Received ▌10 Mars 2019; Accepted : 5 May 2019 ▌Published : 10 May 2019
DOI : 10.20870/oeno-one.2019.53.2.2392

OENO One
VINE AND WINE OPEN ACCESS JOURNAL

Single and cumulative effects of whole-vine heat events on Shiraz berry composition

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This article is published in cooperation with the 21th GIESCO International Meeting, June 23-28 2019, Thessaloniki, Greece. Guests editors: Stefanos Koundouras and Laurent Torregrosa

ABSTRACT

Aim: The aim of the study was to test the effect of a single heat event (HE), and the additive effects of repeated HEs at whole-vine level, on Shiraz berry composition, including detailed tannins.
Methods and results: In a UV-transparent glasshouse, a system was developed to individually heat the above-ground parts of well-irrigated potted Shiraz vines without changing fruit and canopy light exposure. At the end of fruit set, and again prior to véraison, selected vines were heated to + 6 °C above ambient temperature for three consecutive days and nights in a combination of treatments to test the effect of a single HE and the additive effects of repeated HEs. A factorial design was used with four treatments (n=6): Control (C), heated at E-L 31 (HW1), heated at E-L 32 (HW2) and heated twice (HW1&2). Berries were sampled from fruit set until maturity at regular intervals, and primary and secondary metabolites, including detailed tannin composition, were analysed by GC-MS and LC-MS/MS, respectively. Temperatures (mean and maximum) inside the glasshouse were influenced by outside weather conditions and the 1st HE (HE1) was more intense than the 2nd (HE2). Photosynthesis was significantly decreased for the heated vines during HE1 where maximum temperature reached 45 °C, affecting both berry weight and titratable acidity (TA). HE2 was less intense with maximum temperature only reaching 40 °C, and had no effect on photosynthesis and less direct impact on composition. A few primary metabolites were affected by either HE1 or HE2 such as valine, leucine, pyruvic and lactic acids. Interactions between the two HEs were found for TA, malic acid and glucose at harvest. Skin tannin composition was significantly impacted by HE1, applied during the main biosynthesis period, but not by HE2. Epicatechin gallate terminal subunit concentration was the most impacted by heat. Seed physiology was also affected by HE1 and HE2 as well as seed tannin composition right after HE1. A small decrease in both total anthocyanins and total soluble solids at the end of véraison suggested that ripening was slightly delayed for HW1&2.
Conclusions: Shiraz grapevines showed an elastic response to short heat stress between fruit set and véraison, with most impacts on physiology and composition observed during early post-treatment no longer evident by harvest.
Significance and impact of the study: Increasing climate variability, with more frequent heatwaves, is a threat for viticulture in Australia where a large proportion of vineyards are located in already warm and hot regions. As grapevines may be exposed to abnormal high temperatures prior to véraison, knowledge on the effect of heat on metabolite biosynthesis occurring during this phase, such as tannins, is needed.

KEYWORDS

berry development, climate change, composition, cumulative events, heatwave, metabolism, tannins

INTRODUCTION

Regardless of the method used to characterise the temperature of a region (e.g. average growing season temperature, heat degree days, mean January temperature, Huglin index), the majority of Australian vineyards are located in areas that fall within the upper temperature range for wine grape production (Coombe, 1987; Hall and Jones, 2010). With climate change, there has been growing recognition of the impact of increasing temperatures on viticulture, and over the past decades records are confirming an increase in extreme weather in Australia (Perkins and Alexander, 2013; Perkins-Kirkpatrick and Pitman, 2018; Perkins-Kirkpatrick *et al.*, 2016). Highlighted in these studies, the increase in frequency of heat events (HEs) could be a threat for viticulture, especially if those happen earlier during the grapevine growing season. Very early HEs have been shown to impact on grapevine reproductive development and yield by affecting budburst (Petrie and Clingeleffer, 2005), flowering and berry setting (Buttrose and Hale, 1973; Dunn and Martin, 2000; Kliewer, 1977; Pagay and Collins, 2017). In addition, after berry set, fruit quality can also be affected as berry growth and metabolism are regulated by several parameters, including bunch microclimate where temperature plays a key role in the accumulation of primary and secondary metabolites (Bonada and Sadras, 2015; Downey *et al.*, 2006; Gouot *et al.*, 2019; Pillet *et al.*, 2015).

Several experiments have already studied berry development and/or metabolite accumulation, but most short-term whole-vine heating treatments were under limited ultra-violet (UV) radiations, e.g. polycarbonate enclosures (Soar *et al.*, 2009; Sweetman *et al.*, 2014), in growth chambers without UV supplement (Greer and Weston, 2010; Sweetman *et al.*, 2014) or in green houses built with glass or plastic filtering most natural light and UV (Lecourieux *et al.*, 2017; Majer and Hideg, 2012; Pillet, 2011). Systems allowing a more natural UV environment such as open-top chambers have been developed, but have mainly been used to study an increase in long-term average temperature (+ 1-3 °C) during several months or the whole grape growing season (Bonada *et al.*, 2015; de Rosas *et al.*, 2017; Sadras *et al.*, 2012; Sadras and Moran, 2012; Sadras and Soar, 2009).

Australian Shiraz has been shown to adapt to short but intense heat stress by increasing leaf gas exchange rates (Soar *et al.*, 2009) as well as maintaining yield under long-term elevated temperature (Sadras and Soar, 2009). Interactions between water status and heat have also been extensively studied with temperature predominant in affecting berry composition (Bonada, 2014; Bonada *et al.*, 2018; Bonada *et al.*, 2015). However, the potential additive effect of consecutive heatwaves on the same vines has never been reported. To our knowledge, this concept has only been studied once with wheat (Wollenweber *et al.*, 2003).

The study described here used well-irrigated potted Shiraz vines inside a UV-transparent glasshouse and an adapted system using fan heaters to deliver hot air to whole-vines without affecting bunch light exposure (Tarara *et al.*, 2000). Free from light artefacts, the effect of early HEs was assessed on a wide range of primary and secondary metabolites. This work was conducted to answer three questions: (i) is there a berry development stage at which a heatwave is more critical, (ii) are the effects of more than one heat event additive and (iii) is tannin accumulation affected when whole-vines are exposed to high temperature? The phenological stage at which the HEs occurred was a central parameter in this experiment with berry development stages, 20 and 35 days after fruit set, targeted.

MATERIALS AND METHODS

1. Plant material

Dormant 7-year-old, own-rooted potted Shiraz grapevines were removed from their containers and replanted in 50-L pots filled with premium potting mix. Dormant plants were stored in a dark cool room (4 °C) from September to November and were then moved to a research glasshouse mid-November to induce budburst. The glasshouse was located at Charles Sturt University, Wagga Wagga (35° S, 147° E), built with UV transmitting Plexiglas (PLEXIGLAS® Alltop SDP 16/980 (/1053, /1200) – 64) and the bay used was orientated SSE. The experiment was carried out during the summer and autumn months of the 2016/2017 growing season. Vines were pruned to 6 shoots and 12 bunches each and trained vertically using bamboo stakes. Fertilisation and pest and disease control were performed according to standard viticulture practices.

2. Experiment

Vines were arranged on two steel mesh benches with 14 vines each, including two buffer vines. Vines were individually watered using a drip system starting every day at 6 AM, 10 AM and 2 PM for 15 min, with the timing controlled by an automatic watering system (Pope SnapTimer). The soil was fully saturated at each watering time and soil moisture was measured regularly along the experiment using a ML2x ThetaProbe

soil moisture sensor connected to a HH2 moisture meter (Delta-T Devices Ltd, Cambridge, UK). Air temperature, relative humidity (RH) and light were monitored above the canopy with two stations in the centre of each bench consisting of a Tinytag Plus 2 TGP-4500 dual channel dataloggers (Gemini dataloggers, West Sussex, UK) and a SQ-110 quantum sensor (Apogee Instruments Inc, Logan, Utah, USA). The glasshouse temperature was controlled with evaporative air conditioning and gas heating and the daily maximum glasshouse air temperature averaged 31 ± 4 °C, the daily minimum was 18 ± 3 °C and the glasshouse RH varied between 25 and 92 %. The photosynthetically active radiation (PAR) varied from 0 at night to above 2000 µmol/m^2/s^{-1} on a sunny day. The CO_2 concentration was measured fortnightly with a LCA-4 portable infrared gas analyser instrument (ADC Bioscientific Ltd., Hoddesdon, Hertfordshire, UK) and ranged 406-430 ppm in the glasshouse bay.

3. Treatment application

Individual vines were wrapped with reflective foil insulation to form an open-topped cylinder from the bottom of the pot to the top of the cordon with major parts of the canopy exposed to natural light (Figure 1A). Based on the design of the chamber-free heating system developed by Tarara *et al.* (2000), two delivery PVC (polyvinyl chloride) tubes of 30 cm were mounted from each side of the vine. They were positioned at the top of the pot with a 35° angle to the left to avoid direct heat on the trunk, itself protected with reflective insulation (Figure 1B). Hot and ambient air was blown (approximately 1 m^3/min per tube) with axial fans installed at the lower end of the delivery tube. The hot air was produced with commercial heaters (2000W) set on medium heat blowing at 6 m^3/min into two insulated boxes (1 m^3 each) and delivered through flexible ducts to the fans. Ambient air was also drawn using fans for non-heated vines.

During berry development, vines were exposed to two HEs at different stages according to a factorial experimental design with four treatments (n=6): Control (C), vines heated only during the first heat event (HW1), vines heated only during the second heat event (HW2) and vines heated during both (HW1&2). Flowering was recorded as the first flower cap fall (E-L 19, Coombe, 1995). The first heat event (HE1) started at the end of berry set (pea size), 21 days on average after the start of flowering, corresponding to E-L developmental stage 31.

