Effect of Heat Waves and Irrigation Practices on Grape Berry Phenolics and Transcriptomics of Cabernet Sauvignon Grapes

By

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE
in

Viticulture & Enology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2023

Abstract

Current climate conditions indicate that California's viticultural regions will experience more frequent heat events, thus leading to greater water stress resulting in lower quality fruit in grapevines. One strategy used to help mitigate the negative effects is irrigating throughout these events to help preserve berry composition. In order to evaluate the usefulness of irrigation throughout heat events, *Vitis vinifera* cv. Cabernet Sauvignon vines in an established vineyard were exposed to three differential irrigation treatments. In 2020, the baseline treatment was under water deficit (60% ET), while the 2x baseline (120% ET) and 3x baseline (180% ET) treatments had double and triple the irrigation of the baseline, respectively. The irrigation treatments were adjusted in 2021 to see if less water could be used without negatively impacting the phenolics results. The treatments were 60% ET, 90% ET, and 120% ET. Differential irrigation started one to two days prior to a heat wave (HW) and continued until the last day of the HW. In this study, a HW was defined as three consecutive days with maximum temperatures at or above 38°C. Throughout the 2020 and 2021 growing seasons, there were major declines in anthocyanin concentration for the 60% ET treatment, which suggests a suppression of anthocyanin synthesis and promotion of degradation at such high temperatures. A common trend seen throughout both years is that the 180% and 120% treatments from 2020 and 2021 respectively, resulted in lower phenolic concentrations than the 120% and 90% treatments. This is important to note as these results suggest that irrigation prior to heatwaves can be beneficial in maintaining fruit quality, but excessive watering may negate the beneficial aspects of deficit irrigation. Transcriptomic analyses identified the down-regulation of genes involved in the phenylpropanoid and flavonoid pathways, which provides a potential explanation at the molecular level for the lower phenolic

concentrations. This study highlights the detrimental effects of too little or excess water application during HWs on grape berry composition and gene expression.

1. Introduction

According to current climate projections, we face an increase in the intensity, frequency, and duration of heat waves (HWs) in the coming years (IPCC, 2013). Therefore, it is imperative that the grape and wine industry study the effects of these heat events on different winegrowing regions around the world. Extreme temperatures can have detrimental effects on grapevines including but not limited to decreases in yield, unwanted changes in berry composition, and decreases in overall grape quality (Venios et al., 2020). High temperatures cause increased water loss via evapotranspirative cooling and overall stress on grapevines, so irrigation practices can be useful in mitigating the negative impacts of HWs by altering vine water status, leaf and berry temperature. Shade cloths, cover crops, rootstock selection, changes in row orientation and trellis system to protect from solar radiation with misting, or increased irrigation throughout HWs, are further strategies being implemented in current wine regions to mitigate their adverse effects (Marigliano et al., 2023).

However, with growing water scarcity, a more efficient use of water and a deeper understanding of the effects of HWs and water use during different grapevine phenological stages will be required (Falkenmark, 2013). Although grapevines are resilient crops that can tolerate drought and extreme temperatures (Martinez-Luscher et al., 2020), it is important to explore alternative grape cultivars that may be better suited for these warmer scenarios.

Two widely used irrigation methods are regulated deficit irrigation (RDI) and partial root-zone drying (PRD) (Chaves et al., 2007). In RDI, irrigation is reduced or completely stopped for specific periods during the growing season. A study done in South Australia showed

that water deficit after flowering resulted in the "greatest reduction in berry weight compared with that of well-watered vines" (McCarthy, 1997). This is important to note because this may not have been the result had water deficit been practiced before flowering, thus showing that timing is crucial. In turn, water deficit after veraison only had a minor effect on berry weight at maturity and berries were not affected by water deficit during the month before harvest. With PRD, half of the root system is maintained in a dry state while the other half is irrigated (Chaves et al., 2007). The theory behind PRD is that the watered roots maintain a favorable plant water status, while the dry roots result in chemical signals, such as increases in abscisic acid (ABA) production, that are transported to leaves to reduce growth and therefore vigor.

Deficit irrigation has two main effects on grape berry composition: a decrease in berry size and the upregulation of genes in the phenylpropanoid and flavonoid pathways. In terms of berry size, the skin to pulp ratio increases, which causes phenolic compounds to become more concentrated. In terms of transcriptomics, the upregulation of genes in the phenylpropanoid and flavonoid pathways causes an increase in anthocyanin synthesis due to signaling from an increase in abscisic acid (ABA) (Roby et al., 2004; Roby & Matthews, 2008). Alternatively, the effect of deficit irrigation on tannins is largely due to a reduction in berry size rather than an impact on their biosynthesis. (Roby et al., 2004; Lizama et al., 2021). It is important to note that the beneficial aspects of deficit irrigation may not be the same in the future due to projected climate warming, and the combined effects of greater heat and water stress with deficit irrigation may be detrimental to berry quality (Bonada et al., 2015).

Berry development follows a double sigmoidal curve, which is divided into three stages (Coombe, 1992). The first phase occurs after fruit set and is characterized by cell division and cell expansion (Panagiotis et al., 2012). This is then followed by a lag phase, which is a period of

little to no growth, but is characterized by a rapid accumulation of organic acids, particularly malic acid (Coombe and McCarthy, 2000). The last stage is characterized by the onset of veraison (Panagiotis et al., 2012). During this second growth phase, berries soften, accumulate sugar, and grow larger. In red varieties, anthocyanins begin to synthesize, allowing for the red and purple pigments to show in the skins (Coombe and McCarthy, 2000). High temperatures affect berry development primarily at post fruit set, veraison, and mid ripening (Greer & Weston, 2010). A decrease in berry size before veraison caused by high temperatures is due to effects on cell division (Kliewer, 1977), whereas post veraison, this decrease is likely due to a stop in cell expansion and an increase in transpiration. Closer to maturity, high temperature seems to be linked to cell death, loss in berry mass, and increased water loss, leading to shriveling and sunburn (Greer and Weedon, 2013).

With global warming, increasing mean temperatures are further correlated with an earlier onset of phenological stages in the grapevine and the shortening of the duration of these stages (Venios et al., 2020). Moreover, since most viticultural regions are currently at or near their optimal growing temperatures for the grape cultivars grown there (Jones et al., 2005), global warming intensifies the pressure of exploring new varieties that better suit these regions. Additionally, the effects of HWs on grapevines will depend on the timing of the heat event during specific phenological stages. Although the effects of elevated temperatures throughout the growing season vary by cultivar, literature has consistently shown that flowering is a period that is more sensitive to heat, and the length of the interval from budburst to flowering is more susceptible to a decrease than other phenological intervals. (Cameron et al., 2022; Merrill et al., 2020). During grape development, fruit set shows resistance to elevated temperatures, whereas veraison and mid-ripening are more sensitive to heat (Greer & Weston, 2010).

From a berry chemistry perspective, exposure to high temperatures has an important impact on primary and secondary metabolite production in the berry. Studies have shown that grape berry metabolism is sensitive to both day and nighttime temperatures and the magnitude of these diurnal temperature changes (Cohen et al., 2008 and Yan et al., 2020). Primary metabolites of grape berries include sugars, amino acids, and organic acids. While they contribute to the support of normal growth and reproduction, secondary metabolites serve ecological functions, such as defense to abiotic or biotic pressures (Blancquaert et al., 2019). Among the secondary metabolites produced by the grapevine, phenolic compounds and aromatic compounds are of major interest due to their impact on grape and wine quality. The Shikimate, phenylpropanoid, and flavonoid pathways are responsible for the biosynthesis of the different phenolic compounds that can be found in grapes (Blancquaert et al., 2019; Dewick & Haslam, 1969; Heller & Forkmann, 1988). Grape phenolics can be divided into two groups: non-flavonoids and flavonoids. Flavonoids are most relevant to wine quality and are divided into three groups: flavan-3-ols, anthocyanins, and flavonols (Waterhouse et al., 2016 and Cohen et al., 2008). Flavan-3-ols are mainly present in the form of proanthocyanidins and contribute to the bitterness and astringency of wine (Cohen et al., 2008). Anthocyanins are responsible for the color of red wine (Cohen et al., 2008), and flavonols act as UV protectants and copigments (Downey et al., 2003). All three groups of flavonoids are affected by environmental factors including high temperature in different ways.

Flavonols are synthesized from the flavonoid biosynthetic pathway and also give rise to anthocyanins and proanthocyanidins (Downey et al., 2003). They are primarily located in the skin and mainly function as UV protectants (Flint et al., 1985; Smith and Markham, 1998) and as copigments with anthocyanins to form stable pigments (Asen et al., 1972; Scheffeldt and

Hrazdina 1978). The major flavonol compounds found in grapes include quercetin, myricetin, and kaempferol (Makris et al., 2006). Flavonol synthesis begins at flowering, reaches peak concentrations after veraison, and decreases during development as the berries increase in size (Flamini et al., 2013). Flavonol synthesis is light dependent, and sunlight has a greater impact on development than temperature does. While shading has modest effects on berry development, it significantly decreases flavonol synthesis (Downey et al., 2008). It has been found that high temperatures don't have a significant impact on flavonol content when compared to other grape berry metabolites (Cohen et al., 2008; Gouot et al., 2019a). Gouot et al., (2019b) studied the combined effects of high temperature duration and intensity on phenolic metabolism of Shiraz berries. They found that flavonol content of berries exposed to 46 °C showed no significant difference to those exposed to 35 °C. However, flavonols were degraded in berries exposed to 54 °C. This shows that high temperature has indirect effects on flavonol levels, while sunlight remains the key influencing factor.

