OENO One vine & wine

DOI: https://doi.org/10.20870/oeno-one.2024.58.2.7847

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Overhead spray water treatment as a mitigation strategy to alleviate vine stress and safeguard grape quality during heatwaves

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Associate editor:
Jorge Queiroz

Received:
30 October 2023

Accepted:
12 March 2024

Published:
16 April 2024

ABSTRACT

Changes in climate are influencing the quality of wine grapes worldwide. The impact of extreme climate events over short periods is increasingly recognised as a serious risk to grape quality and yield quantity. In this study, the mitigation effects of a pulsed water spray treatment on vine canopy during heatwave (HW) events were evaluated in relation to vine condition during the growing season and grape quality. In the UNCuyo experimental vineyard, vines of the three cultivars Malbec (ML), Bonarda (BO) and Syrah (SY) were treated with an overhead pulsed water spray. Heatwaves were defined as days with a minimum temperature of 21 °C and a maximum temperature of 35 °C. Two heat waves were identified during the growing season. Samples were collected at weekly intervals from veraison to harvest. On five sample dates, Leaf and Stem Water Potential (LWP, SWP), Stomatal Conductance (gs), Leaf Temperature (LT), Berry Temperature (BT), Chlorophyll Content (CC), Fluorescence (F_v/F_m) and Performance Index (PI) were recorded at several time points during the day to evaluate the physiological responses of the vine. Berries were collected on each sample date and at harvest. Berry weight, soluble solid content and pH were recorded. In the treated vines (Trt), LWP, SWP, F_v/F_m, PI and gs were significantly higher and LT was lower than in the control vines (Ctl) during the second heatwave, which was longer and more intense than the first one. One week after the more severe heatwave, LWP, SWP and gs remained significantly higher in Trt than in Ctl, displaying reduced physiological stress in Trt. At harvest, the anthocyanin profile, total polyphenol index (TPI), fruit yield, number of bunches and their average weight, berry weight, soluble solid content and pH were also recorded. Bunch weight was significantly higher in Trt for all cultivars. No differences were found in total anthocyanin concentration. These findings indicate that the vines subjected to targeted overhead water treatment during heatwaves underwent less physiological stress and yielded higher grape production, without increasing the risk of potential fungus diseases, in the Mendoza climate. Consequently, this practice could serve as a valuable strategy for mitigating the adverse effects of heatwaves.

KEYWORDS: heatwaves, Malbec, Syrah, Bonarda, mitigation strategy, climate change

INTRODUCTION

Although climate change is often associated with an expected gradual increase in global average temperatures of between 1.5 and 4 ° C (Intergovernmental Panel on Climate Change*,* 2007), it is becoming increasingly clear that of greater concern are the short-term extreme weather events (Field *et al.,* 2012). These events include heavy rainfall, strong winds, hail, late frosts, drought and the topic of this research, heatwaves. The number of observed heatwave events are increasing, as are their duration and severity (Perkins-Kirkpatrick and Lewis, 2020). Although grapevine can adapt well to various environmental pressures, long-lasting and extremely high temperatures or heatwaves can permanently affect yield attributes and vine physiology (Jones and Alves, 2012). The ideal temperature range for optimal photosynthesis in grapevines is typically between 25 and 35 °C (Zhang *et al.,* 2018). When temperatures drop below 10 °C, the majority of physiological processes decline, while temperatures exceeding 35 °C trigger heat acclimation mechanisms (Ferrandino and Lovisolo, 2014). Extremely high temperatures, such as those surpassing 40 °C, can have profound effects on photosynthesis, primarily because they disrupt the photosynthetic apparatus, affecting electron transport rates and provoking stomatal closure to conserve water, and consequently lowering leaf water potential (Carvalho *et al.,* 2015). When stomatal closure occurs transpiration rate also decreases (Carvalho *et al.,* 2015; Greer and Weedon, 2014). Extended periods of exposure to extreme heat conditions can disrupt these processes and thus cause vine health and berry quality to decline (Rogiers *et al*., 2022; Venios et al., 2020; Zhang et al., 2018). At even higher temperatures (> 45 °C) significant injury is possible (Zha *et al.,* 2018), with an inhibition of photosystem II (PSII) activity (the main driver of photosynthesis). The duration and severity of heatwaves are both important factors that impact plant health and production, but the rate of increase in temperature can also pose a threat due to the reduced potential for the plant to acclimate (Carvalho *et al.,* 2015; Webb *et al.,* 2010). Furthermore, a vine's response to heat stress can vary depending on the specific grape cultivar and phenological stage (Zha *et al.,* 2018).

Indicators of optimal vine health include physiological measurements such as leaf water potential, stomatal conductance, chlorophyll content, chlorophyll fluorescence and performance index (Tuccio *et al.,* 2019). These measurements help to characterise plant performance in terms of respiration, transpiration and photosynthetic activity. Under heat stress conditions, these measurements often indicate a deterioration in vine health (Wahid *et al.,* 2007; Zhang *et al.,* 2005; Zhang *et al.,* 2018).

Stomatal conductance (gs) is used to estimate the rate of gas exchange by measuring the degree of stomatal aperture. Stomatal conductance can be used to estimate functions such as transpiration, photosynthetic activity and respiration (Cotthem, 2018), while leaf water potential (LWP) is a direct measure of plant water status. These characteristics can be affected by available soil water and vapour pressure deficit (VPD), as well as canopy size, temperature and radiation exposure (Choné, 2001). Stomata serve two essential roles: they contribute to the regulation of canopy temperature and play a pivotal role in controlling gas exchange and water use efficiency (Sadras and Moran, 2012). In the case of heat stress, increased transpiration from stomata, can impact leaf and stem water potential (Keller, 2015). Normally, transpiration increases up to a certain threshold to help maintain a lower canopy (leaf) temperature, as has been observed by Millan *et al*. (2023). Vines under severe water deficit (below -16 bar SWP) can experience increased tension in the water column, leading to cavitation of the xylem, leaf shedding and possibly vine mortality (Gambetta *et al.,* 2020).

Chlorophyl *a* fluorescence is a measure of the maximum quantum efficiency of PhotoSystem II photochemistry (Force and Critchley, 2003; Ju *et al.,* 2018). Many parameters are associated with Chlorophyl *a* fluorescence including the F_v/F_m ratio and the Performance Index (PI), which quantify the functionality of the electron flow through photosystem II (Ceusters, 2019). Chlorophyll *a* fluorescence is a recognised abiotic stress indicator in grapevine and is known to be negatively influenced by drought conditions, extreme light exposure and extreme heat (Ju *et al.,* 2018, 2021; Su *et al.,* 2015). Relative chlorophyll content is a measure of the concentration of chlorophyll cells in vine leaves, which can be used to determine photosynthetic capacity and plant health (Cogato *et al*., 2021). Heat stress has been known to alter chloroplast structure, which become more globular in shape, while also inducing the structural disorganisation of thylakoid membranes within the chloroplasts. This has been identified as the main area of potential injury in the plant during exposure to high temperatures, leading to a reduction in photosynthetic activity (Bensalem-Fnayou *et al.,* 2011; Wahid *et al.,* 2007; Zhang *et al.,* 2005). Zhang *et al.* (2005) observed changes to chloroplasts after 4 to 10 hours of exposure to a temperature of 45 °C. However, Bensalem-Fnayou *et al.* (2011) only observed changes to grapevine chloroplasts after extended exposure (3 months) to increased temperatures of 35 °C. Extreme heat has also been associated with the reduction in activity of Rubisco, an enzyme in the chloroplast that is associated with carbon metabolism (Carvalho *et al.,* 2015; Liu *et al.,* 2019).

Berries are susceptible to heat stress, which affects their composition and, ultimately, wine quality (Rogiers *et al.,* 2022). In previous studies and in commercial practice, heatwaves have been known to negatively impact both yields and berry quality. Furthermore, increasing temperatures have been associated with an increase in total soluble solid (TSS) and a decrease in acid content, particularly malic acid, along with a corresponding increase in pH (Rogiers *et al.,* 2022; Van Leeuwen and Darriet, 2016). However, extreme heat stress is known to reduce TSS accumulation, slow down berry growth and increase berry dehydration, thus reducing yields and delaying ripening (Bindi *et al.,* 1996; Rienth *et al.,* 2021).

It has been well established that high temperatures reduce anthocyanin concentration because of a reduction in anthocyanin biosynthesis and degradation (de Rosas *et al.,* 2022; Rienth *et al.,* 2021; Tarara *et al.,* 2008). However, the decrease in anthocyanin concentration due to extreme temperature exposure can vary depending on the cultivar (de Rosas *et al.,* 2022). Higher temperature exposure has also been known to shift anthocyanins from glucosylated to acylated and *p*-coumarylated forms (de Rosas *et al.,* 2022; Mori *et al.,* 2007), which are more stable during the winemaking process. In addition, extreme temperature has been observed to cause a decoupling of anthocyanins from sugar due to increased sugar accumulation rates (Sadras and Moran, 2012).

From a wine quality perspective, flavonols are known to stabilise anthocyanins through co-pigmentation (Asen *et al.,* 1972) and they can impart bitter flavours (Ferrer-Gallego *et al.,* 2016) to wine. Both stable colour and bitterness can be associated with enhanced wine quality. The effect of extreme temperature on flavonols is not very clear. However, Gouot *et al*. (2019b) found that at extreme temperatures (>50 °C) flavonol concentrations in Shiraz berries were significantly negatively impacted; meanwhile, they found that temperatures as high as 46 °C did not impact anthocyanins content, but modified their profile.

