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**CICLO: XXXVI**

# The Identification of Climate Change Derived Risks to the Terroir Driven Grape and Wine Production of Nebbiolo in Barolo DOCG with the Development of a Prevention and Mitigation Strategy

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# Abstract

It is well established that the climate is changing globally. It is impacting wine regions with negative consequences for some existing regions. Increased vine stress, reduced yields, and lower quality fruit leading to lower quality wines are all current concerns with increasing temperatures, drought, and extreme climate events such as high-volume rainfall events, hailstorms, and late spring frosts. Climate change also threatens the typicality of wines that are renowned for their terroir driven character. Barolo DOCG wines are among the world's highest quality, terroir driven wines.

The purpose of this research was to identify the influence of some geomorphological traits associated with resulting berry characteristics of a high-quality wine grape growing region (Barolo DOCG). Further, research was carried out to determine the potential for increased risk associated with these geomorphological traits on some quality parameters of grape berries in a changing climate, specifically under increased temperature or decreased UV exposure. In this research, the growing region associated with the production of Barolo DOCG wines has been used due to the complexity of the topography. Barolo DOCG grapevines are situated on slopes of varying aspects ranging from 45° NE through 180° S to 45° NW and on elevations between approximately 200 m ASL to a maximum of approximately 500 m ASL

Finally, the investigation of the efficacy of a possible mitigative strategy focused on maintaining vine health and berry quality during exposure to extremely high temperatures.

# 1. Thesis Introduction

## 1.1. Climate change risks to viticulture

Globally, the climate has been changing. Records are showing increasing temperatures with expected global average temperatures to increase between 1.5

to 4.0 ℃ depending on projections of mitigative efforts (Calvin et al., 2023). In fact, the year 2023 was a record year for global mean surface temperatures set, in part, due to anthropogenic climate change (Adler & Gu, 2024). Beyond increasing average temperatures, an increase in short term exposure to extreme temperatures (heatwaves) as well as the duration and temperature of these events is being observed globally (Perkins-Kirkpatrick & Lewis, 2020). Precipitation is also changing globally; however, the patterns are less clear. In some regions of the world increases in annual precipitation are being observed while in other areas, total precipitation is decreasing (Adler & Gu, 2024). Further, in some areas, although total precipitation is not changing significantly, the volume per event and the number of events are changing with the prior increasing and the latter decreasing (Calvin et al., 2023). With these global changes in climate, particularly on temperature and water availability, concerns in the viticultural sector have increased regarding risks of earlier harvests, higher wine alcohol levels, altered flavour profiles and increased risk of extreme weather events, including hail storms, wind storms, high volume precipitation events, and heatwaves resulting in significant yield losses and possible vine death. Exposure to high (> 35℃) or extreme temperatures (>40℃) can impact berry quality with reduced anthocyanin production as well as possible effects on flavonols and other phenols and volatile aromas with the possibility of reducing the overall quality of the wine and its regional typicality (Mori et al., 2007). Further, exposure to high temperatures can lead to yield losses with reduced berry size, shrinkage, and sunburn damage (Venios et al., 2020). A combination of low precipitation and high temperatures can exacerbate risks of yield loss and reduced plant health as well as the potential for vine death. Financial losses associated with lost or lowered yields can run into billions of euros and can impact rural based jobs and rural society (Parker et al., 2020).

### 1.2. Terroir

This reduction in quality parameters associated with climate change also runs the risk of impacting the terroir. The International Organisation of Vine and Wine (OIV, 2010) defines terroir as the “concept which refers to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied vitivinicultural practices develops, providing distinctive characteristics for the products originating from this area.” (Leturcq, 2020). Natural terroir considers a region’s parent material (geology); soil; geomorphology (topography) and climate while biological terroir includes man made choices in the vineyard such as clone, rootstock, row orientation, and vineyard management. The focus of this research was specific to natural terroir.

### 1.3. Steep Slope Viticulture

Viticulture practiced on steep slopes has a long history in Europe and is associated with increased quality predominantly due to increased insolation (Hofmann & Schultz, 2015). Climate change associated risks to the viticulture sector are more pronounced in steep slope viticultural regions (Tarolli et al., 2023). Not only do steep slopes increase the potential for serious soil erosion in significant rainfall events but also water management issues in case of drought (Wang et al., 2023). Further, with increased solar radiation reception on slope faces, plant evapotranspiration rates are increased (Hofmann & Schultz, 2015), and with increasing temperatures associated with a changing climate this can lead to severe water deficits, as well as damage to berries from extreme sun exposure (Hofmann & Schultz, 2015; Strack et al., 2021).

### 1.4. Barolo Terroir

Barolo DOCG (*Denominazione di Origine Controllata e Garantita*) is a type of wine produced from 100 % Nebbiolo grapes grown in a specific geographical area. This region is located in the northwest of Italy in the Piemonte region approximately 55 km southeast of the city of Torino. The growing region covers close to 70 $km^2$ area with a topographically complex environment. The region is a part of a synorogenic tertiary Langhe sub-basin produced from the collision of the European and Adriatic continental plates and the eventual indentation from this collision which occurred in the middle-late Eocene (38 – 33.9 Ma (Megaannum)) (Piana et al., 2017). The geology of the region is sedimentary shallow marine deposits predominantly marl and silty carbonates and claystone (Dela Pierre et al., 2011). These deposits occurred as the Adriatic Sea encroached on the current day Po Valley reaching the base of the Appenine Mountain chain in the west. As the Apennine range uplifted during the quaternary, the hilly Langhe region was created (Bartolini, 2010). This was in part due to the mountain building process and partly due to the recession of the Adriatic Sea (Cyr & Granger, 2008). As the sea receded, it eroded the clay, marl, and sandy marl stone deposits that had previously been the seabed into an extremely tortuous environment with steep sloped hills with aspects ranging from 0 to 360° gradient. Slopes can reach 45° gradient with a variation in elevation from the valley floor at approximately 190 m ASL to almost 550 m ASL in the growing region. The soils of the area largely reflect the parent material deposits of shallow sea, high and low velocity deposits of carbonates, silts, sands and clays.

The climate of Barolo is elaborated in papers 1 and 2. However, not discussed in either paper is the observation of temperature increase in the region. The Huglin Index, a measure of the suitability of a region’s climate for viticulture taking into account factors like temperature and sunlight, was calculated at two weather stations: Castiglione Falletto (CF) (44°37'44.0"N 7°58'38.3"E); La Morra (LM) (44°37'22.3"N 7°56'20.2"E).

$$HI = k * \sum_{01.04}^{30.09} \left(\frac{Tmean + Tmax}{2}\right) - 10$$

Tmean = daily mean temperature

Tmax = daily max temperature

K = latitude dependent parameter (1.04 for Barolo DOCG area)

Calculated between April 1 and September 30 (Huglin, 1978).

Although there was variation between years in the Huglin Index, indicating that climate conditions can fluctuate, it has increased by approximately 400 DD (Degree Days) between 1996 and 2023 which supports global observations of temperature increase (Figure 1A). The $R^2$ value of 0.4209 for the trend line suggests a moderate correlation between time and the Huglin Index, meaning that while there is a general upward trend, there might be other factors influencing the variability. Total precipitation (mm) in the region had no observable temporal trend in the last 28 years (Figure 1B).