Anthocyanins are pigmented molecules responsible for the color of red wines and begin their synthesis in grape berries after veraison, reach maximum values close to maturity, and then decrease (Bautista-Ortín et al., 2016; Pastore et al., 2017). Previous studies have shown that high temperatures affect anthocyanin levels in two ways: inhibiting anthocyanin biosynthesis and promoting degradation (Mori et al., 2007; Movahed et al., 2016; Ryu et al., 2020). Literature suggests that anthocyanin accumulation in grapes is more influenced by temperature rather than light when photon fluxes are above 100 μmol/m^2/s (Downey et al., 2006). Anthocyanins reach critical metabolic temperature for synthesis around 30ºC (Spayd et al., 2002), but signs of inhibition begin beyond this temperature, where reduction in the activity of enzymes involved in the phenylpropanoid and flavonoid pathways, such as phenylalanine ammonia lyase (PAL),

VIMYBA2, and UDP-glucose flavonoid-3-O-glucosyltransferase (UFGT), have been observed (Ryu et al., 2020). Additionally, it has been found that while anthocyanins were suppressed at transcriptional and enzymatic levels, peroxidase activity had increased, suggesting that peroxidase plays a key role in degradation (Movahed et al., 2016). Studies carried out in Cabernet Sauvignon by Mori et al., (2007) found that biosynthesis of anthocyanins was not affected by high temperatures and the decrease of anthocyanin levels was mainly due to chemical and enzymatic degradation.

Flavan-3-ols are the most abundant phenolic compounds in grapes and wine (Teixeira et al., 2013), and they are composed of monomeric catechins and oligomeric or polymeric proanthocyanidins (Padilla-Gonzalez et al., 2022). In terms of sensory effects, proanthocyanidins are responsible for astringency and bitterness perceived in grapes and wine (Teixeira et al., 2013). These compounds also have antioxidant properties (Hanlin et al., 2010) and interact with anthocyanins to form stable pigments (Waterhouse et al., 2016). Proanthocyanidins are found in both the skin and seeds of grape berries (Hanlin et al., 2010). Tannin synthesis in the grape berry starts at flowering, continues in the early stages of grape development, and reaches its maximum around veraison (Downey et al., 2003; Teixeira et al., 2013). It then decreases throughout ripening due to a reduction in the extractability of tannins related to their interactions with cell wall components, rather than a decrease related to degradation (Blancquaert et al., 2019; Cheyneir et al., 1997 Kennedy et al., 2001). The effect of high temperatures on skin and seed tannins have shown inconsistent results across different studies. Cohen et al., (2008) found a linear relationship between the increase of skin tannin content and heat accumulation during phase I of grape development. However, Gouot et al., (2019) found an increase of total skin tannin concentration in vines exposed to short periods of heat stress before veraison, but found

no differences in skin tannin concentration and content at maturity. Studies where heat was applied after veraison have shown no effect on tannin levels, which could be associated with the stop of tannin synthesis after this point in development (Mori et al., 2004; Pastore et al., 2017). Lastly, Bonada et al., (2015) conducted a field experiment where vines were exposed to elevated temperatures throughout the entire growing season and found a decrease in total tannin content and concentration at maturity. This was mainly related to a decrease of seed tannins while skin tannins remained unresponsive to elevated temperatures. The different experimental conditions as well as differences in heat treatment design and time of application could be possible explanations for the discrepancies of these findings, as well as whether concentration or content of tannins was studied.

Flavonols, anthocyanins, and flavan-3-ols are derived from the same precursors and are produced from the flavonoid biosynthetic pathway (Gouot et al., 2019a). These precursors are derived from the Shikimate and phenylpropanoid pathways. Each step of these pathways are catalyzed by specific enzyme families. Flavonoid enzymes include flavone synthase (FS), flavanone-3ß-hydroxylase (F3H), and flavonol synthase (FLS). Two enzymes, flavonoid-3'-hydroxylase (F3'H) and flavonoid-3'-5'-hydroxylase (F3'5'H), are particularly important because they catalyze the reactions that form di- and tri-hydroxylated flavonoids from monohydroxylated precursors. The genes that code for F3'H and F3'5'H are expressed in the berry skin and contribute to the production of di- and tri-hydroxylated flavonols, anthocyanins, cyanidins, and delphinidin derivatives (Gouot et al., 2019). The main enzymes for flavan-3-ol production are anthocyanidin reductase (ANR) and leucoanthocyanidin reductase (LAR). However, the enzymes involved in producing proanthocyanidins are unknown. The enzyme that converts anthocyanidins to anthocyanins is UDP-glucose flavonoid-3-O-glucosyltransferase

(UFGT). It is also important to note the MYB transcription factors that are involved in the regulation of the phenylpropanoid pathway. MYBF1 regulates FLS for flavonol production. MYBA1&2 and MYB4 regulate UFGT for anthocyanin synthesis, with MYBA1&2 also being involved in several other steps in the pathway (Vogt 2010).

The effects of high temperatures on the phenylpropanoid and flavonoid pathways is variable, and dependent upon the phenological stage during which heat events occur. Veraison is the most sensitive stage for anthocyanins, while flowering is the most sensitive stage for tannins (Lorrain et al., 2011). Although it has been widely reported that high temperatures impact anthocyanin synthesis, gene expression does not consistently reflect concentration. A study done by Mori et al. (2005) on the variety Darkridge showed that high night temperature resulted in lower anthocyanin concentration due to a downregulation of genes including F3'H and UFGT. Similar results were seen in Kyoho grapes in that high day and night temperatures down-regulated UFGT (Mori et al., 2004). However, when the berries were exposed to high temperatures only at night, there was an initial decrease then sudden increase in UFGT activity during ripening. In contrast, a study done on Cabernet Sauvignon showed that UFGT was not strongly down-regulated in grapes that were exposed to high temperatures (Mori et al., 2007). Studies show that the effects of high temperatures on the expression of MYB transcription factors remains inconsistent, particularly with MYBA1. Some studies have shown that the expression of MYBA1 was unaffected by high temperatures (Carbonell-Bejerano et al., 2013 and Lecourieux et al., 2017), whereas other studies showed an extreme down-regulation of this transcription factor (Movahed et al., 2016 and Yamane et al., 2006).

Currently, there is a lack of published research that investigates the potential effects of HWs on berry temperature. While there have been studies published that look at how elevated

temperatures affect berry development, primary metabolism, and secondary metabolism, they did not collect data measuring temperature of the berries themselves. For example, in a study done by Cohen et al. (2008), individual cluster temperatures were manipulated to assess the impact of temperature on phenolic metabolism. While this study provided insight to the direct effects of high temperatures, it failed to show whether heat events result in higher berry temperature under different irrigation regimes, and if so, to what extent.

The objective of this study was to evaluate the impact that different irrigation practices have on berry phenolics and gene expression carried out prior to and during HWs in a commercial vineyard of Cabernet Sauvignon. The results from this study provide a better understanding of how irrigation during heat events can help mitigate the effects of extreme temperatures and lead to more efficient water use. In addition, by studying how HWs and irrigation practices influence the production of metabolites in the berry at the chemical and molecular levels provides the wine industry new knowledge that can be used to direct future efforts at preserving berry and wine quality under climate change conditions.

2. Materials and methods

Field Site

This study was conducted during the 2020 and 2021 growing seasons in a commercial vineyard located in northeastern Lodi, CA (latitude: 38° 17' 37.1" N, longitude: 121° 06' 39.7" W, elevation: 40-50 masl). This site holds 23 acres of *Vitis vinifera* cv. Cabernet sauvignon grafted on 1103 Paulsen. Rows are oriented East-West with a vine spacing of 1.8 m and a row spacing of 3 m. The trellis system is a bilateral cordon with a single high wire. The canopy is not held by any support wires, so it falls to the sides creating natural shading for the fruit. Pruning, hedging, and harvest are mechanized.

The 23 acres are divided into a total of 98 pixels of 30 m x 30 m with a variable rate drip irrigation (VRDI) system allowing the application of a differential irrigation to each individual pixel (Sanchez et al., 2017). Each pixel has an average of 170 vines and the drip irrigation hoses are situated on the ground along the pixels and are connected to controller boxes that have valves which regulate the flow of irrigation on each pixel (Fig. S2). The addition of nutrients is carried out through the drip irrigation system.

Experimental Design and Treatment Application

In this experiment, a HW was defined as three consecutive days with maximum temperatures at or above 38 °C (Fig. S1). Rather than creating artificial HWs with a heating system, this study relied on the occurrence of natural HWs. There were four HWs during the 2020 growing season, and there were two HWs during the 2021 growing season.

Differential irrigation treatments were applied when HWs occurred and started one or two days before each HW and continued until the last day of the HW. There were three irrigation treatments: a baseline, which was exposed to deficit irrigation and held at 60% ET, a second treatment where irrigation was double the baseline (2x baseline, 120% ET), and a third treatment where irrigation was triple the baseline (3x baseline, 180% ET). In 2021, the irrigation treatments were adjusted in order to fine tune the amount of water used to see if less water could be used while maintaining the positive aspects of increased irrigation during HWs. The baseline treatment stayed the same at 60% ET, the second treatment was 1.5x baseline (90% ET), and the third treatment was 2x baseline (120% ET). Four pixels (30 m x 30 m) per treatment were randomly selected and distributed along the site using the VRDI system to provide differential irrigation to the individual plots. All measurements performed were carried out on three of the

four plots of each treatment. ET was estimated throughout the season using Landsat data, normalized difference vegetation index (NDVI) and crop coefficient.

Variations in pre-treatment irrigation were implemented at the beginning of the growing season on the plots to even out vigor based on NDVI and thermal imaging due to the heterogeneous soil profile of the site. After, differences in irrigation schedules were based completely on implemented treatments.