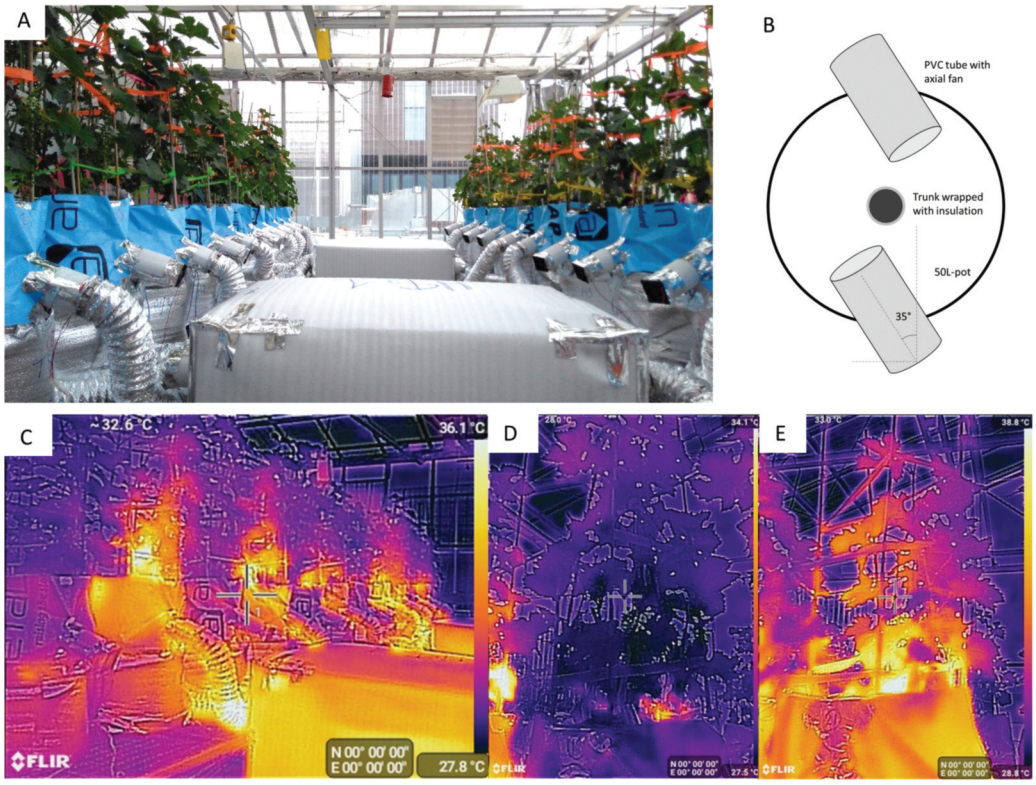


FIGURE 1. Whole-vine heating system in a UV transparent glasshouse

The second one (HE2) started 14 days after the end of HE1, corresponding to E-L developmental stage 32. Treatments were maintained for three consecutive days and nights (72 h), starting at 7 AM on day 1 and stopping at 7 AM on day 4.

Experimental set-up with heating system and vines organised in two rows (A), disposition of the fan heaters (B) and examples of pictures taken with the FLIR One thermal camera of a row of vines (C), a non-heated vine (D) and a heated vine (E).

The air temperature around each vine was estimated by two thermocouples (PVC/PVC TX stranded 24F 7/0.2 Extension Wire, ECEfast, Australia) joined to bare fine-wire (0.13 mm diam.; Type T copper-constantan, ECEfast, Australia) and positioned within the canopy, 30 cm from the bottom of the pot and at the top (80 cm). Sensor signals were scanned every 10 s and the average recorded every 5 min by two data acquisition systems (AM-25T and CR-1000, Campbell Scientific, Logan, UT, USA) from flowering until the end of the treatment application. Whole-vine temperature was also monitored using a thermal imaging camera (FLIR One for Android, FLIR systems, OR, USA) at several times of the day and night during the experiment (Figure 1C, D&E).

4. Photosynthesis measurement

Photosynthesis was regularly measured with a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, Nebraska, USA) set at a PPFD of 2 000 µmol/m^2/s^{-1}, air flow set at 500 mol/s^{-1}, with a reference gas CO_2 concentration of 410 ppm and temperature set to 30 °C. One leaf (directly opposite a bunch) per vine was used to measure net photosynthesis and stomatal conductance: the day before each HE; twice during each HE (day 1 and day 3); and two weeks after the last HE, when all vines returned to ambient temperature. Measurements were all conducted between 11 AM and 1 PM.

5. Berry sampling

Berries were sampled one day before HE1, before the start of the HE2 two weeks later, at the onset of véraison, twice during ripening, and at maturity. Each sampling date is referenced to the number of days after flowering F+20, 34, 55, 76, 97 and 117, respectively. For each sampling, two berries were collected per bunch and pooled together per vine (12 bunches). After determination of the fresh weight of each 24-berry sample, they were snap-frozen in liquid nitrogen (N) and stored at -80 °C until processing. Then, berries were slightly thawed on ice and skin and seeds were quickly separated from pulp and blotted dry. Their fresh mass was determined before being snap-frozen in liquid N and stored at -80 °C. Pulp and juice were homogenised and immediately manually ground into a fine powder using mortar and pestle under liquid N. Total soluble solids (TSS) and sugar content were determined for the last three sampling dates. Ground powder (around 5 g) was thawed for 90 min at 20 °C, vortexed and then centrifuged at 4,000 rpm for 4 min. TSS was determined for each sample from the supernatant with a refractometer (PR-101, Atago, Tokyo, Japan). The remaining juice was combined to give two 10mL-samples per treatment for analyses of pH and titratable acidity (TA), measured by titration with sodium hydroxide (0.1 M) using an autotitrator (Metrohm Fully Automated 59 Place Titrando System, Metrohm AG, Herisau, Switzerland) run by Tiamo (version 2.3). A sub-sample of pulp powder was kept frozen and then freeze-dried until constant weight (Gamma 1-16 LSC, Christ, Osterode am Harz, Germany). Prior to analysis, skin and seeds were manually ground with a mortar and pestle and freeze-dried until constant weight.

6. Chemical analysis

6.1 Pulp primary metabolite analysis

Extraction of pulp primary metabolites was adapted from Reshef *et al.* (2017) as follows: 1 mL of a pre-chilled mixture of methanol: chloroform:water (2.5:1:1, v/v) was added to 20 mg of freeze-dried pulp powder. An internal standard (60 µL of 0.2 mg/mL of ribitol in 50 % (v/v) aqueous methanol) was subsequently added to each sample. The mixture was briefly vortexed, sonicated on ice for 10 min and extracted with a Ratek rotary mixer (RSM7DC, Ratek Instruments Pty Ltd, Australia) for 10 min in a dark cool room. Samples were then centrifuged for 10 min at 14,000 rpm and 1 mL of supernatant transferred into new tubes. The supernatant was then mixed with 300 µL of chloroform and 300 µL of MilliQ water, briefly vortexed and centrifuged at 14,000 rpm for 2 min. Then, 70 µL of the water/methanol phase were dried under a constant flow of pure N_2 gas and stored at -80 °C until derivatisation, conducted as per Rossouw *et al.* (2017).

The GC-MS system consisted of a 7890A gas chromatograph and 5975C mass spectrometer with an electron impact ionisation source and a quadrupole analyser (Agilent Technologies, Agilent, Santa Clara, CA). GC-MS conditions were as described in Rossouw *et al.* (2019). Sample volumes of 1 µL were injected into a GC column (30 m × 0.25 mm, 0.25 µm HP-5MS UI,

Agilent, Santa Clara, CA) using a Gerstel MPX autosampler and tray cooler (10 °C). All samples were injected twice for low and high abundance metabolites as described in Rossouw *et al.* (2019).

Spectral deconvolution (signal-to-noise ratio threshold = 5; mass absolute height ≥ 500; compound absolute area ≥ 1000) allowed the identification of co-eluting peaks and was conducted through the MassHunter Workstation software (Quantitative Analysis for GCMS, version B.08.00, Agilent, Santa Clara, CA). Compounds were identified with reference standards or by using the NIST library and their retention index calculated using a series of alkanes (C10–C40) (Rossouw *et al.*, 2019). Samples from F+20, 34, 55 & 117 were analysed. The data set acquired by GC-MS was normalised to internal standard and tissue dry weight (DW).

6.2 Skin and seed secondary metabolite analysis

The extraction protocol was adapted from Pinasseau *et al.* (2017). Freeze-dried skin samples of 14 mg and seed samples of 10 mg were used for extraction. First, 200 µL of methanol were added, followed by 1.4 mL of 0.05 % (v/v) trifluoroacetic acid (TFA) in acetone/water (70/30, v/v) and 40 µL of an internal standard solution containing corticosterone and ampicillin at 0.5 g/L in 50 % (v/v) aqueous methanol. Samples were sonicated in ice for 10 min and then, shaken in a cool dark room using a Ratek rotary mixer for 20 min before being centrifuged (5 min, 4,000 rpm, 4 °C). An aliquot of 500 µL was dried under constant flow of pure N_2 gas for polyphenol analysis. Additional aliquots of 500 µL were transferred into separate tubes and dried with Genevac (EZ-2 Plus, SP Scientific, Ipswich, England) for tannin analysis.

Skin and seed samples for tannin analysis were prepared by phloroglucinolysis as adapted from Pinasseau *et al.* (2016). First, 350 µL of phloroglucinol reagent (50 g/L phloroglucinol and 10 g/L ascorbic acid in methanol with 0.2 mol/L HCl) were added to the residue obtained after Genevac drying and the mixture sonicated on ice for 30 min. Samples were then placed into a water bath at 50 °C for 20 min and then, immediately transferred on ice for 1 min. The reaction was stopped by adding 350 µL of ammonium formate (12.6 g/L) and samples were centrifuged (10 min, 14,000 rpm, 4 °C), filtered (Regenerated cellulose, 0.22 µm, Phenomenex) and transferred into vials. Skin samples for polyphenol analysis were prepared by adding 250 µL of 1 % (v/v) formic acid in methanol/water 50/50 (v/v) to the N_2 gas-dried residue. Samples were sonicated in ice for 30 min, centrifuged (14,000 rpm, 10 min, 4 °C), filtered and transferred into LC vials with inserts.