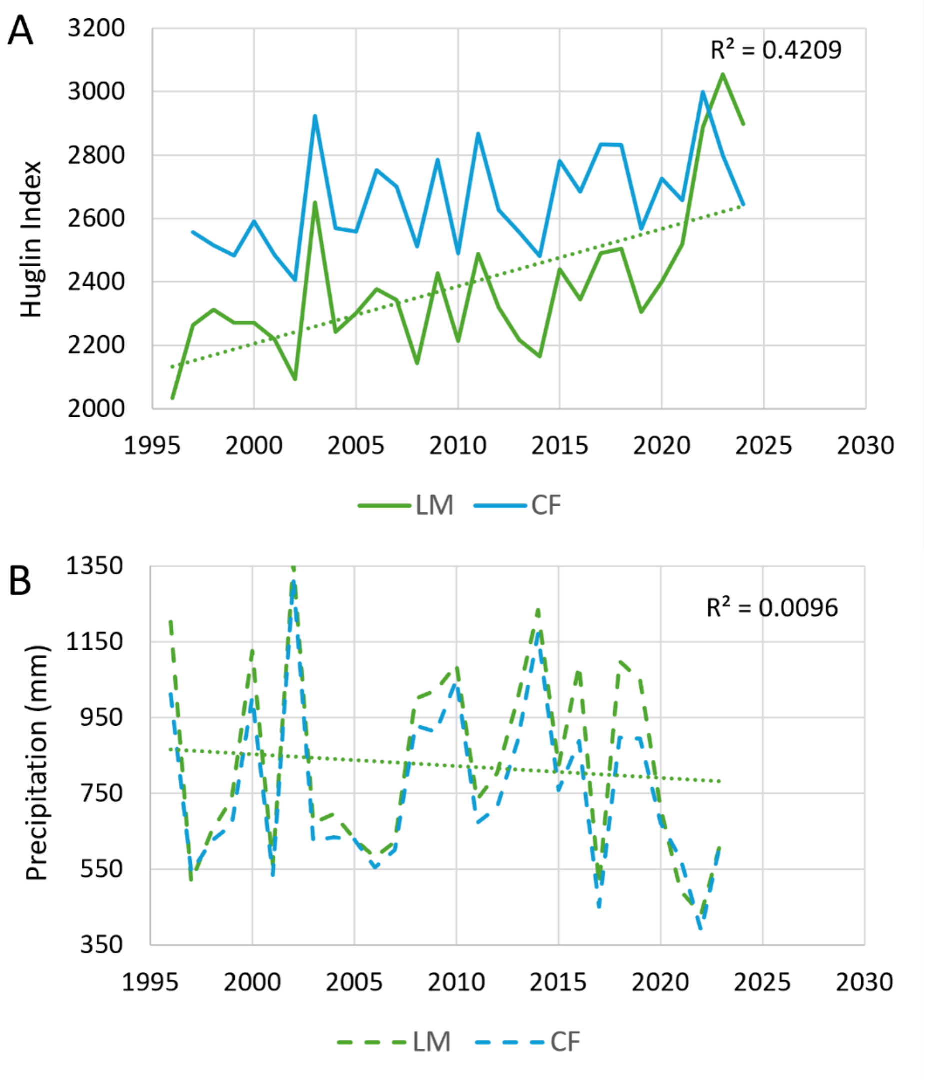

Figure 1: A) Annual Huglin Index for La Morra (LM) (solid green) and Castiglione Falletto (CF) (solid blue) weather stations from 1996 to 2024 with linear regression and $R^2$ (dotted green) for LM weather station and B) Annual precipitation (mm) LM (dashed green) and CF (dashed blue) with linear regression and $R^2$ (dotted green) for LM weather station.

### 1.5. Geostatistical analysis of meso-scale climate behaviour

Geostatistical calculations were applied in the region of study to determine if any topographical features influenced specific climatic indices consistently. Further, this analysis was performed to establish if mesoscale climatic indices behaved in a manner that followed macro-scale expectations of the region including the Barolo DOCG growing area and surrounding area. It also sought to identify any potential climatic behaviour at the mesoscale which did not comply with macroscale expectations associated with topographical traits including elevation.

Geostatistics is a set of statistical techniques that incorporate a spatial element to predict point data on two, three, or four dimensions (Hengl, 2007). Originally developed by the mining industry to locate ore deposits, it is commonly used in several environmentally based industries. Geostatistical processes have been utilized to map soil, geological, and climatic characteristics for enhanced comprehension of regional processes and spatial variation (Hudson & Wackernagel, 1994; Minasny & McBratney, 2010). In the viticultural field, geostatistical methods are recommended as an approach to terroir identification (International Organisation of Vine and Wine, 2012). There are several examples of various geostatistical methods being used to interpolate climate, soil, and phenological point data across a three or four dimensional plain. Regression kriging has been utilized to interpolate daily minimum and maximum temperature in the Bordeaux growing region as well as several bioclimatic indices and phenological forecasting (Bois et al., 2018).

Geostatistical evaluation at the mesoscale of specific climate, soil, or geological characteristics in a region can be utilized as a low-cost, the first step to a better understanding on a multi-dimensional plane how a dependent variable is influenced by topographical features such as elevation and aspect (Bois, et al., 2018).

For reliable geostatistical analysis, it is recommended that 50 data points be utilized in the region of interest (Hengl, 2007). Within the immediate region legally defined for Barolo DOCG wines, there are from 3 (1996) and 7 (2019) active open-source weather stations. Within a 40 km radius of the center of the Barolo DOCG growing region, 50 active weather stations were identified in

2019. Extending the analysis beyond a 40 km radius risked the inclusion of weather stations situated in the pre-Appenine mountains at elevations above 1000 m ASL which could have introduced macroscale influences. Of the 50 weather stations within the mesoscale region, 5 were pluviometers. Ultimately, open-source data from 45 weather stations was collected between the years 1996 (13 active weather stations) and 2019 (45 active weather stations). Weather stations are managed by Sisteme Piemonte (*RAM - Banca Dati Agrometeorologica - Sistemapiemonte*, 2019) and Agenzia Regionale per la Protezione Ambientale (ARPA) (Arpa, 2019) (Figure 2). All weather stations measured TMax and TMin at an hourly rate while some also measured precipitation without differentiation between rain and snow. Due to the scale of the work and the limited number of weather stations that collected more than temperature data, only temperature values were analyzed. Further, monthly average values were used rather than daily or hourly.

Linear models were developed between climatic indices (Huglin Index (HI); Average Monthly Maximum Temperature (TMax); Average Monthly Minimum Temperature (TMin); and Diurnal Range (DR) and topographical variables associated with the location of the weather stations. Topographical values that were utilized as independent variables included elevation (ELEV) [m above sea level (ASL)], aspect (° from N), slope (° gradient) and degrees latitude. Model performance was evaluated using Akaike Information Criteria (AIC) and Bayesian Information Criterion as well as Root Mean Square Error (RMSE) to determine the best prediction and performance.

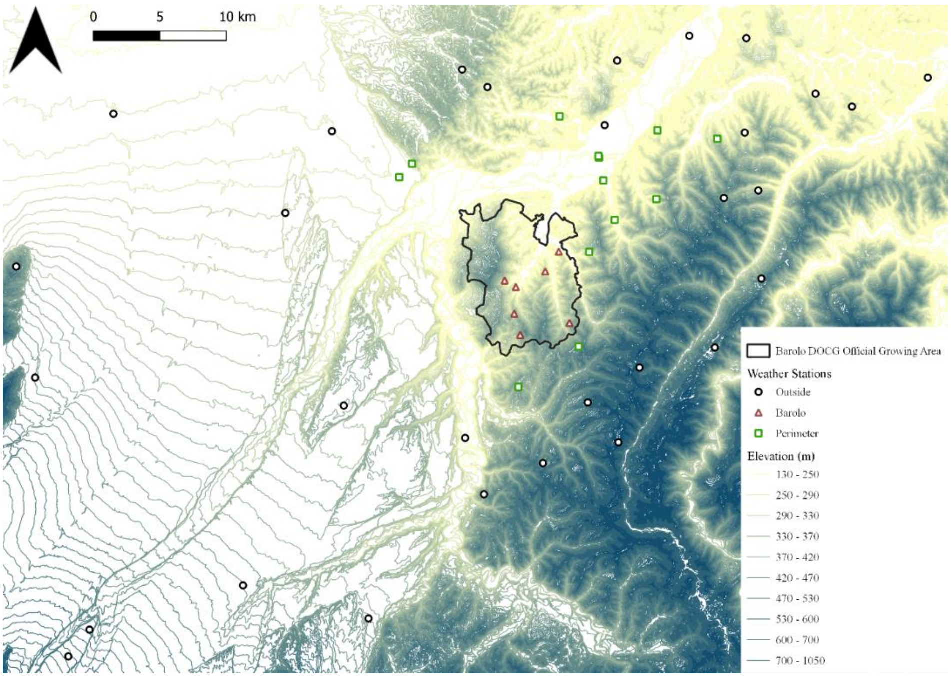

Figure 2: Weather station location within 40 km radius of the center of the Barolo DOCG growing region. In Barolo DOCG (red triangle); proximate to Barolo DOCG (green square); distal to Barolo DOCG (black circle). Elevation extracted from Digital Elevation Model at 10 m resolution (Tarquini et al., 2007).

If a relationship was found between a topographical trait (or combination of topographical traits) and a bioclimatic index, universal kriging otherwise known as kriging with extended drift was utilized to interpolate the bioclimatic indices over the entire region of study.