Berry Temperature

Berry temperature was measured using 0.076 mm diameter type 'E' thermocouples (OMEGA Engineering, Stamford, CT, USA). There were a total of 20 thermocouples placed in individual berries within three of the treatment blocks. The thermocouples were inserted into the center of the berries in exposed clusters facing the east and west side of the vine, and at each side of the vine 4 thermocouples were placed in different berries within the cluster. Because berries could develop necrosis from being punctured by the thermocouple, thermocouples were relocated to adjacent exposed berries at least every 12 days to maintain relatively fresh conditions (Bailey & Ponce de Leon, 2021).

Berry Sampling

When HWs occurred, berries were sampled before, during, and after the heat event. Forty berries per vine (20 berries on each side of the canopy) were collected from different clusters and sides of the cluster for a total of 360 berries (9 vines) per treatment plot for each sampling point during the HW.

After sampling, a subset of 180 berries was divided into three replicates of sixty berries. The pulp and seeds were removed by squeezing the berries, and the skins were flash-frozen in liquid nitrogen and stored at -80 °C until they were ground for molecular extractions.

Reagents for phenolic extractions and analysis

Acetone (99.9% HPLC grade), acetonitrile (99.9% HPLC grade), (-)-epicatechin (90% Purity by HPLC) from Sigma-Aldrich (St. Louis, MO, USA), malvidin-3-*O*-glucoside chloride from Sigma-Aldrich (St. Louis, MO, USA), quercetin-3-*O*-glucoside from from Sigma-Aldrich (St. Louis, MO, USA), acetaldehyde (99% Reagent Plus), methyl cellulose, sodium metabisulfite, and ammonium sulfate were acquired from Sigma-Aldrich (St. Louis, MO, USA). Ethanol 190 Proof (95%) was purchased from DLI (King of Prussia, PA, USA), and trifluoroacetic acid (HPLC grade) from Sigma-Aldrich (St. Louis, MO, USA).

Exhaustive Extractions

A subset of grape samples were stored at -20 °C for phenolic extraction. Three sets of sixty berries were weighed and volume occupied in water was recorded using a graduated cylinder.Skins were prepared for extraction by separating pulp and seed by hand. The skins were homogenized using a T25 digital ULTRA-TURRAX and S 25 N-18 G Dispersing tool. The skins were homogenized for one minute at 14 speed x 1000 rpm with 100 mL of a 66% (v/v) acetone and transferred to an opaque polypropylene jar. The jar's headspace was filled with nitrogen gas, closed with a screw cap lid, and sealed with parafilm. The skins were extracted for 24 hours on a orbital shaker (150 rpm). The next day, samples were centrifuged for 10 minutes at 10,000 rpm. Samples were then poured through a Buchner funnel into a Buchner flask and filtered through Whatman filter paper with a pore size of 1. Each filtered sample was transferred to a round-bottom flask and put onto the rotovap for 10 minutes with the water bath at 33 °C. Acetone was removed under reduced pressure. The extracts were brought back to 50 mL with milli-q water, and volume was recorded using a graduated cylinder. The samples were transferred

to centrifuge tubes and stored at -20 °C until analysis. The next day, the samples were filtered, and acetone was separated from the solution using a rotovap. The samples stayed on the rotovap for 10 minutes with the water bath at 33 °C. The concentrated extracts were diluted with milli-q water, and volume was recorded using a graduated cylinder. The samples were transferred to falcon tubes and stored at -20 °C until analysis.

Methyl Cellulose Precipitation (MCP)

Triplicate grape extracts were analyzed by methyl cellulose precipitation, which is the method used for analyzing tannin in extracts and wine, as previously published (Mercurio et al., 2007; Sarneckis et al. 2006). Briefly, 25 µL of extraction sample was placed into 1200 µL deep well plates and combined with 300 µL of 0.04% (w/v) methyl cellulose (1500 cps) (Sigma Aldrich, St. Louis, MO, USA) solution or water (Control), and mixed on an Thermomixer-C (Eppendorf AG, Hamburg, Germany) for 5 minutes at 1500 RPM and left to stand for 3 minutes. Following the mixing, 200 µL of saturated ammonium sulfate was added to the wells to prevent the re-release of proanthocyanidin (PA) material into solution following precipitation. Water was then added to the wells (475 µL for treatment and 775 µL for the control) and again it was mixed for 5 minutes. The deep well plate was then allowed to stand for 10 minutes before being centrifuged for 5 minutes at 2,272 × g (5804R Eppendorf AG, Hamburg, Germany). Both the treated and control samples were taken (200 µL) and placed in a 96 half-area well plate (Corning, Corning, NY, USA). (-)-Epicatechin (Sigma Aldrich) was used as a quantitative standard. Proanthocyanidin quantification was conducted by calculating Δ280 nm (Control − Treatment) with the linear regression from an (-)-epicatechin standard curve. The samples were analyzed on the SpectraMax iD3 microplate reader. Endpoint analysis was at 280 nm, and a pathway correction made up for volume differences.

Monomeric Anthocyanin and Flavonol Analysis

Anthocyanin and flavonol analysis were conducted using a previously published method with modifications. Briefly, a Phenomenex Kinetex PFP 150 × 3.0 mm column was used with a particle size of 2.6 μm. Samples were injected at a volume of 10 μL with a column temperature of 50 °C. Mobile phase A consisted of milli-q water with 0.2% TFA; mobile phase B consisted of acetonitrile with 0.2% TFA. Eluting peaks were monitored at 365 and 520 nm. Elution conditions were 0.5 mL/min; 12.5% of solvent B for the first 3 minutes; a linear gradient from 12.5% to 20% from 3 to 14 minutes; 20% to 27.3% from 14 to 26 minutes; 27.3% to 70% from 26 to 26.02 minutes; held at 70% for 2 minutes; 70% to 12.5% from 28 to 28.02 minutes. The column was then washed and re-equilibrated for 4 minutes before the next injection. Anthocyanins were quantified using a malvidin-3-*O*-glucoside standard curve (Sigma Aldrich, St. Louis, MO, USA). Flavonols were quantified using a Quercetin-3-*O*-glucoside standard curve (Sigma Aldrich, St. Louis, MO, USA).

Molecular Extractions

A subset of berry skins were stored at -80 °C for molecular extraction. Pulp and seeds were removed prior to freezing. The skins were removed from storage and kept frozen using liquid nitrogen. Using a Tissuelyser II grinder (Qiagen N.V., Hilden, Germany), the skins were ground to a powder, transferred to falcon tubes, and stored at -80 degrees Celsius until RNA extraction was performed.

RNA Sequencing

A subset of the August 2020 HW samples were sent for RNA sequencing. This subset included three replicates of each treatment from the northwest blocks sampled before, during,

and after the HW. The pre-HW date is August 13th, the HW date is August 16th, and the post-HW date is August 22nd.

cDNA libraries were sequenced using an Illumina HiSeqX Ten system as paired-end (PE) 150-bp reads (IDseq, Davis, CA, USA). On average, 19.9 ± 6.5 million sequencing reads were generated per sample (Raw reads; **Table 1**).

Gene Expression Analysis

Removal of adapter sequences, quality trimming, and length filtering were performed using only the first mate with Trimmomatic v.0.36 (Bolger *et al*., 2014) and the following settings: LEADING:3 TRAILING:3 SLIDINGWINDOW:10:20 MINLEN:36 CROP:75. Because sample 1_NW_B1R3 was composed of a higher number of reads compared to the other samples (**Table 1**), the sequencing reads of this sample were randomly down-sampled to 20 million reads with seqtk v.1.0-r57-dirty (https://github.com/lh3/seqtk) and the option -s 100 before trimming. Transcript abundance was quantified with Salmon v.1.5.1 (Patro *et al*., 2017) and the options: --gcBias --seqBias --validateMappings. Transcriptome index file was built using *Vitis vinifera* cv. Cabernet Sauvignon coding sequences (Massonnet *et al*., 2020), Cabernet Sauvignon genome as decoy, and a k-mer size of 31. The sample 1_NW_B3_R2 was removed from the RNA-seq analysis because of its low mapping rate (46.8%; **Table 1**). Quantification files were imported using the R package tximport v.1.20.0 (Soneson *et al*., 2015), and counts were summarized at gene level. Statistical testing of differential expression was performed using DESeq2 v.1.34.0 and default parameters (Love *et al*., 2014). Genes with a Benjamini-Hochberg adjusted *P*-value inferior to 0.05 were considered as significantly differentially expressed between two consecutive dates of collection.

Statistical Analysis

Statistical analysis using one-way ANOVA for total skin proanthocyanidins,, berry skin anthocyanins, and flavonol concentration and two-way ANOVA for berry temperature were carried out using GraphPad Prism 10 software. Interaction term was included and fit a full model. Significant differences among treatments were tested with $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***) as significance levels. Then, a post-hoc Tukey's HSD test was carried out for mean comparison with $p \leq 0.05$ used for minimum significance. Error bars and variability shown in tables and graphs represent mean standard error (SE).

3. Results

Berry Temperature

Berry temperature was recorded across the August 2020 and September 2020 heat waves (Figs. 2 & 3). Berry temperatures from 60% ET treatment were significantly different from the 120% ET and 180% ET treatments for the pre-HW date of August 13th (Fig. 2). There were no significant differences in berry temperature between the three treatments for the August 2020 HW and post-HW dates. For the August 13th pre-HW date, peak berry temperature occurred at 13:00 hours for the 180% ET treatment. For the HW date, peak berry temperature was at 15:00 hours for the 120% ET treatment. For the post-HW date, peak berry temperature was at 17:00 hours for the 60% ET treatment. There were no significant differences among the three treatments during the September 2020 HW (Fig. 3**)**. For the pre-HW date, peak berry temperature was at 15:00 hours for the 60% ET treatment. Similarly, for the HW and post-HW dates, peak berry temperature was at 15:00 hours for the 180% ET treatment. Berries in the August 2020 HW consistently reached higher temperatures than berries in the September 2020 HW, which may be due to differences in the extremity of the respective HWs.