Both tannin and polyphenol analyses were carried out on an Agilent LC system which consisted of a binary pump G1312B, a HiP autosampler G1367E maintained at 8 °C, an Agilent G4212B diode array detector (DAD) recording spectra from 210 to 600 nm (resolution of 1.5 nm) and an Agilent G6470A triple quadrupole tandem mass spectrometer (QQQ). An Acquity UPLC® BEH C18 column (1.7 µm, 2.1*50 mm, Waters, Dundas, Australia), protected by a BEH Shield RP18 Vanguard pre-column (1.7 µm, 2.1*5 mm, Waters, Dundas, Australia), was maintained at 40 °C. The mobile phase consisted of 1 % (v/v) formic acid in MilliQ water (solvent A) and 1 % (v/v) formic acid in methanol (solvent B). For tannin analyses, the injection volume was 1 µL, the flow rate was 0.4 mL/min, and the gradient as follows (expressed in solvent B followed by cumulative time): 5%, 0.00 min; 5%, 1.04 min; 20%, 3.12 min; 40%, 5.73 min; 90%, 6.77 min; 90%, 10.50 min; 5%, 10.94 min; 5%, 15.00 min. For the polyphenol analyses, the flow rate was 0.34 mL/min. The gradient was as per Pinasseau *et al.* (2017) with an additional 2 min of equilibration at the end of the run. Samples, after véraison (F+76 onwards), of 2 µL were injected.

The optimum MS fragmentor and collision energies were determined for each compound in both methods with the Agilent Optimizer software (B08.00.00, Agilent, Santa Clara, CA). MassHunter software Quant for QQQ (B08.00.00, Agilent, Santa Clara, CA) was used to process the data. Polyphenol data required smoothing prior to integration (Smoothing function width: 15, Smoothing Gaussian width:5).

Tannin subunits were measured using multiple reaction monitoring (MRM) MS and DAD at 280 nm and terminal and upper subunits were quantified in MS and UV, respectively, according to Pinasseau *et al.* (2016) and Kennedy and Jones (2001) using (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate and (-)-epigallocatechin gallate standards (Extrasynthese, Genay, France). Anthocyanins were quantified using the MRM mode with malvidin-3-O-glucoside (Extrasynthese, Genay, France) as standard (Lambert *et al.*, 2015; Pinasseau *et al.*, 2017). The data sets were normalised to tissue dry weight.

7. Statistical analysis

In the factorial design, each factor, i.e., heat events (HE1 and HE2), was assigned two levels: non-heated with ambient air blown by the fans, and heated with hot air blown by the fans. Before HE2 application, treatments (n=12) were simply compared using t-test to examine the effect of HE1 on parameters measured at F+34, with heated vines (HW1 and HW1&2) versus non-heated (C and HW2). After HE2 application (F+55 onwards), the number of treatments rose to four with six replicates each. For each variable at a single sampling date, both treatments and factors were of interest and each parameter was analysed by 2-way ANOVA with HE1 and HE2 as factors and one-way ANOVA followed by post-hoc Tukey HSD test and mean comparison (R software, version 3.5.1.).

RESULTS

1. Temperature treatment and system performance

The mean temperature in the glasshouse throughout the study varied between 15.8 and 31.1 °C, with a maximum temperature of 38.7 °C recorded on January 23 (Figure 2A). Air temperature values from the upper and lower thermocouples were averaged to provide a mean bunch-zone temperature and graphed every 30 min for the two HEs (Figure 2B&C). The day average temperatures were 31.6 °C during HE1 and 31.5 °C during HE2 for the non-heated treatments (C and HW2) versus 37.2 and 36.0 °C for the heated treatments (HW1 and HW1&2). The night average temperatures were 26.7 °C during HE1 and 24.8 °C during HE2 for the non-heated treatments (C and HW2) versus 31.7 and

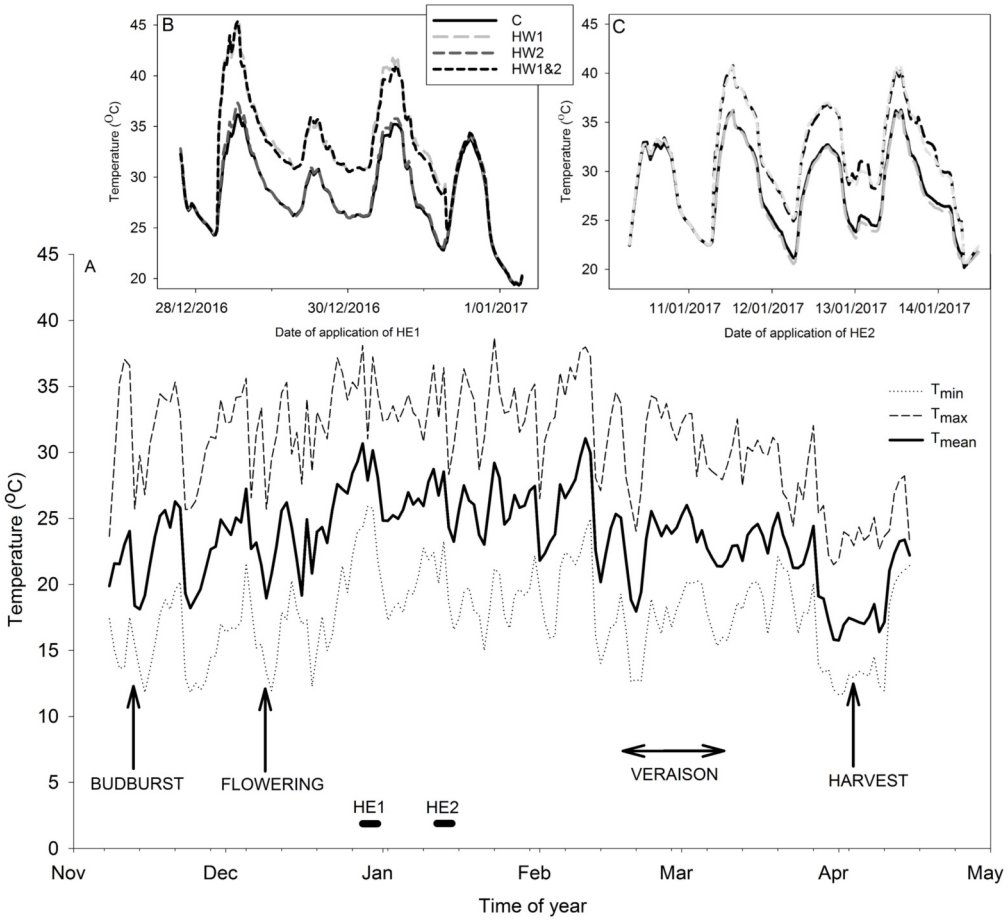


FIGURE 2. Average temperature during the experiment and heat events
Daily mean (T_{mean}), minimum (T_{min}) and maximum (T_{max}) temperatures in the glasshouse during the experiment (A) with average temperature records (every 30 min) during the 1st heat event at E-L 31 – HE1 (B) and the 2nd heat event at E-L 32 – HE2 (C) for the four treatments (Control (C), heated during HE1 (HW1), heated during HE2 (HW2) and heated twice (HW1&2)).

29.2 °C for the heated treatments (HW1 and HW1&2). The system maintained an average temperature difference of 5.6 °C during the day and 5 °C at night during HE1 and 4.5 and 4.4 °C during HE2, respectively. The maximum temperature reached during HE1 by the heated treatments was 45.5 °C which occurred on day 1 (Figure 2B) while the highest temperature recorded during HE2 was only 40.9 °C, also observed on day 1 (Figure 2C). In addition, the gradient of temperature from bottom to top of the vine was sizeable due to the design of the heating system. For example, the difference between the bottom and top thermocouple temperatures averaged 11.3 and 10.2 °C during HE1 and HE2, respectively (data not shown). Cordon, bottom of the shoots and the zone with the bunches were consequently more heated than the tip of the shoots and the leaves at the top of the canopy as shown on thermal pictures (Figure 1C, D&E). Furthermore, vapour pressure deficit (VPD), which is a function of temperature and RH, was increased for the heated vines during the HEs (data not shown) as RH was the same for all vines in the glasshouse. Soil moisture varied between 20 and 45 %, and no significant differences were found between heated and non-heated vines at any point of measurements (data not shown). Soil temperature was also monitored and averaged 30 °C at the hottest time of day with no differences between treatments.

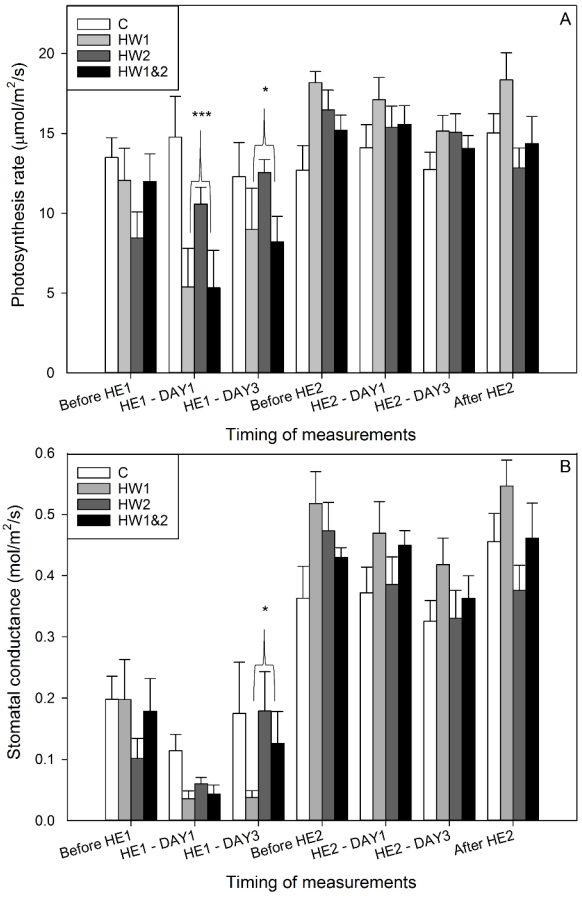


FIGURE 3. Leaf gas exchange
Photosynthesis response (A) and stomatal conductance (B) (mean ± SE) at key timings before, during and after heat events (HEs) measured for each treatment (C: Control; HW1: heated during HE1; HW2: heated during HE2; HW1&2: heated twice). Effect of HE1 was tested by t-test (n=12) for the first four measurements and * indicates significant effect of HE1 (*p<0.05, **p<0.01, ***p<0.001). The effect of HE1 and HE2 was tested by 2-way ANOVA (n=6).