Elevation, aspect, latitude, and slope values from a rasterized Digital Elevation Model (DEM) were extracted onto a digital point grid with grid-nodes at 100 m spacing covering the same area as the weather stations used for the linear models. This grid was used in conjunction with weather station point data to perform universal kriging. Different kriged models were compared using Leave One Out Cross Validation (LOOCV). LOOCV recalculates the model repeatedly, each time excluding one weather station 'real' value and interpolating a value based on the linear model (Bois et al., 2018).

Geostatistical analysis of the regional historical temperature behaviour as it is associated with the local topography showed some significant relationships. The most important linear relationship is a negative relationship between ELEV and HI, GST, and TMax. This relationship was consistent for all months in all years of the study.

The Huglin Index (Figure 3A) showed a consistent negative relationship with ELEV for all years between 1996 and 2019. The $R^2$ values ranged from 0.30 to approximately 0.70. In some years HI was also impacted by latitude with higher values in the north due to a decrease in elevation trending northeast. Linear models for all weather station values suggest that within the growing region of Barolo DOCG, a parabolic behaviour for the Huglin Index may exist, with low values occurring at low and high altitudes with the middle altitudes having higher HI values (Figure 3A).

No consistent relationship between HI and Aspect was identified although in some cases an additive model including Elevation and Aspect was used due to improved model performance. The lack of a consistent relationship with aspect may be attributed to a scale issue and deserves further exploration for its role as a dominating topographical characteristic in berry quality as was determined by (Mania et al., 2021). In this research, the scale was too large (the distance between weather stations was too large to capture granular detail from changes in aspect) to consistently capture the effect of differing aspects on insolation levels and thus temperature exposure (Hengl, 2007).

The same negative relationship between elevation and maximum monthly temperature was observed for all months (Figure 3B). The average GST also showed a negative relationship with ELEV with approximately 0.41 °C decline per 100 m (data not shown). No relationship between Elevation and TMin was observed in any year during months of the growing season (Figure 3C). Considering that the minimum daily temperature is almost always observed at night, the monthly minimum temperature can be considered a night temperature and the average monthly diurnal range can be calculated between the Maximum and Minimum Temperatures. Due to the lack of a relationship between the minimum temperature and elevation, the Diurnal Range appeared to have a negative relationship with elevation meaning that the lower elevation areas had a higher difference between maximum and minimum temperatures (Figure 3D). No relationship was observed between PRECIP and any topographical character in any year of analysis (data not shown).

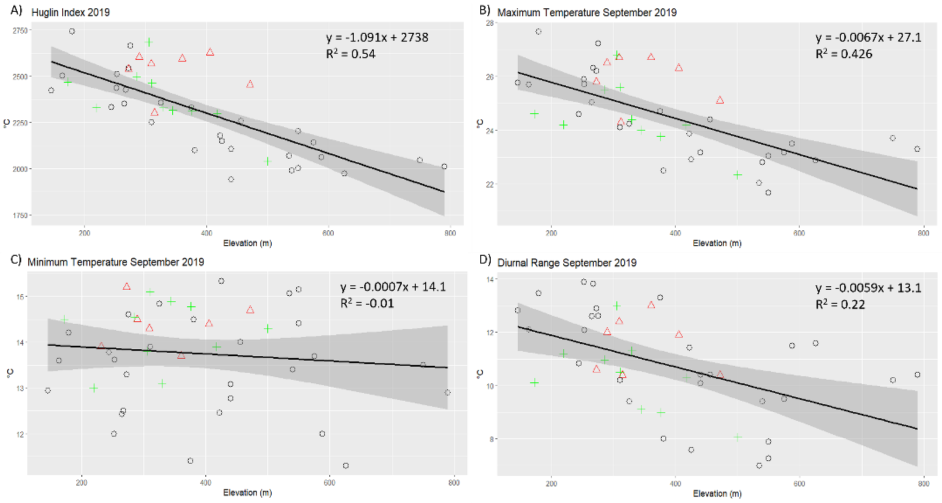

Figure 3: Linear models of HI (A) in 2019; TMax (B); TMin (C) and DR (D) in September of 2019 by elevation calculated at 45 weather stations within a 40 km radius from the center of Barolo DOCG growing region: Δ - indicates weather stations within the growing region of Barolo DOCG; + - weather stations proximate to Barolo DOCG region; ○ – weather stations within 40 km of Barolo DOCG region.

## 1.6. Thesis Structure

This thesis is a compilation of three research papers (3 published). The following is a brief introduction to the concepts researched in each paper.

### *1.6.1. Terroir Units and Aspect as a terroir component*

The first paper (Chapter 2: Managing Vineyard Topography and Seasonal Variability to Improve Grape Quality and Vineyard (https://doi.org/10.3390/agronomy11061142)) investigates the difference in grape yield parameters and secondary metabolites in the Barolo DOCG growing region based on variation in topography. The research identified that the slope aspect played a significant role in variation in quality parameters likely predominantly associated with variation in solar radiation incidence. This field research was carried out during the 2012 and 2013 vegetative seasons, in 17 different vineyards throughout the Barolo area. Results showed a correlation between solar radiation and exposure. SW and SE-facing vineyards had higher radiation than E and W vineyards with SW vineyards being the hottest in both years and showing increased total soluble solids and higher pH and lower anthocyanin concentration than vineyards at other slope aspects. Elevation did not appear to factor in the cluster analysis in a meaningful way, although all SE facing vineyards were at lower elevations than all other vineyards (except two of the W-facing vineyards) (Chapter 2 Table 1).

### *1.6.2. Berry quality at different elevations under different temperature and UV exposure*

Due to the geostatistical findings described in Chapter 1.5, the second paper (Chapter 3: The effect of temperature and UV manipulation on anthocyanins, flavonols and hydroxycinnamoyl-tartrates in cv Nebbiolo grapes (*Vitis vinifera* L.) (https://doi.org/10.3390/plants13223158)) was the result of a research project designed to determine the effect of elevation on berry quality parameters. Often elevation is recognized as an important determinant of berry quality. Typically, at macro-scale, temperatures decrease with increased temperature between 0.3 °C and 1.0 °C per 100 meters increases in altitude. This is called the lapse rate (Fairbridge & Oliver, 2005). Therefore, higher elevation is often linked to cooler climates which result in delayed phenological timing and allows for prolonged ripening. Exposure to short wavelength UV-irradiation (200 - 400 nm) also increases with increased elevation up to more than 20 % per 1000 m altitude, specifically 310 – 340 nm (Blumthaler et al., 1997). This can lead to increased production of secondary metabolites potentially enhancing berry quality since UV light can induce its synthesis (Mansour et al., 2022).

Interestingly, during the experiment presented in the second paper, an unexpected phenomenon occurred wherein the climate behaviour altered for the first time in almost 30 years of measurements. Typically, since the first readings in 1996, the La Morra weather station observed the lowest annual Huglin Index calculations compared to all other weather stations in the Barolo growing region. However, between the 2022/2023 growing seasons, this trend reversed with La Morra (314 m ASL, SE aspect) observing much higher Huglin Index values than the neighbouring weather station at Castiglione Falletto (309 m ASL; SW aspect) (Figure 1A). The reason for this reversal is unclear but noteworthy. A similar response was observed in the three vineyards with the middle vineyard observing a higher Huglin Index value in 2022 and a lower value in 2023 than the higher and lower vineyards. This sudden and significant climate alteration reduced the clarity of the impact of elevation on grape quality parameters. Further, no significant increase in flavonol concentrations was observed at the higher elevation vineyard suggesting that although there was a 200 m difference between the highest and lowest vineyards, the increase in UV exposure is negligible at 400 m ASL in this region.

This piece of research also incorporated treatments reflecting possible alterations to UV and temperature exposure to determine the potential impact on berry quality associated with increased temperature and alterations in UV exposure. Due to the erratic behaviour of climate at the different elevations, the results of this research focus predominantly on the response of the berries to the UV and temperature treatments.

### *1.6.3. Mitigative strategies to protect vine health and berry quality during heatwaves*

The third paper (Chapter 4: Overhead spray water treatment as a mitigation strategy to alleviate vine stress and safeguard grape quality during heatwaves (https://doi.org/10.20870/oeno-one.2024.58.2.7847)) discusses one potential mitigative application to reduce vine stress and protect berry quality during heatwaves.