Berry Skin Anthocyanins

Anthocyanins in both growing seasons were measured from pre-veraison until commercial harvest. As seen in Figure 4, in the 2020 growing season, the 60% ET treatment started out with the lowest total anthocyanin concentration and continued throughout the rest of the season. During HW3 and HW4, we see significant differences between the treatments. At harvest, there were no differences between the 120% ET and 180% ET treatments, but both treatments had significantly higher anthocyanin concentrations than the 60% ET treatment, suggesting that the additional water prior to the heat waves had mitigated the loss of anthocyanin material.

Looking at Figure 4, again in 2021, the 60% ET treatment had consistently lower anthocyanin concentrations throughout the season. Unlike the 2020 growing season, the 60% ET treatment remained significantly different from the other two treatments starting at August 17th until close to harvest. However, at harvest, there were no significant differences between the three treatments.

In both 2020 and 2021, there were HWs that occurred mid to late August. Looking at anthocyanin concentration, there was more variation in concentrations among treatments in 2020. Additionally, concentrations were higher in 2021 among the three treatments. The higher anthocyanin concentrations may be due to less extreme HW temperatures, thus less anthocyanin degradation.

Flavonol Concentration

Looking at Figure 5, in 2020, there were no significant differences between the treatments during HW2, but looking at HW3, the 60% ET treatment suffered the most significant losses. At the start of HW4 the 120% ET treatment had significantly higher flavonol

concentrations than the 60% or the 180% treatments. Nevertheless, at harvest there were no significant differences between the three treatments.

In 2021 as seen in Figure 5, there were no significant differences in flavonol concentration until HW2 where there were differences between the 60% and the 120% ET treatments. Interestingly, there was a major decline in concentration for the 120% ET treatment at the start of HW2, and by commercial harvest, this treatment has the lowest concentration. This may be due to differences in light exposure because flavonols develop when exposed to light. The 120% treatment may have had the lowest flavonol concentration at harvest because these vines had the most water applied during the growing season, so there was likely more foliage covering the berries.

Similar to anthocyanin concentration, flavonol concentrations reached higher peaks in the 2021 growing season than in the 2020 growing season. Flavonol concentration went beyond 0.20 mg/B for the 60% ET treatment in 2021, but did not exceed 0.15 mg/B in 2020. This may be due to less extreme HW temperatures in 2021 and more direct sunlight exposure that allowed for increased flavonol development.

Total Skin Proanthocyanidins

The particular effects of temperature on PA biosynthesis is still being explored, but research has shown that temperature impacts the expression of key biosynthetic genes, including ANR and LAR (Poudel et al., 2020). In this study, initial work done in 2019 showed significant differences in PA concentration between irrigation treatments. Therefore, sampling began pre-veraison in the 2020 and 2021 growing seasons to capture data to elucidate differences during PA synthesis.

As seen in Figure 6, the 2020 results show that, following HW2, there was a significant difference between the 60% and 120% treatments. This is also seen at the start HW3, and all treatments saw a marked decrease in PAs leading to no significance between them for the rest of the season until at harvest where there was a difference between the 60% and 180% treatments.

Also seen in Figure 6, in 2021, there was a significant difference between the 90% and 120% ET treatments at the start of HW1 and at the start of HW2. After HW2, significant differences were seen between the 60% and the other two treatments.

Overall, there were more significant differences between the treatments during the 2021 growing season compared to the 2020 growing season. In 2020, PA concentrations were on a similar trend among the three treatments. The larger variations in PA concentrations between treatments in 2021 may be attributed to the severity of the HWs and the impact it had on the grapevines. During the 2021 growing season, the berries got sunburnt due to high exposure, and therefore more irrigation was applied in order to preserve quality.

Differential Gene Expression

Table 1 shows the summary statistics of the RNA sequencing. Sample 1_NW_B3R2 was an outlier and removed from the analysis due to having a higher number of reads compared to the other samples.

For each irrigation treatment, the berry transcriptomes of two consecutive dates of collection were compared to evaluate the number of differentially expressed genes (DEGs; Fig. 7). The purple represents the 60% ET treatment, the teal represents the 120% ET treatment, and the yellow represents the 180% ET treatment. As seen in the figure, there were significantly more genes differentially expressed between the HW vs. pre-HW dates than the HW and

post-HW dates for the 60% ET and 120% ET treatments. Interestingly, it was the opposite for the 180% ET treatment.

For each pairwise comparison of collection dates, the DEGs detected for the three irrigation treatments were compared (Fig. 8). Looking at the top two diagrams, there is a larger number of shared genes unique to the 60% and the 120% treatments compared to those uniquely shared with the 180% treatment. Specifically, there were 550 up-regulated genes shared only between the 60% and 120% treatments versus the 96 (60% ET) and 38 (120% ET) genes only shared with the 180% treatment. Looking at the bottom two diagrams, there is an overall decrease in the number of genes being differentially expressed when comparing the post-HW date relative to the HW date. There is also a higher number of genes being differentially expressed for the 180% treatment with 478 being up-regulated and 605 being down-regulated.

4. Discussion

Effect of Differential Irrigation on Grape Berry Temperature and Composition Across Heat Waves

In terms of berry temperature, significant differences among the treatments were only observed on August 13th, which is the pre-HW date for the August 2020 HW. Measurements recorded throughout the remaining HW dates showed that berry temperature remained similar among the three treatments. These results may be due to the berries being in the shade. In a study done by Ponce de Leon and Bailey (2021), they found that shaded berries tended to have similar temperatures to the ambient air temperature and could reach over 10 °C above ambient temperature when in direct sunlight. There is currently a lack of published literature that explores

the effects of HWs and irrigation on berry temperature, and the results from this study suggest that the two factors do not play a significant role in berry temperature.

There were major declines in anthocyanin concentration in 2020 and 2021 for the 60% ET treatment, which suggests a suppression of anthocyanin synthesis and promotion of degradation at such high temperatures. This finding remains consistent with published literature, such as the work done in Yan et al. (2020). In their study, three experiments were conducted on *Vitis vinifera* L. cv. Merlot, cl. 347 in which the grapevines were exposed to three different temperature regimes. Their results consistently showed that the low-temperature regimes and high-temperature regimes had the highest and lowest anthocyanin levels, respectively. Further evidence of anthocyanin synthesis suppression and promotion of degradation at high temperatures is found in Mori et al. (2004). Similar to the experimental design in Yan et al. (2020), Kyoho grape berries in this study were grown under different temperature conditions. Anthocyanin levels were consistently lower in berries grown under high temperature conditions (30 °C) compared to berries grown at 25 °C or berries grown at 30 °C during the day and 15 °C at night. These results were attributed to a decrease of UFGT activities in the flavonoid pathway, as previously mentioned.

Particular attention should be given to the anthocyanin results seen in 2020 (Fig. 4). By harvest, the 120% ET treatment was significantly different from the 60%, but not significantly different from the 180% treatment. This is important because similar results were seen in anthocyanin concentrations with much less water used for irrigation. These results further support research that has proven the beneficial aspects of deficit irrigation. As seen in Bucchetti et al. (2011) and Roby et al. (2004), deficit irrigation consistently increased anthocyanin concentrations by reducing berry size and thus increasing content per berry. That being said, the

results from this study show that overwatering during HWs is unnecessary when trying to compensate for berry phenolics.

In terms of flavonol concentration, particular attention should be given to the 120% ET treatment in the 2021 growing season (Fig. 5). By harvest, there was a major decline in concentration for this treatment. As previously mentioned, flavonol synthesis is light-dependent, and shading can have notable effects on flavonol concentration (Downey et al., 2008). Since sunlight is a key influencing factor, the decline in flavonol concentration for the 120% ET treatment may be attributed to a denser canopy that provided more shading to the fruit, which inhibited flavonol synthesis.

In the 2020 and 2021 growing seasons, total PA concentration was significantly lower in the 60% ET treatment than the other two treatments by harvest (Fig. 6). Since HWs occurred in both seasons pre-veraison, the treatment differences likely happened during PA synthesis, which occurs from flowering to veraison. The pre-veraison HWs could have impacted PA synthesis in the 60% ET treatment, which was exposed to higher water and heat stress, thus decreasing PA concentration. Although studies have shown variable results on the effects of heat events on PA concentration, PAs are unlikely to degrade due to the high stability of their chemical structure (Teixeira et al., 2013).

A common trend seen throughout both years is that the 180% and 120% treatments from 2020 and 2021 respectively resulted in lower phenolic concentrations than the 120% and 90% treatments. This is important to note because these results suggest that irrigation prior to HWs can be beneficial in maintaining fruit quality, but excessive watering may negate the beneficial aspects of deficit irrigation. In terms of the lower anthocyanin biosynthesis in the 3x and 2.5x treatments, it may be related to plant water status since moderate deficit irrigation has been

shown to increase anthocyanin biosynthesis and promote ripening (Castellarin et al., 2007). Additionally, this may also be due to insufficient light exposure of the clusters since the canopies were more dense with foliage.

Effect of Differential Irrigation on Gene Expression Across Heat Waves

As seen in Figure 7 and Figure 8, there were significantly more genes differentially expressed between the HW vs. pre-HW dates than the HW and post-HW dates for the 60% ET and 120% ET treatments, and it was the opposite for the 180% ET treatment. This may potentially be a combined response to the heat stress and too much irrigation being applied.