With the increasing frequency, length and severity of heatwaves and their potential negative impacts on vine health and berry quality, mitigative strategies to reduce the associated risks to plant health and berry quality are being investigated. Perhaps the most common current mitigative practice is to increase irrigation prior to and during heatwaves to offset evapotranspiration (ET) losses and to maintain plant physiological function (Naulleau *et al*., 2021; Previtali *et al*., 2023; Savi *et al*., 2018; Webb *et al*., 2010). Irrigation can use a lot of water. Although calculations vary depending on multiple factors, including degree days, canopy size, vine density, soil type, soil depth and irrigation efficiency, maintaining an evapotranspiration rate of 0.85 when a canopy is at its largest can equate to 10.6 L of water per vine per day (Hellman, 2019). In the case of heatwave mitigation, ET is often maintained above 1.00. In some regions, water is a scarce resource, which can lead to limited capacity for protecting vines when applying an irrigation strategy (Webb *et al.,* 2010).

Historically, aerial sprinkler systems have also been used to mitigate both increasing average temperatures and extreme short-term heatwaves (Gilbert *et al.,* 1971). Kliewer and Schultz (1973) investigated the effects of using sprinklers on leaf and berry temperature, with treatments applied at a threshold temperature of 30 °C; their results showed a notable reduction in the temperature of the treated vine canopies along with increased berry weights, without using a large amount of water. Aljibury *et al.* (1975) used a threshold temperature of 32 °C to initiate treatment application and observed a notable reduction in leaf and berry temperature and an increase in berry weight in treated vines. In both papers it appears that canopy irrigation was untargeted. Caravia *et al.* (2017) focused on heatwave mitigation with treatment application applied at a threshold temperature of 38 °C.

The goal of the research in this paper was to assess the effectiveness of applying a reduced volume of water via a targeted and pulsed spray, to the upper portion of the vine canopy when aiming to sustain vine physiological functions during and after heatwaves and preserving crop yields and berry quality.

MATERIALS AND METHODS

1. Experimental design

1.1. Experimental site

The experiment was conducted in an experimental vineyard at the Faculty of Agricultural Science of the National University of Cuyo located in Luján de Cuyo in Mendoza province in western Argentina (33°00’30.2”S and 68°52’20.9”W). Eleven-year-old-own-rooted *Vitis vinifera* cultivars were used for the experiment: Malbec (ML) (clone N° 2), Syrah (SY) (clone N° 84) and Bonarda (BO) (clone N° 9). The vines were all oriented north-south and spaced at 1m intervals within rows and 2.2 m between rows. They were managed using spur pruning, employing a fruit load adjustment tailored to the individual vigour of each plant and utilising a bilateral cordon system with vertically positioned shoots.

Prior to the budbreak, flowering, fruit set and post-veraison stages, all the vines were drip-irrigated for 48 h. The drip distance was 1 m and the water application rate was 2 L/h. The air temperature was measured by two temperature sensors (iButton 1 Wire® Thermochron® Maxim Integrated USA) inside plastic boxes to avoid direct sun exposure and installed at each end of each row. The experiment was performed during the growing season of 2023 from veraison until harvest (January to February), as the onset of veraison is known to be a sensitive period for flavonoid development (Gouot *et al.,* 2019a).

1.2. Experimental design and sampling

The experimental design consisted of a randomised plot of 5 rows: 3 rows of treated vines and 2 rows of control vines, with 6 and 9 plants per row respectively. The experimental unit consisted of two plants of the same cultivar per row, and there were three replicates of each treatment (Supplementary Figure 1).

Two jet nozzles were installed at the end of each treated row (Trt) (n = 3). The jets were aligned longitudinally in relation to the canopy (Supplementary Figure 2) and were activated during heatwaves only. Each jet emitted a pulsed spray of water to the top of the canopy for 15 min (1 s on, 2 s off) for 12 h from 8:00 to 20:00. Each jet emitted an average of 200 ml/h, resulting in a daily total of 2.4 L per heatwave day (Hwday) per jet or 0.53 L per plant per HWday. The treatment was applied to the canopy only, with a minimal amount of water coming into contact with the soil.

Leaf wetness sensors (PHYTOS 31, Meter Group) were installed in two treatment rows to monitor leaf surface wetness; this was done by measuring the dielectric constant of the sensor's upper surface and thus determining the evaporative time between treatment application. No pulsed spray was applied to the control rows (Ctl) ($n = 3$).

The research was performed during natural heatwaves. These were defined as two or more consecutive days of an expected maximum temperature equal to or higher than 35 ºC and an expected minimum temperature equal to or higher than 21 ºC, as anticipated from regional weather forecasts.

1.3. Physiological and berry sampling protocol

Physiological sampling was done on five dates during the season: 20 January (S1), 25 January (S2), 2 February (S3), 9 February (S4) and 16 February (S5). Berry characteristics and berry anthocyanin and total polyphenol concentrations were monitored on the same five sampling dates, as well as at harvest (23 February for Malbec and Syrah (S6) and 28 February for Bonarda (S7)) (Figure 1). All the samples were collected from the sun-exposed side of the vine (east-facing before noon, west-facing in the afternoon)

On all the sample dates, leaf water potential (LWP) was measured at 5:00 (pre-dawn), 8:00, 11:00, 14:00 and 17:00. Completely healthy, dry and mature leaves were randomly selected from the middle of the canopy at the first training wire and outside of the limit of direct exposure to the water droplets, which were distributed over the top third of the canopy area. Each leaf was placed in a plastic bag, which was sealed prior to cutting off the leaf at the petiole using a razor blade. The bagged leaves were immediately placed in a pressure chamber operated in the field (Model 4, Biocontrol).

The relative chlorophyll content (CC) of the leaves was measured using the SPAD-502Plus, (Konica Minolta, Osaka, Japan) in duplicate on each replicate at 8:00, 11:00, 14:00 and 17:00. Leaf (LT) and Berry temperature (BT) were measured on the fully exposed leaves and clusters of each vine. These were measured in duplicate per replicate at the same four time points per day as the CC using an infra-red thermometer. Stomatal Conductance (gs) (SC1 Leaf Porometer, Decagon Devices, Pullman, WA, USA) was measured on dry leaves four times per day from 8:00 to 17:00 on S4 (during HW2) and S5 (after HW2). Chlorophyll *a* fluorescence measurements (F_v/F_m ratio and Performance Index on absorption basis (PI_{abs})) were done after 20 min of dark exposure and calculated as follows:

$$F_v/F_m = (F_m - F_o)/F_m$$

where:

F_m = maximum fluorescence yield after dark adapted leaves

F_o = dark fluorescence yield (minimal fluorescence)

F_v = variable fluorescence

And:

$$PI_{abs} = (RC/abs)[\Psi Po/(1 - \Psi Po)]\,[\Psi o/(1 - \Psi o)]$$

where:

RC/abs= fraction of active reaction centers of PSII relative to the total light absorbing chlorophyll.

$\Psi Po = (Fm - Fo)/Fm$ = maximum quantum yield of PSII,

Ψo = efficiency with which a trapped exciton can move an electron to the downstream of QA^- on the electron transport chain.

These two variables were measured at 11:00 and 14:00 from the same leaves on each sample date using a fluorometer (Pocket PEA; Hansatech Instruments, England) to quantify plant stress.

All the non-destructive leaf-related measurements (CC, F_v/F_m, PI, LT, and gs) were carried out on the same leaves (pre-marked with a ribbon) at each sample date and time during the experiment. Stem Water Potential (SWP) was measured from 14:00 to 15:00 after the intact leaves had been covered with aluminium foil for 30 min prior to leaf removal and measurement.

At each sample date two sets of twelve berries were collected at 17:00 (approximately the hottest time of the day). The berries were cut above the pedicel, stored in an insulated environment cooled with ice (not in direct contact with the berries) and transported directly to the place of refrigeration within one hour of sampling. Twelve berries were weighed, manually crushed and placed in 100 mL test tubes to measure total soluble solid (TSS) by refractometry (°Brix) (Atago®, Master –T Japan) and pH (Altronix®). Additionally, twelve berries were frozen at -20 °C for further phenolic analysis.

The final harvest dates (S6 and S7) were determined when the berries had reached a median TSS of 24 °Brix. Malbec and Syrah reached maturity five days earlier than Bonarda and were harvested on 23 February (S6), while Bonarda reached 24 ºBrix on 28 February (S7). Each replicate was harvested separately. The average yield per plant (kg), number of bunches per plant and bunch weight (g) were measured.

2. Phenolic Analysis

2.1. Grape berry phenolic extraction

The frozen berries were immersed briefly in water and their skins immediately removed. The skins were dried and ground to powder using liquid nitrogen. The phenolic compounds were then extracted using a method according to Revilla *et al.* (1998) with minor modifications (de Rosas *et al.,* 2022). To summarise, 150 mg of skin was macerated with 1850 µl of MeOH:HCl (99:1, *v/v)* (HCl 10 N) (Sintorgan, Buenos Aires, Argentina) in the dark at 20 ºC for 24 h. This was followed by centrifugation for 20 min at 14,000 rpm and a constant temperature of 4 ºC (Z 326K, Hermle Labortechnik GmbH, Wehingen, Germany). The supernatant was collected and stored at -20 ºC, and a second extraction (as described above) was performed on the residual skins. Equal parts of both extracted supernatants were incorporated and filtered with 45 µm pore cellulose acetate membranes (Sartorius, Gottingen, Germany).