Considering the increase in the number of heat waves as well as the increase in their duration and intensity (Perkins-Kirkpatrick & Lewis, 2020) researchers, producers and proprietors have been establishing mitigative methods to protect grape quality and vine health in pre-existing vineyards. Research on mitigative strategies to protect current vineyards from the effects of a changing climate is underway globally. Strategies for new vineyard development will not be discussed in this brief synopsis as they are considered adaptative.

Briefly, some of the main current strategies for pre-existing vineyards include:

Kaolin applications are frequently utilized as a mitigative treatment to reduce sunburn from excessive exposure (Cataldo et al., 2021). Kaolin is an inert clay mineral which increases reflectance of ultraviolet and infrared radiation from grape leaves and berries thus reducing temperature (Conde et al., 2016; Glenn & Puterka, 2004). It has been established that kaolin applications can increase the concentration of phenolic compounds including anthocyanins and flavonols in fully mature berries through stimulation of the phenylpropanoid (flavonoid) pathways (Conde et al., 2016). Further, foliar kaolin applications have been observed to protect the structure and function of photosystem II while enhancing photosynthesis (Dinis, Ferreira, et al., 2016). Further, this treatment has been observed to reduce the amount of Reactive Oxygen Species (ROS) in Touriga Nacional (Dinis et al., 2016) as well as less negative water potential in Sauvignon Blanc (Cataldo et al., 2022).

The application of shade netting is frequently utilized to reduce both temperature and sun exposure. Increased shading is known to alter grape composition and can lead to a reduction in berry quality (Cataldo et al., 2022). Shade netting can also induce changes in physiological response with a significant decrease in net photosynthesis as was observed by Cataldo et al., (2022) under 30 % and 70 % shade in Sauvignon Blanc vines as compared to control, as well as a decrease in stomatal conductance (which was lower than both control and foliar kaolin application. In the same research, no clear differences were observed between shade netting applications, foliar kaolinite and, the control for either Fv/Fm or SPAD measurements suggesting that the treatments did not impact Photosystem II in any meaningful way. In this research, 70 % shade netting (along with 30 % shade netting and foliar kaolin treatments all showed a significantly lower TSS (°Brix) than the control treatment at harvest. However, only 70 % of shading and kaolin applications showed a significantly higher tartaric acid concentration than other treatments (Cataldo et al., 2022). Further, differences in pH or berry weight were observed between treatments with 70 % shading having lower pH and higher berry weight than 30 % shading and control in the second year of the study which had slightly higher maximum temperatures and significantly less precipitation than the first year. This suggests that 70 % shade netting could be effective in reducing plant stress in years with lower precipitation and higher temperatures. Increased shading is known to reduce the concentration of flavonols as well as di-hydroxylated anthocyanins (Chorti et al., 2010). Shade netting application (60 % shading) has previously been observed to reduce the concentration of cyanidin, peonidin and, petunidin while increasing the concentration of malvidin and enhancing total anthocyanins as compared to a control (Martínez-Lüscher et al., 2020). In severe heatwaves, anthocyanins in both treatments declined but exposed berries observed a greater decline than shaded. In the same research, shaded vines observed an increase in myricetin and a corresponding decrease in the proportion of quercetin.

Canopy management including leaf thinning is often used to increase airflow in the canopy, reducing relative humidity and thus decreasing the risk of disease (Guidoni et al., 2008). However, leaf removal also has the potential to increase the temperature in the canopy, as well as exposure to solar radiation and UVB radiation (Anić et al., 2021). These effects can result in reductions in the concentration of anthocyanins and an increase in the concentration of flavonols (Chorti et al., 2010; Guidoni, et al., 2008). On the contrary, vine leaves not only reduce the temperature in the canopy through transpiration cooling but also act as a natural sunscreen with the ability to block ultraviolet, visible and infrared radiation (Keller, 2015). Thus, increasing leaf area by minimal pruning, no leaf removal, or other pruning or training strategies can aid in reducing exposure to increased heat and UVB.

Management of inter-row space is also being considered with the aim of manipulating the micro-climate near the lower portion of the vine. Minimizing soil tillage or practicing ‘no-tillage’ can help to protect soil structure and increase soil organic matter thus increasing soil moisture retention which can reduce the temperature of the micro-climate close to the soil (Blanco-Canqui & Ruis, 2018; Penfold & Collins, 2012). This is also believed to work by altering the surface albedo or possibly through alteration of evapotranspiration rates (Davin et al., 2014).

Cover crops and no or reduced tillage are practices often utilized to reduce erosion, particularly on steep slopes. They can also increase soil organic matter (SOM), as well as microbial population and diversity (Abad et al., 2021). However, cover crops are known to alter the micro-climate at ground level by reducing the temperature during warm season while also increasing soil available water (Parker et al., 2020; Xyrafis et al., 2023). There have been historical concerns over increased competition for water resources and a general negative effect on grapevine water status. However, these results were realized under specific conditions (Zumkeller et al., 2023). Further, tillage has been shown in some studies, to increase grapevine water status, particularly in semi-arid regions during the early season (Zumkeller et al., 2023).

The main strategy to reduce the impact of heatwaves is to increase irrigation before and during a heatwave to maintain leaf water potential and stomatal conductance to sustain vine health and yields (Parker et al., 2020;

Previtali et al., 2023). Irrigation as a strategy to mitigate against heatwaves can be effective but it is resource intensive, and many regions are dealing with significant water supply limitations.

Overhead spray systems have also been classically applied to reduce canopy temperatures during periods of extreme high temperatures (Gilbert et al., 1971). However, this treatment was traditionally untargeted and utilized a significant amount of water. Currently, investigations into the efficacy of nebulized sprays in the bunch zone are underway (Caravia et al., 2017). Targeted spray systems show promise in maintaining vine health and maintaining yields (Valentini et al., 2024). Berry quality can be altered but is varietal dependent.

The third paper of this thesis investigates a potential mitigative strategy of targeted overhead pulsed spray for vines exposed to heatwave conditions. This research was performed in an experimental vineyard in Mendoza Argentina with cv Malbec, cv Syrah, and cv Bonarda.

### 1.7. Aim and objectives of the thesis

This thesis aims to explore the relationship between geomorphological traits in Barolo DOCG with climate behaviour through a combination of field research and geostatistical analysis in order to determine the variation in berry quality parameters that may be attributed to variation in some natural terroir traits of the region. Further, the research includes passively amplified temperature and alteration of UV exposure to vines at three different elevations in order to understand the potential risks to berry quality and expression of terroir associated with two climate-based variables on the Nebbiolo grape in Barolo. Finally, research was performed on a novel approach to risk reduction in a changing climate focusing on maintaining vine health and berry quality parameters in cases of exposure to extreme temperatures in three different cultivars.

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# 2. Managing Vineyard Topography and Seasonal Variability to Improve Grape Quality and Vineyard Sustainability

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**Abstract:** Topography may induce variability in meteorological conditions at the mesoscale level and could influence grape quality. Understanding the impact of topography on grape ripening allows for the development of sustainable management practices that include topographical influences in their consideration. This is also important for applying proactive strategies to counter the expected changes of climate. This study was conducted on cv. Nebbiolo vineyards in North-West Italy. The topographic traits were performed in 17 vineyards within the region, which had previously been identified as belonging to different terroir units. An analysis of historical meteorological data series was also carried out to characterize the units from the meteorological point of view. The grape composition was investigated in 2012 and 2013. Based on the topography traits, a Cluster Analysis classified the 17 vineyards into four groups. Differences among groups mainly concerned insolation and heat accumulation. Topography influenced the individual components of grape quality differently depending on the seasonal weather trend. Interactions between topography and vintage were observed for a few parameters. Better understanding the grapevine reaction to external factors/site characteristics can allow for improved site and season-specific management decision-making and can contribute to improving vineyard sustainability while maintaining winery objectives and wine typicity.