The RNA sequencing results showed that there were several down-regulated genes from the phenylpropanoid and flavonoid pathways shared between the pre-, during, and post-HW dates and irrigation treatments. What is potentially being observed is a general repression of core and peripheral phenylpropanoid pathways, which are normally triggered in red-skinned grape berries throughout ripening (Blacno-Ulate et al., 2017). Some of the genes that deserve particular attention include trans-cinnamate 4-monooxygenase (C4H), 4-coumarate-CoA ligase (4CL), chalcone synthase (CHS), flavonol synthase, F3’5’H, and UFGT. C4H and 4CL play key roles in the phenylpropanoid pathway. C4H catalyzes the reaction that forms p-coumaric acid, and 4CL converts p-coumaric acid to 4-Coumaroyl-CoA (Wang et al., 2020). The down-regulation of C4H and 4CL are important to note because 4-Coumaroyl-CoA is an important precursor for compounds produced in the flavonoid pathway, and decreased production of this precursor may lead to overall decreased levels of phenolic compounds. Chalcone synthase is the first key enzyme in the flavonoid pathway, and therefore plays a central role in initiating flavonoid biosynthesis (Wang et al., 2016). The down-regulation of this gene may lead to reduced flavonol levels in grape berries, which is crucial in times of excessive heat and light exposure due to their

nature as UV protectants. Flavonol synthase plays an important role in converting dihydroflavonols to flavonols (Liu et al., 2019). F3'5'H catalyzes flavonoid hydroxylation, which leads to the formation of flavonols, anthocyanins, and PAs (Bogs et al., 2006). UFGT converts anthocyanidins to anthocyanins (Gouot et al., 2019a). The down-regulation of these genes lead to decreased production of phenolic compounds, and such effects have been well-documented (Lecourieux et al., 2017; Mori et al., 2007; Movahed et al., 2016).

5. Conclusion

Implementation of differential irrigation prior to and during HWs were shown to have a major impact on berry composition and gene expression. Damaging effects on berry quality were observed from underwatering and overwatering. Significant changes in berry composition occurred throughout the HWs, including a suppression of anthocyanin synthesis and promotion of degradation, and lower flavonol and PA concentrations in highly irrigated treatments. These berry composition results remained consistent with the gene expression results. The down-regulation of key enzymes involved in the phenylpropanoid and flavonoid pathways, suggest a possible mechanism for the lower phenolic concentrations.

The frequency and duration of extreme heat events predicted for the upcoming decades indicates the need for further field research looking at potential strategies to mitigate the negative effects of heat waves on berry composition and gene expression. Misting, shade cloths, cover crops, canopy management, and trellis systems that protect fruit exposure remain as options to help cope with the effects. Furthermore, studying acclimation within and across seasons of different cultivars to extreme heat events will help guide the grape and wine industry towards cultivars with better adaptations and ultimately higher quality fruit and wine.

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Figures

Figure 1. Borden Hills vineyard with the locations of the selected pixels for each treatment. Baseline treatment (60% ET) is represented by the yellow pixels, 2x Baseline ET the blue, and 3x Baseline ET in purple.

Figure 2. Berry temperature measurements taken from 20 thermocouples as outlined in Ponce de Leon & Bailey (2021) on the three treatments (Baseline, 2x, and 3x) throughout the August 2020 heatwave. pre-HW (August 13th), during HW (August 16th), and post-HW (August 22nd)

Figure 3. Berry temperature measurements taken from 20 thermocouples on the three treatments (Baseline, 2x, and 3x) throughout the September 2020 heatwave. pre-HW (September 4th), during HW (September 8th), and post-HW (September 12th)

Figure 4. Total anthocyanin concentrations reported as mg/Berry during the 2020 and 2021 growing seasons. A, b, and c indicate significance groups, and standard error bars are recorded. The star on HW3 indicates the HW that was used for RNA sequencing.

Figure 5. Total flavonol concentrations reported as mg/Berry during the 2020 and 2021 growing seasons. A, b, and c indicate significance groups, and standard error bars are recorded. The star on HW3 indicates the HW that was used for RNA sequencing.

Figure 6. Total proanthocyanidin concentrations reported as mg/Berry during the 2020 and 2021 growing seasons. A, b, and c indicate significance groups, and standard error bars are recorded. The star on HW3 indicates the HW that was used for RNA sequencing.

Figure 7. The berry transcriptomes of two consecutive dates of collection were compared to evaluate the number of differentially expressed genes. The two bar graphs on the top are showing the number of upregulated and downregulated genes between the three treatments when comparing the heatwave date which is August 16th to the pre-heatwave date which is August

13th. The two bar graphs on the bottom are showing us the same thing but comparing the post-heatwave date which is August 22nd to the heatwave date of August 16th.

Figure 8. The berry transcriptomes of two consecutive dates of collection were compared to evaluate the number of differentially expressed genes. The venn diagrams show the number of overlapping genes between the treatments.

Figure 1.

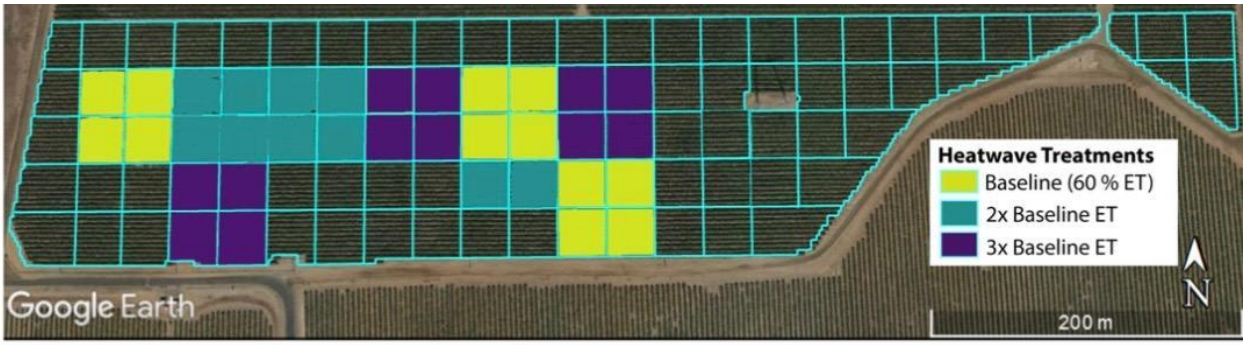


Figure 2.

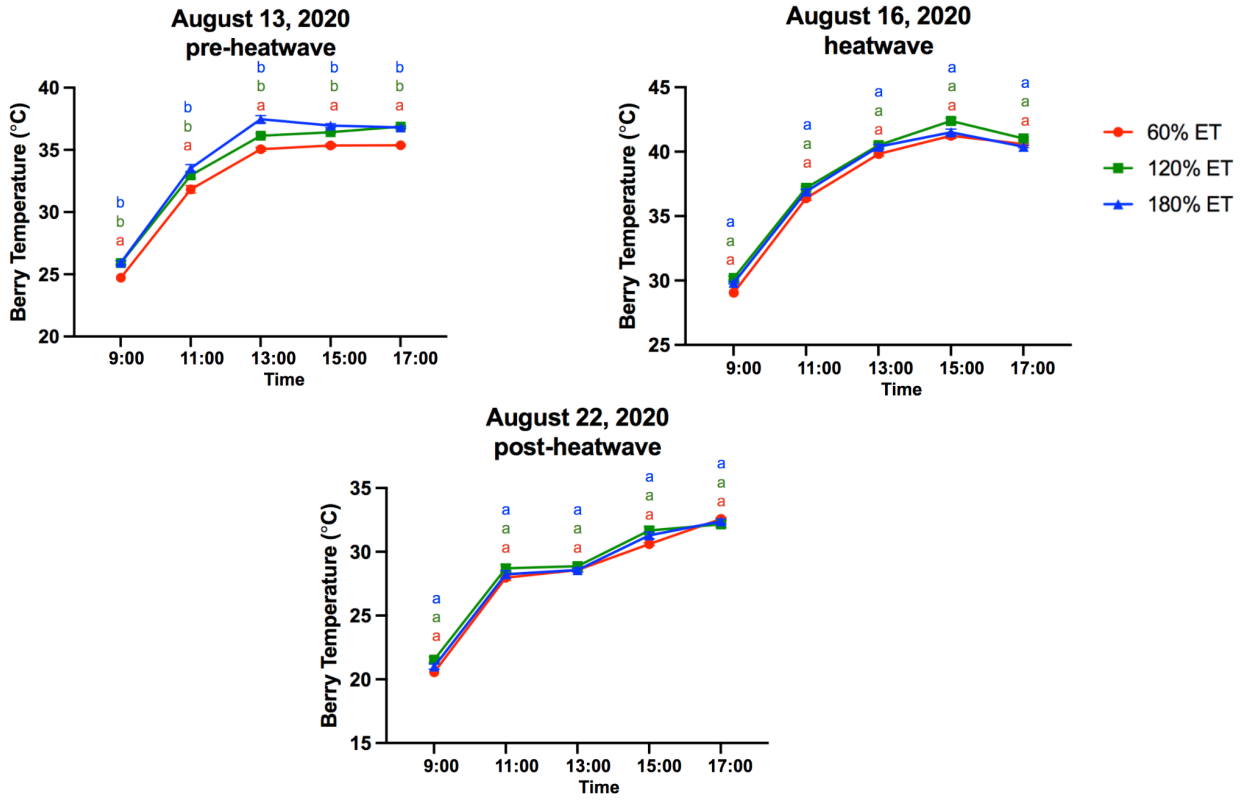


Figure 3.