2.2. Anthocyanin HPLC-DAD Analysis

An anthocyanin analysis of the phenolic extractions was performed by High Performance Liquid Chromatography coupled to a Diode Array Detector (HPLC-DAD) (Thermo Fisher Scientific UltiMate 3000). The anthocyanin measurements were carried out on a Restek (ROC) C18 column of 5 µm, measuring 250 mm x 4.6 mm. The mobile phases were A: 87 % H_2O, 3 % acetonitrile, and 10 % formic acid, and B: 40 % H_2O, 50 % acetonitrile, and 10 % formic acid. Quantification was performed at 520 nm by constructing a 5-point calibration curve using a commercial standard of malvidin-3-glucoside chloride (Sigma, St. Louis, MO, USA). The concentrations (in mg/g of berry) of the following compounds were determined: Delphinidin-3-glucoside (Df), Cyanidin-3-glucoside (Cn), Petunidin-3-glucoside (Pt), Peonidin-3-glucoside (Po), Malvidin-3-glucoside (Mv), Peonidin-acetyl-glucoside (PoAC), Malvidin-acetyl-glucoside (MvAc), Peonidin-coumaroyl-glucoside (PoCu) and Malvidin-coumaroyl-glucoside (MvCu). Anthocyanin concentrations were expressed as mg/g of berry skin fresh weight (mg/g FW). Using these concentrations, total anthocyanin content (TAC) was calculated as the sum of the 9 anthocyanins. Compositional ratios were calculated by comparing content to TAC. The variations in tri- vs di-substitution, acylation, acetylated and coumaroylated ratios were calculated as follows:

$$\%\,Glucosylated = ((Df + Pt + Mv + Cn + Po)\ x\frac{100}{TAC})$$

$$\%\,Acetylated = ((PoCu + MvCu)\ x\frac{100}{TAC})$$

$$\%\,Coumaroylated = ((PoCu + MvCu)x\frac{100}{TAC})$$

$$\%\,Acylated = (\ \%\,Acetylated +\ \%\,Coumaroylated)$$

$$Trisubstituted/Disubstituted\ (Tri/Di) = (\frac{Df + Pt + Mv}{Cn + Po})$$

2.3. Total Polyphenol Index

Total polyphenols were measured using the Ribéreau-Gayon method. The unfiltered supernatant that had been extracted as described above was diluted with demineralised water at a ratio of 1:100. The dilution was placed in a UV quartz cuvette with a 10 mm path length and then inserted into a spectrophotometer (E-1000UV, Peak Instruments, Houston, USA). Total polyphenol index (TPI) was estimated as absorbance at 280 nm multiplied by 100 (Ribéreau-Gayon *et al.,* 1970).

3. Statistical Analysis

The results were subjected to a two-way ANOVA to examine the effects of the treatments, cultivars, and treatment and cultivar interaction. Shapiro-Wilk Test was performed to determine normality and Breusch-Pagan Test to determine homoscedasticity. The data are presented as means of three replicates (n = 3) with 2 vines as an experimental unit per replica. Mean comparisons were performed by the Tukey test, considering $p \leq 0{,}05$ (*) to be significant, $p \leq 0.01$ (**) highly significant, $p \leq 0.001$ (***) very highly significant and 'NS' not significant. T-tests were performed on the means of maximum daily in-row temperatures of Ctl and Trt. The resulting p values were transformed (-10log) for graphical purposes.

The statistical analysis was performed using XL-STAT extension (Addinsoft, 2019) and R with RStudio (RStudio Team, 2019). Graphics were created using GGPlot2 (Wickham, 2016) and Microsoft Excel.

RESULTS AND DISCUSSION

1. Heatwaves

There were two heatwaves during the 2023 growing season (Figure 1). The first heatwave (HW1) was shorter than the second, lasting only two days. It occurred from 23 to 24 January, with an average maximum daytime temperature of 37.2 ºC measured in the canopy of the control vines. The second heatwave (HW2) began on 5 February and ended on 12 February; the average maximum daytime temperature was 38.9 ºC and over 40.0 ºC was measured for two days in the Ctl vines. During each of the heatwaves the water spray treatment was applied for a consecutive 2 and 8 days respectively. Regarding the 3-day period just before the onset of the heatwaves, Figure 1 illustrates a more gradual incline for HW1, with a daily temperature increase of less than 1 ºC over the three days; by contrast, for HW2, an average daily increase of 2 ºC per day can be observed (Figure 1).

The average daytime canopy temperature of the Trt was lower than that of the Ctl during the treatment (Figure 1). During HW1, the average daytime maximum temperature for the Ctl was 2.8 ºC higher than that of the Trt, while during HW2 it was 3.8 ºC higher. This trend continued after the treatment was no longer applied (i.e., after the heatwaves), particularly after HW2, with an average difference of 3.1 ºC, which increased in significance, as indicated by the increased -log10 value. Minimum (night) temperatures for Trt and Ctl were similar throughout the experiment, except for those measured on two nights during the second heatwave (8 and 9 February).

In this growing region, humidity is quite low due to the rain shadow effect caused by the region's close proximity to the Andes mountain range. Humidity values rarely rise above 50 % after 8:00 and consistently drop to below 20 % in the afternoon in February in the Mendoza area. In the present study, the data obtained from the leaf wetness sensor (Phytos 31/LWS) show that water from the treatment application had completely evaporated within 30 min of the completion of each treatment cycle, thus allowing the vines to dry for 30 min prior to the next treatment application cycle, and creating non-predisposing conditions for pathogenic fungi at the canopy level (Supplementary Figure 3). For this reason, risk of disease was not a concern in this study. This mitigative strategy is best applied in regions with lower relative humidity to reduce the risk of pathogenic fungal infection. The quality of the accessible water used in this treatment must also be considered, since, according to Kliewer and Schultz (1973), water that has high total salt

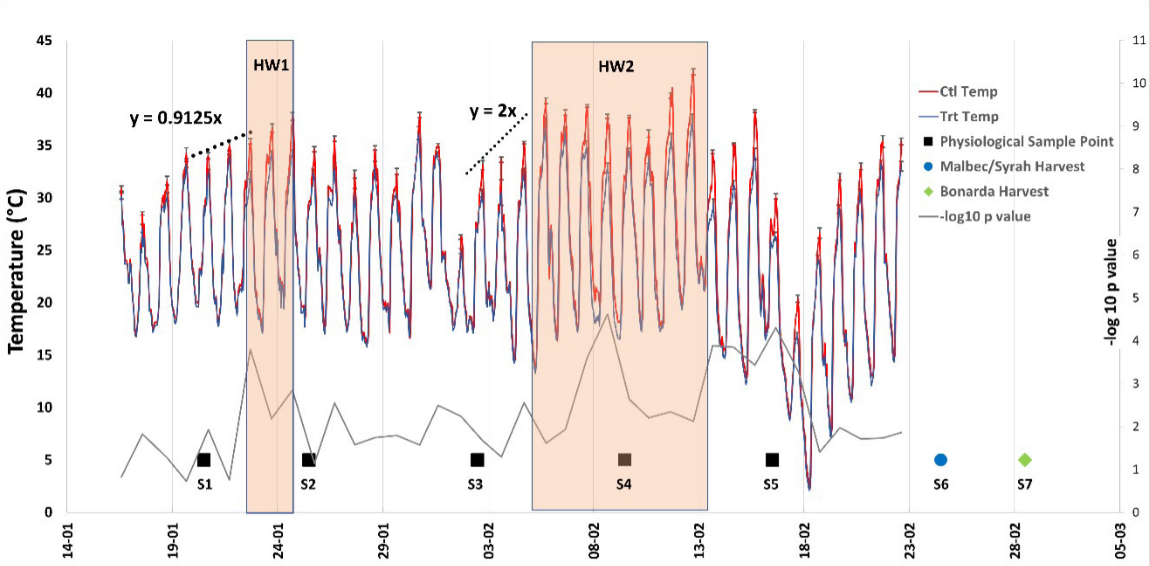


FIGURE 1. Average in-canopy temperature for treated (Trt) and control (Ctl) with standard error. HW1 and HW2 events (orange), sample dates (S1 to S7). -log10 transformation of *p* values from t-test for each daily maximum Ctl and Trt temperature (> 1.3 is ≤ 0.05 *p*-value). Average temperature increase for three days preceding each heatwave (black dotted lines) include regression coefficient.

content can lead to salt deposits being left on the leaves and berries, and can provoke necrosis of leaf tissue; moreover, it can clog the pipes and sprinklers.

2. Physiological Response

2.1. Leaf and Stem Water Potential

Trt and Ctl were significantly different in terms of pre-dawn water potential and the 14:00 and 17:00 LWP of all the cultivars (S2) after the first heatwave (Figures 2a, d and e). These differences were no longer observed at any of the time points, except for at 17:00 (Figure 2e) on S3; i.e., 8 days after the end of HW1. The S4 samples were taken in the middle of HW2: all the cultivars showed significantly higher LWP in the Trt vines at all sample time points during the day. These significant differences were still observed on S5 (three days after the end of HW2) with a greater difference between Trt and Ctl than on S4; this indicates that the longer and more severe heatwave led to extended periods of water stress, with values of -15 bar at 11:00, 14:00 and 17:00 for the Ctl vines on S5. The higher water potential exhibited by the plants of the 3 cultivars irrigated by overhead sprinklers may have been due to an increase in the relative humidity of the surrounding canopy environment, which reduced the vapour pressure deficit, thus decreasing water loss through transpiration. Water uptake through the leaves was ruled out, because during aerial irrigation the water droplets remained on the leaf surface for only 30 min before evaporating (Supplementary Figure 3).