**Keywords:** intercepted surface solar radiation (insolation); slope aspect; temperature and rainfall time series; grape quality; anthocyanin; Nebbiolo; terroir; climate change; vineyard management; sustainability

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## 2.1. Introduction

Winegrowing areas are characterized by different landforms. In Italy, more than 50% of viticulture is located in hilly areas; in these conditions, high topo-climatic heterogeneity in terms of exposure, elevation, and slope is evident [1]. The heterogeneity of the land morphology creates unique and complex landforms that can influence berry ripening and can require specific knowledge to carefully manage with suitable agronomic choices. The need to improve the sustainability of agriculture and reduce the impact of climate change [2] further complicates the farmer's decision-making process. Climate elements (temperature, rainfall, etc.), soil features (parent material, soil evolution and composition, etc.) and site topography (i.e., slope aspect, elevation, etc.) of an individual geographic area, that often is individuated as a "terroir", justify specific management choices over time. Among these choices, slope settling, soil management practices, training systems, choice of variety and rootstock, winemaking techniques, and oenological objectives are the most important [3,4]. It is difficult to investigate the synergistic influence exerted by all these factors on grape and wine composition [1]. Recent studies statistically examined the potentiality of soil and topo-climatic variables for winegrowing area zoning [5,6]. Other studies also concerned the influence of topography on grape ripening and composition [7–10]. The climate variability within a vineyard at a meso or micro scale has been studied [11–14] also evidencing its relationship with grape ripening [15–17]. The interaction between the environment, soil characteristics (e.g., texture and lithological origin), and cultural choices (e.g., row orientation and soil management) may influence the interception of solar radiation, soil water holding capacity, and the microclimate of a vineyard, and vines [16,18–22]. These factors, in turn, can influence vine vigor and productivity as well as ripening processes and grape composition [7,8,11,23–31]. In two studies conducted in flat vineyards at different elevations in Mediterranean climates, air temperature and canopy exposure to sunlight, and therefore vine photosynthetic efficiency, depended on row orientation [22] and vineyard elevation [8]. Hunter et al. [22] did not observe differences in terms of hourly mean temperature, between NW–SE and NE–SW row orientations, while the E canopy side of N–S row orientation achieved the highest temperature during the morning. Similar behaviour was observed in hilly vineyards facing east, while temperatures peaked in the afternoon in west facing vineyards [32]. From another study conducted in the Barolo region, it emerged that in south-facing vineyards air temperature above the canopy varied inversely according to their elevation, whereas in terms of heat accumulation (calculated as Growing Degree Days) west facing vineyards were cooler than those facing south [12,28,29]. Furthermore, the correlation between berry temperature and air temperature depended on the vineyard aspect [32]. Because berry temperature may hugely affect biosynthesis and degradation of primary and secondary metabolites in the berries [33–40], vineyard aspect and elevation, which may influence berry temperature, may also influence berry composition [28,29,32,41,42]. In a study carried out in 18 vineyards of the Douro region (Portugal) growing cv. Touriga National, a significant influence of vineyard elevation and slope aspect on vine behaviour and grape quality emerged [43]. In particular, the lowest yield and berry mass and the highest anthocyanin concentration were found in the vineyards at higher elevation with a SW aspect. In Switzerland, a study performed on 23 plots of cv. Chasselas confirmed the influence of elevation on the precocity of phenological stages with the vineyards at higher elevation being the latest ripening plots [44]. Moreover, the latest ripening plots accumulated the highest amount of soluble solids per berry. The highest sugar concentrations were achieved in the less vigorous plots that also received the highest potential solar radiation regardless of altitude. This was in agreement with the findings of a recent study conducted in South Tirol (northern Italy) that revealed a direct relationship between the must sugar content and a “Solar Radiation Identity index”. This last also showed to be a useful descriptor of the vineyard topo-climate [7]. Although vineyard aspect and elevation influence the vineyard microclimate and grape ripening, the vintage might exhibit greater effects on metabolic composition of grape berry and grape must than the effects of topographical variables [45–47].

Improving the understanding of the influence of topography on grape ripening processes is an important undertaking to develop viticultural management practices that include topographical influences in their consideration. This understanding is also important for implementing precision management practices that can contribute to the enhancement of the growing environment and grape quality while in the longer term aiming to reduce the impacts of climate change [2,48]. The aim of this work was to further understand the role of the topographic elements on climate at a meso-scale level, and on berry ripening and composition in an extremely hilly wine growing region.

## 2.2. Material and Methods

The study was carried out in 2012 and 2013 in the hilly winegrowing area of Langhe (North-West Italy) that is situated on marine grey marls (Marne di Sant'Agata Fossili) [49]. This is an intensively cultivated area, where elevation, gradient and aspect of the slopes vary considerably due to the heterogeneity of the territory. Here, viticulture covers most of the cultivated surface, thus it is strongly linked to the landscape and to the local economy. The study was conducted on vineyards belonging to the hilly premium wine growing region where "Barolo DOCG" wine is produced (Figure 1).

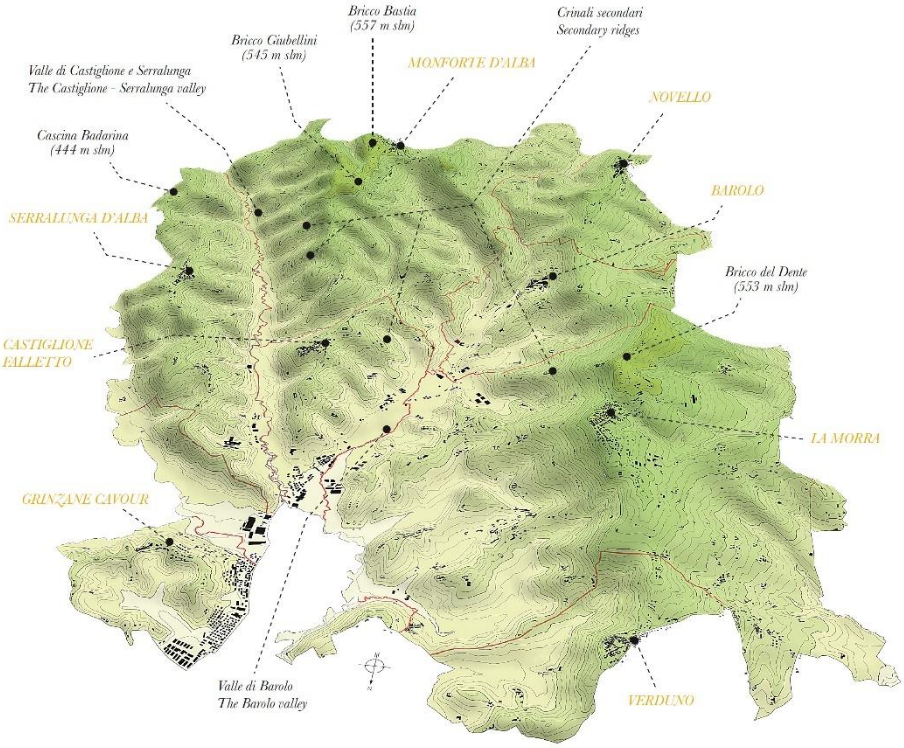


**Figure 1.** The study area (about 8000 ha) includes 2100 ha of vineyards planted with cv. Nebbiolo for the production of the premium wine "Barolo DOCG. Figure courtesy of Alessandro Masnaghetti Editore ENOGEA [50].

### *2.2.1. Climatic Conditions of the Area and Seasonal Trend of Temperature and Rainfall*

Following the Köppen–Geiger classification, the climate of the area (coordinates of the Barolo village: 44° 36′ 42” 84 N 07° 56′ 38” 04 E) is warm temperate, humid with hot summers (Cfa) (http://koeppen-geiger.vu-wien.ac.at/) [51]. In the area five public weather stations belonging to the Regional AgroMeteorological survey system (RAM) were present. They were located at: Serralunga Boscareto (SB) (405 m above sea level, ASL), La Morra (LM) (326 m ASL), Castiglione Falletto (CF) (309 m ASL), Barolo (B) (360 m ASL), and Serralunga Fontanafredda (FF) (309 m ASL). To synthetically describe the winegrowing area from a climatic point of view, the average minimum, maximum and mean temperatures, the average annual accumulation of Growing Degree Days (GDD, base temperature 10 °C), and rainfall (mm) were calculated for each of the five stations based on a time series of 14-20 years, depending on the station (readings beginning from 1999 to 2005 and ending in 2018). For every parameter, the average value of each site was then compared to the average value of the area calculated using all the available historical data series. Furthermore, to evaluate the two studied vintages, the annual GDD and rainfall of 2012 and 2013 were calculated for each weather station and compared with the historical average values for that weather station.