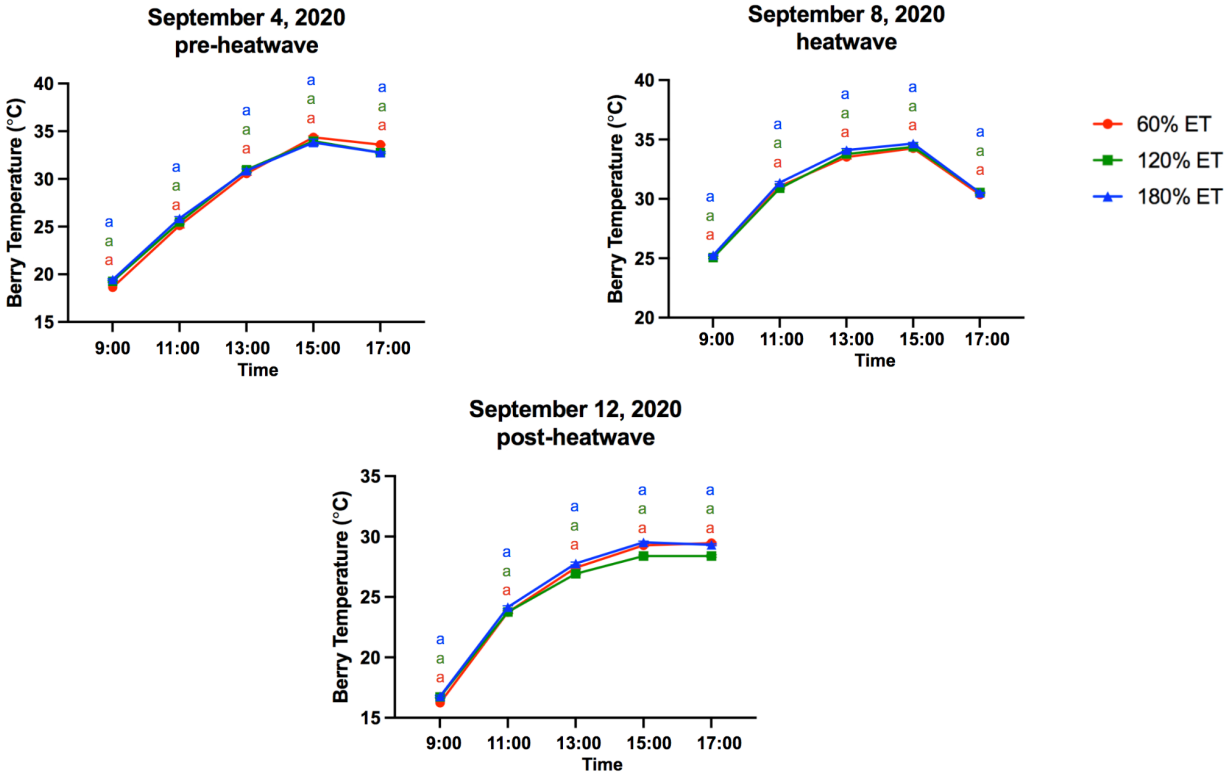


Figure 4.

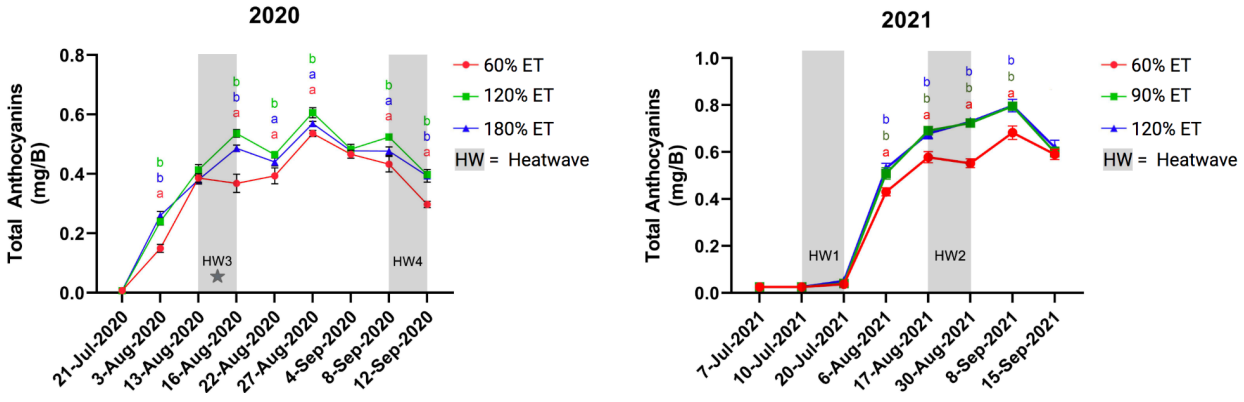


Figure 5.

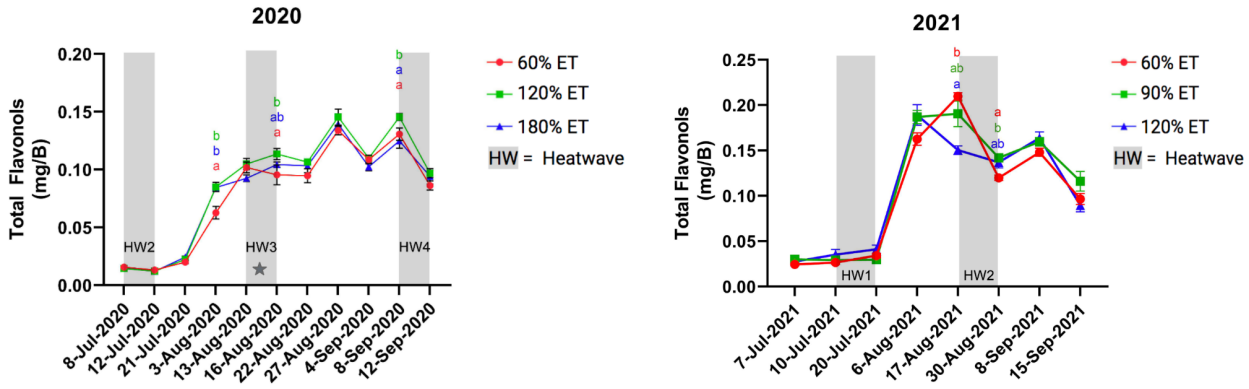


Figure 6.

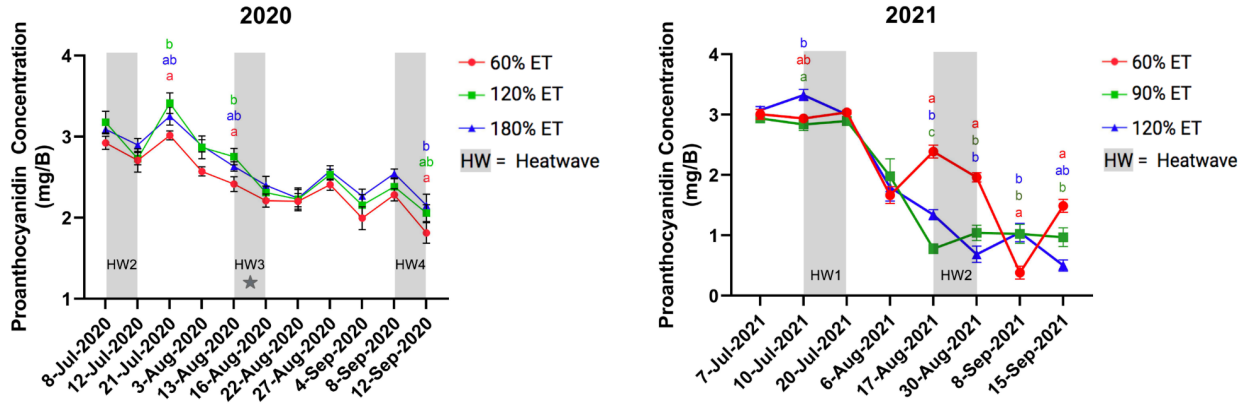


Figure 7.

