose strongly; possibly because of still conditions at dusk, allowing CO_2 to build up in the air above the canopy.

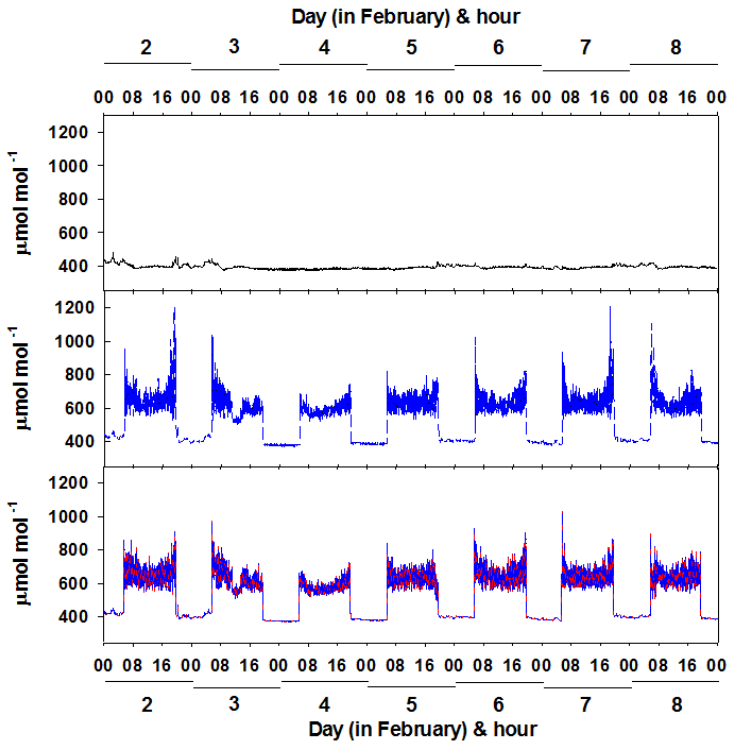


Figure 41 Typical 1-minute average [CO_2] measured at the top of the middle vine in an OTC Control (top), an eCO_2 (middle) and an eCO_2+eTemp OTC (bottom)

Measurements cover the period 2 – 8 February 2014. The daytime means [CO_2] was 661 µmol mol^{-1}, with standard deviation of 66.

Spatial variation of [CO_2] within OTCs

Establishing that [CO_2] was uniform throughout the canopy was one aspect of having confidence that the system was achieving what it was designed to do; namely, increasing [CO_2] to the desired level in and around the canopy. The means at the 10 locations (Figure 3) across the transect through each vine canopy showed that the [CO_2] was variable (Table 15). In the heated OTC (*i.e.* eCO_2+eTemp), the mean [CO_2] was above the 90% of the target (585 µmol mol^{-1}) at all locations. In the unheated OTC (*i.e.* eCO_2), the [CO_2] at 40% of the locations in the transect did not meet this criterion. However, the overall mean [CO_2] for both OTCs was within ± 10% of the target (585 – 715 µmol mol^{-1}), complying with the set performance standard.

Table 15 Mean ($\bar{x}$) and standard deviations (SD) of [CO_2] measurements at 10 locations across a transect through a canopy in two elevated CO_2 chambers in February, 2014

Locations described in Figure 3.

	OTC			
	eCO_2		**eCO_2+eTemp**	
Locations	$\bar{x}$	**SD**	$\bar{x}$	**SD**
1	700	245	760	380
2	798	403	832	478
3	826	392	766	399
4	642	168	665	306
5	561	134	599	236
6	521	73	627	227
7	525	92	604	173
8	546	114	601	167
9	594	158	601	196
10	623	229	596	194
Overall	**634**	**201**	**665**	**276**

The [CO_2] measurements across the 10 locations in each canopy of the adjacent non-chambered vines, the OTC Control vines and the eTemp vines varied between 384 and 388 µmol mol^{-1} with standard deviations of approximately 10 µmol mol^{-1}.

Temperature and [CO_2] in elevated CO_2 OTCs

Temperature traces for two days when the temperature in the canopies of non-chambered vines reached 30 and 40°C respectively are presented in Figure 42. Figure 42 also features

traces for incoming solar radiation on both of those days. Firstly, irrespective of whether the temperature was increasing or decreasing, the +2°C differential was maintained in the OTCs supplied with warmed air. Secondly, on the 40°C day, a strong drop in solar radiation at midday was accompanied by a strong drop in canopy air temperature in all OTCs. A smaller drop in solar energy on the 30°C day was not accompanied by a decrease in canopy air temperature in any OTC. The drop in solar radiation was most likely due to intermittent cloud cover, but the intermittent nature of direct solar radiation did not appear to exert an overly strong influence on the canopy air temperatures for most of the day. At midday, however, some influence was obvious, but was possibly dependent on the ambient temperature and possibly on the thickness of any intermittent cloud cover. As mentioned though, the +2°C differential was maintained, irrespective of solar radiation, direct or otherwise.

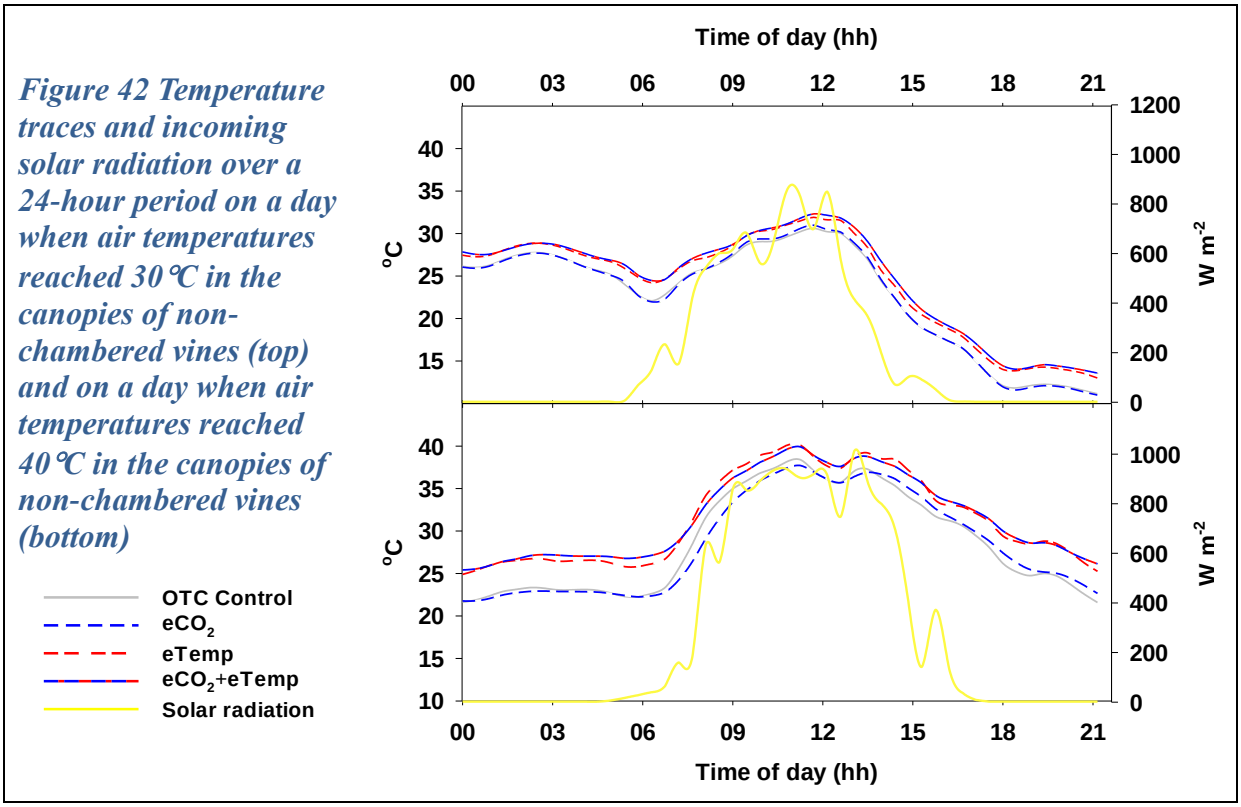


Figure 42 Temperature traces and incoming solar radiation over a 24-hour period on a day when air temperatures reached 30℃ in the canopies of non-chambered vines (top) and on a day when air temperatures reached 40℃ in the canopies of non-chambered vines (bottom)

The impact of daytime temperature variation and imposed [CO_2] stability

The air temperature in the OTCs over the course of a day did not impact on the stability of the imposed [CO_2]. This is illustrated in Figure 43 for a single day in February 2014. On a day when the air temperature in the canopy of a non-chambered vine, in an OTC Control and in an eCO_2 OTC rose to nearly 40°C and the temperature in an eCO_2+eTemp OTC rose to 42°C, following the rapid rise at dawn, the steady state [CO_2] of approximately 650 µmol mol^{-1} was unaffected by the rise in temperature from 06:00 h and, the temperature maxima had no impact on that steady state [CO_2].

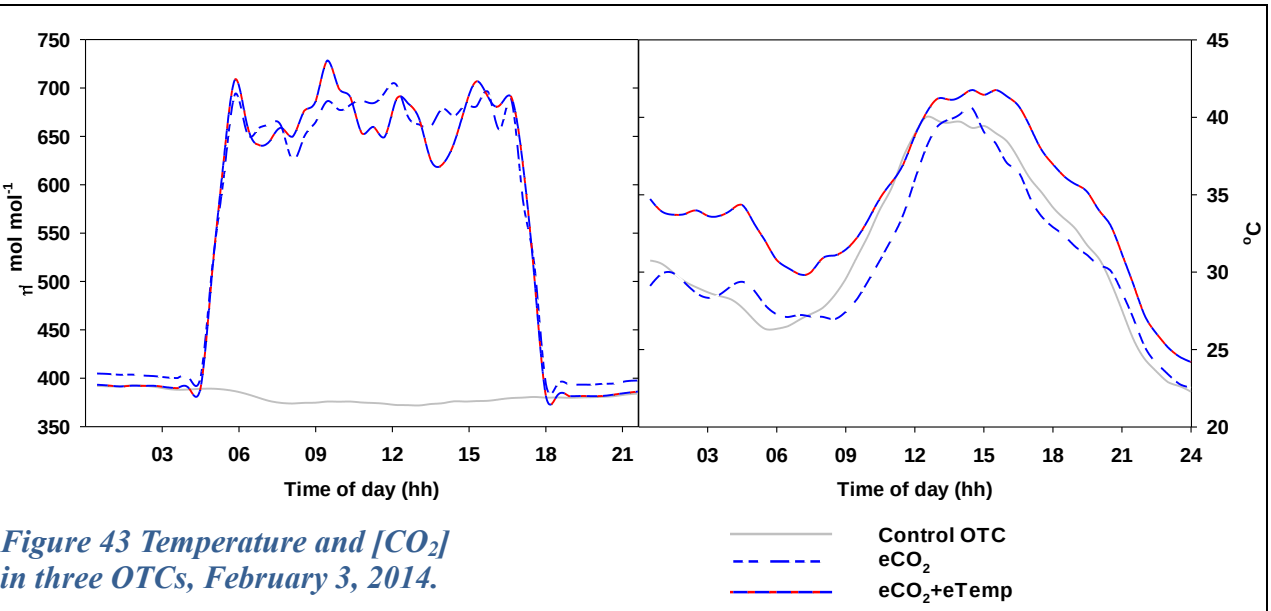


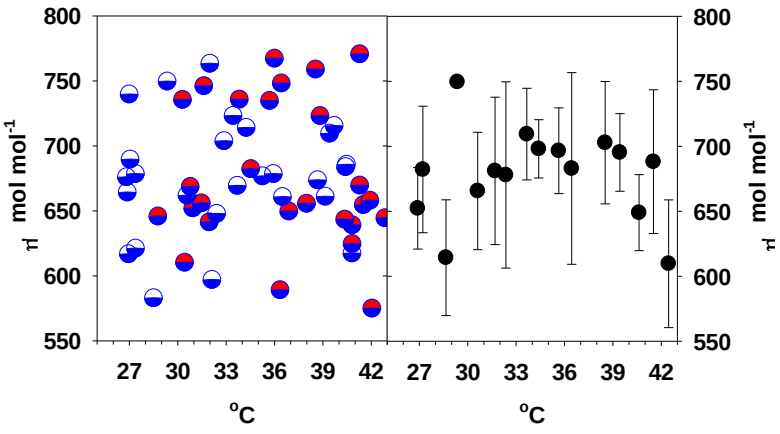
Figure 43 Temperature and [CO_2] in three OTCs, February 3, 2014.

Data plotted are spline-smoothed rolling means.

The lack of correlation between OTC air temperature and [CO_2] is illustrated in a scatter plot of [CO_2] above 550 µmol mol^{-1} versus temperature for that particular day (Figure 44). Also presented in Figure 44 are the [CO_2] mean and standard deviations for each °C. The scatterplot indicates no structure, neither in the means nor in the distribution of the [CO_2] deviation across the temperature range on that day in those two OTCs. In other words, OTC [CO_2] was independent of OTC temperature.

Figure 44 Scatter plot of [CO_2] greater than 550 µmol mol^{-1} versus temperature in two elevated CO_2 OTCs on February 3, 2014 (left panel) and mean [CO_2] and standard deviation for each °C

The same 30-minute mean temperature and [CO_2} data were used for both graphs.



Long Term System Performance

Establishing the stability of the imposed climates over the full term of the experiment was critical to correctly identifying a true response as opposed to random biophysical noise. Having validated the systems during the initial season, an objective assessment of the long-term performance of the systems over the over the course of the experiment was undertaken.

OTC [CO_2] over four seasons

Over the four seasons the mean daytime [CO_2] at the centre of the elevated CO_2 OTCs varied between 628 and 643 µmol mol^{-1} (Table 16); within 96% of the target [CO_2] of 650 µmol mol^{-1}, and well within the performance criteria of ±10% of the target [CO_2]. Thus, the system successfully controlled the [CO_2] at the centre of each elevated CO_2 OTC over the course of the experiment.

The [CO_2] in the eCO_2 chambers was marginally more variable than the [CO_2] in the eCO_2+eTemp OTCs (see SD in Table 16). It is possible that the warmer air caused more turbulence in the heated OTCs which resulted in faster CO_2 distribution within the canopy.

Table 16 OTC daytime [CO_2] statistics (mean, $\bar{x}$; standard deviation, SD; % coefficient of variation, CoV)

Measurements collected from 16 September 2013 – 22 June 2014, 19 August 2014 – 17 June 2015, 14 August 2015 – 20 July 2016 and 25 August 2016 – 10 July 2017.

**µmol mol^{-1}*

		Treatment		
Season	Statistic	OTC Control	eCO_2	eCO_2+eTemp
2013/14	$\bar{x}$*	390	635	640
	SD	29	71	67
	CoV	7	11	11
2014/15	$\bar{x}$	390	642	642
	SD	29	73	70
	CoV	7	11	11
2015/16	$\bar{x}$	356	640	642
	SD	17	72	71
	CoV	5	11	11
2016/17	$\bar{x}$	355	638	629
	SD	17	73	74
	CoV	5	11	12
Overall	$\bar{x}$	373	639	638
	SD	23	72	70
	CoV	6	11	11

Typical sets of [CO_2] measurements for the same elevated [CO_2] chambers over a 24-hour period in December, 2015, and the same calendar day in 2017, are presented in Figure 45. In both sets, [CO_2] in the OTC Control chamber was stable at approximately 400 µmol mol^{-1} over each 24-hour period, and [CO_2] in the eCO_2 and eCO_2+eTemp chambers were at similar levels when the CO_2 supply was switched off between sunset and sunrise. During the daylight hours [CO_2] in the eCO_2 and eCO_2+eTemp chambers were consistently kept above 550 µmol mol^{-1} CO_2.