2. Photosynthesis

Leaf gas exchange was monitored regularly throughout the experiment. In practice, leaf temperature, measured in the LiCOR chamber, ranged between 30 and 35 °C with small differences between heated and non-heated vines (data not shown). Photosynthesis rates averaged 12 ± 4 µmol/m²/s before any treatment application. On the first day of HE1, heated vines (HW1 and HW1&2 all together) had a significantly lower photosynthesis rate than non-heated vines (C and HW2) ($p<0.001$). On day 3 of HE1, this decline was less pronounced but still significant ($p=0.047$). Measurements made before HE2 showed that all vines returned to a normal photosynthetic rate of about 16 ± 3 µmol/m²/s. Stomatal conductance was also recorded (Figure 3B) and varied between 0.1 and 0.2 mol/m²/s before HE1. On day 1 of HE1, all conductances decreased compared to prior HE1 and no differences between heated and non-heated were found. However, on day 3, heated vines (HW1 and HW1&2) exhibited a significantly lower conductance than non-heated (C and HW2). No clear effect of the heated treatment on photosynthesis rates and stomatal conductance was observed during HE2 as well as after HEs.

3. Berry physiology and basic composition

Berry fresh weight was recorded at every sampling date and exhibited a typical double sigmoid pattern with the first phase of berry development between F+20 and F+50, followed by the lag phase until F+70 to finish with the ripening phase. Prior to the application of HE1, at F+20, berries were small with an average of 120 mg per berry and grew rapidly to reach 0.7 g by véraison and 1.4 g at harvest. The effect of HE1 was immediate with berry weight of the heated vines smaller on average than for non-heated vines, and HW1&2 significantly smaller than C at F+34. After the application of HE2, differences were still observed due to an effect

of HE1 at F+55 and F+97 but no effect of HE2 was found at any sampling date (Figure 4).

Basic parameters, such as TSS, sugar per berry, pH and TA, were measured from the pulp after véraison with TSS, sugar content and pH not significantly affected by any factors or treatments. However, TA was significantly affected by HE2 with HW1&2 significantly higher compared to C at F+76, while at F+97, a significant effect of HE1 was found. All treatments were picked on the same date at a similar average ripeness level of about 20.5 °Brix and the acidity of these samples was found to be significantly affected by both HE1 and HE2 with interactions between the two. TA for both HW1 and HW1&2 was significantly higher than C by harvest (Figure 4).

Pulp and skin moisture, and proportion of skin per berry were not impacted by any of the HEs or treatments. While the proportion of pulp was not affected until F+117, HE2 led to a significantly smaller proportion of pulp by harvest with HW2 being significantly lower compared to C (Table 1).

The average number of seeds per berry was 1.7 without differences between treatments at any sampling date (data not shown). In general, seeds were the most affected with the proportion of seeds (% fresh mass) per berry significantly increased by HE2 from F+76 until maturity

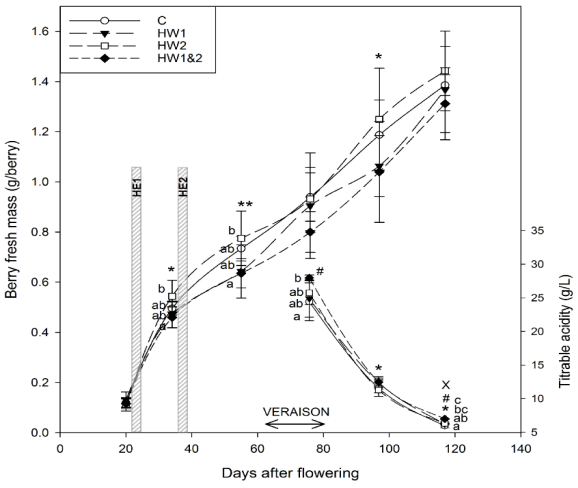


FIGURE 4. Berry basic parameters
Berry fresh mass and pulp titratable acidity (mean ± stdev) measured for each treatment (C: Control; HW1: heated during HE1; HW2: heated during HE2; HW1&2: heated twice). Effect of factors (HE1 and/or HE2) were tested by 2-way ANOVA (n=6) for each sampling date and * indicates significant effect of HE1, # indicates significant effects of HE2 and X for interactions (*, **, *** indicate significant effects at $p<0.05$, 0.01, 0.001, respectively).

(Table 1). Seed moisture was only significantly impacted by HE1 and HE2 at F+76 with HW1&2 significantly higher than C and seed fresh mash was increased by HE1 at F+55 and by HE2 at F+117.

4. Pulp primary metabolites

Several sugars, amino acids, organic acids and other compounds were analysed from the pulp by GC-MS. Pulp from the samples collected at F+20, 34, 55 and 117 were examined and about 80 compounds were detected. Only a few compounds exhibited significant differences between treatments or were affected by one of the HEs with most differences found at harvest (Figure 5). The most abundant metabolites found in berry pulp were sugars: glucose, fructose and sucrose; sugar alcohol: *myo*-inositol; and organic acids: tartaric and malic acids. The latter were both slightly decreased in HW1 and HW2, whereas malic acid was increased in HW1&2 and interactions between HEs were found for this compound. Both glucose and fructose were increased by 1.3 for HW1 and by 1.6 for HW2 compared to C, while HW1&2 glucose and fructose levels were the same as C, and a significant interaction between the two HEs was found for glucose ($p=0.0441$). Sucrose was in similar abundance in HW1 and HW1&2 but was 1.4 times higher in HW2 than C. However, no treatments were found to be significantly different from C for any of those high abundance compounds.

Low abundance metabolites, pyruvic and lactic acids, were both decreased in all treatments (Figure 5). HE1 led to a lower abundance of pyruvic acid in HW1 and HW1&2, while HE2 significantly lowered lactic acid in HW2 and HW1&2. The abundance of various amino acids at harvest was affected with higher abundance of valine, leucine, isoleucine and glycine induced by HE1 and higher tyrosine and cysteine induced by HE2. Arginine was slightly increased in both HW1 and HW2 but was unchanged in HW1&2 as interactions between HEs counteracted each other. HE2 was also associated with a small decrease in cellobiose and an increase in palmitic acid.

5. Skin secondary metabolites

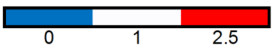
The proportion of skin per berry spanned 6-12 % and after véraison, the main compounds giving the skin its red colour, the anthocyanins, were measured. Individual anthocyanins were analysed by LC-MS with delphinidin, cyanidin, petunidin, peonidin, malvidin and pelargonidin detected in their monoglycosylated forms as well

TABLE 1. Berry physiology parameters

Parameters	Sampling date	Treatments				Factors		
		C	HW1	HW2	HW1&2	HE1	HE2	Int
Proportion of pulp per berry (% fresh mass)	F+55	84.1 ± 0.5	83 ± 2	84 ± 1	84 ± 1	NS	NS	NS
	F+76	85 ± 1	85 ± 2	84.8 ± 0.9	84 ± 1	NS	NS	NS
	F+97	84 ± 3	84 ± 4	84 ± 2	83 ± 2	NS	NS	NS
	F+117	86 ± 1 b	85 ± 1 ab	83 ± 2 a	84 ± 1 ab	NS	**	NS
Proportion of seed per berry (% fresh mass)	F+55	8.5 ± 0.7	9 ± 2	9 ± 1	8.7 ± 0.6	NS	NS	NS
	F+76	7 ± 1	6.9 ± 0.8	8 ± 1	8 ± 1	NS	*	NS
	F+97	4.5 ± 0.4	4.5 ± 0.5	4.9 ± 0.6	5.0 ± 0.6	NS	*	NS
	F+117	4.8 ± 0.5	4.8 ± 0.4	5.5 ± 0.6	5.5 ± 0.6	NS	**	NS
Seed moisture (% fresh mass)	F+55	41 ± 1	42 ± 4	43 ± 1	44 ± 2	NS	NS	NS
	F+76	36 ± 2 a	37 ± 3 ab	38 ± 3 ab	40 ± 2 b	*	*	NS
	F+97	31 ± 3	32 ± 3	32 ± 4	33 ± 1	NS	NS	NS
	F+117	26 ± 1	26 ± 1	26 ± 2	27 ± 1	NS	NS	NS
Seed fresh mass (mg)	F+55	37.3 ± 4	35 ± 6	41 ± 6	34 ± 4	*	NS	NS
	F+76	37 ± 4	35 ± 4	41 ± 6	36 ± 4	NS	NS	NS
	F+97	32 ± 2	32 ± 3	37 ± 5	31 ± 4	NS	NS	NS
	F+117	31 ± 2	33 ± 2	36 ± 5	33 ± 3	NS	*	NS

Berry parameters (mean ± stdev) measured for each treatment (C: Control; HW1: heated during HE1; HW2: heated during HE2; HW1&2: heated twice) and effect of the heat events (HE1 and HE2) tested by 2-way ANOVA (n=6). Significant differences between treatments for a given sampling date, indicated as day after flowering (F+), are indicated with lower case letters ($p<0.05$). Significant effect of the 1st or the 2nd heat event as well as interactions (Int) for each sampling date are indicated with asterisks (*$p<0.05$, **$p<0.01$, ***$p<0.001$, NS: Not Significant).