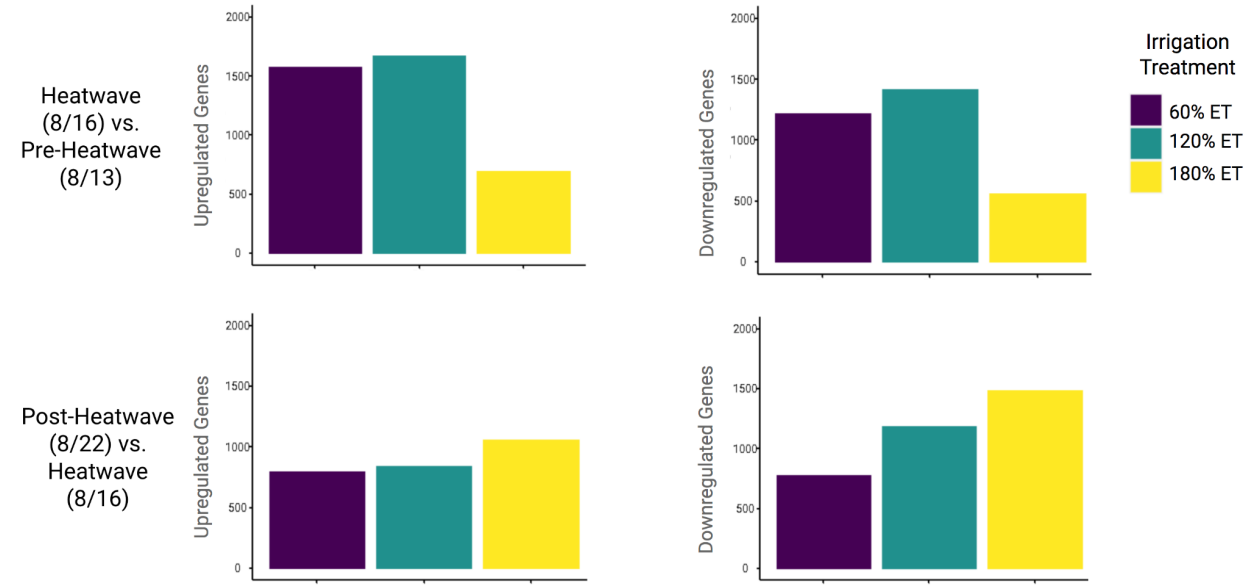
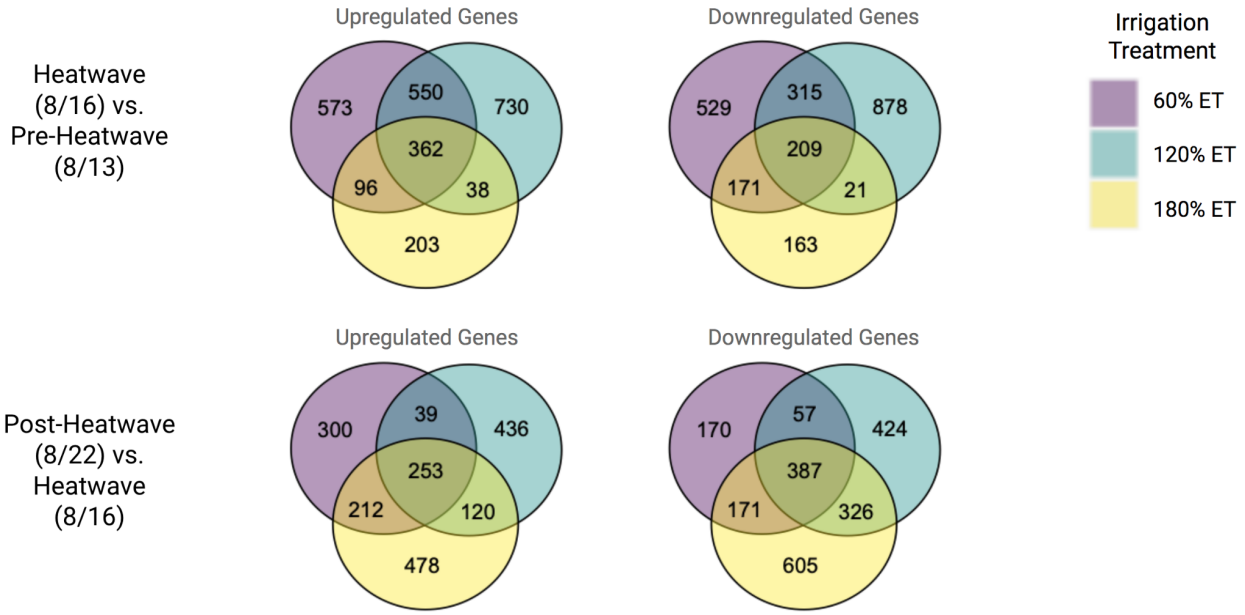


Figure 8.



Tables

Table 1. Summary statistics of RNA sequencing performed on the subset of samples from the August 2020 HW. Sample 1_NW_B3R2 was an outlier and removed from the analysis due to having a higher number of reads compared to the other samples.

Table 1.

Date	Sample_ID	Raw reads (PE 150bp)	High-quality reads (SE 75 bp)	%	Mapped on CS CDS	%
8/13/20	1_NW_B1R1	19,688,584	19,657,453	99.8	13,451,623	68.4
8/13/20	1_NW_B1R3	45,090,827	19,966,733	99.8	13,330,617	66.8
8/13/20	1_NW_B1R4	21,633,088	21,597,126	99.8	14,728,068	68.2
8/13/20	1_NW_B2R1	16,126,486	16,084,954	99.7	10,534,314	65.5
8/13/20	1_NW_B2R2	21,650,959	21,610,210	99.8	14,403,659	66.7
8/13/20	1_NW_B2R3	30,336,355	30,284,473	99.8	20,293,363	67.0
8/13/20	1_NW_B3R1	15,779,910	15,743,042	99.8	11,009,878	69.9
8/13/20	1_NW_B3R2	28,756,826	28,714,641	99.9	13,428,270	46.8
8/13/20	1_NW_B3R3	24,082,787	24,043,315	99.8	15,599,369	64.9
8/16/20	2_NW_B1R1	16,389,361	16,363,102	99.8	11,071,308	67.7
8/16/20	2_NW_B1R3	15,723,910	15,699,195	99.8	10,612,535	67.6
8/16/20	2_NW_B1R4	17,110,873	17,085,405	99.9	10,943,572	64.1
8/16/20	2_NW_B2R1	19,469,089	19,440,240	99.9	13,071,888	67.2
8/16/20	2_NW_B2R2	17,002,773	16,978,735	99.9	11,644,896	68.6
8/16/20	2_NW_B2R3	15,471,088	15,442,595	99.8	10,335,843	66.9
8/16/20	2_NW_B3R1	20,070,084	20,036,507	99.8	13,630,065	68.0
8/16/20	2_NW_B3R2	14,631,717	14,610,005	99.9	9,862,962	67.5
8/16/20	2_NW_B3R3	13,579,074	13,558,979	99.9	8,520,975	62.8
8/22/20	3_NW_B1R1	14,061,615	14,034,822	99.8	9,583,917	68.3
8/22/20	3_NW_B1R3	18,384,151	18,322,562	99.7	13,228,597	72.2
8/22/20	3_NW_B1R4	16,976,839	16,951,914	99.9	11,696,777	69.0
8/22/20	3_NW_B2R1	21,929,998	21,898,355	99.9	15,160,216	69.2
8/22/20	3_NW_B2R2	14,684,192	14,658,967	99.8	10,077,461	68.7
8/22/20	3_NW_B2R3	20,629,437	20,599,863	99.9	14,334,996	69.6
8/22/20	3_NW_B3R1	16,767,841	16,732,797	99.8	11,339,845	67.8
8/22/20	3_NW_B3R2	22,053,897	22,018,608	99.8	14,968,309	68.0
8/22/20	3_NW_B3R3	18,280,532	18,251,572	99.8	12,606,675	69.1

Supplemental Figures

Figure S1. Number of 3-day heat waves with maximum temperatures above 38 ºC for the years 2070-2099 relative to data from 1981-2010 (CMIP5 multi model mean – RCP 8.5). Areas highlighted in blue correspond to Paso Robles, Lodi, and Napa/Sonoma.

Figure S2. Diagram of variable drip irrigation system at Borden Hills (Sanchez et al., 2017).

Figure S1.

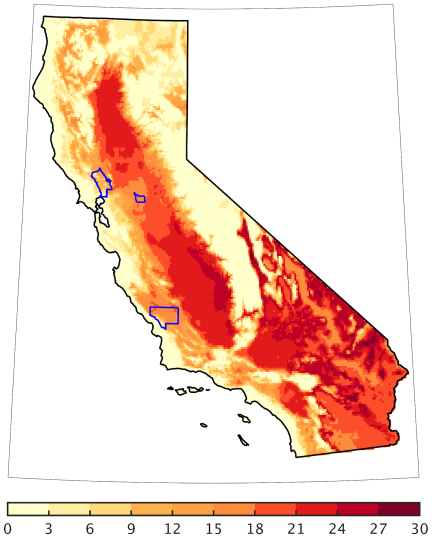
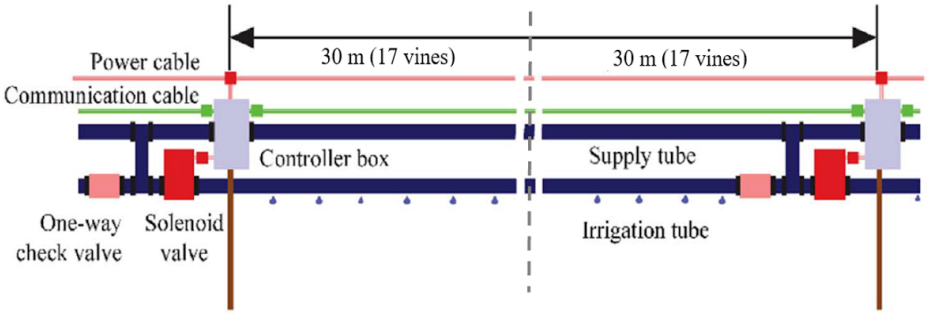


Figure S2.



Supplemental Tables

Table S1. ANOVA results of the effect of differential irrigation treatments on berry total anthocyanin concentration on August 3, 2020.

Table S2. ANOVA results of the effect of differential irrigation treatments on berry total anthocyanin concentration on August 16, 2020.

Table S3. ANOVA results of the effect of differential irrigation treatments on berry total anthocyanin concentration on August 22, 2020.

Table S4. ANOVA results of the effect of differential irrigation treatments on berry total anthocyanin concentration on August 27, 2020.

Table S5. ANOVA results of the effect of differential irrigation treatments on berry total anthocyanin concentration on September 8, 2020.

Table S6. ANOVA results of the effect of differential irrigation treatments on berry total anthocyanin concentration on September 12, 2020.

Table S7. One-way ANOVA results of the effect of differential irrigation treatments on berry total proanthocyanidin concentration on July 21, 2020.

Table S8. One-way ANOVA results of the effect of differential irrigation treatments on berry total proanthocyanidin concentration on August 13, 2020.