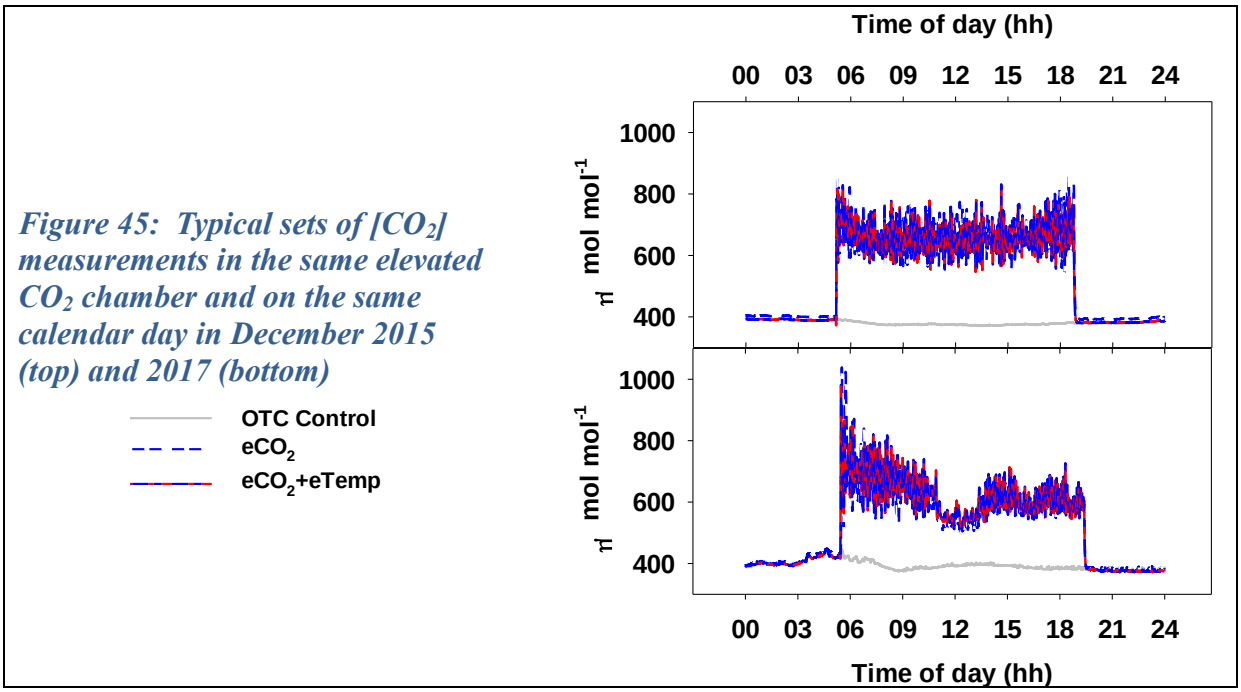


Figure 45: Typical sets of [CO_2] measurements in the same elevated CO_2 chamber and on the same calendar day in December 2015 (top) and 2017 (bottom)

OTC temperatures over four seasons

Over the four seasons, the mean canopy air temperature in the non-heated chambers was 0.2°C higher than the mean air temperature measured in the canopies of the non-chambered vines (Table 17). The pumping of air heated by 2°C relative to ambient air into the eTemp and eCO_2+eTemp chambers elevated the temperature in the canopies by a further 1.9°C and 2.0°C above ambient, respectively, on average.

Table 17 Vintage (September – March) average canopy air temperature statistics (mean, $\bar{x}$; standard deviation, SD; % coefficient of variation, CoV)

*Includes 2014, 2015 and 2016 dormancy periods (April – August)

		Treatment				
			Chamber			
Season	Statistic	Control	OTC Control	eCO_2	eTemp	eCO_2+eTemp
2013/14	$\bar{x}$	21.6	21.6	21.6	23.8	23.9
	SD	0.50	0.36	0.49	0.57	1.02
	CoV	2	2	2	2	4.28
2014/15	$\bar{x}$	21.4	21.9	21.7	23.0	23.1
	SD	0.45	0.89	0.52	1.27	1.30
	CoV	2	4	4	4	4
2015/16	$\bar{x}$	22.5	22.7	22.9	25.0	25.0
	SD	0.44	0.40	0.64	0.79	1.27
	CoV	2	2	4	3	5
2016/17	$\bar{x}$	21.5	21.6	21.5	23.5	24.1
	SD	1.27	1.12	1.32	1.31	1.68
	CoV	6	5	6	6	7
Overall*	$\bar{x}$	17.8	18.0	18.0	19.9	20.0
	SD	0.51	0.53	0.55	0.91	1.19
	CoV	3	3	3	5	6

The statistics for the daily temperature minima (Table 18) showed little evidence of the chamber effect mentioned above. The mean daily minima in the eTemp and eCO_2+eTemp chambers were 1.8°C and 2.1°C respectively, higher than the mean daily temperature minima in the Control, OTC Control and eCO_2 chambers (assuming very little difference between the non-heated treatments). Examining the temperature ranges over a season, the mean monthly minimum was very stable, with the temperature in the heated chambers consistently 2°C above the non-heated chambers. This was much more evident in the mean monthly minimum than it is in the mean monthly maximum (data not shown).

Table 18 Vintage (September – March) daily canopy air temperature minima statistics (mean, $\bar{x}$; standard deviation, SD; % coefficient of variation, CoV)

**Includes 2014, 2015 and 2016 dormancy periods (April – August)*

		Treatment				
			Chamber			
Season	Statistic	Control	OTC Control	eCO_2	eTemp	eCO_2+eTemp
2013/14	$\bar{x}$	13.4	13.2	13.2	15.3	15.3
	SD	0.26	0.15	0.32	0.46	0.74
	CoV	2	1	2	3	5
2014/15	$\bar{x}$	13.3	13.3	13.2	14.6	14.5
	SD	0.22	0.66	0.22	1.26	1.12
	CoV	2	5	2	9	8
2015/16	$\bar{x}$	14.2	14.3	14.2	16.3	16.8
	SD	0.22	0.33	0.22	0.71	0.87
	CoV	2	2	2	4	5
2016/17	$\bar{x}$	12.9	13.1	13.1	14.5	15.5
	SD	0.85	1.04	1.03	1.09	1.43
	CoV	6	8	8	7	9
Overall*	$\bar{x}$	13.4	13.5	13.4	15.2	15.5
	SD	0.39	0.54	0.45	0.88	1.04
	CoV	3	4	3	6	7

Overall mean daily canopy air temperature maxima of the chambered vines were approximately 1.0°C higher compared to non-chambered vines (Table 19). When the vines were enclosed, the mean daily canopy air temperature maxima were 1.6°C higher in the eTemp and eCO_2+eTemp chambers compared to the OTC Control chambers. The cooler conditions during the 2015 growing season may have contributed to the lower mean maximum temperature when compared to non-heated treatments. The mean daily temperature minima varied less than the overall average and the mean daily temperature maxima. Direct solar radiation can have a strong effect on canopy and canopy air temperature; intermittent cloud cover may result in large fluctuations in canopy air temperature over a short time frame, but daily maxima would be largely unaffected.

Table 19 Vintage (September – March) daily canopy air temperature maxima statistics (mean, $\bar{x}$; standard deviation, SD; % coefficient of variation, CoV)

*Includes 2014, 2015 and 2016 dormancy periods (April – August)

		Treatment				
			Chamber			
Season	Statistic	Control	OTC Control	eCO_2	eTemp	eCO_2+eTemp
2013/14	$\bar{x}$	30.9	31.3	31.8	33.5	33.4
	SD	0.80	0.76	0.79	0.67	1.04
	CoV	3	2	2	2	3
2014/15	$\bar{x}$	30.3	31.7	31.8	32.5	33.1
	SD	0.52	0.90	1.08	1.00	1.41
	CoV	2	3	3	3	4
2015/16	$\bar{x}$	31.8	33.1	33.0	34.6	33.7
	SD	0.88	0.81	0.83	0.86	0.84
	CoV	3	2	3	2	2
2016/17	$\bar{x}$	30.3	30.9	30.6	32.7	33.0
	SD	1.27	1.19	1.52	1.50	1.60
	CoV	4	4	5	5	5
Overall*	$\bar{x}$	30.8	31.7	31.8	33.3	33.3
	SD	0.87	0.91	1.05	1.01	1.22
	CoV	3	3	3	3	4

Summary: Objective 4

In all OTCs, on average, the systems injecting CO_2 maintained the [CO_2] at the centre of the OTC to at least 95% of the target concentration; the original aim being to achieve 90% of the target. The CO_2 injection system performed satisfactorily over a wide range of daytime temperatures with an overall standard deviation varying between 85 – 112 µmol mol^{-1}, providing confidence in the design. The [CO_2] in OTCs equipped with only CO_2 injection but no heating were more variable than the [CO_2] in OTCs equipped with CO_2 injection and heating. The most likely reason for this is that CO_2 from the delivery tubes in heated OTCs reached the sensor more quickly due to the warmer air causing more turbulence and lifting

CO_2-enriched air to the top of the canopy sooner than in the OTCs only supplied ambient air. The spatial distribution of [CO_2] within vine canopy was also more even in heated OTCs.

On average over 43 months of temperature logging the heating system maintained an elevated temperature of 2.03°C above the non-heated treatments. Further, there was no or very difference in canopy air temperature between the Control, OTC Control and the eCO_2 treatments.

Finally, the initial validation conducted during the 2013/14 season provided confidence that the heating and CO_2 injection systems could independently impose the desired climates on vines in the field. The assessments of the stability of the climates in the OTCs over the four seasons of the experiment provide confidence that the vines' responses can be attributed to the climatic variables manipulated.

Outcomes and conclusions

Many resources are being invested worldwide trying to predict what the likely impact of the projected rise in atmospheric temperatures might mean for the geographic distribution of grape production in the future, and what the varietal mix might be in those locations and what the impact might be on grape composition. Some of this work has been conducted using fruiting cuttings in pots under controlled environment conditions (*e.g.* Martínez-Lüscher *et al.* (2016), some have used historical data (*e.g.* (Chuine *et al.*, 2004, Menzel, 2005, Petrie and Sadras, 2008), and others have built complex empirical models to provide geo-specific predictions (*e.g.* (Nendel, 2010)). The situation for elevated atmospheric [CO_2] is similar to some extent (*e.g.* fruiting cuttings under controlled conditions (Martínez-Lüscher *et al.*, 2016)), but some field work has been carried out (Bindi *et al.*, 2001a, Bindi *et al.*, 2001b, Gonçalves *et al.*, 2009) albeit not at a scale that would impart confidence in the observations. The imposition of both elevated [CO_2] and elevated air temperature — the former a driver of the latter — had only been considered under controlled conditions with fruiting cuttings (Martínez-Lüscher *et al.*, 2016).

Recognition of the lack of field-scale data that would help guide the Australian wine industry as the climate changes over the coming decades, coupled with knowledge of the effects both atmospheric [CO_2] and temperature have on grapevine physiology and phenology, and grape development, was the basis for the establishment of the experiment reported herein. The lack of field scale data may be partly due to the cost and logistical difficulties associated with consistently and reliably imposing higher concentrations of CO_2 and warmer air around vines in the field, and on a scale that would give the exercise scientific rigour. The "free air CO_2 enrichment" (FACE) approach has been utilised for grapevines, but the issue of scale is difficult to solve, and without containment it would be extremely difficult to impose warming. Gonçalves *et al.* (2009) reported the use of OTCs to contain CO_2-enriched air around grapevines in the field, and Sommer *et al.* (2012) reported the use of OTCs at Irymple to compare the effects on vines of a +2°C differential relative to ambient temperatures. Combining the two environmental variables reliably and on a suitable scale was the challenge.

Overcoming those difficulties was a major part of the project. Designing, building and maintain a system that combined CO_2 enrichment and heating, and then validating the combination in the first season of the experiment was a significant technical achievement. The assessment of the system's performance over four years conferred confidence that the combinations of planned climate settings were reliably imposed and, *ergo*, the observations and measurements could be ascribed to the planned climate variations.

Growing Shiraz vines in elevated atmospheric [CO_2] resulted in more grapes being produced overall, but the effect was confined to the last season of the experiment. The effect was mainly due to more bunches per vine, and was independent of air temperature. A lack of response in the first season was to be expected because bud fertility was already set. The lack of response in the second and third seasons may indicate that the effect of elevated [CO_2] was incremental. The yield increase in the final season suggests the incremental changes reached a threshold by the time bud fertility for the final season was set in November and December of the third season. Production cycles of perennial crops stretch over seasons, and it is scarcely surprising that it effectively took four seasons for an effect of elevated [CO_2] to manifest. In a sense, the experimental timeframe of four seasons may have been barely adequate. In contrast, OTC-based elevated [CO_2] experiments on other woody perennial crops and forest trees have been run for more than a decade or are projected to run for that

timeframe[6] (Ellsworth *et al.*, 2017, Kimball *et al.*, 2007). The implication for the Australian wine industry is that, provided inputs such as water are not limiting, the projected climate of the future will not impose a productivity penalty. Indeed, vine productivity may be enhanced by higher atmospheric [CO_2]; that enhancement may be more obvious in some seasons than others, and season will probably continue to be the major driver of yield variability.

The physiology of the effect of 650 μmol CO_2 mol^{-1} may be related to the elevated assimilation rates and higher carbohydrate levels found in leaves on vines in the elevated [CO_2] OTCs. The higher rates of CO_2 fixation could be expected to alter the whole vine carbohydrate balance during the growing season. The linkage between carbohydrate supply at the time of bud initiation is well established (Candolfi-Vasconcelos and Koblet, 1990). The winter pruning weight and trunk diameter data do not support the idea of a carbohydrate-driven increase in vine biomass, and the trunk and cane winter wood did not indicate an increase in dormant seasons vine carbohydrate reserves. The former may be related to the adequate, but not excessive, water supply not promoting excessive shoot growth. The latter may be due to looking at the wrong part of vine; on a mass balance basis, more carbohydrates are stored in the roots than in the above ground parts (Holzapfel *et al.*, 2010).

Consistent with that argument, growing vines in +2°C warmer air had no impact on assimilation rates, leaf carbohydrates, or yield. While photosynthesis is very temperature dependent, over the range of temperatures that measurements were conducted in this experiment, that temperature dependence was not apparent. The frequency of weather extremes in the future notwithstanding, it is reasonable to conclude that on the evidence available, a generally warmer climate will not impact on Shiraz productivity.

In the generally warmer higher [CO_2] climate of the future, pressure on surface water supplies is likely to be greater than is currently the case. Water use efficiency (*i.e.* Mg fruit ML^{-1} water transpired will likely be a very important consideration. Based on the two seasons for which data are available, whole season water use by vines in eTemp OTCs was almost double that of OTC Control and eCO_2 vines. The effect of warmer air on season water use was strongly modulated by elevated [CO_2]. The basis of that modulation is not readily apparent because stomatal conductance measurements do not necessarily point to eTemp vines transpiring more than vines in all the other treatments. Irrespective of whether the modulating effect of elevated [CO_2] can be explained, and irrespective of whether water availability for irrigation in the future is limiting, the data collected as part of this project do not suggest the need for wine grape producers and water supply authorities to be concerned that wine grape production in the future will require more water than is currently the case.

The effects of seasonal weather patterns on grape vine phenology are generally considered *a posteriori*; after the event, in other words. The climates that were imposed here, coupled with the four unique weather patterns afforded by the seasons, effectively imposed eight unique weather patterns. While the seasonal conditions were unpredictable, within each season a physiologically significant consistent temperature differential was imposed that was reflected in the season × heat interaction term for bud burst and season × [CO_2] × heat interaction term for harvest date. Those effects weren't matched by significant equivalent terms in the analyses of variance for the temperature indices.

Elevated [CO_2] and +2°C across the course of the experiment resulted in marginally earlier veraison and harvest. The effect of elevated [CO_2] was much smaller than the effect of +2°C, and marginal compared to the effect of season. Nonetheless, that fact that the effect was detectable suggests some shift in the physiology of the vine such that maturation was

[6] http://www.bbc.com/news/science-environment-39472425

accelerated, and, although marginal, indicating that possibly the effect of elevated [CO_2] may take time to accrue. The effect of warmer air on maturation, again, smaller than the effect of season, is not attributable to assimilation rates or leaf carbohydrates, which were unrelated to air temperature in the weather patterns of the four seasons of the experiment.

Given the critical nature of maturation timing with respect to vintage logistics, it is not surprising that most climate change related research has focused on this developmental point (*e.g.* Petrie and Sadras (2008) The observations made as part of this experiment suggest that the temperature-related maturation advancement effect has its genesis at or about flowering. Earlier flowering due to warmer temperatures was followed by earlier veraison and earlier harvest. The effect appeared to accelerate; the spread of flowering was smaller than the spread of veraison, which was smaller than the spread of maturation.

As with phenology and yield, season was the dominant factor in determining the composition of grapes at maturity. The composition of grapes at maturity generally determined the composition of the finished wine. Beyond bottling, however, season, [CO_2] or heat were not important sources of variation in the changes in wine quality during storage out to two years. The implication for the wine industry is that the post-vinification storage and orderly release of wine onto markets need not change as the climate changes.

After season, air temperature was the next most important factor; +2°C was associated with less tannin in grape skins at maturity, and +2°C combined with 650 μmol mol^{-1} of CO_2 was associated with less anthocyanins and flavonols. The implication for the Australian wine industry is that as average temperature increases, there will be a commensurate drop in red wine grape quality. That drop in red wine quality is most likely due to the negative effect that higher temperatures have on tannin, anthocyanin and flavonol biosynthesis being greater than the reduced likelihood of degradation of those compounds due to temperature-related earlier maturity.