	Metabolites	m/z[a]	RI[b]	Treatments HW1	HW2	HW1&2	Factors HE1	HE2	Int[c]
Major compounds	MALIC ACID	233	1510	0.86	0.78	1.33			*
	TARTARIC ACID	292	1676	0.88	0.78	0.99			
	FRUCTOSE[d]	73	1920	1.30	1.58	1.05			
	GLUCOSE[d]	73	1957	1.34	1.63	1.06			*
	Myo-INOSITOL	217	2139	0.88	1.07	0.95			
	SUCROSE	361	2719	1.02	1.41	1.01			
Minor compounds	Pyruvic acid	174	1067	**0.53**	0.76	**0.50**	**		
	Lactic acid	117	1084	0.93	**0.73**	**0.72**		***	
	Valine (2tms)	144	1233	1.40	1.08	2.27	*		
	Leucine (2tms)	158	1284	1.40	1.17	2.34	*		
	Isoleucine (2tms)	158	1308	1.32	1.11	1.85	*		
	Glycine (3tms)	276	1319	1.14	1.00	1.26	*		
	Arginine (3tms)	157	1315	1.33	1.21	1.04			*
	Tyrosine (3tms)	218	1966	0.97	1.02	1.01		**	
	Palmitic acid	313	2139	1.17	1.49	1.47		*	
	Cysteine (4tms)	218	2211	1.11	1.70	1.62		*	
	Cellobiose	361	2787	0.90	0.91	0.95		*	



[a] Major fragment mass (m) to charge number of ions (z)
[b] Retention Index
[c] Interactions between HE1 and HE2
[d] Sum of two peaks

FIGURE 5. Pulp metabolic changes at harvest due to prior heat stress
Fold changes (Treatment/Control) of berry pulp metabolites measured at maturity (F+117) for each treatment (C: Control; HW1: heated during HE1; HW2: heated during HE2; HW1&2: heated twice). Colours represent a decrease (blue), no change (white) or an increase (red) of metabolite abundance. Bolded numbers represent significant differences between treatment and control, tested by post-hoc Tukey HSD test and mean comparison ($p<0.05$). Effects of factors (HE1 and/or HE2) were tested by 2-way ANOVA (n=6) and *, **, *** indicate significant effects at $p<0.05$, 0.01, 0.001, respectively.

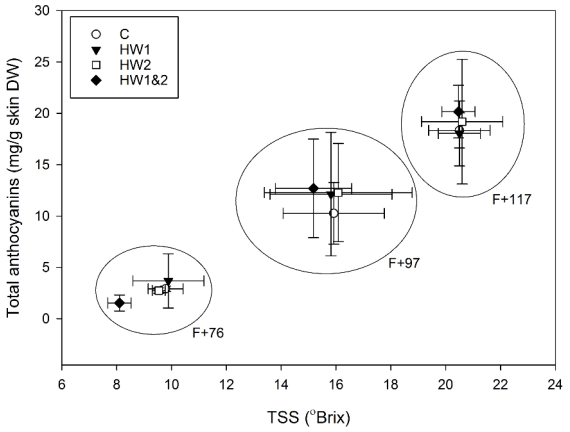


FIGURE 6. Skin anthocyanins and Total Soluble Solids
Average total skin anthocyanin concentration plotted against average Total Soluble Solids (TSS) measured in corresponding pulp samples for each treatment (C: Control; HW1: heated during HE1; HW2: heated during HE2; HW1&2: heated twice) at three sampling dates (F+76, 97, 117). Bi-directional error bars correspond to standard deviations (n=6).

as acylated: acetyl, coumaroyl and caffeoyl forms.

From F+76 onwards, the total anthocyanin content per gram dry skin was calculated from the sum of all peaks and plotted against TSS (Figure 6). At F+76, berries around 8-10 °Brix exhibited 1.5 to 3.7 mg anthocyanins/g dry skin, then increased to around 12.1 mg for an average of 16 °Brix and reached an average of 19.1 mg at harvest. No significant differences were found for total anthocyanins or TSS at any sampling date, however both parameters were found to be slightly lower for HW1&2 just after véraison (F+76). Individual anthocyanins and families were also examined for differences. Malvidins were the most abundant compounds detected with about 66 %, followed by peonidins, 18 %. The percentage of tri-hydroxylated anthocyanins was significantly increased by HE2 at harvest with 81.7 % for HW2 and HW1&2 combined versus 79.6 % for C and HW1 combined.

6. Skin and seed tannins

The total skin tannin content varied between flowering and maturity with stable concentrations at around 3.0-3.5 mg per berry until F+55 and then decreasing rapidly around véraison to stabilise during ripening at about 1.5 mg per berry (Figure 7A).

Seeds only represented 4.5 to 9.0 % of the berries on a fresh weight basis (Table 1) but were a rich source of tannins. The total seed tannin content increased rapidly during berry development from 0.7 mg to 5 mg per berry and stabilised during the maturation/drying phase with values spanning 3.2-5.0 mg (Figure 7B). Despite several effects of the heating factors on seed moisture and fresh mass, no differences in total seed tannins were found at any sampling dates.

Total skin tannins were also expressed on a DW basis to explore their accumulation pattern after HEs (Figure 8). Total skin tannin concentration exhibited an average of 204 mg/g skin at F+20 before the first treatment application (Figure 8A), and then steadily decreased during berry development and until maturity, reaching 37 mg (Figure 8A). HE1 was found to significantly increase the total tannin concentration of both HW1 and HW1&2 by about 12 % at F+76 and

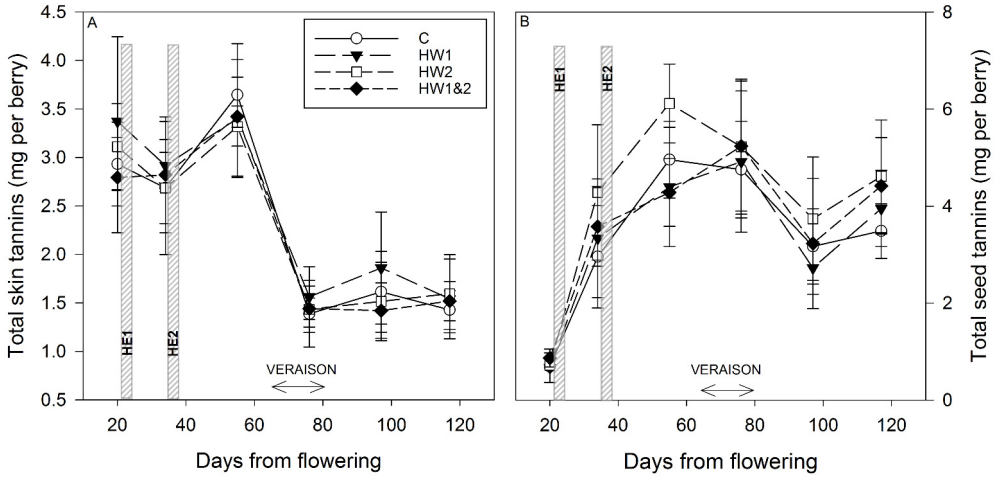


FIGURE 7. Total tannin concentrations in berry skin and seeds
Total skin (A) and total seed (B) tannins (mean ± stdev) measured for each treatment (C: Control; HW1: heated during HE1; HW2: heated during HE2; HW1&2: heated twice) at every sampling date from 20 days after flowering (F+20) until maturity (F+117). Effects of factors (HE1 and/or HE2) were tested by 2-way ANOVA (n=6).

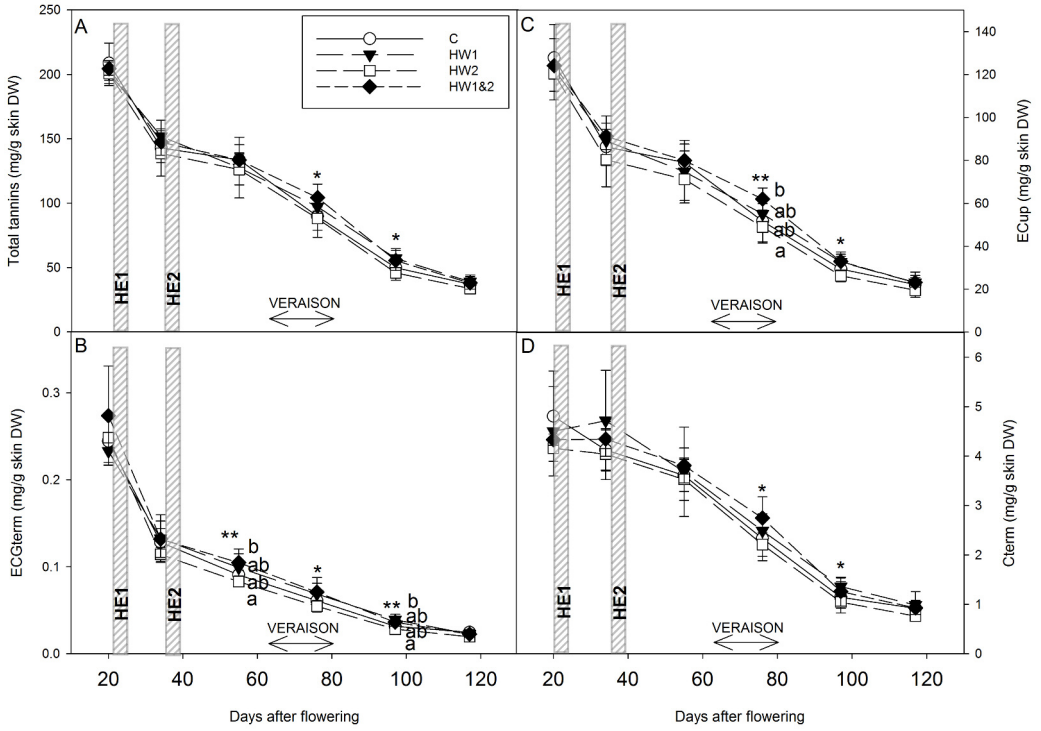


FIGURE 8. Skin tannin detailed composition

Total skin tannins, and upper (up) and terminal (term) subunit evolution (mean ± stdev) of epicatechin gallate (ECG), epicatechin (EC) and catechin (C) from baseline sampling, 20 days after flowering (F+20) until harvest (F+117) measured for each treatment (C: Control; HW1: heated during HE1; HW2: heated during HE2; HW1&2: heated twice). Effects of factors (HE1 and/or HE2) were tested by 2-way ANOVA (n=6) for each sampling date and * indicates significant effect of HE1, # indicates significant effect of HE2 and X for interactions (*, **, *** indicate significant effect at $p<0.05$, 0.01, 0.001, respectively).