No significant differences were found in stem water potential between cultivars on any of the sample dates. After HW1 (S2), only the Malbec Trt was significantly lower than their Ctl. On S3 there were no observable differences between Trt and Ctl for all cultivars (Supplementary Table 1). During HW2 (S4), all three cultivars of Trt showed an upward trend, with Bonarda and Syrah showing a significant response between Trt to Ctl (Figure 3a). Regarding the response of Malbec on S4, a similar trend of higher values for Trt than Ctl can be observed, but with no significant difference. After HW2 (S5), all three cultivars of Trt vines showed clear significantly higher SWP than those of Ctl vines, and all of the cultivars showed much lower variance in their response than on S4 (Figure 3b). These findings are consistent with those from other research focusing on heatwave mitigation via irrigation or sprinkler application (Cogato *et al.,* 2021; Martínez-Lüscher *et al.,* 2017). On S5, the SWP of all the Ctl vines was below -16 bar, indicating that the water deficit was extreme enough to trigger xylem cavitation (Gambetta *et al.,* 2020). In the Ctl vines, water potentials of below -16 bar were also observed for LWP at 11:00 and 14:00 on S5, while the Trt vines maintained an LWP and SWP above -15 bar during and after the heatwaves.

2.2. Stomatal Conductance

The stomatal conductance results for S4 (during HW2) show no significant differences at 8:00; however, the Trt and Ctl vines show increasingly significant differences during the day, with higher values obtained for Trt than Ctl (Figure 4a). This indicates that Trt vines were better able to maintain transpiration rates with increasing heat during the heatwave than the vines with no treatment. Similar findings were obtained by Cogato *et al. (*2021), with midday gs being higher in the vines undergoing a sprinkler treatment than in the control vines both during and after the heatwave. As observed by Sadras *et al.* (2012), increasing leaf temperature at constant relative humidity increases the

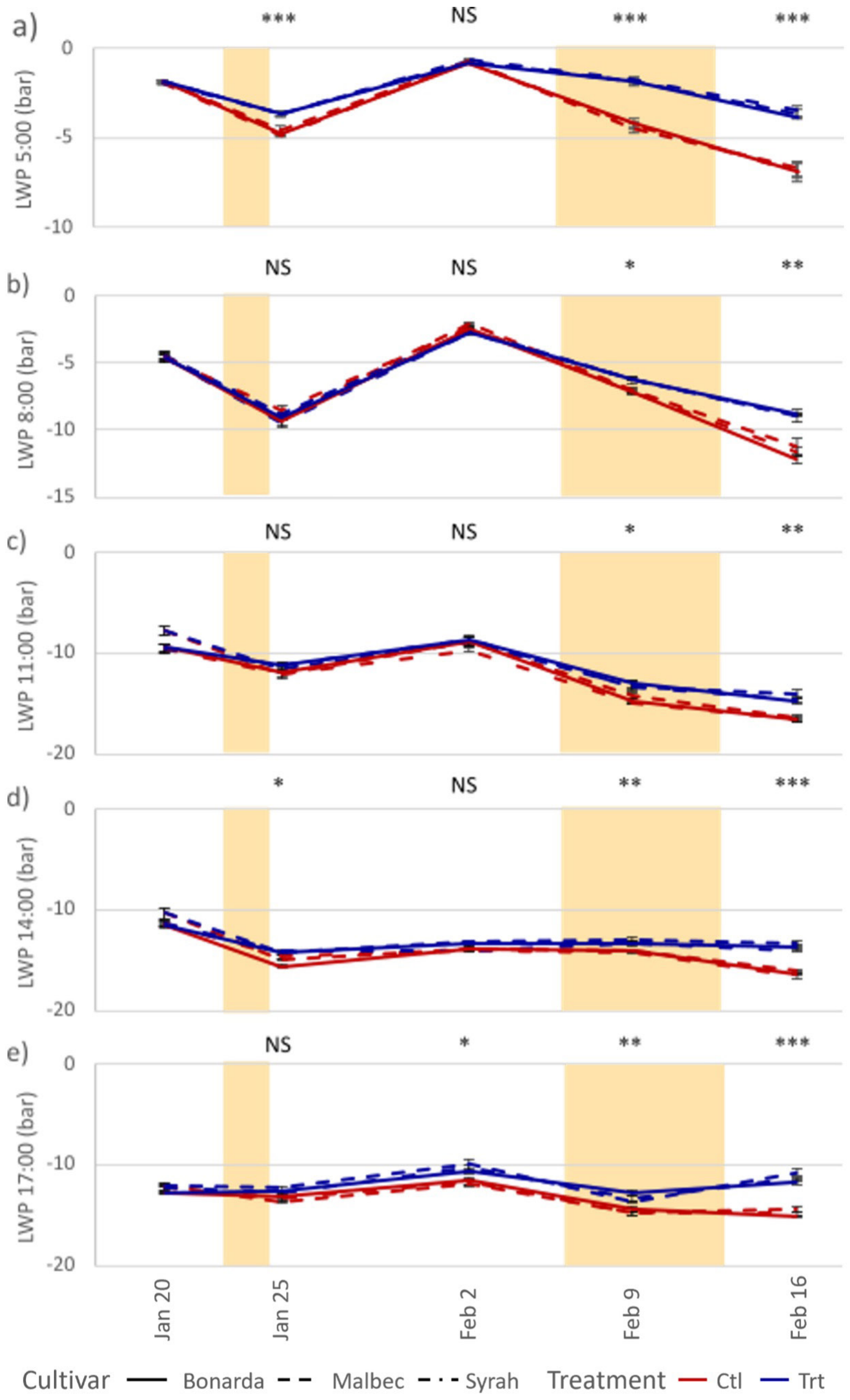


FIGURE 2. Leaf water potential variation for Bonarda, Malbec and Syrah cultivars during the maturity stages (S1-S5) at different daytime hours: a) pre-drawn (5:00), b) early morning (8:00), c) late morning (11:00), d) early afternoon (14:00), and e) afternoon (17:00). Data points are means ±SE (n = 3). Statistical significance determined by two-way ANOVA with Tukey Test post-hoc. $p \leq 0.05$ '*', $p \leq 0.001$ '**', $p \leq 0.0001$ '***', NS = not significant.

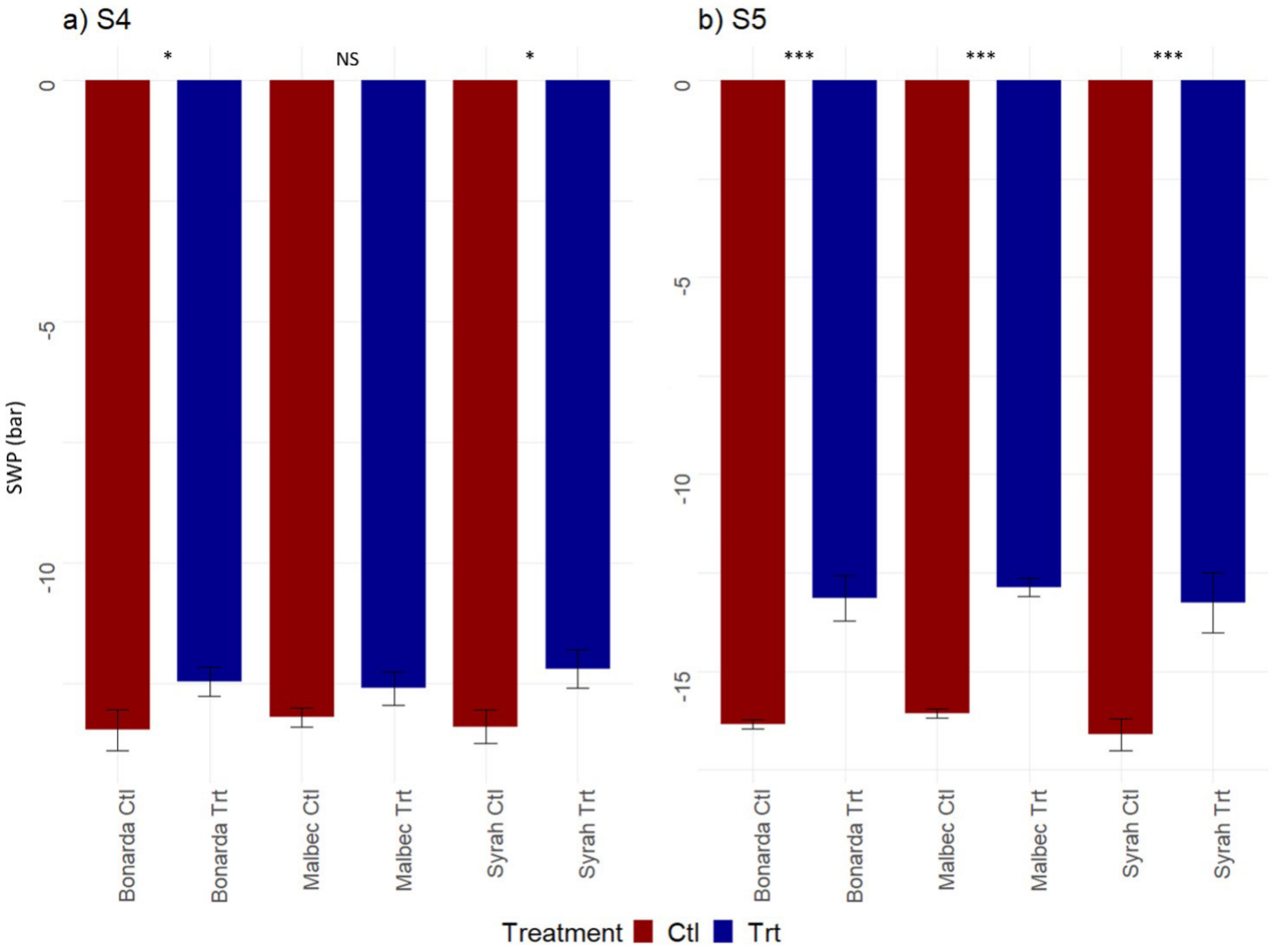
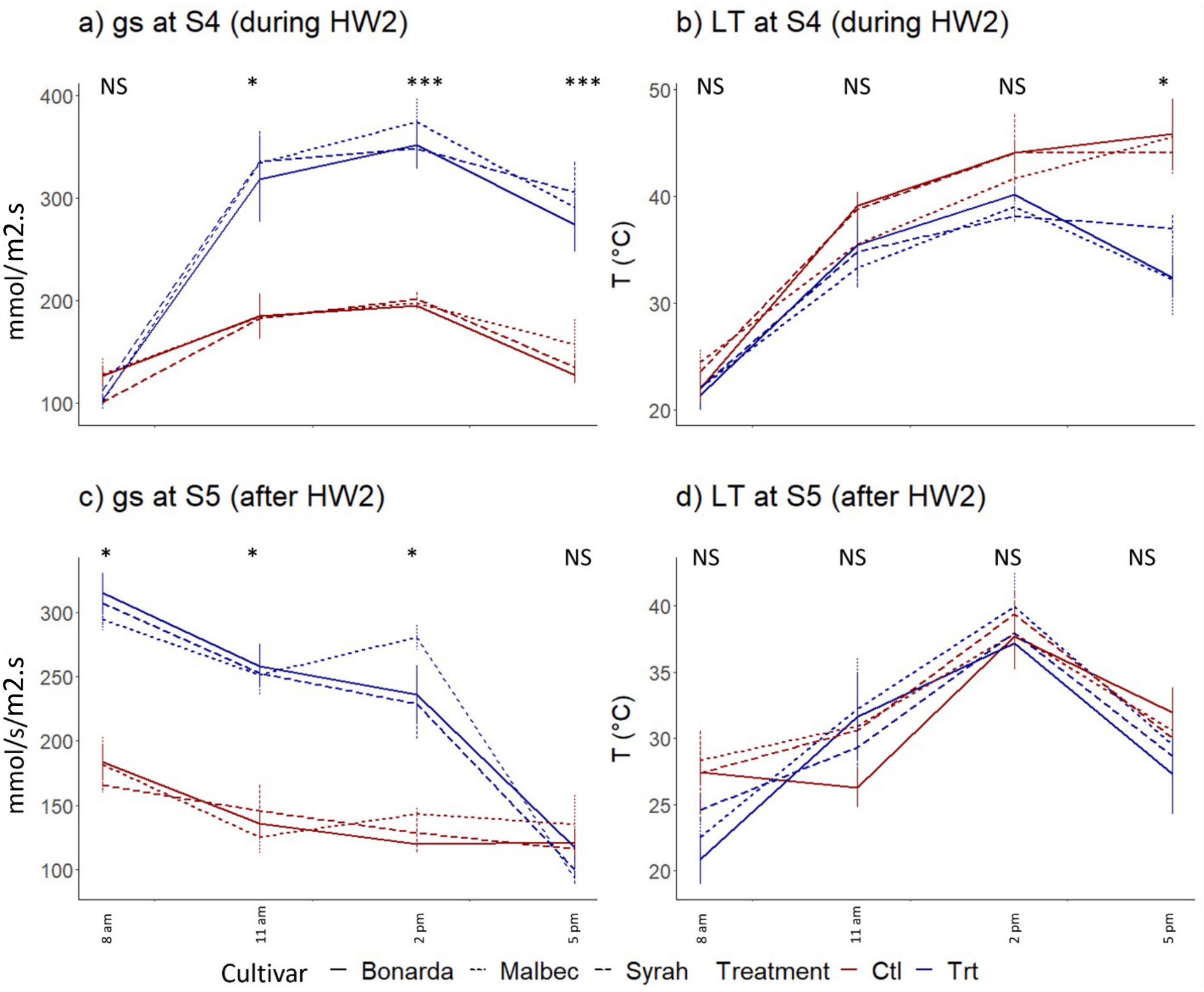