### *2.2.2. Vineyard Characteristics*

The area of the study consists of a complex hilly system crossed by two valleys which run from NNE–SSW and NW–SE, respectively, dividing one central and two outer hills (Figure 1). Because of this morphology of tortuous aspects along with a significant slope gradient, vineyard topography varies considerably. In this area, 17 commercial vineyards were selected to represent all the “terroir units” identified during a previous zoning study of the area [52]. The vineyards belonged to different growers but in each of them cv. Nebbiolo was cultivated by vertical shoot positioned trellis system and by Guyot pruning system (8-10 buds/cane). The row orientation was perpendicular to the slope gradient, which is the tradition in this area. Vineyard management was quite similar for all vineyards according to the know-how of the area and included yield control by cluster thinning. On average, the vineyards were 20 years old and all of them were planted at a density of 4,500 plants/ha. The soil between vines was managed by tillage or chemical weeding; the soil between rows was covered by resident vegetation and tilled in autumn every second year by harrow. In every vineyard, all the field and grape quality assessments were conducted on three replicates of 15 plants each, randomly distributed within the vineyard.

Vineyard topography, specifically elevation, slope aspect and gradient, and geographical coordinates were obtained by a Global Positioning System (GPS) instrument (GARMIN eTrex 20x, Olathe, USA). The intercepted surface solar radiation (insolation) was estimated using the “Area Solar Radiation” tool of ArcGIS Pro 2.1 software (ESRI, USA): the average annual insolation was calculated for a circular surface of 60 m diameter centered in each vineyard.

During the 2012 season, a pedological survey was carried out in each vineyard by a protocol proposed by IPLA [53]. The soils were classified according to the USDA Soil Taxonomy [54]. For each horizon, soil samples were collected and analyzed for texture, pH in water, calcium carbonate [55], and organic carbon [56]. Soil from the topsoil (0-20 cm) was also analyzed for nitrogen content and cation exchange capacity (CEC) [55]. Available water capacity (AWC) was calculated on samples from topsoil and from the horizon explored by most of the root system (30 to 70 cm) by application of official soil analysis methods [57,58].

### *2.2.3. Field Measurements and Grape Quality*

Seasonal grape ripening evolution, grape quality at harvest, vine vigor, and yield were monitored in both seasons, on 15 vines per replicate. Three consecutive berry samples of 200 berries were collected at 30, 45, and 65 days after veraison (dav); the harvest was made on the 65th dav. Sampled berries were weighed and crushed, and the must analyzed to determine the main technological parameters. Total soluble solids (TSS), pH, and titratable acidity were analyzed by an FT-IR method (WineScan$^{TM}$, Foss, Denmark). Malic and tartaric acids were measured by HPLC (LC-920 Varian, Palo Alto, CA) equipped with the column Phenomenex Rezex ROA Organic Acid H+, 300 × 7.8 mm, 5 μm [59]. Total anthocyanin concentrations were analyzed by spectrometry (Helios Squamate UV-VIS 9423 Aqua 2200E, Thermospectronic, Waltham, MA) [60–62]. At harvest the number of bunches per plant was counted and the yield was weighed with a dynamometer (KERN HCB 20K10, KERN & Sohn GmbH, Germany); then the average weight per cluster was calculated. At winter pruning, the shoot number per plant was counted and the pruning weight was measured with the dynamometer; the average weight per shoot and the Ravaz index were then calculated.

### *2.2.4. Statistical Analysis*

A grouping of the 17 vineyard sites was performed by Cluster Analysis (CA) calculated on slope (°), aspect (°), elevation (m ASL), and insolation (kW h $m^{-2}$). After grouping the vineyards based on CA results, a one-, or two-way ANOVA was carried out on vine and grape quality parameters, using “sampling date”, “group”, and “year” as factors to determine if differences existed among the groups. Before proceeding with the ANOVA, normal distribution of the data (Shapiro–Wilk test) and homogeneity of the variances (Levene test) were verified. Significant differences among groups were determined by the Duncan test at a significance level of 5%. A Principal Component Analysis (PCA) was carried out on quality berry variables (berry weight, Total Soluble Solids (TSS), Total Acidity (TA), pH, malic acid, and anthocyanin concentration) at harvest. Statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

## 2.3. Results

### *2.3.1. Development of Mesoclimatic Units and Weather Conditions of the Study Years*

The mean annual temperature (13.3 °C, ranging from 12 to 14.2 °C), rainfall (800 mm, ranging from 488 to 1250 mm) and seasonal accumulation of GDD were calculated based on data from all five weather stations over about 20 years. The average values and seasonal accumulation of GDD and rainfall significantly differed depending on the weather station location (Figure 2). Serralunga Boscareto (SB) and La Morra (LM) accumulated the highest and the lowest seasonal GDD, respectively. An intermediate situation was observed for Barolo (B), Serralunga Fontanafredda (SF) and Castiglione Falletto (CF) where the seasonal pattern and annual total amounts were similar to each other and to the GDD average calculated for the whole area (Figure 2a). Rainfall volumes were greater in LM; while the other stations registered similar average values and accumulation patterns (Figure 2b). Due to these observations of historical weather conditions, the area of study was divided into three mesoclimatic units (MU) corresponding to SB, LM, and B (which included SF and CF). Weather related data recorded from the respective weather stations were used to describe each MU from the climatic point of view, and the weather conditions of the two years of the study, 2012 and 2013 (when berry sampling occurred), which was then compared to the long-term averages.

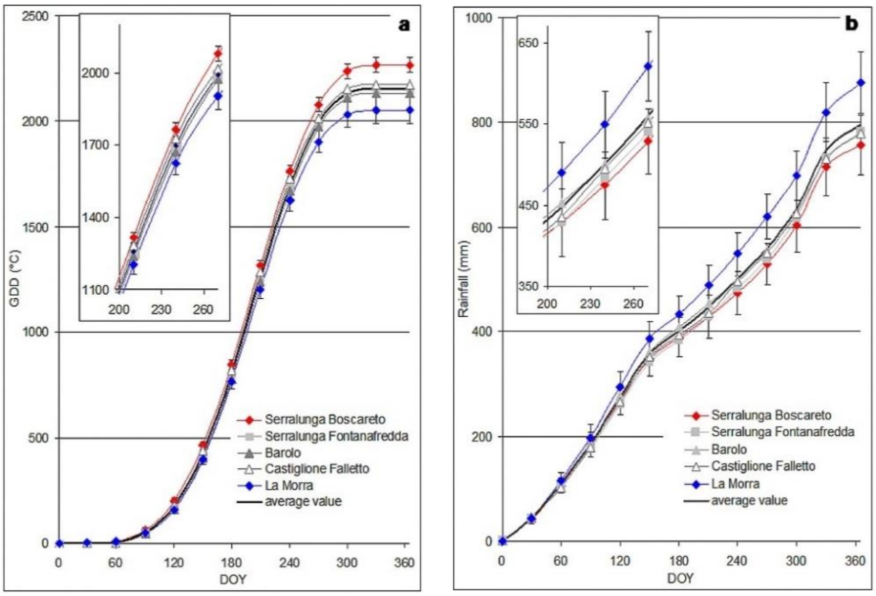
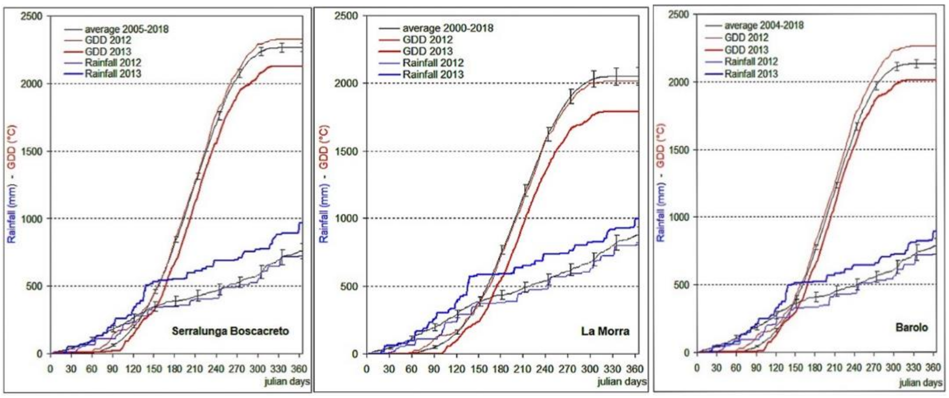


**Figure 2.** Yearly cumulated growing degree days (GDD, a) and rainfall (mm, b) calculated for the five weather stations of the area and average value for the whole area. Lines are the mean values of 20 year-observations for Castiglione Falletto and Serralunga Fontanafredda, 19 years for La Morra, 16 years for Barolo, and 14 years for Serralunga Boscareto. In the insert, the enlarged details of the period between veraison (Julian day 210) and harvest (Julian day 275) are shown. Data of Figures 2 and 3 were recorded by Piedmont Agrometeorological Network, Agrometeorology Section of Agriculture Department—Regione Piemonte (www.sistemapiemonte.it/agricoltura/ram/).