F+97 compared to C. This increase was concomitant with an increase in four out of the nine tannin subunits, analysed after polymer cleavage, upper (up) and terminal (term): (-)-epicatechin (ECup), (+)-catechin (Cup and Cterm) and (-)-epicatechin gallate (ECGterm). ECGterm was the most impacted by HE1 for three consecutive sampling dates (F+55, 76, 97) with significant increases of HW1&2 at F+55 and HW1 at F+97 compared to C (Figure 8B). ECup, which was the most abundant subunit detected in skin with concentrations spanning 120-20 mg/g skin, was significantly increased by HE1 at F+76 and F+97 and HW1&2 exhibited 1.2 times more ECup than C at F+76 (Figure 8C). Cterm was significantly increased by HE1 at F+76 and F+97 (Figure 8D) and Cup was also significantly increased by HE1 at F+76 (data not shown). Despite the increase of ECGterm, the percentage of galloylation which is the proportion of ECG (upper and terminal) was not significantly affected and spanned 4 to 5.1 % (data not shown). In addition, tannin size, characterised by the mean degree of polymerisation (mDP), which averaged 36 at F+20, decreased to 28 at F+34 and then stabilised around 26-30 from F+55 until maturity, was not affected by any of the HEs (data not shown). HE2 did not affect skin tannins at any sampling dates and any effect observed on any tannin composition and parameters during the experiment had disappeared by harvest.

Detailed seed tannin composition was also examined on a DW basis and observed differences are shown in Figure 9. At F+20, prior to HE1, non-heated (C and HW2 combined) and heated vines (HW1 and HW1&2 combined) exhibited a similar total concentration of 364 and 367 mg, respectively. When measured at F+34, 10 days after HE1 and before HE2 was applied, small significant differences in total tannins were found with an increase of about 10 % for heated vines compared to non-heated. At this sampling date, (-)-epicatechin subunits represented about 65 % of the subunits found in seeds and both ECup and ECterm were significantly increased in heated vines, while no differences were observed prior to HE1 at F+20. Many parameters (total, mDP, galloylation) were also examined after HE2 but no other effects of HE1 were found and HE2 did not seem to have affected seed tannins at all.

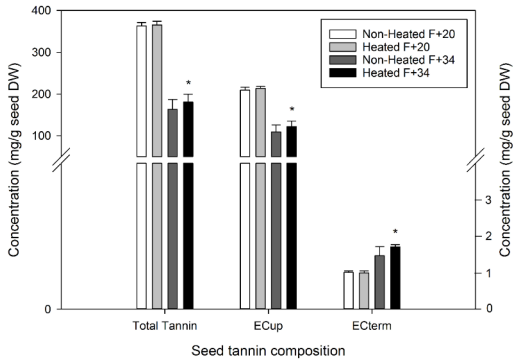


FIGURE 9. Seed tannin detailed composition
Total seed tannin and epicatechin upper (ECup) and terminal (ECterm) subunit evolution from baseline sampling 20 days after flowering (F+20) to after the 1st heat event (HE1) at F+34. The treatments (C: Control; HW1: heated during HE1; HW2: heated during HE2; HW1&2: heated twice) were combined and the effect of HE1 was tested by t-test (n=12) between heated (HW1 and HW1&2) and non-heated (C and HW2) and * indicates significant effect at $p<0.05$.

DISCUSSION

1. System performance and relevance of temperature treatments

The system was designed to heat whole individual potted grapevines without affecting leaf or bunch exposure to direct sunlight. Stable temperature differences over day and night were maintained between heated and non-heated vines while the heating was active. In previous studies, a number of effective heating systems have been used, but a significant reduction in, or an absence of, UV reaching the vines could have affected the accumulation rate of the secondary metabolites of interest (Greer and Weston, 2010; Soar *et al.*, 2009; Sweetman *et al.*, 2014) as light quantity and quality are known to play an important role in flavonoid biosynthesis (Downey *et al.*, 2006; Downey *et al.*, 2004; Ristic *et al.*, 2007; Spayd *et al.*, 2002). The design of the current system, with hot air blown in at the base of the vine and vented upwards, did create a vertical temperature gradient of about 10 °C. While heat damage to trunks was prevented with insulation, shoots were not protected and a small number of leaves and bunches were damaged (not sampled).

Although vines in this study were offset relative to the phenology of field-grown vines due to extended dormancy in a cool room, vines in the surrounding Riverina region of New South Wales are considered to be exposed to unusual high temperature during berry development when maxima above 32-35 °C are recorded in November and 35-36 °C in December (Perkins, 2015). November 2009 experienced one of the most intense springtime heatwaves observed in the last decade in the Riverina, with temperatures between 35 and 42 °C recorded for several days in a row in Wagga Wagga (Bureau of Meteorology). The temperatures tested during this experiment were slightly higher to simulate more intense HEs as predicted by climate scientists but remained realistic with seasonal field conditions.

2. Physiology and composition parameters affected by the first heat event

HE1 was applied after flowering, right after the start of berry setting (E-L 31), targeting an important berry growth stage of cell division and expansion (Ollat *et al.*, 2002). Berries, which were very small and green, were immediately affected in several ways with heat stress modifying both primary and secondary metabolism in all tissues. While Soar *et al.* (2009)found that field-grown Shiraz upregulated gas exchange under heat stress, our potted vine photosynthesis was reduced as previously observed in controlled environment experiments (Edwards *et al.*, 2011; Greer and Weston, 2010). For the first time, a direct effect of high temperature at whole-vine level is reported on detailed tannin composition with skin tannins the most affected and a small effect on seed tannins. Studies looking at the effect of high temperature at this particular phenological stage on whole-vines are rare and results on tannin accumulation are inconsistent depending on the heating system, intensity, duration and grape variety (Gouot *et al.*, 2019). Some studies reported there was no effect on tannins when berries were heated during the whole season (Pastore *et al.*, 2017) or for 30 days (Mori *et al.*, 2004). However, Bonada *et al.* (2015) found that skin and seed total tannins were decreased at harvest by long-term elevated temperature regardless of the irrigation regime. In our study, some responses were immediate after HE1 such as seed total tannins and epicatechin concentrations but then disappeared during berry development. The decrease in epicatechin upper subunit, the most abundant in seeds, observed in heated vines was not as pronounced as in non-heated vines, hence a significantly higher total seed tannin concentration was observed in heated vines. This finding suggests that seed development might have been slowed down during the three days of heat stress where photosynthesis was significantly reduced and disrupted tannin decline rather than biosynthesis. Skin tannin also responded to HE1 but changes appeared later during berry ripening with an increase in total tannins. Cohen *et al.* (2008) also found an increase in total skin tannins when Merlot bunches were heated at night for six weeks

during berry development but did not find the effect consistent across seasons (Cohen *et al.*, 2012b). Here, Shiraz grapevines showed an elastic response to short heat stress in regards to tannin accumulation as both skin and seeds were affected at some point but differences were no longer found at maturity. Finally, no effects on anthocyanin concentration and profile were found as biosynthesis was not yet triggered in the berries when HE1 was applied. This is in agreement with other studies on Cabernet Sauvignon reporting that anthocyanins were not affected by high temperature when applied during early berry development (Buttrose *et al.*, 1971; Lecourieux *et al.*, 2017).

3. Moderate impact of the second heat event

Despite a similar average temperature for heated vines during both HEs, the maximum temperature reached during HE2 was just above 40 °C compared to 45.5 °C during HE1. HE2 was subsequently less intense than HE1 and did not disturb berry physiology, with no photosynthetic differences and berry weight responses to heat stress. As a result, the effect on primary and secondary metabolisms was minimal. It is suggested that the lack of response in berry growth is more likely due to a lower heating intensity rather than the phenological stage targeted for this second heat application. Whole Semillon vines heated for four days/nights at 40/25 °C at fruit set did not exhibit any berry weight differences (Greer and Weston, 2010) and a previous study on Shiraz reported a reduction in berry weight when maximum temperatures spanned 42.1-47.5 °C during one season but no effect the 2nd season with maxima ranging only 35.3-41.5 °C (Soar *et al.*, 2009). Except for some minor primary metabolites (tyrosine, cysteine, palmitic acid) which increased due to high temperature, and lactic acid and cellobiose that decreased, HE2 had no effect on major primary metabolites nor any of the secondary metabolites, such as skin and seed tannins. While HE1 had a minor effect on tannin accumulation, it is not surprising that these parameters were not affected under less extreme high temperature, especially on 3-year old Shiraz already acclimated to very hot climate. In addition, HE2 was applied later in berry development, four weeks after the start of flowering, when tannin biosynthesis slows for both skin and seeds (Bogs *et al.*, 2005). Nevertheless, HE2 may have impacted anthocyanins and led to an increase in tri-hydroxylated anthocyanins by harvest. The ratio of di- to tri-hydroxylated flavonoids can be modulated by the regulation of two biosynthetic genes known as flavonoid hydroxylase *F3'H* and *F3'5'H* (Bogs *et al.*, 2006); hence results observed in our study could be explained by an increase of gene expression of *F3'5'H* under high temperature. However, in the literature, most studies reported a down-regulation of *F3'5'H* and subsequent lower proportion of tri-hydroxylated anthocyanins or no effect (Azuma *et al.*, 2012; Carbonell-Bejerano *et al.*, 2013; Lecourieux *et al.*, 2017; Mori *et al.*, 2007). In only one study, the *F3'5'H-F3'H* ratio was increased prior to véraison after high night temperature during the whole berry development period (Cohen *et al.*, 2012a).