FIGURE 3. Stem Water Potential (SWP) (bar) on S4 (during HW2) (a) and S5 (after HW2) (b) for the cultivars Bonarda, Malbec and Syrah treated (Trt) or not (Ctl). Statistical significance determined by two-way ANOVA with Tukey Test post-hoc. '*' $p \leq 0.05$, '***' $p \leq 0.0001$; NS = Not significant.

VPD exponentially, thus potentially inducing stomatal closure. At 8:00 on S5 (after HW2), the Trt vines had a significantly higher gs than the Ctl vines. The Trt vine gs gradually decreased until 17:00, at which point there were no significant differences between Trt and Ctl for all cultivars (Figure 4c), with a substantial decrease in Trt gs compared to Ctl gs. The response of Trt on S5 is similar to that observed in previous research by Sabir and Yazar (2015) on the diurnal gs range under normal growing conditions (25 °C to 30 °C air temperature, 60 to 70 % relative humidity). This suggests that on S5 the Trt vines were regaining a lower stress gs function, while the Ctl vines were still restricted in terms of stomatal activity. Stomatal conductance was higher in the treated vines for all three cultivars due to a reduction in their canopy temperature; this resulted in lower water loss from transpiration and higher stomatal conductance, allowing the Trt vines to remain hydrated and sustain higher LWP and SWP than the Ctl vines. The wetting of the canopy of the treated plants allowed a more humid environment to be maintained around the leaves; as a consequence, the physiological response of these plants was to increase stomatal conductance, allowing the plant to maintain a higher water potential. Meanwhile, the significant decrease in Ctl gs was likely a response to the stressful atmospheric conditions during the heatwave, in order to diminish vulnerability to cavitation. (Hochberg *et al.,* 2017; Gambetta *et al.,* 2020). Foliar absorption of the sprayed water during the treatment is highly unlikely due to the short time the small water droplets remained on the leaves (30 min/h, Supplementary Figure 3) before evaporating. Additionally, no surfactant was used, which decreased the capacity for water to enter through hydathodes.

2.3. Leaf and Berry Temperature

Significant responses for all cultivars were only observed during the second heatwave (S4) at 17:00 (Figure 4b), with higher temperatures in the Ctl vines than in the Trt vines: this is considered an indicator of leaf cooling due to the treatment. On S4, an inverse relationship between LT and gs response can be observed (Figure 4a) due to the direct and significant relationship between increased stomatal activity and decreased canopy temperature. After the second heatwave, gs was significantly higher in the Trt vines between 8:00 and 14:00, likely due to the rehydration and recovery of


a) gs at S4 (during HW2)

b) LT at S4 (during HW2)

c) gs at S5 (after HW2)

d) LT at S5 (after HW2)

FIGURE 4. Stomatal Conductance (gs) (a), (c) and Leaf Temperature (LT) (b), (d) measured at 08:00, 11:00, 14:00 and 17:00 (during HW2) (S4) (a, b) and S4 (three days after HW2) (c, d) in Trt and Ctl vines of cultivars Bonarda, Malbec and Syrah. Statistical significance determined by two-way ANOVA (n = 3) with Tukey test post-hoc. '*' $p \leq 0.05$, '***' $p \leq 0.0001$, NS = Not Significant.

the vines after the second heatwave, as well as water balance regulation in order to adapt to the cooler and moister morning conditions. No relationship between gs and LT was found on S5, LT was not significantly different for any cultivar at any time during the day (Figure 4c and d). Early leaf abscission - documented only qualitatively - was also observed in the Ctl vines of the three cultivars during and after HW2 due to the higher temperature stress. This is another indication that the water deficit was severe enough to reduce turgor and to trigger xylem cavitation in the Ctl vines.

Under high air humidity, such as that occurring in the morning, grapevine stomata can be wide open (Sabir and Yazar, 2015), as was observed in all S5 vines in the present study (Figure 4c). The higher stomata closure of the Ctl vines compared to the Trt vines was probably a response to excessive transpiration and direct dehydration of the stomatal cells, a process known as hydro passive stomatal closure.

When the leaf temperature of plants exceeds 40 ºC, stomatal conductance tends to decrease due to heat stress and higher VPD, leading to stomatal closure and a reduction in photosynthetic rate and photosystem II efficiency, as was observed in the Ctl vines during HW2. These mechanisms help protect the plant against damage caused by high temperatures by maintaining its water balance (Gambetta *et al.,* 2020).

At 17:00 during the second heatwave (S4), the Trt vines of all three cultivars showed significantly lower BT (36.02 ºC) than the Ctl vines (45.22 ºC; $p \leq 0.05$) due to the evaporative cooling of the Trt vines having the effect of dissipating the heat. Also at 17:00 on S5 during the recovery phase, the difference between the Trt and Ctl vines was significantly lower (28.92 ºC and 31.23 ºC respectively; $p < 0.1$). While directly cooling the berries was not the primary aim of the treatment during the heatwave, it had an unexpected evaporative cooling effect on them at 17:00, when the temperature was at its highest. By contrast, other studies