In both years, the highest GDD was recorded in SB, the lowest in LM (Figure 3). Furthermore, the 2012 values in LM did not differ from the local average of the 1999-2018 series; however, in SB and B, the GDD were higher than the average of their respective time series (of 14 and 16 years, respectively). In 2013, all GDD's were lower than 2012 GDD's and lower than the time series averages, particularly in LM.; however, the relationships among the stations appeared constant (Figure 3). The average annual rainfall was higher in LM than in SB or B. In 2012, the amount of rainfall did not differ from the average of the time series for any of the three mesoclimatic areas; while in 2013, rainfall was more abundant than the average, especially in early spring, and particularly in SB; in all cases, B was the least rainy MU (Figure 3). Due to its meteorological characteristics, 2013 can be considered an anomalous year.



(a) (b) (c)

Figure **3.** Yearly cumulated growing degree days (GDD, °C—red lines) and rainfall (mm, blue lines) of 2012, 2013 (bold lines), and average values of the time series of both parameters (black lines) ±standard errors, calculated for the sites used as a reference for the three mesoclimatic units in the study area. Average values were calculated for 14 years for Serralunga Boscareto (SB, a), 19 years for La Morra (LM, b), and 15 years for Barolo (B, c).

### *2.3.2. Vineyards Characteristics*

Based on intercepted surface solar radiation, elevation, slope aspect, and gradient (Table 1), the 17 vineyards considered for this study, were grouped into four homogenous groups by a Cluster Analysis and were named after the dominant slope aspect of their group. The vineyards located in the LM unit were separated into groups facing east north-east (E) and east south-east (SE); the vineyards located in the B unit faced west north-west (W), and those located in the SB unit faced south-west (SW) (Figure 4). SE and SW facing vineyards intercepted similar amounts of solar radiation, but SE vineyards were at a lower elevation. E and W facing vineyards intercepted the lowest amount of radiation and were at the same elevation (Table 1).

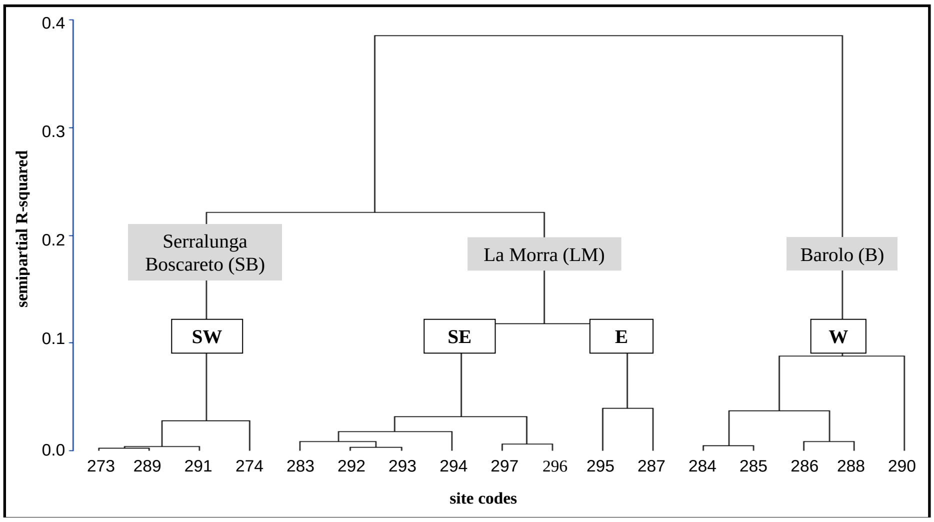


**Figure 4.** Grouping of the vineyards based on the Cluster Analysis carried out on the geographical variables (i.e., aspect, elevation, slope gradient, and intercepted surface solar radiation), dominant slope aspect of each group and mesoclimatic units to which groups belong are also indicated.

**Table 1.** Vineyard codes, urban district, mesoclimatic unit (MU), group membership obtained by cluster analysis, and main topographic features of the vineyards investigated; average values were calculated for each group.

| Code | Urban District | MU | Group | Aspect (°) | Elevation (m ASL) | Slope Gradient (°) | Intercepted Surface Solar Radiation (kW h m$^{-2}$) |
|---|---|---|---|---|---|---|---|
| 287 | Castiglione F. | LM | E | 70 | 313 | 7 | 981 |
| 295 | Verduno | LM | E | 96 | 365 | 15 | 1137 |
| 296 | Verduno | LM | SE | 82 | 264 | 11 | 1107 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 297 | Verduno | LM | SE | 115 | 283 | 20 | 1158 |
| 294 | La Morra | LM | SE | 135 | 217 | 17 | 1195 |
| 293 | La Morra | LM | SE | 165 | 249 | 2 | 1204 |
| 292 | Diano d’Alba | LM | SE | 146 | 274 | 18 | 1223 |
| 283 | Barolo | LM | SE | 148 | 307 | 15 | 1217 |
| 273 | Novello | SB | SW | 190 | 398 | 18 | 1263 |
| 289 | Serralunga | SB | SW | 209 | 384 | 13 | 1238 |
| 291 | Sinio | SB | SW | 190 | 361 | 25 | 1251 |
| 274 | Novello | SB | SW | 235 | 338 | 7 | 1159 |
| 284 | Barolo | B | W | 279 | 282 | 14 | 1056 |
| 285 | Castiglione F. | B | W | 270 | 252 | 13 | 1095 |
| 286 | Castiglione F. | B | W | 320 | 333 | 14 | 973 |
| 288 | Serralunga | B | W | 305 | 323 | 9 | 1051 |
| 290 | Sinio | B | W | 280 | 432 | 15 | 1054 |
| | Mean values of the groups | | E | 83 d[1] | 339 ab | 11.0 a | 1059 b |
| | | | SE | 132 c | 266 b | 13.8 a | 1184 a |
| | | | SW | 206 b | 370 a | 15.8 a | 1228 a |
| | | | W | 291 a | 324 ab | 13.0 a | 1046 b |

[1] For each column, different letters indicate significant differences among groups at $p \leqslant 0.05$ according to the Duncan test.

Despite differences among units and groups in terms of topography and climate, the area showed a higher homogeneity with regard to soil and lithological substratum (mainly marly) and soil classification that, in particular, evidenced a low degree of soil evolution for all vineyards (Table 2). Very few differences emerged among groups in terms of the main soil chemical–physical characteristics, both when topsoil and deeper soil horizons were examined (Table 2 and Supplementary Table S1). For these reasons soil characteristics were not considered in the effect on vine growth and grape ripening.

Entisols and Inceptisols were the most widespread soil types, whereas only in one vineyard the soil was an Alfisol (Table 2). Topsoil textures were mainly silty loam without skeleton; the percentage of silt was rarely less than 45%, the percentage of clay was always above 20% (in most cases over 25%), and the percentage of sand averaged 24% (Table 2). The C/N ratio was, on average, around six and the Cation Exchange Capacity (CEC) averaged 11.4 meq/100 g. No significant differences emerged among groups, regarding topsoil composition and properties, with the exception of the C/N ratio which was higher for group E. Available water capacity was similar in both upper and deeper soil layers in every vineyard (Table 2). The soil was alkaline at every site and horizon: pH values ranged from 7.8 to 8.7 and the average percentage of calcium carbonate was 21.7%. The differences identified in the subsoil properties were not consistent with the vineyard groupings (Supplementary Table S1).