4. Interaction between the two heat events

Despite a very small effect of HE2 due to lower extremes recorded, vines which experienced two consecutive HEs (HW1&2) were significantly affected for some parameters. In this instance, it is more difficult to interpret the effect of the interaction as the effect of the 2nd factor (HE2) depends on the response after HE1. To our knowledge, there has been no studies on cumulative effects, within season, of repeated HE on grapevines. Most recent studies have focused on testing a single heat stress applied at different phenological stages (Degu *et al.*, 2016; Greer and Weston, 2010; Koshita *et al.*, 2015; Lecourieux *et al.*, 2017). Only one study, designed as an experiment to test the additive effect of heat stress on wheat, applied consecutive treatments to the same plants and could not find any interactions between the two heat events (Wollenweber *et al.*, 2003). In our experiment, an increase in malic acid was observed for HW1&2 which was, in total, heated for six non-consecutive days and nights. A previous experiment on Australian Shiraz found that day and night heating for 11 days at E-L 31, which are the closest conditions to our double HEs, increased malic acid accumulation (Sweetman *et al.*, 2014). In these sets of experiments, the increase was associated with high night temperature pre-véraison. The increase in titratable acidity at harvest was probably related with the increase in malic acid for HW1&2 as no other organic acids (tartaric and minor acids) were affected. Based on these findings, high temperature pre-véraison would potentially have some positive impact on berry composition for winemaking as rising temperatures associated with climate change will likely result in a more rapid loss of acidity after véraison and higher juice pH. The double HEs seemed to have also slightly delayed the onset of véraison without impacting on the phenolic profile and sugar ripeness by harvest, similar to a long-term heating experiment conducted on Australian Shiraz (Sadras and Moran, 2012) and 14 days of heating prior to véraison on Cabernet Sauvignon (Lecourieux *et al.*, 2017).

CONCLUSION

HE1 was more extreme than HE2 and had consequently more effect on berry physiology and composition. Berry physiology and primary and secondary metabolisms were immediately affected after HE1 where a maximum temperature of 45 °C was recorded on day 1. While HE2 had a very small effect on composition, the additive effect of the two HEs was mainly evident for sugar and acid maturity. HW1&2 berries seemed to be delayed and exhibited a higher acidity by harvest than C berries. In addition to previous work on short-term heat stress, skin and seed tannins were examined in detail during berry development and ripening, with skin tannin composition significantly impacted by HE1, but not HE2. HE1 was imposed at the end of the main tannin biosynthesis period in skin and seemed to have affected epicatechin gallate in particular. Seed physiology and tannin composition were mostly affected right after HE1 but without further effect of either HE1 or HE2.

Increasing climate variability, with more frequent extreme events such as heatwaves can be a threat for viticulture in Australia. However, solutions are available to minimise the effects of heat and well-irrigated Shiraz grapevines showed that they could adapt to a short-term heat stress of 45 °C during berry development and remain unaffected at a temperature just above 40 °C. The application of shade cloth or hydrocooling system (overhead or undervine sprinklers) in addition to irrigation and soil and cover crop management could be used to mitigate effects of extreme heat by reducing the temperature by 3 to 5 °C. Protecting bunches from direct sunlight which could lead to higher berry surface temperature is also critical and canopy management practices as well as avoiding as much canopy damage as possible will ensure natural shading of berries.

Acknowledgements: This work was supported by Charles Sturt University Postgraduate Research Scholarship (CSUPRS) and the National Wine and Grape Industry Centre (NWGIC). The NWGIC is a research centre within CSU in alliance with the Department of Primary Industries New South Wales (DPI NSW) and the NSW Wine Industry Association. The authors would like to thank Winifred Holzapfel, Lorenzo Pazzi and David Foster for their help throughout the glasshouse trial with sampling and measurements and Mandy Schoeler for her assistance in the laboratory.

REFERENCES

Azuma A., Yakushiji H., Koshita Y. and Kobayashi S., 2012. Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. *Planta, 236*(4), 1067-1080. doi:10.1007/s00425-012-1650-x

Bogs J., Downey M.O., Harvey J.S., Ashton A.R., Tanner G.J. and Robinson S.P., 2005. Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevine leaves. *Plant Physiology, 139*(2), 652-663. doi:10.1104/pp.105.064238

Bogs J., Ebadi A., McDavid D. and Robinson S.P., 2006. Identification of the flavonoid hydroxylases from grapevine and their regulation during fruit development. *Plant Physiology, 140*(1), 279-291. doi:10.1104/pp.105.073262

Bonada M., 2014. *The impact of water deficit and high temperature on berry biophysical traits and berry and wine chemical and sensory traits*. PhD thesis, The University of Adelaide. https://digital.library.adelaide.edu.au/dspace/bitstream/2440/86745/8/02whole.pdf

Bonada M., Buesa I., Moran M.A. and Sadras V.O., 2018. Interactive effects of warming and water deficit on Shiraz vine transpiration in the Barossa Valley, Australia. *OENO One, 52*(2), 189-202. doi:10.20870/oeno-one.2018.52.2.2141

Bonada M., Jeffery D.W., Petrie P.R., Moran M.A. and Sadras V.O., 2015. Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. *Australian Journal of Grape and Wine Research, 21*(2), 240-253. doi:10.1111/ajgw.12142

Bonada M. and Sadras V.O., 2015. Review: critical appraisal of methods to investigate the effect of temperature on grapevine berry composition. *Australian Journal of Grape and Wine Research, 21*(1), 1-17. doi:10.1111/ajgw.12102

Bureau of Meteorology. Daily Extremes. 25/02/2019. Retrieved from http://www.bom.gov.au/cgi-bin/climate/extremes/daily_extremes.cgi

Buttrose M.S. and Hale C.R., 1973. Effect of temperature on development of the grapevine inflorescence after bud burst. *American Journal of Enology and Viticulture, 24*(1), 14-16.

Buttrose M.S., Hale C.R. and Kliewer W.M., 1971. Effect of temperature on the composition of 'Cabernet Sauvignon' berries. *American Journal of Enology and Viticulture, 22*(2), 71-75.

Carbonell-Bejerano P., Santa María E., Torres-Pérez R., Royo C., Lijavetzky D., Bravo G., Aguirreolea J., Sánchez-Díaz M., Antolín M.C. and Martínez-Zapater J.M., 2013. Thermotolerance responses in ripening berries of *Vitis vinifera* L. cv Muscat Hamburg. *Plant*

and Cell Physiology, 54(7), 1200-1216. doi:10.1093/pcp/pct071

Cohen S.D., Tarara J.M., Gambetta G.A., Matthews M.A. and Kennedy J.A., 2012a. Impact of diurnal temperature variation on grape berry development, proanthocyanidin accumulation, and the expression of flavonoid pathway genes. *Journal of Experimental Botany, 63*(7), 2655-2665. doi:10.1093/jxb/err449

Cohen S.D., Tarara J.M. and Kennedy J.A., 2008. Assessing the impact of temperature on grape phenolic metabolism. *Analytica Chimica Acta, 621*(1), 57-67. doi:10.1016/j.aca.2007.11.029

Cohen S.D., Tarara J.M. and Kennedy J.A., 2012b. Diurnal temperature range compression hastens berry development and modifies flavonoid partitioning in grapes. *American Journal of Enology and Viticulture, 63*(1), 112-120. doi:10.5344/ajev.2011.11015

Coombe B.G., 1987. Influence of temperature on composition and quality of grapes. *Acta Horticulturae, 206*, 23-35. doi:10.17660/ActaHortic.1987.206.1

Coombe B.G., 1995. Growth stages of the grapevine: adoption of a system for identifying grapevine growth stages. *Australian Journal of Grape and Wine Research, 1*(2), 104-110. doi:10.1111/j.1755-0238.1995.tb00086.x

Degu A., Ayenew B., Cramer G.R. and Fait A., 2016. Polyphenolic responses of grapevine berries to light, temperature, oxidative stress, abscisic acid and jasmonic acid show specific developmental-dependent degrees of metabolic resilience to perturbation. *Food Chemistry, 212*, 828-836. doi:10.1016/j.foodchem.2016.05.164

de Rosas I., Ponce M.T., Malovini E., Deis L., Cavagnaro B and CavagnaroP., 2017. Loss of anthocyanins and modification of the anthocyanin profiles in grape berries of Malbec and Bonarda grown under high temperature conditions. *Plant Science, 258*, 137-145. doi:10.1016/j.plantsci.2017.01.015

Downey M.O., Dokoozlian N.K. and Krstic M.P., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *American Journal of Enology and Viticulture, 57*(3), 257-268. https://www.ajevonline.org/content/ajev/57/3/257.full.pdf

Downey M.O., Harvey J.S. and Robinson S.P., 2004. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Australian Journal of Grape and Wine Research, 10*(1), 55-73. doi:10.1111/j.1755-0238.2004.tb00008.x

Dunn G.M. and Martin S.R., 2000. Do temperature conditions at budburst affect flower number in *Vitis vinifera* L. cv. Cabernet-Sauvignon? *Australian Journal of Grape and Wine Research, 6*(2), 116-124. doi:10.1111/j.1755-0238.2000.tb00169.x