As mentioned, those effects mostly flowed through to the finished wine, but not so for tannin. As a main effect +2°C was associated with less tannin in skins at maturity, and elevated [CO_2] did not modulate that effect. The heat main effect was not consistent through to the final wine. Given that tannin levels in the skin and tannin extraction during vinification determine wine tannin levels, the different relativities between the treatments before and after vinification point to extraction involvement. Extraction is known to be affected by cell wall composition, and possibly the cell walls developed differently in responses to higher temperatures and/or elevated [CO_2]. Previous research on the relationship between grape and wine tannin has drawn attention to the complexity of this connection (Kilmister *et al.*, 2014).

Recommendations

The project has provided significant new knowledge about the interaction between grapevine physiology and the environment and what that might mean for wine quality. On one level that knowledge confirms the trend observed by wine industries around the world, but highlighted a knowledge failure that forms part of that industry experience. On another level, by virtue of the experimental infrastructure developed and used over four seasons, the project provides a cautionary note to researchers seeking to identify what the implications of future climate scenarios might be on grapevines, and delivers some positive news for wine grape producers contemplating projections of diminished surface water supplies.

Industry

Despite the gradual advancement of vintage, seasonal effects are still strong (see Table 3 for an example of the extent), and likely to remain so. Scheduling vintage activities is difficult because of those strong seasonal influences that are — apart from long term trends — largely unpredictable in any quantitative sense. It would be in industry's best interest to have available better means to predict likely maturity dates. An appreciation of the deficiency of the current understanding of the link between temperature and phenology was an outcome of the project. Considering the issue in the context of the new science presented herein, the new concepts in thermal environment/phenological behaviour of woody perennial plants may be a useful point to develop better means to predict maturity. Equally, underpinning the efficacy of any new predictive framework would be the availability of accurately recorded growth stages.

Although observations only cover a four season timeframe, the project has demonstrated that the impact of rising CO_2 concentrations is unlikely to be catastrophic to quality and may even enhance yield. This is a significant positive for industry. Also, the impact of rising CO_2 was still much less than season-to-season variability. There were strong indications that leaf carbohydrate balance was perturbed, and the effect of that disturbance over the life of the vine is difficult to predict. Carbohydrate balance is known to be a good predictor of biennial bearing in other woody perennials, and this tendency may warrant monitoring as [CO_2] increases.

The effect of warmer temperatures on grape composition is well established, and the project confirmed that in a structured way by applying a uniform +2°C increase in air temperature relative to ambient to some vines and comparing them with vines under natural conditions. The implications are that the average increase in temperatures across all regions will result in a similar detrimental effect on grape composition. The adoption of vineyard mesoclimate cooling during critical stages of fruit development may need to be considered by wine grape producers, but knowing the thresholds for the effect would be of benefit, as would the timing.

Research

Levels of the various classes of phenolic compounds important in wine quality in grapes at harvest is a balance between their biosynthesis and their temperature-related degradation. The temporal and environmental nature of the trade-off is unclear. Knowing when the various classes of compounds are synthesised and knowing what the environmental circumstances that are conducive to their synthesis and degradation would inform the timing of mesoclimate modification or the development of alternative canopy management strategies. The research focus should be more on the processes; accumulation is the balance of the two (synthesis and degradation).

The inadequacies of the currently accepted vine heat requirements were clear. Across

perennial horticulture and forestry, the thermal drivers of phenology are an active area of research given their importance in predicting the behaviour of crops and deciduous forests; some of the work in these sectors may potentially benefit the wine industry. The use of OTCs makes it possible to impose demonstrably modified climates around vines in the field. This should enable a change in the investigation of vines' thermal requirements from qualitative to quantitative, as well as allowing other factors, such as the changes in CO_2 concentration used here, to be studied in combination with temperature. In a sense, the use of the OTCs would move the science from an empirical basis to evidence-based. Using this approach to test empirically derived models of vines' thermal requirements would be strong step forward in providing better timing estimates for phenology-dependent viticultural and wine making operations.

Further to the point above, every season is different due to the almost limitless combinations of high and low temperatures of varying duration. Trying to capture that season-to-season variation in single independent variable models is a tall order. Perhaps consideration needs to also be given to employing alternative modelling techniques such as neural networks and the like, to develop better predictive models. Although these modelling approaches tend to be "black box" and essentially empirical in nature, they nevertheless may enable the capture of the dynamism of weather in relation to phenology, such that any need for a deeper understanding is redundant.

Whilst one outcome of the project has been to put a bound on the scale of medium-term effects likely to be seen as atmospheric CO_2 increases, the work has also highlighted the improvement in water use efficiency at the leaf level. Although this was contrasted by limited effects at the whole vine level, it proffers an opportunity to alter vine management to find means to express the leaf level effect at the vine or vineyard scale.

Appendix 1 Communication

Refereed publications submitted for publication

- Mollah, M., Unwin, D., Fitzgerald, G. (2018). A CO_2 injection system inside an open-top chamber to study the effects of future climates on mature, field-grown grapevines. *Transacations of the ASABE*. (Submitted for publication)

Industry journal articles

- Sommer, K., Unwin, D., Kilmister, R., Edwards, E., Mollah, M., Fitzgerald, G., Downey, M. (2013), Tank installation aids research at Mildura. *Australian and New Zealand Grapegrower and Winemaker*, 598, pp. 28 – 29.
- Unwin, D., Sommer, K., Mollah, M., Edwards, E., (2015). Shiraz in a future climate. *Australian and New Zealand Grapegrower and Winemaker*, No. 613, Feb 2015: 30 – 31.
- Kilmister, R., Unwin, D., Treeby, M., Edwards, E., Krstic, M. (2016). Effect of elevated CO_2 and temperature on phenology, carbohydrates, yield and grape composition — preliminary results. *Wine & Viticulture Journal* 31, 38 – 42.

Scientific Conferences/Seminars/Workshops

- Mollah, M., Edwards, E., Unwin, D., Fitzgerald, G., Kilmister, R. (2014) 'A cost-effective system for injecting pure CO_2 into open top chambers: design and performance'. International Conference of Agricultural Engineering, Zurich, Switzerland.
- Kilmister, R (2015). Effect of elevated CO_2 and temperature on grapevine physiology. AWRI Webinar Series, November 12, 2015. http://www.awri.com.au/industry_support/courses-seminars-workshops/webinars/
- Edwards, E., Unwin, D., Kilmister, R.L. & Treeby, M (2016). Multi-seasonal effects of warming and elevated CO_2 on the physiology, growth and production of mature, field grown, Shiraz grapevines. Proceedings of the 2016 ClimWine Conference, Bordeaux, France.
- Edwards, E.J., Unwin, D.J., Sommer, K.J., Downey, M.O. & Mollah, M. (2016). The response of commercially managed, field grown, grapevines (*Vitis vinifera* L.) to a simulated future climate consisting of elevated CO_2 in combination with elevated air temperature. *Acta Horticulturae* 1115, 103–110.

Posters

- Unwin, D. Dale (2014) "Increases in carbon dioxide and temperature; a study on commercially grown grapevines" at the 7th International Table Grape Conference in Mildura 11 – 14 November.
- Edwards, E., Unwin, D., Mazza, M., Downey, M. (2014). Hot and getting hotter – how will a warming climate affect warm climate viticulture? Mildura Field Days.
- Edwards, E., Unwin, D., Mazza, M., Treeby, M. (2015). Climate change & regional wine grape production. Mildura Field Days.

- Edwards, E., Unwin, D., Mazza, M., Treeby, M. (2016). Effect of climate change on grape maturation and wine composition. Mildura Field Days.
- Mazza, M., Treeby, M. (2017). Wine production in a changing climate. Mildura Field Days.

Site visits and other media

Site visits (12) by various national and international industry bodies, government agencies and private companies including:

- USDA
- 7th International Table Grape Symposium – Conference delegates
- AWRI/MVWI Seminar Delegates
- NSW DPI
- SARDI
- Ministry of Agriculture, Bhutan
- HIA
- NCEA, USQ
- AWRI
- AGWA
- EJ Gallo

Appendix 2 Intellectual Property

This information generated by this project will enter the public domain and will be accessible for future reference.

Appendix 3 References

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Appendix 4 Staff

Name	Position/Role	Dates	
Dr Mark Downey	Chief Investigator	Feb-13	Oct-13
Dr Ian Goodwin	Chief Investigator	Oct-13	Mar-18
Dr Michael Treeby	Senior Research Scientist	Feb-15	Mar-18
Dr Karl Sommer	Senior Research Scientist	Feb-13	Sep-14
Dr Everard Edwards	Senior Research Scientist	Jun-14	Mar-18
Dr Mahabubur Mollah	Senior Research Scientist	Jun-14	Mar-18
Dr Rachel Kilmister	Research Scientist	Feb-13	May-16
Dr Belinda Kerridge	Research Scientist	Feb-17	Mar-18
Marica Mazza	Research Scientist	Jun-14	Mar-18
Dale Unwin	Research Scientist	Feb-13	Mar-18
Alex Lawlor	Research Project Officer	Feb-13	May-16
Annette Boettcher	Research Project Officer	May-16	Mar-18
Chris Davies	Technical Officer	Feb-13	Feb-14
Sue Williams	Casual Technical Assistant	Feb-13	Mar-18

Appendix 5 Sensory Analysis Reports

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Sensory Evaluation Report

Marica Mazza
Research Scientist -Viticulture & Oenology
Agriculture Research - Horticulture
Department of Environment and Primary Industries
PO Box 905, Mildura, Victoria 3502

Samples received: 23-11-2014 Report date: 17-12-2014

Introduction

The aim of this study was to determine whether there were significant differences between a series of treatment wines and a control wine. In total 150 bottles of Shiraz wine were provided (Table 1). Associated chemical test data was provided regarding the samples, with the specific comparisons of pairs of wines conducted as requested.

Materials and Methods

The balanced reference triangle test was applied, following the Australian Standard: Sensory analysis of foods - Specific methods – Triangle Test AS 2542.2.2. The triangle test is a forced choice test and is not valid for determining preferences, nor the character or degree of any perceived difference.

There were three fermentation replicates provided for each of the control and treatment wines.

Table 1. The wines studied with bottle codes.

	Bottle Code	Number of bottles
Control	Rep 1 #39	12
	Rep 2 #40*	12
	Rep 3 #41	6
Treatment 3	Rep 1 #32	12
	Rep 2 #33*	8
	Rep 3 #34	10
Treatment 4	Rep 1 #20	10
	Rep 2 #21	10
	Rep 3 #22*	10
Treatment 5	Rep 1 #4	10
	Rep 2 #5	10
	Rep 3 #6	10
Treatment 6	Rep 1 #1	10
	Rep 2 #2	10
	Rep 3 #3	10

*Replicates excluded from difference tests

AWRI

From a preliminary assessment involving several AWRI staff with extensive experience in wine sensory evaluation, it was noted that there were differences among fermentation replicates of the Control (#40), Treatment 3 (#33) and Treatment 4 (#22) wines. As a result, these were excluded, mainly due to perceived oxidation characteristics. There were no noticeable differences between replicates of Treatment 5 and Treatment 6. The replicates were blended on the day of analysis and blended wines poured into laboratory bottles prior to pouring for the sensory analysis.

The tests were conducted on different days as shown in Table 2, with two tests per session.

Table 2. Dates of testing.

Date	Comparison
25 November 2014	Control vs. Treatment 3 Control vs. Treatment 4
27 November 2014	Control vs. Treatment 5 Control vs. Treatment 6
2 December 2014	Treatment 3 vs. Treatment 4 Treatment 3 vs. Treatment 5
9 December 2014	Treatment 3 vs. Treatment 6 Treatment 4 vs. Treatment 5
11 December 2014	Treatment 4 vs. Treatment 6 Treatment 5 vs. Treatment 6

All tests were supervised by Wies Cynkar and Alice Barker.

A panel of experienced, screened and qualified assessors was asked to evaluate the wines for aroma and palate, selecting the sample in the set that was different to the other two. A minimum of 30 assessors was used for each test. Panellists were also given the opportunity to comment on any differences between the wines. An example of the instructions provided to the assessors is presented in Appendix 1. There was a forced rest of 60 seconds between sets.

Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered ISO standard wine glasses at 22 – 24 °C, in isolated booths under colour masking sodium lighting, with randomised presentation order across judges. Data were collected using a computerised data acquisition system (Fizz 2.46A, Biosystemes, France).

Data analysis to determine statistical significance was carried out using the binomial model as specified in AS 2542.2.4

Results

Table 3 provides the results from the triangle tests for all comparisons.

Table 3: Results of the triangle tests.

Test number	Comparison	Answers taken	Correct Answers	Significance
1	Control vs. Treatment 3	31	12	n.s.
2	Control vs. Treatment 4	31	15	n.s. (P=0.059)
3	Control vs. Treatment 5	30	21	***
4	Control vs. Treatment 6	30	24	***
5	Treatment 3 vs. Treatment 4	32	10	n.s
6	Treatment 3 vs. Treatment 5	32	20	***
7	Treatment 3 vs. Treatment 6	32	20	***
8	Treatment 4 vs. Treatment 5	32	17	*
9	Treatment 4 vs. Treatment 6	31	19	**
10	Treatment 5 vs. Treatment 6	31	8	n.s.

n.s. – not significant at P < 0.05, *: significant at P<0.05; **: significant at P<0.01; ***: significant at P<0.001.

From Table 3 it can be seen that Treatment 3 and Treatment 4 were not significantly different from the Control. These treatments were also not significantly different from each other.

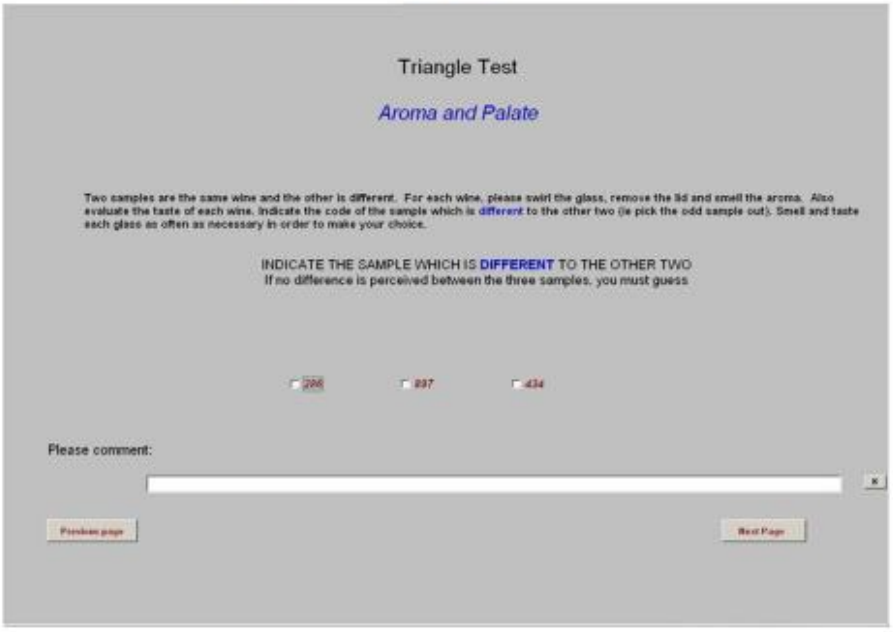
Treatment 5 and Treatment 6 were highly significantly different from the Control and they were both highly significantly different from Treatment 3.

Treatments 5 and 6 were not significantly different from each other. There was a significant difference between Treatment 4 and both Treatments 5 and 6.

Comments from the assessors suggested there may have been some differences in sulfidic aromas in Test 6, and differences in perceived fruitiness in Test 9.

Appendix 1

Screen shot of an example of the test page provided to the assessors.



Leigh Francis

Research Manager - Sensory

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AWRI

Sensory Evaluation Report

Attention: Marica Mazza

Samples received: 04-12-15 Report date: 20-01-16

Introduction

The aim of this study was to determine whether several viticultural treatments had an effect on the aroma and flavour properties of wine.

Materials and Methods

The balanced reference triangle test was applied, following the Australian Standard: Sensory analysis of foods - Specific methods – Triangle Test AS 2542.2.2. The triangle test is a forced choice test and is not valid for determining preferences, nor the character or degree of any perceived difference.

Samples consisted of four treatments and a control, with each sample having three winemaking replicates. Prior to testing all replicates were assessed by AWRI sensory team members and each set was found to be consistent across winemaking replicates. Consequently, winemaking replicates for each set were combined for the difference testing. Each of the treatments was compared to each other, as well as the control, as outlined in Table 1.