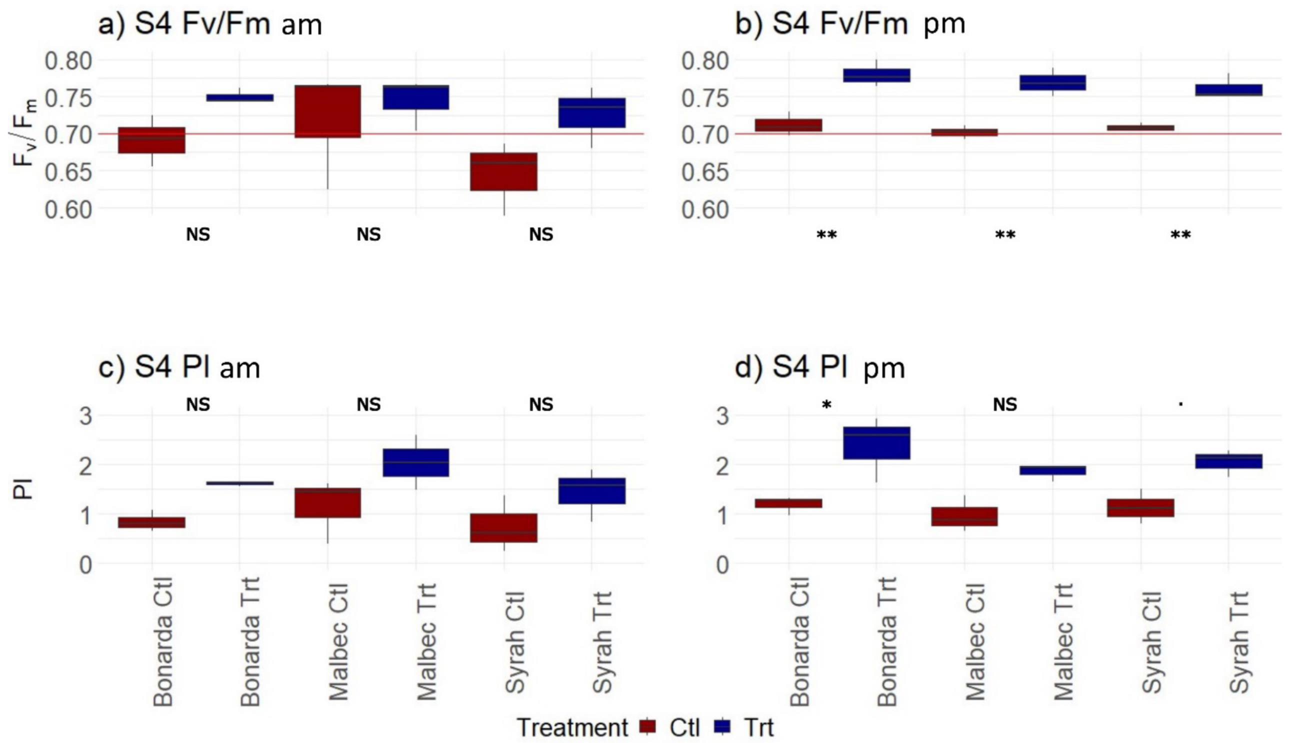


FIGURE 5. Chlorophyll Fluorescence (F_v/F_m) (a), (b) and Performance Index (PI) (c), (d) measured on S4 (during HW2) for cultivars Bonarda, Malbec and Syrah treated (Trt) and control (Ctl). Red line = F_v/F_m threshold for PSII normal functioning (Bolhar-Nordenkampf *et al.*, 1994). Based on two-way ANOVA (n = 3). NS = Not Significant; $p \leq 0.1$ '.', $p \leq 0.05$ '*', $p \leq 0.001$ '**'.

have directly applied spray to the bunch zone and observed a decrease in berry temperature (Paciello *et al.*, 2017).

2.4. Chlorophyll Content (CC), Chlorophyll *a* Fluorescence (F_v/F_m) and Performance Index (PI)

No significant differences were found in Chlorophyll content between Trt and Ctl for all cultivars at all time points on all sample dates (Supplementary Figure 4).

Photosystem II (PSII) is usually suspended or destroyed before the disruption of other cellular functions (Zhang *et al.*, 2018). Chlorophyll *a* Fluorescence of photosystem II - considered to be the physiological system of the grapevine that is the most sensitive to heat stress, and represented here by the F_v/F_m ratio - was not significantly affected after HW1 (S2 and S3) in any of the cultivars (Supplementary Table 2). During HW2, the F_v/F_m morning values were not affected by the treatment in any of the cultivars (Figure 5a); however, the F_v/F_m afternoon values were significantly different between Trt and Ctl for all three genotypes, with Trt showing higher values (Figure 5b). The differences in both the morning and afternoon values were once again non-significant on S5 (Supplementary Table 2). The Performance Index (PI) was also not significant for any of the cultivars in neither the morning nor the afternoon on S2, S3 and S5 (Supplementary Table 3). During HW2 (S4), although not significant, the morning PI values of all cultivars showed higher values in the Trt vines. In the afternoon during HW2, the higher PI value in Trt was significantly different for Bonarda and Syrah ($p \leq 0.05$) (Figure 5 c and d). This response coincides with the significantly lower LT found in all Trt vines at 17:00 during HW2 (Figure 4b). The significantly higher F_v/F_m and PI values during HW2 in the treated vines, particularly during the hottest part of the day, indicates that the treatment supported vine physiological performance during the period of extreme temperatures, thus reducing vine stress.

The maximum efficiency of photosystem II (as measured by the dark-adapted F_v/F_m fluorescence ratio) has been demonstrated to decrease significantly under severe drought conditions (Zhang *et al.*, 2018). This was temporarily observed during HW2 in the Ctl vines, indicating that the treatment supports PSII functioning during prolonged heatwaves.

During heatwaves, vines should be watered regularly to maintain transpiration and cool the canopy and bunches (Hayman *et al.*, 2012). Heatwaves can induce a vine "shut down," halting photosynthesis due to stomatal closure to conserve water, and resulting in slower ripening (Greer and Weedon, 2013). Depending on the heatwave's timing, severity and duration, it may take several weeks for vines to resume normal photosynthetic activity, resulting in generally lower fruit quality. Furthermore, extreme heatwaves can lead to cavitation fatigue, resulting in vines becoming more prone to xylem cavitation (Hochberg *et al.*, 2017) or, even worse, vine mortality. Treatments to reduce canopy temperatures have shown significant results, with temperatures being lowered by approximately 4-5 °C (Cogato *et al.,* 2021; Paciello *et al.,* 2017). Similar effects were observed in this treatment in the current study while using 5 % of the volume of water applied in traditional mitigative irrigation practices. In order to determine how to further decrease demand on water when applying this mitigative application, further research is required on the water volume thresholds at which treatment can still support vine physiological performance and on increasing trigger temperatures for treatment activation.

The physiological results obtained in this study indicate that administering a controlled dosage of water droplets onto the canopy's surface during the daytime hours of extreme heat events occurring at least 15 days before harvest can significantly influence the short-term physiological responses of the vines. Some physiological responses were sustained at non-critical levels in the Trt vines, such as LWP, SWP and gs on S5, three days after the second heatwave. Although grapevine demonstrates high resilience to diverse environmental stresses, prolonged periods of excessively high temperatures or heatwaves can have lasting impacts on both yield attributes and vine physiology. After the second heatwave in the present study, there were significant differences between the treated vines and control vines, with the former displaying healthier physiological traits than the latter. This shows that the treatment acted to protect the plants from stress, not only during the treatment but also on the days following it. Further research is required on the positive longer-term impacts, such as reduced cavitation fatigue and vine mortality associated with the application of a small volume of water to the top of the vine canopy during heatwaves.

Some cultivars are more sensitive to heat stress than others. The Semillon cultivar is particularly sensitive to heat stress during flowering and ripening, reducing photosynthesis due to both stomatal and non-stomatal limitations and requiring up to two weeks to recover (Greer and Weedon, 2014). In the Greer and Weedon study, hydrocooling, activated at a threshold temperature of 35 °C, extended the period of leaf and berry expansion, resulting in larger berries. This method also led to lower canopy temperatures, increased net CO_2 assimilation and slightly elevated berry total soluble solid (TSS) levels. Although the three cultivars in the current research showed similar physiological responses to the treatment, other varietals may benefit depending on their sensitivity to heatwaves.

3. Harvest Parameters

3.1. TSS and pH

No statistical differences were found between Trt and Ctl in terms of berry soluble solid content and pH on all the sample dates (S1-S7) and for all three cultivars (Figures 6a and 6b). Similar results were previously reported by de Rosas *et al*. (2022), who tested Malbec, Merlot and Pinot noir cultivars under heat temperature treatment. The results of the present work are also in agreement with those obtained in studies by Greer and Weedon (2014) and Caravia *et al.* (2017), in which TSS did not differ between heatwave mitigation treatments: the threshold temperatures at which the sprinkler was activated in these two studies were 35 °C and 38 °C respectively. Paciello *et al.* (2017) found a significant difference between treated and control grapes in TSS after a nebulised spray had been applied to the canopy and bunch zone: the grapes from the treated vines had lower sugar content compared to the control. It is important to note that the threshold temperature at which the nebulized spray was activated was 30 °C; i.e, 5 degrees below the threshold temperature commonly used to identify a heatwave. Paciello *et al.* (2017) also observed a significant difference in pH, with a lower pH in the treated vines than in the control vines. These results are similar to findings made by Kliewer and Schultz (1973) and Aljibury *et al.* (1975), who applied threshold temperatures of 30 °C and 32 °C respectively. This indicates that applying a sprinkler treatment as a mitigative practice for heatwave damage is unlikely to reduce TSS or pH, whereas when it is applied using a lower temperature threshold than those associated with heatwaves, berry ripening can be delayed with an associated reduction in TSS and pH. Furthermore, reducing the temperature threshold for treatment application would increase water usage due to the increased frequency of the treatment application. Therefore, the temperature threshold for treatment application needs to be carefully considered if berry quality and yield parameters are to be preserved.

3.2. Berry weight, Number of Bunches per plant, Bunch weight and Fruit yield

Table 1 shows some of the berry physical traits at harvest. The post-hoc comparison done by Tukey test after the two-way ANOVA showed that berry weight, bunch weight and fruit yield (kg/plant) were significantly higher in Trt (1.89, 62.94, and 2.22 respectively) than in Ctl (1.49, 38.44 and 1.68 respectively). The fruit yield of the Trt plants increased by approximately 27 % relative to Ctl, indicating that the higher LWP of the Trt vines likely contributed to greater water availability for sustaining cell elongation growth during stage III of berry development (Keller, 2015). The number of bunches did not differ significantly between Trt and Ctl, as the vines of each cultivar had been pruned to an equal number of buds prior to budbreak, and the heat stress was not high enough during the study to initiate bunch loss. This therefore demonstrates that the increased fruit yield of Trt vines was related to higher berry and bunch weight.