Table S9. One-way ANOVA results of the effect of differential irrigation treatments on berry total flavonol concentration on August 3, 2020.

Table S10. One-way ANOVA results of the effect of differential irrigation treatments on berry total flavonol concentration on September 8, 2020.

Table S11. One-way ANOVA results of the effect of differential irrigation treatments on berry total anthocyanin concentration on August 6, 2021.

Table S12. One-way ANOVA results of the effect of differential irrigation treatments on berry total anthocyanin concentration on August 17, 2021.

Table S13. One-way ANOVA results of the effect of differential irrigation treatments on berry total anthocyanin concentration on August 30, 2021.

Table S14. One-way ANOVA results of the effect of differential irrigation treatments on berry total anthocyanin concentration on September 8, 2021.

Table S15. One-way ANOVA results of the effect of differential irrigation treatments on berry total proanthocyanidin concentration on July 10, 2021.

Table S16. One-way ANOVA results of the effect of differential irrigation treatments on berry total proanthocyanidin concentration on August 17, 2021.

Table S17. One-way ANOVA results of the effect of differential irrigation treatments on berry total proanthocyanidin concentration on August 30, 2021.

Table S18. One-way ANOVA results of the effect of differential irrigation treatments on berry total proanthocyanidin concentration on September 8, 2021.

Table S19. One-way ANOVA results of the effect of differential irrigation treatments on berry total proanthocyanidin concentration on September 15, 2021.

Table S20. One-way ANOVA results of the effect of differential irrigation treatments on berry total flavonol concentration on August 17, 2021.

Table S21. One-way ANOVA results of the effect of differential irrigation treatments on berry total flavonol concentration on August 30, 2021.

Table S22. Two-way ANOVA results of the effect of differential irrigation treatments and time on berry temperature for August 13, 2020.

Table S23. Two-way ANOVA results of the effect of differential irrigation treatments and time on berry temperature for August 16, 2020.

Table S24. Two-way ANOVA results of the effect of differential irrigation treatments and time on berry temperature for August 22, 2020.

Table S25. Two-way ANOVA results of the effect of differential irrigation treatments and time on berry temperature for September 4, 2020.

Table S26. Two-way ANOVA results of the effect of differential irrigation treatments and time on berry temperature for September 8, 2020.

Table S27. Two-way ANOVA results of the effect of differential irrigation treatments and time on berry temperature for September 12, 2020.

Table S1.

Date		Df	Sum Sq	Mean Sq	F	P value
3 Aug 2020	Between Treatments	2	0.06274	0.03137	21.26	<0.0001
	Residual (within treatments)	24	0.03542	0.001476		
	Total	26	0.09816			

Table S2.

Date		Df	Sum Sq	Mean Sq	F	P value
16 Aug 2020	Between Treatments	2	0.1290	0.06449	17.41	<0.0001
	Residual (within treatments)	23	0.08519	0.003704		
	Total	25	0.2142			

Table S3.

Date		Df	Sum Sq	Mean Sq	F	P value
22 Aug 2020	Between Treatments	2	0.02367	0.01183	3.994	0.0318
	Residual (within treatments)	24	0.07111	0.002963		
	Total	26	0.09477			

Table S4.

Date		Df	Sum Sq	Mean Sq	F	P value
27 Aug 2020	Between Treatments	2	0.02203	0.01101	8.257	0.0019
	Residual (within treatments)	24	0.03201	0.001334		
	Total	26	0.05404			

Table S5.

Date		Df	Sum Sq	Mean Sq	F	P value
8 Sep 2020	Between Treatments	2	0.03769	0.01885	6.768	0.0047
	Residual (within treatments)	24	0.06683	0.002785		
	Total	26	0.1045			

Table S6.

Date		Df	Sum Sq	Mean Sq	F	P value
12 Sep 2020	Between Treatments	2	0.05801	0.02901	14.2	P<0.0001
	Residual (within treatments)	24	0.04904	0.002043		
	Total	26	0.1070			

Table S7.

Date		Df	Sum Sq	Mean Sq	F	P value
21 July 2020	Between Treatments	2	0.7214	0.3607	3.782	0.0373
	Residual (within treatments)	24	2.289	0.09537		
	Total	26	3.010			

Table S8.

Date		Df	Sum Sq	Mean Sq	F	P value
13 Aug 2020	Between Treatments	2	0.5323	0.2662	4.004	0.0316
	Residual (within treatments)	24	1.595	0.06647		
	Total	26	2.128			

Table S9.

Date		Df	Sum Sq	Mean Sq	F	P value
3 Aug 2020	Between Treatments	2	0.002903	0.001451	9.291	0.0010
	Residual (within treatments)	24	0.003749	0.0001562		
	Total	26	0.006652			

Table S10.

Date		Df	Sum Sq	Mean Sq	F	P value
8 Sep 2020	Between Treatments	2	0.002026	0.001013	4.427	0.0236
	Residual (within treatments)	23	0.005263	0.0002288		
	Total	25	0.007289			

Table S11.

Date		Df	Sum Sq	Mean Sq	F	P value
6 Aug 2021	Between Treatments	2	0.05029	0.02514	6.371	0.0060
	Residual (within treatments)	24	0.09472	0.003947		
	Total	26	0.1450			

Table S12.

Date		Df	Sum Sq	Mean Sq	F	P value
17 Aug 2021	Between Treatments	2	0.06966	0.03483	8.725	0.0014
	Residual (within treatments)	24	0.09581	0.003992		
	Total	26	0.1655			

Table S13.

Date		Df	Sum Sq	Mean Sq	F	P value
30 Aug 2021	Between Treatments	2	0.1366	0.06831	45.25	<0.0001
	Residual (within treatments)	21	0.03170	0.001509		
	Total	23	0.1683			

Table S14.

Date		Df	Sum Sq	Mean Sq	F	P value
8 Sep 2021	Between Treatments	2	0.07096	0.03548	7.287	0.0037
	Residual (within treatments)	22	0.1071	0.004869		
	Total	24	0.1781			

Table S15.

Date		Df	Sum Sq	Mean Sq	F	P value
10 July 2021	Between Treatments	2	1.188	0.5942	8.662	0.0015
	Residual (within treatments)	24	1.646	0.06859		
	Total	26	2.835			

Table S16.

Date		Df	Sum Sq	Mean Sq	F	P value
17 Aug 2021	Between Treatments	2	12.02	6.011	82.11	<0.0001
	Residual (within treatments)	24	1.757	0.07321		
	Total	26	13.78			

Table S17.

Date		Df	Sum Sq	Mean Sq	F	P value
30 Aug 2021	Between Treatments	2	6.009	3.004	24.12	<0.0001
	Residual (within treatments)	21	2.615	0.1245		
	Total	23	8.624			

Table S18.

Date		Df	Sum Sq	Mean Sq	F	P value
8 Sep 2021	Between Treatments	2	2.284	1.142	7.239	0.0041
	Residual (within treatments)	21	3.312	0.1577		
	Total	23	5.596			

Table S19.

Date		Df	Sum Sq	Mean Sq	F	P value
15 Sep 2021	Between Treatments	2	4.395	2.198	16.77	<0.0001
	Residual (within treatments)	24	3.146	0.1311		
	Total	26	7.541			

Table S20.

Date		Df	Sum Sq	Mean Sq	F	P value
17 Aug 2021	Between Treatments	2	0.01643	0.008215	11.74	0.0003
	Residual (within treatments)	24	0.01680	0.000700		
	Total	26	0.03323			

Table S21.

Date		Df	Sum Sq	Mean Sq	F	P value
30 Aug 2021	Between Treatments	2	0.001824	0.0009118	4.299	0.0272
	Residual (within treatments)	21	0.004454	0.0002121		
	Total	23	0.006278			

Table S22.

	Sum Sq	Mean Sq	F	P value
Time x Berry Temperature	7.359	0.9198	1.470	0.1693
Time	5187	1297	2072	<0.0001
Berry Temperature	70.01	35.01	37.74	<0.0001
Subject	51.94	0.9276	1.482	0.0243
Residual	140.2	0.6258		

Table S23.

	Sum Sq	Mean Sq	F	P value
Time x Berry Temperature	8.418	1.052	2.638	0.0088
Time	5675	1419	3557	<0.0001
Berry Temperature	34.37	17.19	17.59	<0.0001
Subject	54.72	0.9772	2.450	<0.0001
Residual	89.35	0.3989		

Table S24.

	Sum Sq	Mean Sq	F	P value
Time x Berry Temperature	15.59	1.949	4.181	0.0001
Time	4672	1168	2506	<0.0001
Berry Temperature	14.16	7.081	2.597	0.0833
Subject	155.4	2.727	5.850	<0.0001
Residual	106.3	0.4661		

Table S25.

	Sum Sq	Mean Sq	F	P value
Time x Berry Temperature	25.64	3.205	4.402	<0.0001
Time	8910	2228	3059	<0.0001
Berry Temperature	0.4999	0.2500	0.1698	0.8443
Subject	80.98	1.472	2.022	0.0002
Residual	160.2	0.7281		

Table S26.

	Sum Sq	Mean Sq	F	P value
Time x Berry Temperature	2.490	0.3112	2.115	0.0357
Time	3023	755.8	5136	<0.0001
Berry Temperature	5.459	2.729	6.036	0.0043
Subject	23.96	0.4522	3.073	<0.0001
Residual	31.20	0.1472		

Table S27.

	Sum Sq	Mean Sq	F	P value
Time x Berry Temperature	16.19	2.023	18.78	<0.0001
Time	6162	1541	14296	<0.0001
Berry Temperature	22.05	11.03	20.53	<0.0001
Subject	28.47	0.5372	4.985	<0.0001
Residual	22.84	0.1078		