Table 1: Triangle Tests Completed

Control 1	vs	Treatment 3
Control 1	vs	Treatment 4
Control 1	vs	Treatment 5
Control 1	vs	Treatment 6
Treatment 3	vs	Treatment 4
Treatment 3	vs	Treatment 5
Treatment 3	vs	Treatment 6
Treatment 4	vs	Treatment 5
Treatment 4	vs	Treatment 6
Treatment 5	vs	Treatment 6

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Alice Barker and Wies Cynkar were the test supervisors and the testing was carried over three days; 12, 13 and 18 January 2016.

Experienced, screened and qualified assessors were asked to evaluate the wines for aroma and palate, selecting the sample in the set that was different from the other two. Panellists were also asked to comment on any differences between the wines if possible. An example of the instructions provided to the assessors and a test page for this session is presented in Appendix 1.

Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered ISO standard wine glasses at 22 – 24 °C, in isolated booths under colour masking sodium lighting, with randomised presentation order across judges.

Data was collected using a computerised data acquisition system (Fizz 2.47B, Biosystemes, France).

Data analysis to determine statistical significance was carried out using the binomial model as specified in AS 2542.2.4.

Results

Tables 2-4 show the results from the tests.

Table 2: Results of the triangle test completed on 12 January 2016, n=35 responses.

Test	Comparison	Correct Answers	Significance
1	Control 1 vs Treatment 3	18	0.020*
2	Control 1 vs Treatment 4	15	ns
3	Control 1 vs Treatment 5	16	ns (p=0.087)
4	Control 1 vs Treatment 6	18	0.020*

Significance levels are as follows:ns: not significant, * $p < 0.05$

Table 3: Results of the triangle test completed on 13 January 2016, n=32 responses

Test	Comparison	Correct Answers	Significance
5	Treatment 3 vs Treatment 4	14	ns
6	Treatment 3 vs Treatment 5	12	ns
7	Treatment 3 vs Treatment 6	22	<0.0001***
8	Treatment 4 vs Treatment 5	13	ns

Significance levels are as follows: ns: not significant, *** $p < 0.001$

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Table 4: Results of the triangle test completed on 18 January 2016, n=33 responses

Test	Comparison	Correct Answers	Significance
9	Treatment 4 vs Treatment 6	18	0.0097**
10	Treatment 5 vs Treatment 6	11	ns

Significance levels are as follows: ns: not significant,** p < 0.01

Conclusion

From Tables 2-4, it can be observed that four of the ten tests completed showed that the sensory properties of the wines differed significantly from each other (P<0.05). A significant difference was observed between the *control and treatment 3* (P<0.05), *control and treatment 6* (P<0.05), *treatment 3 and treatment 6'* (P<0.0001) *and treatment 4 and treatment 6* (P<0.01). For the control and treatment 5 tests, while there was no significant difference, there was a trend that these wines were perceptibly different.

Inspection of comments from the assessors did not provide any information regarding the nature of any differences perceived.

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Appendix

Screen shot of an example of the instruction and test pages provided to assessors.





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The Australian Wine Research Institute

Sensory Evaluation Report

Attention: Marica Mazza

Samples received: 07/2016 Report date: 19/07/2016

Sample Description: 15 x 2014 Shiraz Wines

Analysis Report: Alice Barker

Sensory properties of Shiraz wines made from grapes subjected to different viticultural treatments

Alice Barker
Wes Pearson
Leigh Francis

Introduction

The aim of this study was to evaluate the sensory differences between Shiraz wines made from grapes subjected to different viticultural treatments; a control and four treatments.

Materials and Methods

Wines were received on 5 July 2016 although they had been previously assessed through the AWRI difference testing panel. Winemaking was completed in triplicate. Table 1 shows the wines included in the sensory study.

Table 1. Wines evaluated in the sensory study

Treatment	Fermentation Replicate
Control	1, 2, 3
Treatment 3	1, 2, 3
Treatment 4	1, 2, 3
Treatment 5	1, 2, 3
Treatment 6	1, 2, 3

Training

A panel of nine assessors (two male, seven female) with an average age of 55 years (SD = 6.7) was convened for this study, all of whom are part of the AWRI trained descriptive analysis panel, with extensive experience.

Assessors attended two training sessions to determine appropriate descriptors for rating in the formal sessions. During these sessions the assessors evaluated wines from the study which represented the full range of sensory properties. These wines were used by the assessors to generate and refine appropriate

descriptive attributes, reference standards and definitions through a consensus based approach. Wines were initially assessed by appearance, aroma and palate.

In session two, standards for aroma attributes were presented and discussed and these standards were also available during the booth practice session and the formal assessment sessions.

Following the training sessions, tasters participated in a practice session in the sensory booths under the same conditions as those for the formal sessions. After the practice session, any terms which needed adjustment were discussed and the final list of terms determined. For the formal session this list was refined to include one Appearance term, fifteen Aroma terms (fourteen defined and "Other") and fourteen Palate terms (thirteen defined and "Other"). These attributes, definitions/synonyms and standards provided are shown in Table 2 including only those attributes which were included in the final attribute list.

Table 2: Attributes, definitions and reference standards evaluated by panellists in formal sessions for the wines

Attribute	Definition/Synonyms	Standard composition
Appearance		
Opacity	The degree to which light is not allowed to pass through a sample	-
Aroma		
Overall fruit	Intensity of the fruit aromas in the sample	3 x frozen raspberries, Sara Lee brand
Red fruits	Intensity of the aroma of red fruits and berries: raspberries, red cherries, pomegranate	3 x frozen blueberries, 1 x frozen Blackberry, Sara Lee brand
Dark fruits	Intensity of the aroma of dark fruits and berries: blackberries, plums, dark cherries	3 x frozen raspberries, Sara Lee brand
Confection	Intensity of the aroma of confection; red lollies	1 x raspberry lollies, Natural Confectionary Company brand
Floral	Intensity of the aroma of flowers; violets, blossoms, talcum powder	40 µL of 100 mg/L linalool, 25 µL of 200 mg/L 2-phenyl ethanol
Green	Intensity of the aroma of green stalks, green grass, capsicum, green herbs	1 tomato stalk and 4 x 1 cm cut up fresh green bean, no wine
Dried Herbs	Intensity of the aroma of dried herbs, dried oregano	1/8 tsp dried mixed herbs
Vegetal	Intensity of the aroma of cooked vegetables and vegetable water	1 Tbsp vegetable water – Edgell green beans tin
Sweet Spice	Intensity of the aroma of sweet spices; cinnamon, nutmeg, mixed spice, cloves, star anise, liquorice	1/8 tsp of each; cinnamon, nutmeg, mixed spice, 1 clove, 2 segments star anise
Savoury	Intensity of the aroma of olives, vegetable oils, vinegar, beef soup	2 olives and 1 Tbsp olive brine
Earthy	Intensity of the aroma of earth, beetroot, dust	25 µL of 4 mg/L geosmin
Barnyard	Intensity of the aroma of a barnyard, animal	40 µL of 10.06 mg/L p-cresol, 40 µL of 5.11 g/L 4-ethyl phenol
Plastic	Intensity of the aroma of plastic, band-aid, chemical	30 µL of 100 µg/L 2,6 dichlorophenol
Pungent	Intensity of the aroma and sensation of alcohol	4 ml ethanol

Attribute	Definition/Synonyms	Standard composition
Palate		
Overall Fruit	Intensity of the overall fruit flavours in the sample	-
Red fruits	Intensity of the flavour of red fruits and berries: raspberries, redcurrants, red cherries, cranberries	-
Dark Fruits	Intensity of the flavour of various dark fruits: blackberries, blackcurrants, plums, dark cherries, blueberries	-
Confection	Intensity of the flavour of confectionary; red lolly	-
Green	Intensity of the flavour of green stalks, green capsicum, green grass, green herbs, vegetal and their associated aftertastes	-
Sweet Spice	Intensity of the flavour of cinnamon, cloves, mixed spice, nutmeg, star anise, liquorice and their associated aftertastes	-
Wood	Intensity of the flavour of wood, oak and their associated aftertastes	-
Viscosity	The perception of the body, weight or thickness of the wine in the mouth. Low=watery, thin mouth feel. High= thick, oily mouthfeel.	-
Acid	Intensity of acid taste in the mouth including aftertaste	-
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning	-
Astringency	The drying sensation and feeling of particles in the mouth. Low=low drying sensation and few particles in the mouth; High=high drying sensation and a large number of particles in the mouth	-
Bitter	The intensity of bitter taste perceived in the mouth, or after expectoration.	-
Fruit AT	The lingering fruit flavour perceived in the mouth after expectorating.	

All aroma standards were added to 30 ml of NV Yalumba Shiraz 2L bag in box wine unless otherwise noted.

Sensory assessment

Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered, ISO standard wine glasses at 22–24 °C, in isolated booths under daylight-type lighting, with randomised presentation order, except in the practice sessions where there was a constant presentation order. All samples were expectorated. The assessors were forced to have a 60 second rest between samples and a ten-minute rest between trays. During the ten-minute break assessors were requested to leave the booths.

Fifteen wines were evaluated during this study, presented to assessors three times in a modified Williams Latin Square incomplete random block design generated by Fizz sensory acquisition software (version 2.47B, Biosystemes, Couternon, France). Formal assessment took place over three sessions. The fifteen wines were split into five blocks of three wines, with panellists assessing five blocks each session.

The intensity of each attribute was rated using an unstructured 15 cm line scale from 0 to 10, with indented anchor points of 'low' and 'high' placed at 10% and 90% respectively. Data was acquired using Fizz sensory software.

Panel performance was assessed using Fizz, Senstools (OP&P, The Netherlands) and PanelCheck (Matforsk) software, and included analysis of variance for the effect of judge and presentation replicate and their interactions, degree of agreement with the panel mean and degree of discrimination across samples. All judges were found to be performing to an acceptable standard.

Analysis of variance (ANOVA) was carried out using Minitab (Minitab Inc., Sydney, NSW). The effects of treatment (T), judge (J, random effect); fermentation replicate (FRep) nested in treatment; treatment and fermentation replicate nested in presentation replicate (PRep); the interaction between judge and treatment (J*T); and the interaction between judge and fermentation replicate nested in treatment were assessed. Following ANOVA, Fisher's least significant difference (LSD) value was calculated (P=0.05). Principal component analysis was conducted with the mean data for each fermentation replicate, using the correlation matrix.

Results

From the ANOVA there were eight attributes that differed significantly between treatments: *opacity (*colour intensity*), overall fruit, red fruit, confection, vegetal* and *barnyard* aroma and *astringency* and *bitterness,* (Table 3). *Floral* aroma was found to be very close to being significant (p<0.10). There were several attributes where differences between fermentation triplicates were found (*opacity, floral and barnyard aromas* and *astringency*). These differences are shown in the Appendix, Figures 1-4.

Table 3. ANOVA p-values for each sensory attribute

	T	FRep(T)	J*T	J*FRep(T)	PRep(T, FRep)	MSE
Appearance						
Opacity (colour intensity)	<0.001 ***	<0.001 ***	0.003	0.453	0.056	0.322
Aroma						
Overall Fruit A	0.041 *	0.068	0.241	0.110	0.848	0.607
Red Fruit A	0.001 ***	0.081	0.636	0.278	0.223	1.197
Dark Fruit A	0.172	0.102	0.088	0.257	0.872	0.865
Confection A	0.043 *	0.069	0.055	0.001	0.195	1.090
Floral A	0.089 ǂ	0.011 *	0.362	0.049	0.077	1.235
Green A	0.617	0.690	0.412	0.214	0.077	0.534
Dried Herb A	0.398	0.950	0.250	0.203	0.749	0.914
Vegetal A	0.001 ***	0.119	0.022	0.054	0.557	1.012
Sweet Spice A	0.287	0.661	0.073	0.029	0.162	0.564
Savoury A	0.134	0.071	0.006	0.997	0.201	1.508
Earthy A	0.382	0.504	0.448	0.130	0.228	0.699
Barnyard A	0.008 **	0.009 **	0.083	0.014	0.617	1.053
Plastic A	0.643	0.104	0.093	0.094	0.221	1.589
Pungent A	0.161	0.255	0.579	0.432	0.259	0.758

Palate						
Overall Fruit F	0.282	0.456	0.397	0.113	0.884	0.399
Red Fruit F	0.296	0.577	0.930	0.002	0.499	0.796
Dark Fruit F	0.275	0.065	0.134	0.550	0.844	0.684
Confection F	0.277	0.998	0.546	0.261	0.884	0.903
Green F	0.959	0.840	0.038	0.321	0.744	0.689
Sweet Spice F	0.443	0.637	0.129	0.071	0.724	0.623
Wood F	0.513	0.472	0.196	0.274	0.731	0.827
Viscosity	0.338	0.587	0.616	0.622	0.927	0.231
Acidity	0.849	0.510	0.968	0.012	0.941	0.639
Hotness	0.748	0.664	0.262	0.269	0.775	1.016
Astringency	0.020 *	<0.001 ***	0.533	0.086	0.377	0.886
Bitterness	0.023 *	0.941	0.312	0.953	0.255	1.237
Fruit AT	0.253	0.779	0.794	0.670	0.693	0.586
df	*4*	*10*	*32*	*80*	*30*	*240*

†Significance levels are as follows: * p < 0.05; ** p < 0.01; *** p < 0.001; ǂ p < 0.10. () = nested factor, df = degrees of freedom. Judge effect (*df=8)* was highly significant (P<0.001) across all attributes. FRep: fermentation replicate. PRep: presentation replicate. T: treatment. J: judge.

A principal component analysis representation of the mean data for each of the fermentation replicates for each treatment is shown in Figure 1, illustrating the similarities and differences among the wines. The attributes that varied across the wines (P<0.1) are plotted.

Figure 1a shows that the Control 1 and 3 wines were rated highest in *vegetal* and *barnyard* aroma, while wines from treatment 6 and 3 were tightly clustered and were rated low in these attributes and highest in *confection*, *red fruit*, *floral* and *overall fruit* along PC1. Wines from Treatment 4 were rated similarly as a group, being intermediate in sensory properties, while two wines from Treatment 5 were relatively high in *opacity* (colour intensity) and low in *bitterness*. Conversely, Control and Treatment 6 wines were relatively high in *bitterness*. In Figure 1b, PC3 is shown, which accounts for variation in *astringency* scores. Most of the wines were rated similarly in this attribute, but treatment 5 replicate 3, Treatment 4 replicate 3, and Control 1 were lower in *astringency* (see Appendix Figure 4).

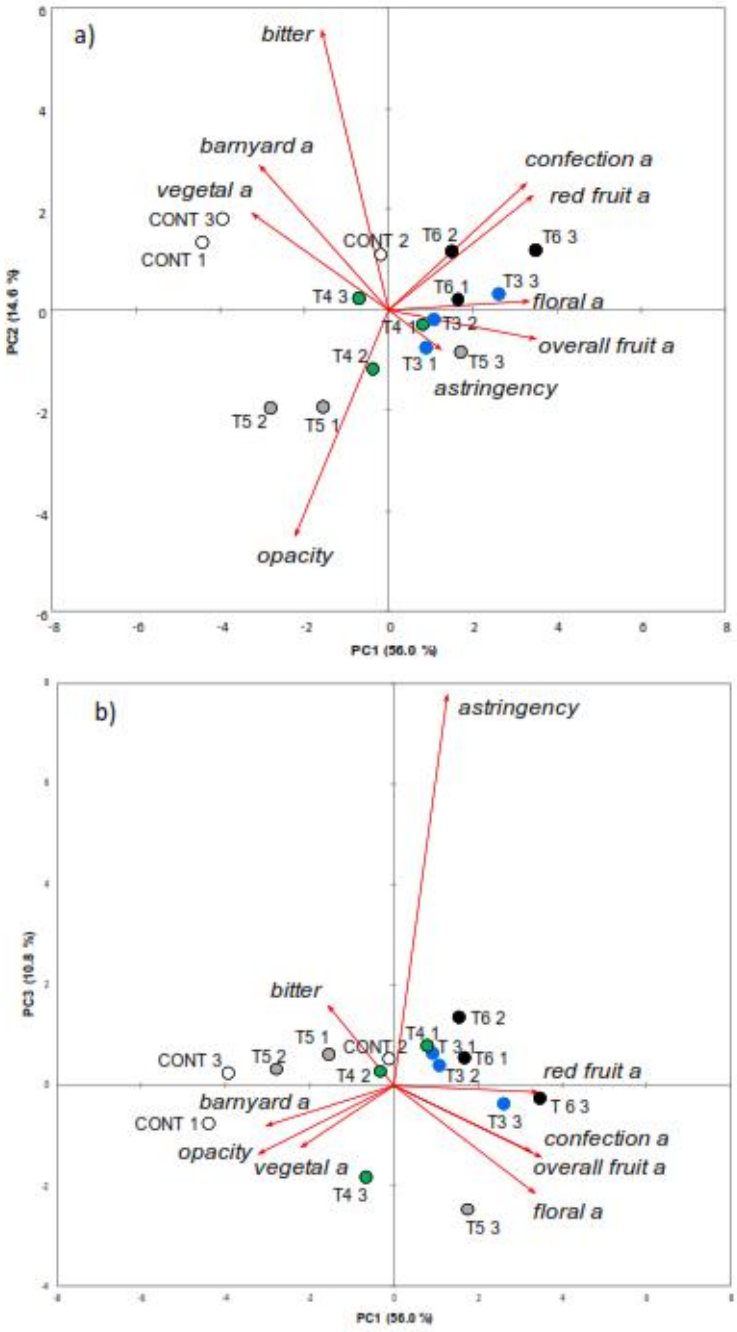


Figure 1. Principal component analysis biplot for a) PC1 and 2, and b) PC 1 and 3, for the fermentation replicates of the treatments investigated (n=9 judges x 3 presentation replicates). a: aroma attribute

It is notable that the Control replicates 1 and 3 showed higher levels of 'barnyard' aroma, which could be an indication of Bretanomyces/Dekkera spoilage or reduction/sulfide.