Edwards E.J., Smithson L., Graham D.C. and Clingeleffer P.R., 2011. Grapevine canopy response to a high-temperature event during deficit irrigation. *Australian Journal of Grape and Wine Research, 17*(2), 153-161. doi:10.1111/j.1755-0238.2011.00125.x

Gouot J.C., Smith J.P., Holzapfel B.P., Walker A.R. and Barril C., 2019. Grape berry flavonoids: a review of their biochemical responses to high and extreme high temperatures. *Journal of Experimental Botany, 70*(2), 397-423. doi:10.1093/jxb/ery392

Greer D.H. and Weston C., 2010. Heat stress affects flowering, berry growth, sugar accumulation and photosynthesis of *Vitis vinifera* cv. Semillon grapevines grown in a controlled environment. *Functional Plant Biology, 37*(3), 206-214. doi:10.1071/FP09209

Hall A. and Jones G.V., 2010. Spatial analysis of climate in winegrape□growing regions in Australia. *Australian Journal of Grape and Wine Research, 16*(3), 389-404. doi:10.1111/j.1755-0238.2010.00100.x

Kennedy J.A. and Jones G.P., 2001. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *Journal of Agricultural and Food Chemistry, 49*(4), 1740-1746. doi:10.1021/jf001030o

Kliewer W.M., 1977. Effect of high temperatures during the bloom-set period on fruit-set, ovule fertility, and berry growth of several grape cultivars. *American Journal of Enology and Viticulture, 28*(4), 215-222.

Koshita Y., Mitani N., Azuma A. and Yakushui H., 2015. Effect of short-term temperature treatment to clusters on anthocyanin and abscisic acid content in the peel of 'Aki Queen' grape. *Vitis, 54*(4), 169-173. doi:10.5073/vitis.2015.54.169-173

Lambert M., Meudec E., Verbaere A., Mazerolles G., Wirth J., Masson G., Cheynier V. and Sommerer N., 2015. A high-throughput UHPLC-QqQ-MS method for polyphenol profiling in rosé wines. *Molecules, 20*(5), 7890-7914. doi:10.3390/molecules20057890

Lecourieux F., Kappel C., Pieri P., Charon J., Pillet J., Hilbert G., Renaud C., Gomès E., Delrot S. and Lecourieux D., 2017. Dissecting the biochemical and transcriptomic effects of a locally applied heat treatment on developing Cabernet-Sauvignon grape berries. *Frontiers in Plant Science, 8*, 53. doi:10.3389/fpls.2017.00053

Majer P. and Hideg É., 2012. Developmental stage is an important factor that determines the antioxidant responses of young and old grapevine leaves under UV irradiation in a green-house. *Plant Physiology*

and Biochemistry, 50, 15-23. doi:10.1016/j.plaphy.2011.09.018

Mori K., Goto-Yamamoto N., Kitayama M. and Hashizume K., 2007. Effect of high temperature on anthocyanin composition and transcription of flavonoid hydroxylase genes in 'Pinot noir' grapes (*Vitis vinifera*). *The Journal of Horticultural Science and Biotechnology, 82*(2), 199-206. doi:10.1080/14620316.2007.11512220

Mori K., Sugaya S. and Gemma H., 2004. Regulatory mechanism of anthocyanin biosynthesis in 'Kyoho' grape berries grown under different temperature conditions. *Environment Control in Biology, 42*(1), 21-30. doi:10.2525/ecb1963.42.21

Ollat N., Carde J.-P., Gaudillère J.-P., Barrieu F., Diakou-Verdin P. and Moing A., 2002. Grape berry development: a review. *OENO One, 36*(3), 109-131. doi:10.20870/oeno-one.2002.36.3.970

Pagay V. and Collins C., 2017. Effects of timing and intensity of elevated temperatures on reproductive development of field-grown Shiraz grapevines. *OENO One, 51*(4), 409-421. doi:10.20870/oeno-one.2017.51.4.1066

Pastore C., Dal Santo S., Zenoni S., Movahed N., Allegro G., Valentini G., Filippetti I. and Tornielli G.B., 2017. Whole plant temperature manipulation affects flavonoid metabolism and the transcriptome of grapevine berries. *Frontiers in Plant Science, 8*, 929. doi:10.3389/fpls.2017.00929

Perkins S., 2015. Scorcher. 02/02/2019. Retrieved from http://scorcher.org.au

Perkins S.E. and Alexander L.V., 2013. On the measurement of heat waves. *Journal of Climate, 26*(13), 4500-4517. doi:10.1175/JCLI-D-12-00383.1

Perkins-Kirkpatrick S. and Pitman A., 2018. Extreme events in the context of climate change. *Public Health Research & Practice, 28*(4), e2841825. doi:10.17061/phrp2841825

Perkins-Kirkpatrick S.E., White C.J., Alexander L.V., Argüeso D., Boschat G., Cowan T., Evans J.P., Ekström M., Oliver E.C.J., Phatak A. and Purich A., 2016. Natural hazards in Australia: heatwaves. *Climatic Change, 139*(1), 101-114. doi:10.1007/s10584-016-1650-0

Petrie P.R. and Clingeleffer P.R., 2005. Effects of temperature and light (before and after budburst) on inflorescence morphology and flower number of Chardonnay grapevines (*Vitis vinifera* L.). *Australian Journal of Grape and Wine Research, 11*(1), 59-65. doi:10.1111/j.1755-0238.2005.tb00279.x

Pillet J., 2011. *Impact du microclimat sur le métabolisme de la baie de raisin*. PhD thesis, Bordeaux 2 University. https://www.theses.fr/2011BOR21863.pdf

Pillet J., Berdeja M., Guan L and Delrot S., 2015. Berry response to water, light and heat stresses. In H. Géros, Chaves, M.M., Medrano Gil, H., and Delrot, S. (Eds.), *Grapevine in a changing environment: a molecular and ecophysiological perspective* (pp. 223-257). Chichester, UK: John Wiley & Sons, Ltd. doi:10.1002/9781118735985.ch10

Pinasseau L., Vallverdú-Queralt A., Verbaere A., Roques M., Meudec E., Le Cunff L., Peros J.-P., Ageorges A., Sommerer N., Boulet J.-C., Terrier N. and Cheynier V., 2017. Cultivar diversity of grape skin polyphenol composition and changes in response to drought investigated by LC-MS based metabolomics. *Frontiers in Plant Science, 8*, 1826. doi:10.3389/fpls.2017.01826

Pinasseau L., Verbaere A., Roques M., Meudec E., Vallverdú-Queralt A., Terrier N., Boulet J.-C., Cheynier V. and Sommerer N., 2016. A fast and robust UHPLC-MRM-MS method to characterize and quantify grape skin tannins after chemical depolymerization. *Molecules, 21*(10), 1409. doi:10.3390/molecules21101409

Reshef N., Walbaum N., Agam N. and Fait A., 2017. Sunlight modulates fruit metabolic profile and shapes the spatial pattern of compound accumulation within the grape cluster. *Frontiers in Plant Science, 8*, 70. doi:10.3389/fpls.2017.00070

Ristic R., Downey M.O., Iland P.G., Bindon K., Francis I.L., Herderich M. and Robinson S.P., 2007. Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. *Australian Journal of Grape and Wine Research, 13*(2), 53-65. doi:10.1111/j.1755-0238.2007.tb00235.x

Rossouw G., Holzapfel B., Rogiers S., Gouot J. and Schmidtke L., 2019. Repercussions of four different herbicides on reproductive and vegetative development in potted grapevines. *Australian Journal of Grape and Wine Research*. doi:10.1111/ajgw.12395

Rossouw G.C., Orchard B.A., Šuklje K., Smith J.P., Barril C., Deloire A. and Holzapfel B.P., 2017. *Vitis vinifera* root and leaf metabolic composition during fruit maturation: implications of defoliation. *Physiologia Plantarum, 161*(4), 434-450. doi:10.1111/ .12604

Sadras V.O., Bubner R. and Moran M.A., 2012. A large-scale, open-top system to increase temperature in realistic vineyard conditions. *Agricultural and Forest Meteorology, 154–155*, 187-194. doi:10.1016/j.agrformet.2011.11.005

Sadras V.O. and Moran M.A., 2012. Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. *Australian Journal of Grape and Wine Research, 18*(2), 115-122. doi:10.1111/j.1755-0238.2012.00180.x

Sadras V.O. and Soar C.J., 2009. Shiraz vines maintain yield in response to a 2–4 °C increase in maximum temperature using an open-top heating system at key phenostages. *European Journal of*

Agronomy, 31(4), 250-258. doi:10.1016/j.eja.2009.09.004

Soar C.J., Collins M.J. and Sadras V.O., 2009. Irrigated Shiraz vines (*Vitis vinifera*) upregulate gas exchange and maintain berry growth in response to short spells of high maximum temperature in the field. *Functional Plant Biology, 36*(9), 801-814. doi:10.1071/FP09101

Spayd S.E., Tarara J.M., Mee D.L. and Ferguson J.C., 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *American Journal of Enology and Viticulture, 53*(3), 171-182. https://www.ajevonline.org/content/ajev/53/3/171.full.pdf

Sweetman C., Sadras V.O., Hancock R.D., Soole K.L. and Ford C.M., 2014. Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *Journal of Experimental Botany, 65*(20), 5975-5988. doi:10.1093/jxb/eru343

Tarara J.M., Ferguson J.C. and Spayd S.E., 2000. A chamber-free method of heating and cooling grape clusters in the vineyard. *American Journal of Enology and Viticulture, 51*(2), 182-188.

Wollenweber B., Porter J.R. and Schellberg J., 2003. Lack of interaction between extreme high-temperature events at vegetative and reproductive growth stages in wheat. *Journal of Agronomy and Crop Science, 189*(3), 142-150. doi:10.1046/j.1439-037X.2003.00025.x