When comparing the cultivars, Bonarda showed a higher berry weight than Syrah. Malbec also showed high berry weight, but Malbec was not significantly different from either Bonarda or Syrah. Fruit yield was linked to bunch weight in the case of the Syrah and Malbec cultivars, with 2.58 and 2.52 kg/plant respectively being recorded - values that were significantly higher than the average yield of Bonarda (0.75 kg/plant). Although larger berry sizes were recorded for the latter cultivar, it had the lowest number of bunches, which resulted in a significantly lower yield/plant compared to Syrah and Malbec (Table 1). Since the

TABLE 1. Berry weight (g), Bunches per plant (number = n), Bunch weight (g) and Fruit yield (kg/plant) at harvest.

Treatment	Berry weight (g)		Bunches per plant (n)		Bunch weight (g)		Fruit yield (kg/plant)	
Trt	1.89	a	35		62.94	a	2.22	a
Ctl	1.49	b	38		45.17	b	1.68	b
p-value	0	***	0.34	NS	0.0001	***	0.028	*
Cultivar								
Syrah	1.41	b	41	b	62.62	a	2.58	a
Malbec	1.68	ab	55	a	45.90	b	2.52	a
Bonarda	1.98	a	14	c	53.64	ab	0.75	b
p-value	0	***	0.0001	***	0.001	***	0.000	***
Treatment x Cultivar								
Trt*Syrah	1.63	bc	40		75.56	a	3.07	
Ctl*Syrah	1.20	c	42		49.68	bc	2.08	
Trt*Malbec	1.88	ab	51		55.02	b	2.78	
Ctl*Malbec	1.49	bc	60		36.78	c	2.26	
Trt*Bonarda	2.16	a	14		58.23	b	0.81	
Ctl*Bonarda	1.79	ab	14		49.05	bc	0.70	
p-value	0.043	*	0.572	NS	0.068	*	0.291	NS

Values are expressed as an average (n = 3). Different letters within the same column indicate significant differences between the treatments (Trt and Ctl), cultivars (Syrah, Malbec and Bonarda) and treatment x cultivar interaction. Tukey test $p \leq 0,05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***) and NS = not significant.

three different cultivars were all managed in the same way, and they were subject to the same climate, the variations in number of bunches/plant among the grapevine cultivars can be attributed to a genetic component.

The combination treatment*cultivar showed that only berry weight and bunch weight were significant, showing a trend of increased values in Trt vines with respect to Ctl vines for each cultivar. Trt*Bonarda showed higher berry weight values than Ctl*Syrah, while Ctl*Bonarda showed lower bunch weight than Trt*Syrah.

Fruit yield was not significantly different for any treatment-cultivar interaction. However, the three genotypes showed higher Trt values than their respective Ctl values. These results are similar to the findings of other research that focused on mitigative water applications for heatwave stress reduction (Caravia *et al.,* 2017; Greer and Weedon, 2014; Martínez-Lüscher *et al.,* 2020; Previtali *et al.,* 2023). By contrast, Paciello *et al.* (2017) did not observe any significant differences between Trt and Ctl vines treated using a nebulised spray in the bunch zone, which was activated at a threshold temperature of 30 ºC.

3.3. Total polyphenol index

In terms of total polyphenol index (TPI), none of the three cultivars showed any significant differences between the Trt and Ctl on any sample date (Figure 6). This is in line with findings in much of the literature, with no significant effects of temperature exposure on total polyphenols concentration being observed (Cohen *et al.,* 2008; Tarara *et al.,* 2008). Some research has found that overall flavonol concentrations decrease in extreme temperatures (> 45 ºC) (Gouot *et al.,* 2019a). Such extreme temperatures were not observed during the two natural heatwave events of the present study, the maximum observed temperature being 42 ºC (on 12 February 2023) and only two days of temperatures above 40 °C during the course of the season (Figure 1).

3.4. Anthocyanins

Table 2 shows the berry anthocyanin content at harvest time for the three tested cultivars. Df was the only anthocyanin that had a significantly higher content in Trt than in Ctl. The rest of the free and conjugated anthocyanins, as well as total anthocyanins, did not show any significant differences between Trt and Ctl. When comparing the anthocyanin content of the three cultivars, Bonarda was found to have the highest Df, Cn, Pt, Po, Mv, MvCu and total anthocyanin content; moreover, no significant differences ($p \leq 0.05$) were found in total anthocyanins between Ctl and Trt at any sample date during the experiment. However, of the three cultivars, Bonarda contained the lowest concentrations of PoAc, MvAc and PoCu. Nevertheless, Bonarda showed significantly higher total anthocyanin content ($p \leq 0.1$) on S5 than Malbec and Syrah (Figure 6d).

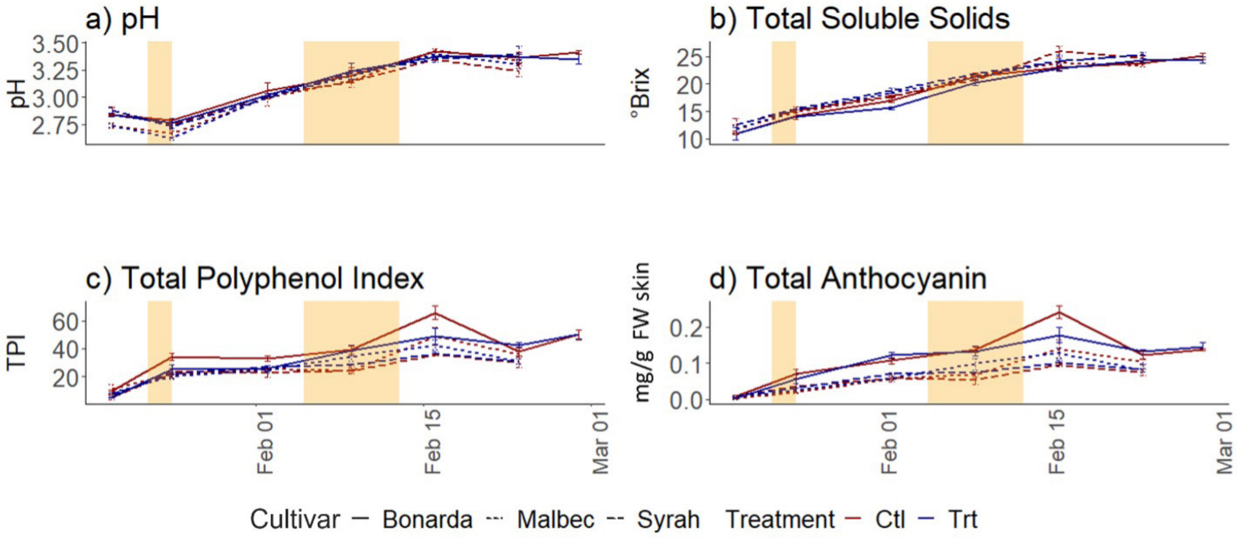


FIGURE 6. a) pH, b) Total Soluble Solid, c) Total Polyphenol Index, and d) Total Anthocyanin evolution during growing season. No significant differences ($p < 0.05$) between Trt and Ctl were identified for any of the cultivars.

The results of the overhead spray water treatment were characterised by a high percentage of simple anthocyanin glycosides (60.16 % of total forms on average), with a higher Df and Cn content than the control, as has already been reported in the literature regarding vines under heat treatment (de Rosas *et al*. 2022). Conversely, in the present study, the highest proportion of acylated and coumaroylated anthocyanins were recorded in the Ctr. These results indicate that the percentage increase in glucosylated forms (3.98 %) in the treated plants occurred as a consequence of the decrease in the same proportion of acylated forms. This is because the treated vines were not subjected to thermal stress, as the VPD was lower due to the overhead spray water, suggesting that both acylated and non-acylated compounds were significantly affected.

No differences were found between Trt and Ctl in terms of percentage of Mv; however, its coumaroylated version was significantly higher in Ctl than in Trt (Table 3).

When comparing the cultivars, Bonarda contained the highest percentage of glycosylated forms: 62.02 % compared to 57.42 and 55.07 % for Syrah and Malbec respectively. The anthocyanin composition of Bonarda also changed, with an observable increase in the percentage of glycosylated anthocyanins in the Trt vines from S5 until S7 (harvest). Inverse behaviour was found between the cultivars in terms of percentage of acylated and coumaroylated forms: from S5 till S7 (harvest), both Syrah and Malbec showed the higher percentages of these forms than Bonarda (Table 3).

Although no significant differences in malvidin-3-*O*-glucoside (Mv) were found between the tested cultivars, it was the most predominant anthocyanin, having an average content of 43.47 %; it was followed by malvidin-3-*O*-glucoside coumaroylated (MvCu), Pt, MvAc, Po, PoCu, Df, PoAc and Cn, all of which (apart from MvCu) differed in behaviour depending on the genotype.

Some differences in the anthocyanin profile between treatment and cultivar were observed. Grapes from Trt Bonarda had the highest content of glycosylated forms, the lowest content of MvCu and coumaroylated anthocyanins (along with Syrah), and the lowest content of acylated anthocyanins.

Finally, Trt Bonarda and Trt and Ctl Syrah all showed the lowest MvCu contents, while Ctl Bonarda and Trt and Ctl Malbec showed the highest contents.