The mean values of each treatment were calculated and a spider plot was created using both the significant and close to significant attributes (P<0.10), in order to assess statistically significant differences among the treatments in more detail (Figure 2).

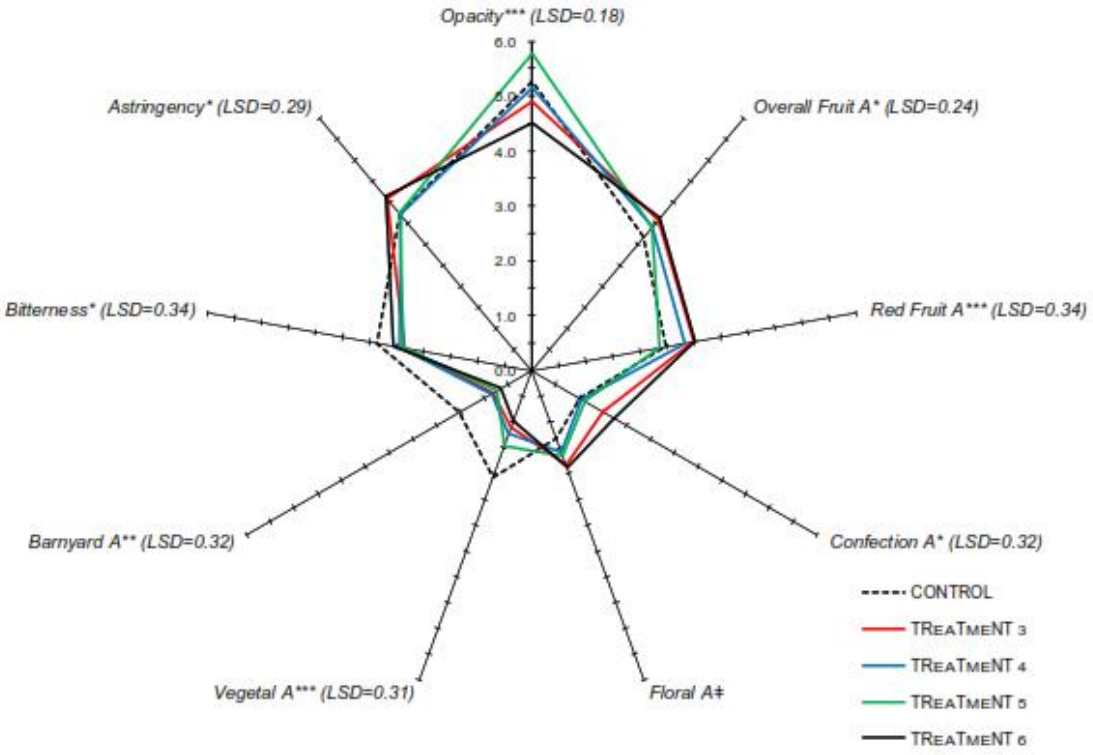


Figure 2: Mean ratings for significant and close to significant attributes for the five treatments studied (‡p<0.10; *p<0.05; **P<0.01; ***p<0.001, n=9 judges x 3 presentation replicates x 3 fermentation replicates). A: aroma attribute.

Compared to the control treatment, Treatments 3 and 6 were rated significantly lower in colour intensity (*opacity*), *bitterness*, *barnyard*, and *vegetal* attributes, and significantly higher in *overall fruit, red fruit, confection*, and *astringency*. Treatment 4 was not significantly different from the control in *opacity* and *astringency*, but was rated higher in *red fruit*. Treatment 5 was rated significantly and substantially higher in colour intensity compared to all the other treatments, but was generally more similar to the control in the *overall fruit*, *red fruit* and *confection* attributes, was significantly different to the other treatments in *vegetal* aroma, and also *astringency*, where it was rated not significantly different from the control or treatment 4.

Conclusion

There were significant differences in sensory properties among the treatments. Treatments 3 and 6 were found to be the fruitiest wines and were also highest in *astringency*, but had relatively low colour intensity. The wines from the Control were found to be the least fruity, and the highest in *bitterness*, *vegetal* and *barnyard* attributes, which may have masked the fruit related attributes. Assessment of the winemaking conditions and compositional data may allow an interpretation regarding whether the sensory attributes of the control were due to the vineyard treatment or were related to winemaking issues.

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Appendix

Table 1. Mean attribute scores for each treatment and their corresponding LSD values (P=0.05)

Yeast	Opacity	Overall Fruit A	Red Fruit A	Dark Fruit A	Confection A	Floral A	Green A	Dried Herb A	Vegetal A	Sweet Spice A	Savoury A	Earthy A	Barnyard A	Plastic A	Pungent A
CONTROL	5.26	3.18	2.50	2.61	1.02	1.33	1.36	1.08	2.09	1.20	1.83	2.23	1.52	1.73	2.96
TREATMENT 3	4.90	3.58	3.00	2.83	1.50	1.83	1.39	0.98	1.10	1.37	1.49	1.96	0.82	1.70	3.01
TREATMENT 4	5.14	3.43	2.84	2.82	1.06	1.58	1.52	0.89	1.22	1.21	1.61	2.15	0.85	2.07	2.96
TREATMENT 5	5.77	3.40	2.38	3.10	1.12	1.66	1.52	0.77	1.46	1.07	1.67	2.19	0.74	1.86	2.81
TREATMENT 6	4.51	3.64	3.02	2.81	1.74	1.89	1.44	0.81	0.97	1.41	1.29	2.18	0.66	1.74	2.71
LSD (5%)	*0.18*	*0.24*	*0.34*	*ns*	*0.32*	*ns*	*ns*	*ns*	*0.31*	*ns*	*ns*	*ns*	*0.32*	*ns*	*ns*

Yeast	Overall Fruit F	Red Fruit F	Dark Fruit F	Confection F	Green F	Sweet Spice F	Wood F	Viscosity	Acid	Hotness	Astringency	Bitter	Fruit AT
CONTROL	3.43	2.46	3.15	0.98	2.58	1.05	2.07	2.91	3.58	3.32	3.73	2.85	2.64
TREATMENT 3	3.63	2.78	3.10	1.03	2.55	1.23	1.90	2.93	3.57	3.15	4.10	2.35	2.84
TREATMENT 4	3.64	2.66	3.31	0.87	2.57	1.37	1.81	2.92	3.47	3.26	3.72	2.44	2.85
TREATMENT 5	3.60	2.60	3.32	0.90	2.62	1.20	2.05	2.99	3.51	3.10	3.76	2.38	2.80
TREATMENT 6	3.48	2.62	3.05	1.19	2.67	1.20	1.93	2.84	3.55	3.26	4.14	2.55	2.76
LSD (5%)	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*0.29*	*0.34*	*ns*

ns =not significant, LSD: Fisher's least significant difference (p=0.05).

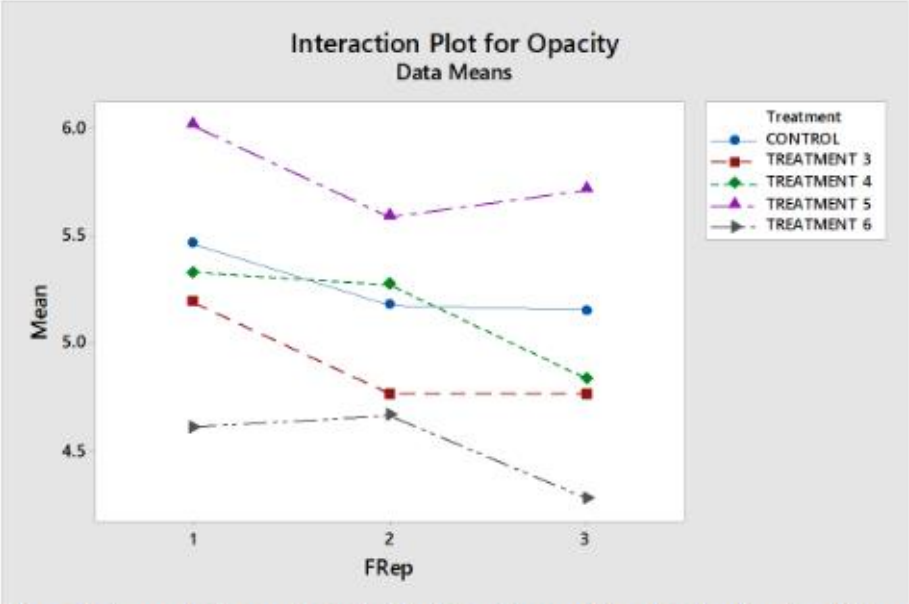


Figure 1. Fermentation replicate by Treatment interaction plot showing the differences in ratings for *opacity* between fermentation replicates

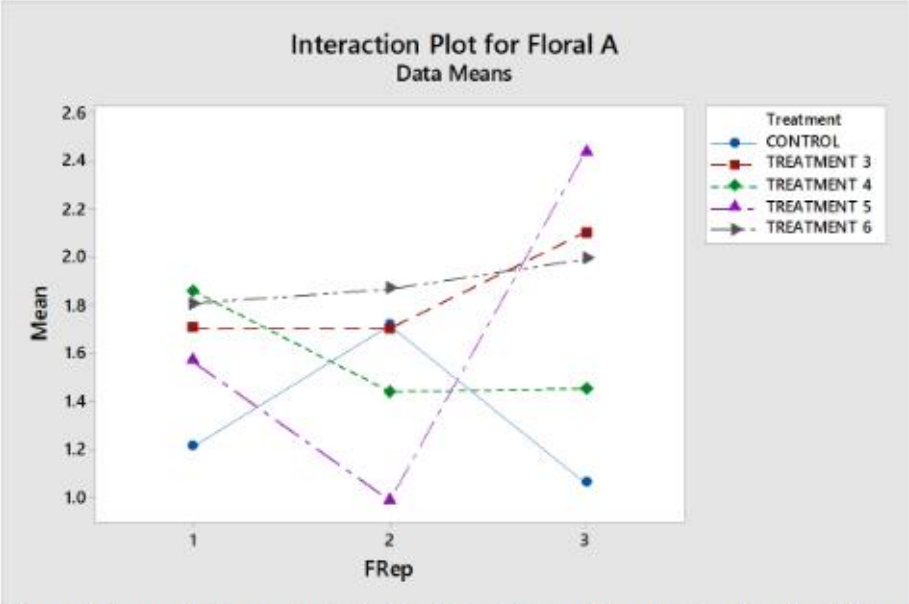


Figure 2. Fermentation replicate by Treatment interaction plot showing the differences in ratings for *floral aroma* between fermentation replicates

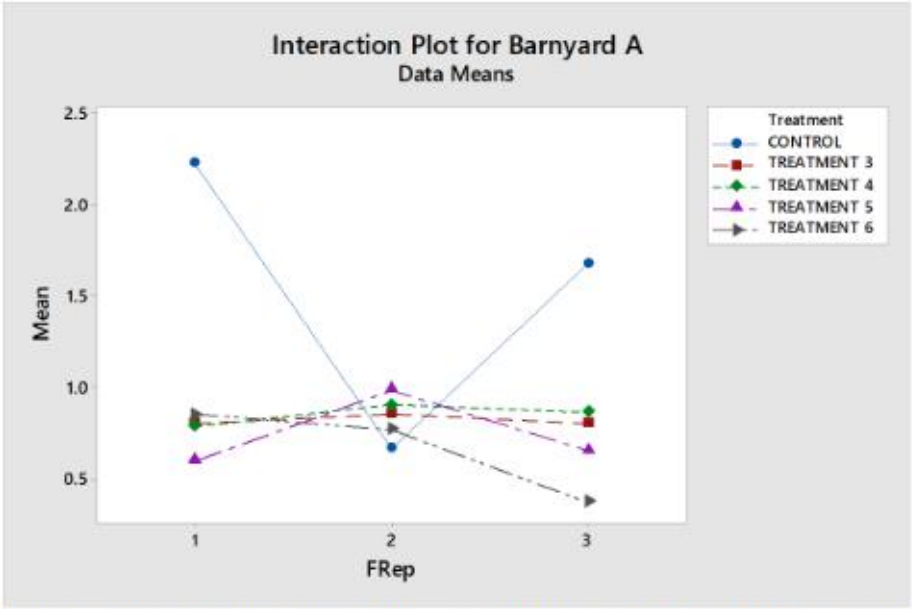


Figure 3. Fermentation replicate by Treatment interaction plot showing the differences in ratings for *barnyard aroma* between fermentation replicates

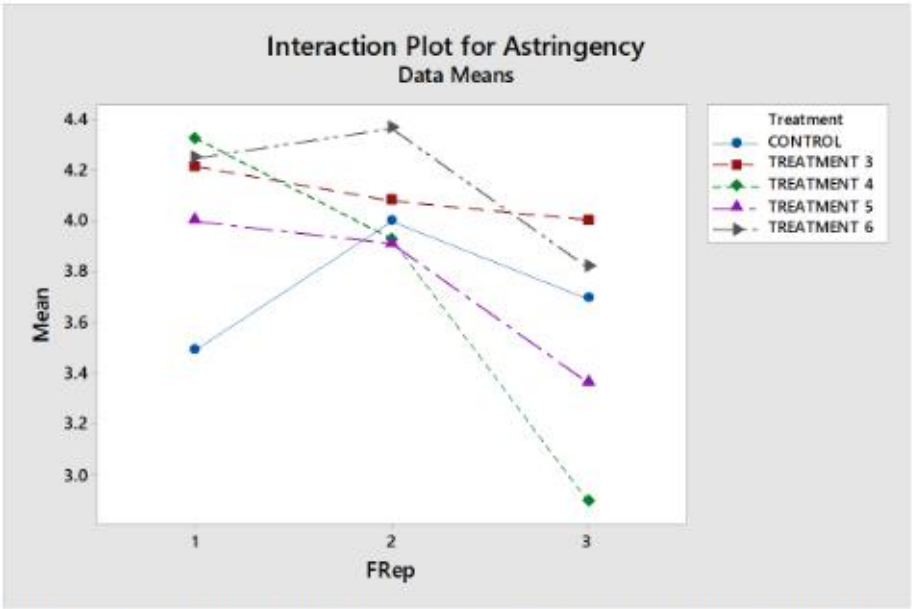


Figure 4. Fermentation replicate by Treatment interaction plot showing the differences in ratings for *astringency* between fermentation replicates

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Sensory Evaluation Report

Attention: Marica Mazza

Sample received: 10-10-16 Report date: 3-11-16

Introduction

The aim of this study was to determine whether several viticultural treatments differed significantly in their sensory properties from one another.

Materials and Methods

The balanced reference triangle test was applied, following the Australian Standard: Sensory analysis of foods - Specific methods – Triangle Test AS 2542.2.2. The triangle test is a forced choice test and is not valid for determining preferences, nor the character or degree of any perceived difference.

There was one control and four viticultural treatments of red wine. All treatments were compared to the control and each other as outlined in Table 1.

Table 1: Difference tests completed

Test no.	Comparison
1	Control 1 vs Treatment 3
2	Control 1 vs Treatment 4
3	Control 1 vs Treatment 5
4	Control 1 vs Treatment 6
5	Treatment 3 vs Treatment 4
6	Treatment 3 vs Treatment 5
7	Treatment 3 vs Treatment 6
8	Treatment 4 vs Treatment 5
9	Treatment 4 vs Treatment 6
10	Treatment 5 vs Treatment 6

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Alice Barker was the test supervisor and the testing was carried out on four separate dates; 13 October (Test 1 and 2), 14 October (Test 3 -6), 18 October (Test 7 and 8), and 19 October (Test 9 and 10) 2016.

Thirty-one to thirty-three experienced, screened and qualified assessors evaluated the wines for aroma and palate, selecting the sample in the set that was different from the other two. Panellists were also asked to comment on any differences between the wines if possible. An example of the instructions provided to the assessors and a test page for this session is presented in Appendix 1.

Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered ISO standard wine glasses at 22 – 24 °C, in isolated booths under colour masking sodium lighting, with randomised presentation order across judges.

Data were collected using a computerised data acquisition system (Fizz 2.47B, Biosystemes, France).

Data analysis to determine statistical significance was carried out using the binomial model as specified in AS 2542.2.4.

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Results

Table 1 shows the results from the tests conducted for this study.

Table 1. Results of the triangle tests for the comparisons between the treatments and the control, and between treatments.

Test	Comparison	Correct Answers	Number of panellists	Significance
1	Control 1 vs Treatment 3	11	32	ns
2	Control 1 vs Treatment 4	10	32	ns
3	Control 1 vs Treatment 5	12	31	ns
4	Control 1 vs Treatment 6	15	31	ns
5	Treatment 3 vs Treatment 4	11	31	ns
6	Treatment 3 vs Treatment 5	16	31	*
7	Treatment 3 vs Treatment 6	12	32	ns
8	Treatment 4 vs Treatment 5	11	32	ns
9	Treatment 4 vs Treatment 6	12	33	ns
10	Treatment 5 vs Treatment 6	17	33	*

ns: not significant. *: significant at P<0.05.

From Table 1 only treatments 3 and 5 and treatments 5 and 6 differed significantly from one another ($p<0.05$). It should also be noted that the comparison between the control and treatment 6 was close to significance ($p<0.10$).

For the comparison between treatments 3 and 4 there were no relevant comments from the panellists. However for the comparison between treatments 5 and 6, some assessors made comments relating to a difference in sweetness and fruitiness of the wine.

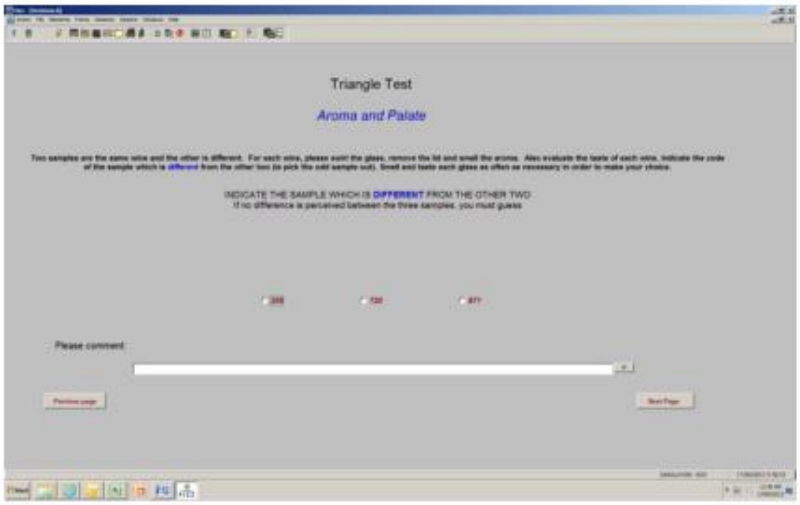
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Appendix

Screen shot of an example of the instruction and test pages provided to assessors.





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Sensory Evaluation Report

Attention: Department of Economic Development Jobs Transport Resources

Samples received: 11/05/2017		Report date:	24/07/2017
Sample Description	20 wines, 5 treatments with 4 individual field replications within each treatment.		
Analysis Report	Damian Espinase Nandorfy, Patricia Williamson, Leigh Francis		

The effect of carbon dioxide and temperature treatments in the vineyard on wine sensory properties.

Executive Summary

The aim of this study was to evaluate the effect of CO_2 and temperature in the vineyard on the sensory properties of 2016 Shiraz wines. Five treatments, each with four field replicates, were examined in this study. With limited knowledge of the design of the experiment and the identity of the treatments, the 20 wines were considered individually. Sensory descriptive analysis of the wines was conducted, with 16 attributes (appearance, aroma, and palate) differing significantly ($P<0.05$) between the wines, with large differences among the field replicates within a treatment. There were large differences among the wines in the attributes *dark fruit*, *opacity* (colour intensity), *viscosity*, *red fruit* and *confectionary*, with some wines rated highly in two attributes related to sulfidic flavour: *drain* and *earthy*.

Introduction

The aim of this study was to evaluate the effect of CO_2 and temperature treatments on the sensory properties of 2016 Shiraz wines.

Materials and Methods

Table 1 lists the wines studied. The wines were assessed in a preliminary session by eight highly experienced members of the sensory team and the AWRI technical quality panel. Samples were independently assessed blind and then discussed. It was evident from this assessment that there were large sensory differences among the wines, with particularly large differences among the field replicates.

Table 1. Summary of treatments, field replicates, wine codes, and wine treatment codes for the wines studied.

Treatment	Field Replicate	Wine code	Wine treatment codes
1	1	38	111
	2	39	141
	3	40	171
	4	41	201
3	1	34	83
	2	35	113
	3	36	173
	4	37	203
4	1	1	114
	2	2	144
	3	3	174
	4	4	204
5	1	5	85
	2	6	145
	3	7	175
	4	8	205
6	1	9	86
	2	10	116
	3	11	146
	4	12	206

Training for sensory descriptive analysis

A panel of ten assessors (two males, eight females) with an average age of 55 years (SD = 7.4) was convened to evaluate the wines. All panellists were part of the external AWRI trained descriptive analysis panel and had extensive experience in wine sensory descriptive analysis.

Assessors attended three two-hour training sessions to determine appropriate descriptors for rating in the formal sessions. All the wines from the study were progressively used during training sessions to generate and refine appropriate descriptive attributes and definitions through a consensus based approach.

Wines were assessed by appearance, aroma and palate. In the second session, standards for aroma attributes were presented and discussed and these standards were also available during subsequent training sessions, the booth practice session and the formal assessment sessions.

Following the third training session, tasters participated in a practice session in the sensory booths under the same conditions as those for the formal sessions. After the practice session, any terms which needed adjustment were discussed and the final list of terms and standards were determined. For the formal sessions, this list was refined to include one appearance term, 11 aroma terms (ten defined and one 'Other' term) and 14 palate terms (13 defined and one 'Other' term). The final list of attributes, definitions/synonyms and reference standards are shown in Table 2.

Evaluation

Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered, ISO standard wine glasses at 22–24 °C, in isolated booths under daylight-type fluorescent lighting. Randomised presentation order was followed except in the practice sessions where there was a constant presentation order. All samples were expectorated. In practice booth sessions, the assessors were presented with four trays of four wines per tray. In the formal booth sessions of two hours duration the assessors were presented with four trays of four wines per tray, except the last formal session where three trays of four wines were presented. The assessors were forced to have a 60-second rest between samples and were encouraged to rinse with water, and a minimum ten-minute rest between trays. During the ten-minute break assessors were requested to leave the booths. Formal evaluation was completed in four sessions.

The 20 wines listed in Table 1 were presented to assessors three times, in a modified Williams Latin Square incomplete random block design generated by Fizz sensory acquisition software (version 2.51, Biosystemes, Couternon, France). A different bottle was used for each of the three presentation replicates.

The intensity of each attribute listed in Table 2 was rated using an unstructured 15 cm line scale (0 to 10), with indented anchor points of 'low' and 'high' placed at 10% and 90% respectively. Data was acquired using Fizz sensory software.

Panel performance was assessed using Fizz software and PanelCheck (panelcheck.com) software, and included analysis of variance for the effect of judge and presentation replicate and their interactions, degree of agreement with the panel mean and degree of discrimination across samples. All judges were found to be performing to an acceptable standard.

Analysis of variance (ANOVA) was carried out using Minitab 18 (Minitab Inc., Sydney, NSW). The effects of wine, judge, presentation replicate and all two-way interactions were assessed for each attribute, treating judge as a random effect. Following ANOVA, Fisher's least significant difference (LSD) value was calculated (P=0.05) for the wine effect. Principal component analysis was conducted for the mean data of the significant attributes (P<0.05) of each of the 20 wines using the correlation matrix.

Table 2. Attributes, definitions and composition of reference standards evaluated by panellists for the Shiraz samples.

Attribute	Definition/Synonyms	Standard
Appearance		
Opacity	The degree to which light is not allowed to pass through a sample	
Aroma		
Overall fruit	Intensity of the fruit aromas in the sample	
Red fruit	Intensity of the aroma of red fruits and berries: raspberries, strawberries and cherries	2 x frozen raspberries (Sara Lee brand) and 1 frozen strawberry (Welch's brand)
Dark fruit	Intensity of the aroma of dark fruits and berries: blackberries, plums, Morello cherries, blueberries and blackcurrant	2 x frozen blueberries, 1 x frozen blackberry (Sara Lee brand), and 100 µL blackcurrant syrup (Ribena)
Confectionary	Intensity of the aroma of red lollies	7 g red snake lolly (Allen's), not in wine
Floral	Intensity of the aroma of flowers, violets	25 µL of 100 mg/L linalool, 25 µL of 200 mg/L 2-phenyl ethanol and 25 µL of β-ionone of 2.11 g/L
Vanilla/Chocolate	Intensity of the aroma of vanilla and milk chocolate	0.5 mL vanilla paste (Queen brand)
Menthol/Eucalypt	Intensity of the aroma of eucalypt, mint, pine, menthol and herbal characters	50 µL of 58.48 mg/L 1,8-cineole and 50 µL Menta Fabbri (mint syrup), Siena foods brand
Green/Stalky	Intensity of the aroma of green stalks, fresh green vegetables, rhubarb stalks and green leaf.	2 g pieces fresh green bean, 2 g rhubarb stalk and 1 g tomato stalk
Earthy	Intensity of the aroma of beetroot and wet earth (two reference standards)	Standard 1. 100 µL of 100 µg/L geosmin. Standard 2. 5 g fresh cut beetroot, not in wine
Medicinal/ Chemical	Intensity of aroma of medical supplies and Band-Aid	20 µL of 80 mg/L of each of *ortho*-, *para*- and *meta* - cresol
Drain	Intensity of the aroma of dirty drains and wet tea towel, reminiscent of cooked vegetables and boiled eggs (two reference standards)	Standard 1. old tea towel. Standard 2. 50 µL of mercaptoethanol 2 % v/v

Attribute	Definition/Synonyms	Standard
Pungent	Intensity of the aroma and effect of alcohol.	4 mL of 95% food grade ethanol (Tarac Technologies)
Palate		
Overall fruit	Intensity of fruit flavours in the sample.	
Red fruit	Intensity of the flavour of red fruits and berries: raspberries, strawberries, cranberries	
Dark fruit	Intensity of the flavour of various dark fruits: blackberries, plums, Morello cherries and blueberries and blackcurrants	
Menthol/Eucalyptus	Intensity of the flavour of eucalypt, mint, pine, menthol and herbal character; including aftertaste.	
Green/Stalky	Intensity of the flavour of green stalks, fresh green vegetables and rhubarb stalks including aftertaste.	
Salt	Intensity of the taste of salt, including aftertaste.	
Viscosity	The perception of the body, weight or thickness of the wine in the mouth. Low = watery, thin mouth feel. High = oily, thick mouth feel.	
Acid	Intensity of acid taste in the mouth including aftertaste.	
Sweetness	Intensity of sweet taste in the mouth including aftertaste.	
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning.	
Astringency	The drying and mouth-puckering sensation in the mouth. Low = coating teeth; Medium = mouth coating & drying; High = puckering, lasting astringency.	
Bitter	The intensity of bitter taste perceived in the mouth, or after expectoration.	
Fruit AT	The lingering fruit flavour perceived in the mouth after expectorating.	

All red wine standards were added to 30 mL of NV Yalumba Classic Dry Red unless otherwise noted.

Results

From the ANOVA, 16 attributes rated by the panel differed significantly ($P<0.05$) among the wines (Table 3). These attributes included: *opacity* (colour intensity), *overall fruit aroma, red fruit aroma, dark fruit aroma, confectionary aroma, floral aroma, vanilla/chocolate aroma, menthol/eucalyptus aroma, earthy aroma, drain aroma (*reductive term*), overall fruit flavour, dark fruit flavour, viscosity, sweetness, hotness* and *fruit aftertaste*. Two attributes; *medicinal/chemical aroma* and *red fruit flavour* were close to being significant ($P<0.10$). The attribute mean scores and post-hoc Fisher's least squares difference values for appearance, aroma, and palate attributes are shown in Tables 4a and 4b.

Table 3. Probability values†, degrees of freedom (df) and mean square error (MSE) from the analysis of variance.

Attribute	W	R	J*W	J*R	W*R	MSE
Opacity	0.000***	0.112	0.000***	0.000***	0.000***	0.224
Overall Fruit A	0.000***	0.681	0.124	0.018*	0.313	0.631
Red Fruit A	0.001***	0.941	0.144	0.004**	0.854	1.353
Dark Fruit A	0.000***	0.491	0.000***	0.084	0.059	0.869
Confectionary A	0.001***	0.620	0.116	0.021*	0.491	1.257
Floral A	0.004**	0.628	0.089	0.005**	0.703	1.200
Vanilla/Chocolate A	0.009**	0.623	0.072	0.356	0.128	1.216
Menthol/Eucalypt A	0.032*	0.111	0.104	0.753	0.492	1.191
Stalky Green A	0.236	0.284	0.119	0.074	0.941	0.726
Earthy A	0.007**	0.344	0.000***	0.057	0.558	0.824
Medicinal/Chemical	0.052ǂ	0.250	0.029*	0.000***	0.961	1.359
Drain A	0.000***	0.139	0.001***	0.024*	0.220	2.186
Pungent A	0.595	0.379	0.002**	0.000***	0.589	0.290
Overall Fruit F	0.002**	0.878	0.488	0.031*	0.292	0.485
Red Fruit F	0.097ǂ	0.332	0.002**	0.006**	0.522	0.854
Dark Fruit F	0.000***	0.308	0.001***	0.001***	0.092	0.915
Menthol/Eucalypt F	0.234	0.442	0.043*	0.000***	0.308	0.976
Stalky Green F	0.202	0.193	0.348	0.007**	0.276	0.902
Salt	0.355	0.430	0.378	0.104	0.885	0.674
Viscosity	0.000***	0.077	0.756	0.101	0.367	0.164
Acid	0.872	0.769	0.340	0.370	0.947	0.322
Sweetness	0.012*	0.409	0.305	0.000***	0.570	0.650
Hotness	0.000***	0.917	0.943	0.016**	0.971	0.961
Astringency	0.143	0.785	0.705	0.025**	0.281	0.753
Bitter	0.349	0.494	0.766	0.000***	0.274	0.615
Fruit AT	0.006**	0.263	0.03*	0.009**	0.450	0.458
df	19	2	171	18	38	342

A: aroma, F: Flavour. †Significance levels are as follows: * $P\leq0.05$; ** $P\leq 0.01$; *** $P\leq 0.001$; ǂ $P\leq 0.10$. df = degrees of freedom. W = Wine, J = Judge, Rep = presentation replicate. Judge effect was significant for all attributes ($P\leq0.001$).

The PCA for the Shiraz wines (Figure 1) was created using the sixteen attributes that were significantly different ($P<0.05$) across the wines. PC1 explained 54.6% of the variation in the data and PC2 explained 26.6% of the variance. The wines do not appear to generally group by treatment. Field replicates 3 and 4 were generally plotted to the right of the figure, being rated highly in those attributes highly positively loaded on PC1, notably *dark fruit aroma* and *flavour*, *viscosity*, *opacity*, *fruit aftertaste*, *overall fruit* flavour and *vanilla/chocolate*, and relatively low in *red fruit* and *confectionary aroma*. The replicate 4 wines for Treatments 5 and 6 were rated highest in the attributes positively loaded on PC1. Conversely, field replicates 1 and 2 were generally plotted to the left of the figure, being lower in *dark fruit aroma* and *flavour*, *viscosity*, *opacity*, *fruit aftertaste*, *overall fruit flavour* and *vanilla/chocolate*, and relatively high in *red fruit and confectionary aroma*. There were several wines rated relatively highly in *drain* and *earthy* aroma, plotted to the upper half of Figure 1, notably wines 35, 4, 9 and 11. These attributes are associated with sulfidic/reduction. This may indicate that a winemaking artefact was influencing some of the wines.

Given the experimental design of this study, it is recommended that a further ANOVA should be run testing for the effects of treatment and field replicate, which would allow better interpretation of the outcomes of the study.