As previously mentioned, the treatment appears to negatively influence the percentage of the most stable forms of anthocyanins, namely acylated -derivatives. An associated rise in the amount of acylated and tri-hydroxylated anthocyanins in Malbec and Bonarda grapes when exposed to elevated temperature conditions was also observed by de Rosas *et al.* (2017). This indicates that pigment acylation is a possible stress-response mechanism that attenuates the negative effects of high temperature. In the present study, there was an increase in more stable forms of anthocyanins as a result of acylation in Bonarda Ctl vines when exposed to higher temperatures during heatwaves; this points to a potential mechanism for coping with temperature stress. These results are also similar to those previously reported by de Rosas *et al.* (2022) in Malbec and other cultivars. The variation in impact of the treatment on the composition and concentration of anthocyanins depending on the cultivar indicates that the cultivars vary in plasticity. Although Syrah, Malbec and Bonarda were exposed to the same treatment, the anthocyanin composition of Bonarda was significantly altered, containing less acylation and coumaroylation, while that of Malbec and Syrah was not. These results are in line with previous observations of cultivar plasticity and

TABLE 2. Total Anthocyanin content (mg/g fresh skin weight) at harvest.

Treatment	Df	Cn	Pt	Po	Mv	PoAc	MvAc	PoCu	MvCu	TAC
Trt	5.274 a	0.411	6.168	3.881	45.783	0.277	4.815	3.629	30.880	102.661
Crt	3.862 b	0.247	7.711	4.163	45.206	0.399	5.399	4.332	35.713	105.488
p-value	0.044	0.067	0.085	0.742	0.884	0.498	0.343	0.309	0.09	0.724
	**	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cultivar										
Syrah	2.133 b	0.309 ab	3.779 b	5.674 a	33.333 b	0.931 a	6.254 a	5.296 a	20.282 c	77.990 b
Malbec	3.278 b	0.077 b	5.133 b	2.069 b	40.201 b	0.082 b	5.448 ab	2.451 b	33.585 b	92.324 b
Bonarda	8.293 a	0.602 a	11.906 a	4.324 ab	62.949 a	0.000 b	3.619 b	4.194 ab	46.023 a	141.909 a
p-value	0.0001	0.001	0.0001	0.013	0.000	0.002	0.01	0.014	0.0001	0.0001
	***	***	***	*	***	**	**	*	***	***
Treatment x Cultivar										
Trt*Syrah	2.903	0.445 ab	4.713	4.971	35.456	0.830	6.238	4.764	21.243	81.562
Ctr*Syrah	1.363	0.173 bc	2.845	6.378	31.210	1.031	6.269	5.829	19.321	74.419
Trt*Malbec	3.070	0.000 c	4.743	1.422	36.366	0.000	4.638	1.744	28.682	80.666
Ctr*Malbec	3.485	0.154 bc	5.522	2.716	44.036	0.165	6.258	3.158	38.488	103.982
Trt*Bonarda	9.848	0.789 a	13.676	5.251	65.526	0.000	3.569	4.379	42.715	145.755
Ctr*Bonarda	6.737	0.414 abc	10.136	3.396	60.372	0.000	3.669	4.009	49.330	138.063
p-value	0.111	0.048	0.138	0.236	0.355	0.883	0.485	0.525	0.209	0.222
	NS	*	NS	NS	NS	NS	NS	NS	NS	NS

Abbreviated pigments names are as follows: Df. delphinidin-3-glucoside; Cn. cyanidin-3-glucoside; Pt. petunidin-3-glucoside; Po. peonidin-3-glucoside; Mv. malvidin-3-glucoside; PoAc. peonidin-3-O-acetylglucoside; MvAc.

malvidin-3-O-acetyl-glucoside; PoCu. peonidin-3-O-coumaroyl-glucoside; MvCu. malvidin-3-O-coumaroylglucoside; TAN. Total anthocyanin content.

Values are expressed as an average (n=3). Different letters within the same column indicate significant differences between treatments (Trt and Ctl), Cultivars (Syrah, Malbec and Bonarda) and Treatment x Cultivar interaction). Tukey test $p \leq 0,05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***) and NS = not significant.

TABLE 3. Anthocyanin composition (percentage) at harvest.

Treatment	% Gluco.	% Acet.	% Cuma.	% Acylated	% Df	% Cn	% Pt	% Po	% Mv	% PoAc	% MvAc	% PoCu	% MvCu	Tri/Di
Trt	60.16 a	5.77	34.06 b	39.84 b	4.58 a	0.37	6.88 a	4.02	44.31	0.4	5.38	3.94	30.12 b	18.4
Ctl	56.18 b	6.24	37.58 a	43.82 a	3.33 b	0.22	5.46 b	4.54	42.63	0.52	5.73	4.58	33 a	17.28
p-value	0.004	0.56	0	0.004	0.02	0.13	0.02	0.631	0.157	0.596	0.582	0.496	0.004	0.731
	**	NS	***	**	*	NS	*	NS	NS	NS	NS	NS	**	NS
Cultivar														
Syrah	57.42 b	9.5 a	33.08 b	42.58 a	2.48 b	0.39 ab	4.56 b	7.62 a	42.36	1.3 a	8.21 a	7.23 a	25.85 c	7.7 b
Malbec	55.07 b	5.98 b	38.95 a	44.93 a	3.56 b	0.07 b	5.58 b	2.17 b	43.7	0.07 b	5.9 b	2.58 b	36.37 a	28.12 ab
Bonarda	62.02 a	2.55 c	35.43 b	37.98 b	5.83 a	0.42 a	8.38 a	3.05 b	44.34	0 b	2.55 c	2.96 b	32.47 b	17.71 ab
p-value	0.001	0	1E-04	0.0001	0	0.02	0	0.002	0.367	0.001	1E-04	0.002	0.0001	0.001
	***	***	***	***	***	*	***	**	NS	***	***	**	***	***
Treatment x Cultivar														
Trt*Syrah	58.68 b	9.17	32.16 c	41.32 a	3.16	0.57	5.34	6.63	42.99	1.19	7.98	6.61	25.55 b	8.77
Ctl*Syrah	56.16 b	9.84	34 bc	43.84 a	1.8	0.22	3.78	8.62	41.74	1.4	8.44	7.86	26.14 b	6.62
Trt*Malbec	56.47 b	5.72	37.81 ab	43.53 a	3.8	0	5.87	1.79	45	0	5.73	2.18	35.62 a	31.56
Ctl*Malbec	53.68 b	6.23	40.1 a	46.32 a	3.31	0.14	5.28	2.54	42.4	0.15	6.08	2.98	37.12 a	24.68
Trt*Bonarda	65.35 a	2.43	32.22 c	34.65 b	6.79	0.55	9.42	3.65	44.93	0	2.43	3.02	29.2 b	14.87
Ctl*Bonarda	58.7 b	2.66	38.64 a	41.3 a	4.87	0.3	7.33	2.46	43.74	0	2.66	2.9	35.74 a	20.55
p-value	0.045	0.97	0.003	0.045	0.48	0.12	0.52	0.48	0.847	0.923	0.988	0.825	0.006	0.3
	*	NS	**	*	NS	NS	NS	NS	NS	NS	NS	NS	**	NS

Abbreviated pigment names are as follows: 'Gluco'.: Glucosylated, 'Acet.': Acetylated, 'Cumar. ': Cumaroylated, 'Tri/Di' trisubstituted/disubstituted. Df. delphinidin-3-glucoside; Cn. cyanidin-3-glucoside; Pt. petunidin- 3-glucoside; Po. peonidin-3-glucoside; Mv. malvidin-3-glucoside; PoAc. peonidin-3-O-acetylglucoside; MvAc. malvidin-3-O-acetyl-glucoside; PoCu. peonidin-3-O-coumaroyl-glucoside; MvCu. malvidin-3-O-coumaroylglucoside. Values are expressed as an average (n=3). Different letters within the same column indicate significant differences between treatments (Trt and Ctl), Cultivars (Syrah, Malbec and Bonarda) and Treatment x Cultivar interaction. Tukey test $p \leq 0{,}05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***) and NS = not significant.

anthocyanin response to temperature (Gilbert *et al*., 1971; de Rosas *et al.,* 2022). Thus, phenotypic plasticity, characterised as the capacity of an organism to alter its phenotype in response to diverse environments, can serve as a mechanism to alleviate adverse effects due to heatwaves. Therefore, the strategy of targeted overhead micro-spray water treatment as a mitigative tool may work better on some cultivars than on others depending on their plasticity linked to their anthocyanin profile.

CONCLUSION

The increase in observed number, severity and duration of heatwaves throughout the world, along with reduced water accessibility, means that strategies for reducing vine stress that do not rely on large volumes of water are needed. It is common practice to apply supplementary irrigation before a heatwave to enhance the cooling effect of transpiration and promote evaporative cooling; such irrigation-based mitigation strategies require an estimated minimum of 10.6 L of water/vine/day. By contrast in the present study, the overhead pulsed micro-spray water treatment administered at canopy level required a significantly lower volume of water: only 0.53 L vine/HW/day, compared to an estimated minimum of 10.6 L/vine/day when applying an irrigation-based mitigation strategy.

This method was applied during heatwaves and showed a clear and prolonged decrease in canopy temperature, leading in turn to a reduction in vine physiological stress metrics associated with extreme heat and drought events. Furthermore, independently of the cultivars used in this study, our method seemed to be an appropriate way of alleviating the negative effects of heatwave stresses on yield during the heatwaves occurring 15 days before harvest, without affecting grape anthocyanin and polyphenol content, pH and TSS. The impacts of the treatment seemed to last longer after the heatwaves of longer duration and that comprised more sudden increases in temperature, indicating that it can help vines adapt to extreme changes in temperature, as well as extreme temperatures themselves. The integration of this technology into vineyard practices could provide viticulturists from warm and dry regions with a mitigation tool for managing heatwaves and proactively adapting to the challenges posed by climate change, without increasing the risk of fungal disease development. This technology could also be used during winter to combat frosts.

ACKNOWLEDGEMENTS

This work was supported by SIIP-UNCuyo (Grant 2022 06/A054-T1) and H2020-MSCA-RISE-2019**:** vWISE Project Nº 872394.

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