Table 4a. Attribute mean scores and post-hoc Fisher's least squares difference values for appearance and aroma attributes.

Wine code	Opacity	Overall Fruit A	Red Fruit A	Dark Fruit A	Confectionary A	Floral A	Vanilla/ Chocolate A	Menthol/ Eucalypt A	Stalky Green A	Earthy A	Medicinal/ Chemical	Drain A	Pungent A
1	4.17	3.57	3.05	2.83	1.82	1.74	1.90	1.40	2.26	2.36	1.07	0.98	2.91
10	4.12	3.49	2.72	2.49	1.49	1.40	1.70	1.42	2.21	2.51	1.56	2.18	3.18
11	4.45	3.56	2.82	2.55	1.35	1.31	1.37	1.02	2.52	2.65	1.03	2.94	3.01
12	6.71	4.02	2.40	3.89	1.12	1.78	2.19	1.71	2.10	3.11	1.58	2.32	3.19
2	4.25	3.67	3.30	2.59	1.63	2.05	1.81	1.43	2.09	2.56	1.26	1.72	2.96
3	6.36	4.10	2.52	3.79	1.34	1.74	2.34	2.13	2.08	2.55	0.95	1.47	3.04
34	4.29	3.88	3.33	2.65	1.88	1.70	1.79	1.63	2.09	2.23	0.75	0.84	2.91
35	6.11	3.74	2.33	3.63	0.81	1.32	1.54	1.24	2.11	3.29	1.57	2.85	3.29
36	6.47	3.88	2.19	3.68	0.97	1.69	2.18	1.72	2.08	2.86	0.81	1.51	2.91
37	6.06	4.05	2.88	3.80	1.24	1.89	2.11	1.39	2.21	2.64	1.07	1.81	3.13
38	4.27	4.11	3.49	2.92	2.04	2.11	2.14	1.36	2.27	2.46	0.64	1.16	2.96
39	6.03	4.02	2.53	3.70	1.33	2.08	2.33	1.71	1.95	2.39	1.02	0.99	2.95
4	5.99	3.65	2.17	3.45	0.79	1.31	2.10	1.16	2.02	3.20	1.21	2.50	3.00
40	5.71	3.98	2.61	3.46	1.21	1.86	1.97	1.35	2.05	2.35	0.86	1.45	2.99
41	6.16	4.09	2.81	3.71	1.71	2.21	2.52	1.69	1.71	2.55	0.59	2.04	3.06
5	3.49	3.71	3.07	2.36	1.94	1.66	1.57	1.21	2.31	2.36	0.85	1.59	3.01
6	5.20	3.96	2.73	3.49	1.43	1.72	1.93	1.21	2.15	2.89	0.82	1.64	3.04
7	5.78	3.78	2.67	3.43	1.25	2.11	1.96	1.66	2.18	2.90	1.53	1.99	3.12
8	6.09	4.33	2.94	3.90	1.32	2.09	2.24	1.71	1.89	2.79	0.99	0.85	2.90
9	3.46	3.17	2.66	2.11	1.21	1.08	1.46	0.97	2.49	2.86	1.22	3.51	3.19
LSD	**0.24**	**0.40**	**0.59**	**0.47**	**0.57**	**0.56**	**0.56**	**0.55**	**ns**	**0.46**	**ns**	**0.75**	**ns**

ns: no significant difference (P>0.05). LSD: Fisher's Least Squares Difference.

Table 4b. Attribute mean scores and post-hoc LSD values for palate attributes.

Wine code	Overall Fruit F	Red Fruit F	Dark Fruit F	Menthol/ Eucalypt	Stalky Green F	Salt	Viscosity	Acid	Sweetness	Hotness	Astringency	Bitterness	Fruit AT
1	3.86	3.56	2.84	1.54	2.81	1.10	3.20	4.93	0.79	3.84	4.99	2.23	2.93
10	4.06	3.56	3.21	1.51	2.76	1.11	3.35	4.91	0.75	3.90	4.79	2.43	2.98
11	3.92	3.51	2.84	1.65	2.73	1.19	3.36	5.05	0.52	4.10	4.56	2.69	2.81
12	4.48	3.00	4.27	1.92	2.55	1.57	3.68	4.86	1.34	4.89	4.80	2.52	3.62
2	4.08	3.84	2.80	1.80	2.78	1.13	3.36	4.80	0.85	3.77	4.51	2.45	3.09
3	4.28	3.23	3.94	1.95	2.72	1.19	3.60	4.78	0.59	4.07	4.93	2.41	3.37
34	4.10	3.59	2.99	1.57	2.75	1.23	3.31	4.78	0.77	3.96	4.62	2.39	3.02
35	4.21	2.89	4.00	1.53	2.90	1.23	3.53	4.82	0.47	4.56	4.86	2.71	3.19
36	4.28	3.34	3.98	1.54	2.70	1.05	3.51	4.84	0.72	4.26	4.88	2.32	3.20
37	4.35	3.49	4.06	1.52	2.60	0.99	3.51	4.91	0.83	4.25	4.81	2.46	3.28
38	4.15	3.64	3.01	1.33	2.99	0.99	3.28	5.02	0.80	4.13	5.07	2.62	3.10
39	4.22	2.93	3.86	1.91	2.86	1.28	3.53	4.99	0.99	4.24	4.71	2.46	3.33
4	4.19	3.26	3.95	1.40	2.65	1.13	3.57	4.85	0.88	4.19	5.04	2.63	3.27
40	4.26	3.45	3.42	1.62	2.79	1.38	3.54	5.00	0.66	3.89	4.52	2.67	3.22
41	4.24	3.17	3.78	1.94	2.47	1.29	3.60	4.85	0.98	4.24	4.62	2.54	3.21
5	3.79	3.49	2.41	1.63	3.10	1.02	3.13	4.85	0.51	3.80	4.91	2.52	2.86
6	4.43	3.51	3.86	1.56	2.86	1.52	3.65	4.91	0.85	4.10	4.78	2.65	3.34
7	4.28	3.29	3.83	1.83	2.41	1.11	3.52	4.80	0.87	4.23	4.75	2.63	3.28
8	4.46	3.31	3.82	2.07	2.29	1.06	3.62	4.76	1.17	4.15	4.44	2.25	3.37
9	3.88	3.53	2.75	1.27	2.98	1.21	3.32	4.85	0.68	4.05	4.78	2.44	2.94
LSD	**0.35**	**ns**	**0.49**	**ns**	**ns**	**ns**	**0.21**	**ns**	**0.41**	**0.5**	**ns**	**ns**	**0.34**

ns: no significant difference (P>0.05). LSD: Fisher's Least Squares Difference.

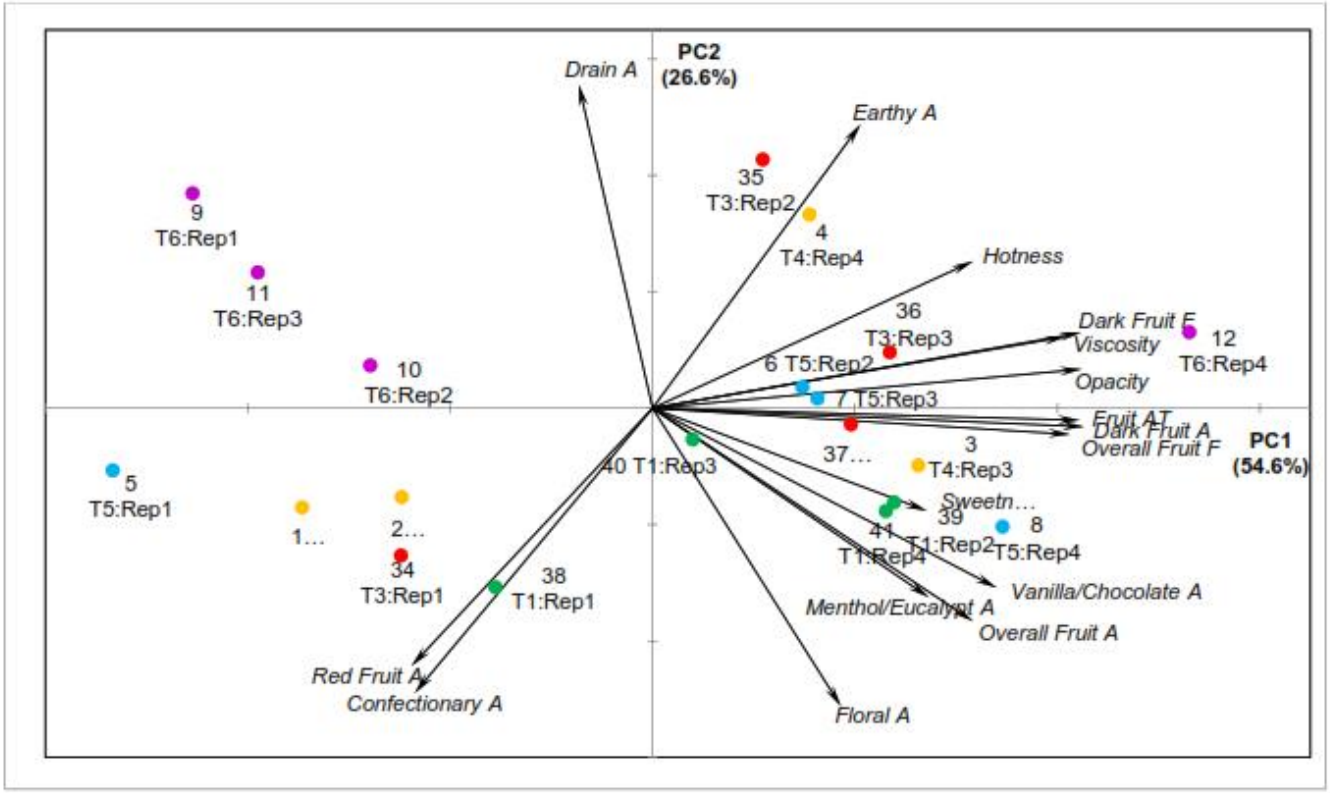


Figure 1. Projection of sensory attributes on principal components 1 and 2 for the 20 Shiraz wines, labelled by wine code, treatment (T) and field replicate (Rep). A: Aroma, F: Flavour, AT: aftertaste.

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Sensory Evaluation Report

Attention: Commercial Services

Samples received: 06/07/17 Report date: 20/07/17

Eleanor Bilogrevic

Introduction

The aim of this study was to determine whether there were significant sensory differences among four treatments (Treatments 3, 4, 5 and 6) and a control for a 2017 Shiraz wine.

Materials and Methods

The balanced reference triangle test was applied, following the Australian Standard: Sensory analysis of foods - Specific methods – Triangle Test AS 2542.2.2. The triangle test is a forced choice test and is not valid for determining preferences, nor the character or degree of any perceived difference.

Eleanor Bilogrevic, Patricia Williamson and Damian Espinase Nandorfy were the test supervisors and the testing was carried out on 18 July, 19 July and 20 July 2017 over five sessions.

Three replicates of each treatment were provided. There were 24 bottles (375 mL) of the control and each of treatments 3, 4, 5 and 6 available for the test. All controls and treatments were blended across the three replicates following preliminary assessment by the test supervisors. The difference tests were carried out according to Table 1, as requested.

Table 1. Wines tested at each session.

Session	Comparison
1	Control vs. Treatment 3
	Control vs. Treatment 4
2	Control vs. Treatment 5
	Control vs. Treatment 6
3	Treatment 3 vs. Treatment 4
	Treatment 3 vs. Treatment 5
4	Treatment 3 vs. Treatment 6
	Treatment 4 vs. Treatment 5
5	Treatment 4 vs. Treatment 6
	Treatment 5 vs. Treatment 6

Between 31 and 33 experienced, screened and qualified assessors evaluated the wines for aroma and palate, selecting the sample in the set that was different from the other two.

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Panellists were also asked to comment on any differences between the wines if possible. An example of the instructions provided to the assessors and a test page for this session is presented in Appendix 1.

Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered ISO standard wine glasses at 22 – 24 °C, in isolated booths under colour masking sodium lighting, with randomised presentation order across judges. There was a forced rest of 60 s between comparisons, with two comparisons per session.

Data were collected using a computerised data acquisition system (Fizz 51, Biosystemes, France). Data analysis to determine statistical significance was carried out using the binomial model as specified in AS 2542.2.4.

Results

Table 2 shows the results from the tests conducted.

Table 2: Results of the triangle tests.

Comparison	Number of responses	Correct Answers	Significance†
Control vs Treatment 3	32	13	ns
Control vs Treatment 4	32	11	ns
Control vs Treatment 5	33	13	ns
Control vs Treatment 6	33	12	ns
Treatment 3 vs Treatment 4	31	10	ns
Treatment 3 vs Treatment 5	31	8	ns
Treatment 3 vs Treatment 6	32	15	ns‡
Treatment 4 vs Treatment 5	32	10	ns
Treatment 4 vs Treatment 6	31	12	ns
Treatment 5 vs Treatment 6	31	10	ns

†ns = not significant at P<0.05. ‡P=0.07

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From Table 2 it can be seen that there was no significant difference ($P<0.05$) for any of the comparisons. It should be noted that for the comparison between Treatment 3 vs Treatment 6 there was some evidence for a perceptible difference ($P=0.07$).

There was no useful information from the comments provided by the judges.

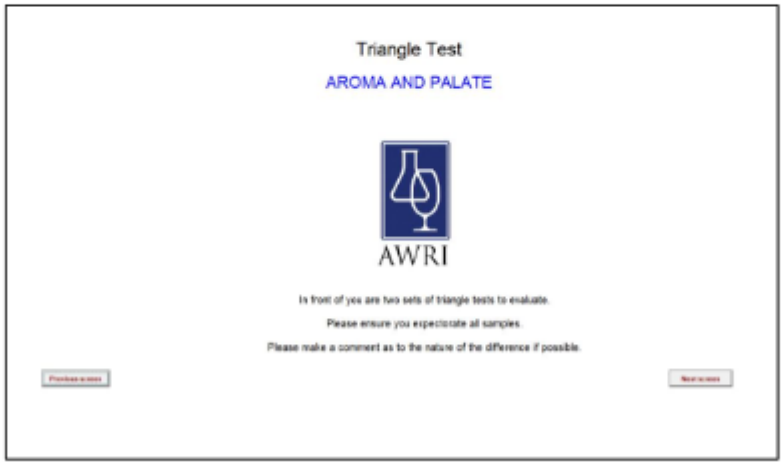
These tests all have strong statistical power, as calculated after the test, with most of the tests having a greater than 0.9 chance of finding a difference if one existed, given 25% of correct answers above chance. In other words, if 25% of assessors could perceive an effect of the treatment, the test would have had greater than 90% likelihood of finding the difference. Appendix 2 shows the statistical power of all tests given 25% of correct answers above chance.

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Appendix 1

Screen shot of an example of the instruction and test pages provided to assessors.





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Appendix 2

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Statistical power of each test assuming 25% of answers above chance.

Comparison	Statistical power
Control vs Treatment 3	0.89
Control vs Treatment 4	0.97
Control vs Treatment 5	0.91
Control vs Treatment 6	0.95
Treatment 3 vs Treatment 4	0.98
Treatment 3 vs Treatment 5	0.99
Treatment 3 vs Treatment 6	0.70
Treatment 4 vs Treatment 5	0.99
Treatment 4 vs Treatment 6	0.92
Treatment 5 vs Treatment 6	0.98

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Sensory Evaluation Report

Attention: Marica Mazza
Samples received: 27/11/2017 Report date: 18/12/2017
Sample Description : 20 wines, 5 treatments with 4 field replications for each treatment

Analysis Report : Damian Espinase Nandorfy, Leigh Francis

The effects of CO_2 and temperature on Shiraz wine sensory properties.

Executive Summary

The aim of this study was to evaluate the effect of vineyard CO_2 and temperature on the sensory properties of 2017 Shiraz wines. Five treatments (one control and four treatments), each with four field replicates, were examined in this study. Data analyses of the study treatments revealed nine attributes: *opacity* (colour intensity), *overall fruit aroma, red fruit aroma, cooked/tinned vegetable aroma, drain aroma* (reduction), *sweaty aroma, overall fruit flavour, red fruit flavour* and *fruit aftertaste* rated by the panel differed significantly ($P \leq 0.05$) among the treatments. Six attributes: *stalky aroma, pungent aroma, dark fruit flavour, viscosity, sweetness* and *astringency* were close to being significant ($P \leq 0.10$). Of the significant ($P \leq 0.05$) and close to significant ($P \leq 0.10$) attributes there were some significant field replicate effects found, indicating variability in the vineyard or due to winemaking. Treatment 5 was free of these effects and was consistently rated highly for overall *fruit flavour, red fruit flavour* and *fruit aftertaste*. Treatments 3, 4 and 6 were rated high in attributes *cooked/tinned vegetable aroma, drain aroma* and *sweaty aroma*, possibly describing winemaking artifacts, and were rated lower in fruit-related attributes. These negative attributes may be masking any underlying fruit flavour differences potentially caused by the different treatments. Chemical analytical data may assist in elucidating these possible masked differences. Treatment 6 was rated lowest in *opacity* (colour intensity).

Introduction

The aim of this study was to evaluate the effects of CO_2 and temperature treatments imposed in the vineyard on the sensory properties of 2017 Shiraz wines. Five treatments (one control and 4 treatments), each with four field replicates, were examined in this study.

Materials and Methods

Training for sensory descriptive analysis

A panel of eight assessors (two males, eight females) with an average age of 50 years (SD=9.1) was convened to evaluate the wines. All panellists were part of the external AWRI trained descriptive analysis panel and had extensive experience in wine sensory descriptive analysis.

Assessors attended three two-hour training sessions to determine appropriate descriptors for rating in the formal sessions. All the wines from the study were progressively used during training sessions to generate and refine appropriate descriptive attributes and definitions through a consensus based approach.

Wines were assessed by appearance, aroma and palate. In the second session, standards for aroma attributes were presented and discussed and these standards were also available during subsequent training sessions, the booth practice session and the formal assessment sessions.

Following the third training session, tasters participated in a practice session in the sensory booths under the same conditions as those for the formal sessions. After the practice session, any terms which needed adjustment were discussed and the final list of terms and standards were determined. For the formal sessions, this list was refined to include one appearance term, fourteen aroma terms (thirteen defined and one 'Other' term) and thirteen palate terms (twelve defined and one 'Other' term). The final list of attributes, definitions/synonyms and reference standards are shown in Table 1.

Evaluation

Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered, ISO standard wine glasses at 22–24 °C, in isolated booths under daylight-type fluorescent lighting. Randomised presentation order was followed except in the practice sessions where there was a constant presentation order. All samples were expectorated. In practice booth sessions, the assessors were presented with four trays of four wines per tray. In the formal booth sessions of two hours duration the assessors were presented with four trays of four wines per tray for the first two days of formal assessments and two trays of four wines per tray for the third day of formal assessment. The assessors were forced to have a 60-second rest between samples and were encouraged to rinse with water, and a minimum ten-minute rest between trays. During the ten-minute break assessors were requested to leave the booths. Formal evaluation was completed in three sessions.

The 20 wines were presented to assessors two times, in a modified Williams Latin Square incomplete random block design generated by Fizz sensory acquisition software (version 2.51, Biosystemes, Couternon, France). A new bottle was used for each of the two presentation replicates.

The intensity of each attribute listed in Table 2 was rated using an unstructured 15 cm line scale (0 to 10), with indented anchor points of 'low' and 'high' placed at 10% and 90% respectively. Data was acquired using Fizz sensory software.

Panel performance was assessed using Fizz software and R with the SensomineR() and FactomineR() packages. The performance assessment included analysis of variance for the effect of judge, wine and presentation replicate and their interactions, degree of agreement with the panel mean, degree of

discrimination across samples and the residual standard deviation of each judge by attribute. All judges were found to be performing to an acceptable standard.

Analysis of variance (ANOVA) was carried out using Minitab 18 (Minitab Inc., Sydney, NSW). The effects of treatment, judge, judge by treatment, field replicate nested into treatment, judge by field replicate nested into treatment, and presentation replicate nested into treatment and field replicate were assessed, treating judge as a random effect. Following ANOVA, Tukey's honest significant difference (HSD) value was calculated (P=0.05) for the treatment effect. Principal component analysis (PCA) was conducted and visualised using Excel (Microsoft) and XLSTAT (ADDINSOFT, Paris, France). Principal component analysis was conducted for each treatment replicate of the significant attributes ($P \leq 0.05$) using the correlation matrix.

Table 1. Sensory attributes, definitions and composition of reference standards.

Attribute	Definition/Synonyms	Standard
Appearance		
Opacity	Colour intensity, the degree to which light is not allowed to pass through a sample	
Aroma		
Overall fruit aroma	Intensity of the overall fruit aromas in the sample	
Red confection and fruit	Intensity of the aroma of red fruits and confection: raspberries, strawberries, red currants and red cherries	1 µL of 2.11 g/L β-ionone, 2 x frozen raspberries (Sara Lee brand) and 1 frozen strawberry (Welch's brand)
Dark fruit	Intensity of the aroma of dark fruits and berries: blackberries, plums, blueberries and blackcurrant	2 x frozen blueberries, 1 x frozen blackberry (Sara Lee brand), 1 mL blackcurrant syrup (Ribena), and 3 g of plum paste (Maggie Beers)
Citrus	Intensity of the aroma of citrus including; lemon and lime sorbet	5 g fresh lemon and lime wedges
Banana lolly	Intensity of the aroma of banana lolly	10 µL of 18.94 g/L isoamyl acetate
Vanilla/Chocolate	Intensity of the aroma of vanilla, chocolate, toffee and caramel	Standard 1. 0.1 g whole vanilla bean (Taylor and Colledge brand) Standard 2. 1 g of 70% dark chocolate chips (Absolute Organic) not in wine and Standard 3. 5 g of caramel (Bonne Maman) not in wine
Floral	Intensity of the aroma of flowers; geranium, violets, rose, and blossoms	40 µL of 10 ml/L geraniol, 20 µL of 10 ml/L linalool
Cooked/tinned vegetable	The intensity of the aroma of cooked or tinned vegetables	1 µL of dimethyl disulfide 0.1% v/v
Earthy	Intensity of the aroma of dusty earth and soil	40 µL of 4 mg/L geosmin
Drain	Intensity of the aroma of dirty drains (natural gas) reminiscent of boiled eggs	40 µL of mercaptoethanol 2 % v/v, and 0.001 g wood ash
Stalky	Intensity of the aroma of stalks, rhubarb stalks, fresh tomato leaf/stalk	0.1 g fresh tomato stalk and 0.5 g fresh rhubarb stalk not in wine
Sweaty	The intensity of the aroma of sweat	40 µL of 10.49 g/L hexanoic acid
Pungent	Intensity of the aroma and effect of alcohol	4 mL of 95% food grade ethanol (Tarac Technologies)
Palate		
Overall flavour	Intensity of overall flavours in the sample.	
Red fruit	Intensity of the flavour of red fruits and confection: raspberries, strawberries, red currants and red cherries	
Dark fruit	Intensity of the flavour of various dark fruits and berries: blackberries, plums, blueberries and blackcurrant	

Attribute	Definition/Synonyms	Standard
Stalky	Intensity of the flavour of green stalks, rhubarb stalks, fresh tomato leaf and stalk.	
Salty	Intensity of the taste of salt, including aftertaste.	1 g/L sodium chloride (brand) in water.
Sweetness	Intensity of sweet taste in the mouth including aftertaste.	8 g/L table sugar (Woolworths essentials) in water
Viscosity	The perception of the body, weight or thickness of the wine in the mouth. Low = watery, thin mouth feel. High = oily, thick mouth feel.	1.5 g/L carboxymethylcellulose sodium salt (Sigma Aldrich) in water
Acidity	Intensity of acid taste in the mouth including aftertaste.	2 g/L L-(+)-tartaric acid (Chem-Supply) in water
Astringency	The drying and mouth-puckering sensation in the mouth. Low = coating teeth; Medium = mouth coating & drying; High = puckering, lasting astringency.	0.43 g/L alum sulfate (Ajax fine Chem Supply PTY LTD in water
Bitterness	The intensity of bitter taste perceived in the mouth, or after expectoration.	0.15 g/L Quinine sulfate (Sigma Aldrich) in water
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning.	8% food grade alcohol (Tarac Technologies) in water
Fruit AT	The lingering fruit flavour perceived in the mouth after expectorating.	

All standards were added to 30 mL of NV Yalumba Shiraz unless otherwise noted.

Results

From the nested ANOVA for the Shiraz wines from the study, nine attributes rated by the panel differed significantly ($P \leq 0.05$) among the treatments (Table 2). These attributes included: *opacity* (colour intensity), *overall fruit aroma, red fruit aroma, cooked/tinned vegetable aroma, drain aroma* (reduction), *sweaty aroma, overall fruit flavour, red fruit flavour* and *fruit aftertaste*. Six attributes: *stalky aroma, pungent aroma, dark fruit flavour, viscosity, sweetness* and *astringency* were close to being significant ($P \leq 0.10$). Of the significant ($P \leq 0.05$) and close to significant ($P \leq 0.10$) attributes there were significant field replicate nested in treatment effects for *opacity, overall fruit aroma, red fruit aroma, cooked/tinned vegetable aroma, drain aroma* (reduction) and *astringency*. These replicate differences indicate differences within some treatments, reflecting variation between field replicates and/or variation in winemaking. Attributes *cooked/tinned vegetable aroma* and *drain aroma* may indicate that some wines could have suffered from reductive winemaking artefacts, possibly masking some of the fruit aromas of the affected replicates. *Sweaty aroma* was significant for presentation replicate nested into treatment and field replicate effect.

Table 2. Probability values[†], degrees of freedom (df) and mean square error (MSE) from the analysis of variance.

Attribute	T	FRep(T)	J*T	J*FRep(T)	PRep (T,FRep)	MSE
Opacity	0.000***	0.000***	0.220	0.098	0.278	0.523
Overall fruit A	0.034*	0.036*	0.414	0.664	0.713	1.379
Red fruit A	0.048*	0.028*	0.073	0.656	0.328	2.550
Dark fruit A	0.426	0.377	0.005**	0.218	0.634	1.691
Citrus fruit A	0.803	0.224	0.025*	0.994	0.812	0.424
Banana lolly A	0.358	0.222	0.177	0.004**	0.398	0.603
Vanilla/chocolate A	0.333	0.825	0.988	0.023*	0.854	1.262

Attribute	T	FRep(T)	J*T	J*FRep(T)	PRep (T,FRep)	MSE
Floral A	0.625	0.387	0.312	0.038*	0.077	1.146
Cooked/tinned vegetable A	0.038*	0.025*	0.002**	0.883	0.568	1.368
Earthy A	0.165	0.257	0.466	0.808	0.182	1.087
Drain A	0.027*	0.006**	0.237	0.162	0.246	1.744
Stalky A	0.068ǂ	0.245	0.908	0.001***	0.209	0.631
Sweaty A	0.017*	0.134	0.183	0.234	0.043*	1.011
Pungent A	0.059ǂ	0.386	0.998	0.129	0.169	0.654
Overall fruit F	0.005**	0.072	0.166	0.314	0.259	1.042
Red fruit F	0.001***	0.110	0.738	0.568	0.798	2.314
Dark fruit F	0.054ǂ	0.333	0.129	0.578	0.311	1.817
Stalky F	0.217	0.032*	0.326	0.005**	0.173	0.774
Salt	0.445	0.690	0.690	0.619	0.936	1.774
Viscosity	0.099ǂ	0.612	0.436	0.365	0.466	0.507
Acidity	0.827	0.354	0.819	0.441	0.505	0.768
Sweetness	0.081ǂ	0.404	0.043*	0.568	0.114	0.620
Astringency	0.068ǂ	0.029*	0.090	0.138	0.237	1.046
Hotness	0.427	0.438	0.768	0.315	0.099	1.132
Bitterness	0.325	0.409	0.002**	0.995	0.962	2.087
Fruit aftertaste	0.024*	0.072	0.440	0.717	0.864	1.067
df	4	15	28	105	20	140

A: aroma, F: Flavour. †Significance levels are as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ǂ $P \leq 0.10$. df = degrees of freedom. Judge effect was significant for all attributes $P \leq 0.01$. T = Treatment, FRep = Field replicate, PRep = presentation replicate, J = Judge.

Of the significant ($P \leq 0.05$) and close to significant ($P \leq 0.10$) sensory attribute treatment means shown in Table 3, three attributes: *overall fruit flavour, red fruit flavour* and *fruit aftertaste* were not affected by field replicate variation. Treatment 5 was found to be rated highly for *overall fruit flavour, red fruit flavour* and *fruit aftertaste*. However, treatment 5 was also rated lowest in attributes *cooked/tinned vegetable aroma*, *drain aroma* and *sweaty aroma*, possibly describing winemaking artifacts. The other treatments and control were rated higher in these negative attributes which may be masking any underlaying fruit flavour differences possibly caused by the different treatments. Further volatile chemical analysis may allow interpretation of these possible masked differences.

Table 3. Means of the significant ($P \leq 0.05$) and close to significant ($P \leq 0.10$) sensory attributes, Tukey's HSD from the ANOVA of 2017 Shiraz samples.

	CO_2 and Temperature Treatments					
	Control	Treatment 3	Treatment 4	Treatment 5	Treatment 6	HSD
Opacity	4.96	4.75	4.81	5.28	3.95	0.35
Overall fruit aroma	5.50	5.21	4.98	5.56	5.09	0.57
Red fruit aroma	4.45	3.88	3.46	4.32	4.03	0.77
Cooked/tinned Vegetable aroma	0.99	1.26	1.34	0.56	1.34	0.56
Drain aroma	0.87	1.10	1.29	0.56	1.47	0.64
Stalky aroma	2.47	2.48	2.74	2.29	2.39	ns
Sweaty aroma	0.72	1.00	1.12	0.37	0.84	0.48

Pungent aroma	3.37	3.53	3.52	3.28	3.36	ns
Overall fruit flavour	5.76	5.56	5.13	5.97	5.34	0.49
Red fruit flavour	4.22	4.21	3.63	4.75	4.00	0.73
Dark fruit flavour	4.97	4.65	4.16	4.56	4.30	ns (P=0.054)
Viscous	3.38	3.51	3.41	3.58	3.22	ns
Sweetness	0.76	0.68	0.73	1.15	0.89	ns
Astringent	4.91	5.24	5.61	5.24	5.48	ns
Fruit aftertaste	3.83	3.81	3.56	4.20	3.81	0.50

HSD = Tukey's honest significant differences ($P \leq 0.05$) value. ns = not significant.

The PCA for the wines (Figure 1) was created using the nine attributes that were significantly different ($P \leq 0.05$) across the control and four treatments. PC1 explained 83.2% of the variation in the data and PC2 explained 10.8% of the variance. Treatment 5 was plotted to the far right of the figure, being rated highly in those attributes highly positively loaded on PC1, notably *overall fruit aroma, overall fruit aroma, red fruit flavour* and *fruit aftertaste*. Conversely, treatments 3, 4 and 6 were plotted to the left of the figure being rated higher in those attributes highly negatively loaded on PC1, notably *cooked/tinned vegetable aroma, drain aroma* and *sweaty aroma*. The Control was intermediate in ratings. While treatment 6 was plotted to the left of the figure, it was rated lower in *sweaty* aroma than treatments 3 and 4, but all three were rated highly in the reductive-like attributes. These attributes to the left of Figures 1, may relate to winemaking artefacts and could have a suppressing effect on perception of fruit flavour attributes. Treatment 6 was also rated lower in opacity (colour intensity) than the other treatments.

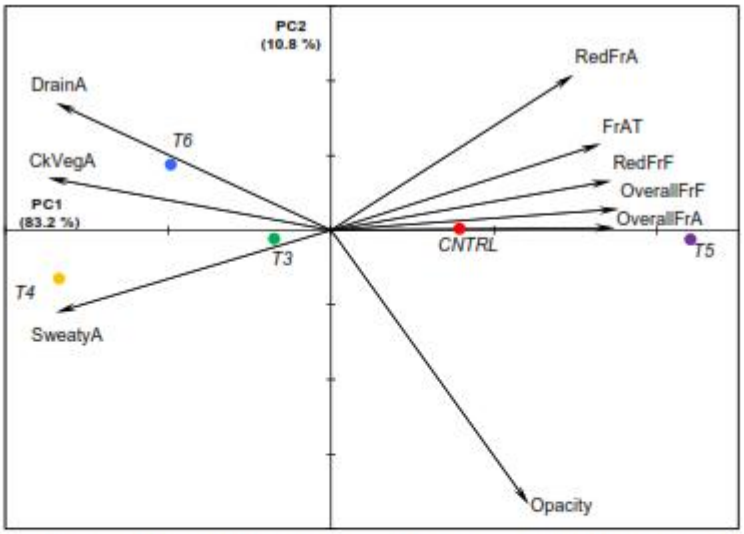


Figure 2. Principal component analysis biplot for 2017 Shiraz control and treatments (n=4 field replicates x 2 presentation replicates x 8 judges). A: Aroma, F: Flavour, AT: aftertaste.

Appendix 6 Budget Reconciliation

Submitted as separate document to AGWA.