**Table 2.** Lithological substratum, soil classification based on USDA Soil Taxonomy, textural fraction, C/N ratio, and Cation Exchange Capacity (CEC) of the topsoil, and Available Water Capacity (AWC) of top and subsoil; average values of the main soil characteristics were calculated for each group.

| Code | Group | Lithological Substratum | Soil Classification [1] | Texture | | | C/N | CEC | AWC (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Sand (%) | Silt (%) | Clay (%) | | meq/100 g | Topsoil 0–20 cm | Subsoil 30–70 cm |
| 287 | E | Marls, claystones, siltstones | Typic Ustorthent fine-silty mixed calcareous mesic | 27.0 | 50.4 | 22.6 | 8.8 | 13.8 | 13.2 | 18.7 |
| 295 | E | Marls | Haplic Ustarent fine-silty mixed calcareous mesic | 22.6 | 48.5 | 28.9 | 11.3 | 13.5 | 21.7 | 17.4 |
| 283 | SE | Marls | Calcic Haplustept fine-silty mixed calcareous mesic | 19.0 | 55.3 | 25.7 | 7.6 | 16.9 | 14.2 | 18.7 |
| 296 | SE | Marls | Calcic Haplustalf fine-silty mixed calcareous mesic | 24.1 | 44.1 | 31.8 | 7.1 | 16.4 | 17.4 | 15.0 |
| 297 | SE | Marls | Haplic Ustarent fine-silty mixed calcareous mesic | 16.1 | 56.0 | 27.9 | 5.4 | 11.9 | 19.5 | 18.0 |
| 294 | SE | Marls | Typic Ustorthent fine-silty mixed calcareous mesic | 29.5 | 48.2 | 22.3 | 4.3 | 5.3 | 15.9 | 16.5 |
| 293 | SE | Marls | Typic Haplustept fine-silty mixed calcareous mesic | 19.3 | 52.5 | 28.2 | 4.3 | 7.5 | 17.1 | 17.6 |
| 292 | SE | Claystones, siltstones | Typic Haplustept fine-silty mixed calcareous mesic | 28.8 | 44.8 | 26.4 | 7.4 | 12.9 | 17.7 | 16.8 |
| 273 | SW | Marls | Typic Haplustept fine-silty calcareous mesic | 29.3 | 45.3 | 25.4 | 6.4 | 9.8 | 15.8 | 16.2 |
| 289 | SW | Marls | Typic Ustorthent fine-silty mixed calcareous mesic | 18.7 | 56.5 | 24.8 | 3.5 | 8.8 | 12.5 | 17.9 |
| 291 | SW | Marls, claystones, siltstones | Typic Haplustept fine-silty mixed calcareous mesic | 36.5 | 40.5 | 23.0 | 4.9 | 8.7 | 15.1 | 16.4 |
| 274 | SW | Marls | Typic Ustorthent fine-silty calcareous mesic | 20.9 | 54.6 | 24.5 | 7.9 | 9.8 | 17.8 | 17.8 |
| 284 | W | Marls | Typic Haplustept fine-silty mixed calcareous mesic | 28.6 | 50.9 | 20.5 | 3.5 | 8.1 | 17.1 | 14.8 |
| 285 | W | Marls | Typic Ustorthent fine-silty mixed calcareous mesic | 25.2 | 51.5 | 23.3 | 6.8 | 18.0 | 19.7 | 19.0 |
| 286 | W | Marls, claystones, | Typic Ustorthent fine-silty mixed calcareous mesic | 19.9 | 54.6 | 25.5 | 6.2 | 13.8 | 18.8 | 17.6 |

|  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  | siltstones |  |  |  |  |  |  |  |  |
| 288 | W | Marls | Typic Haplustept fine-silty mixed calcareous mesic | 19.5 | 51.9 | 28.6 | 5.1 | 13.8 | 17.1 | 16.6 |
| 290 | W | Marls | Typic Ustorthent fine-silty mixed calcareous mesic | 19.8 | 51.8 | 28.4 | 3.0 | 7.6 | 17.1 | 17.2 |
|  |  | Mean values of the groups | E | 24.8 a[2] | 49.5 a | 25.8 a | 10.1 a | 13.7 a | 17.5 a | 18.1 a |
|  |  |  | SE | 22.8 a | 50.2 a | 27.1 a | 6.0 b | 11.8 a | 17.0 a | 17.1 a |
|  |  |  | SW | 26.4 a | 49.2 a | 24.4 a | 5.7 b | 9.3 a | 15.3 a | 17.1 a |
|  |  |  | W | 22.6 a | 52.1 a | 25.3 a | 4.9 b | 12.3 a | 18.2 a | 17.0 a |

[1]. The suffixes ept, ent, alf refer, respectively, to Inceptisols, Entisols, and Alfisols; [2]different letters indicate significant differences among groups at P ≤ 0.05 according to the Duncan test.

### *2.3.3. Berry/Must Parameters*

Year, group, and sampling date influenced berry and must parameters (Tables 3). On average, berry size was larger and must total soluble solids, titratable acidity, malic acid, and anthocyanins concentration were higher in 2013 than in 2012, whereas pH and tartaric acid concentration were lower (Table 3). Regardless of date and year, berry weight varied depending on vineyard group (Table 3). In both years, the average berry weight was smallest in the SW group while berries from E and SE groups were the largest. Berry weight regularly increased from 30 dav to harvest, except in the E group in 2012, where berries reached a large size at 30 dav (Table 3). For this parameter, significant differences between years were observed (Table 3) but no interaction between group and year was observed. In 2012, Total Soluble Solids content (TSS) was similar for all groups at 30 dav; at harvest it achieved the highest value in SW (also in 2013), coherently with a lower berry weight. In 2013, SE and E vineyards had the lowest TSS at any sampling date (Table 3). In the SW and W vineyards, TSS was significantly higher in the wet and cool anomalous 2013 than in 2012 especially at the first two samplings; however, the seasonal trend of accumulation depended on both year and group (Table 3). The interaction between group and year was significant for TSS (Table 3). In both years, at harvest, SW grouped vineyards reached the highest pH, and the lowest titratable acidity (Table 3). At 30 dav, the pH achieved by each group was similar in both years; afterwards, in 2012, an increase of pH larger than in 2013 was observed; this led to a significantly higher pH at harvest for all groups in 2012. Consistently with what was observed for pH, titratable acidity was lower in 2012 than in 2013 at all sampling dates (Table 3). The interaction between group and year was significant for pH but not for titratable acidity. At all sampling dates, significant differences were observed among groups for malic and tartaric acid concentration. From the first sampling onwards, the must from E and W vineyards had the highest and the lowest malic acid concentration, respectively, in both years (Table 3). Furthermore, at 30 dav, malic acid achieved a similar concentration in both years, but its seasonal decrease was more accentuated in 2012 than in 2013 regardless of vineyard group; this led to significantly higher malic acid concentration at harvest in 2013. On the contrary, tartaric acid concentration was higher in 2012 than in 2013. On average, SE and E groups had the highest concentration of both acids, while SW and W had the lowest (Table 3). For both acids, the interaction between year and group was not significant. The berry anthocyanin concentration was highest in the cooler season (2013), and in both years in the vineyards of the cooler MUs (E and W groups); the concentration was higher from 30 dav onwards, regardless of year (Table 3). The anthocyanin concentration increased during ripening, peaking around 45 dav in both years, on average; however, in 2013 a greater synthesis in the early phases was followed by a final plateau in all vineyard groups. The differences among groups observed in the earlier phase were still evident at harvest even with different relationships among groups. In the case of the hottest vineyards (SW), and especially in the hottest year, the anthocyanin concentration at 30 dav was significantly lower than that expressed by the cooler vineyards even though there was no significant difference in TSS. The interaction between year and group was significant for anthocyanin concentration.

**Table 3.** Berry weight and must quality parameters assessed on 30, 45, 65 days after veraison (dav) in 2012 and 2013, and average value of the two years at harvest. GxY = interaction group x year. E = vineyards facing east, SE = vineyards facing south-east, SW = vineyards facing south-west, W = vineyards facing west.

| | | **2012** | | | | | | | | | **2013** | | | | | | | | | **Mean of the Years at Harvest** | | **Significance between Years** | **GxY** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sampling Date** | **Group** | **30 Dav** | | | **45 Dav** | | | **65 Dav** | | | **30 Dav** | | | **45 Dav** | | | **65 Dav** | | | | | | |
| Berry weight (g) | **E** | 1.99 | a[1] | *a*[2] | 2.00 | a | *a* | 2.09 | a | *a* | 1.92 | a | *b* | 2.03 | a | *b* | 2.18 | a | *a* | 2.14 | **a**[3] | ns[4] | ns[5] |
| | **SE** | 1.93 | a | *b* | 1.97 | ab | *ab* | 2.04 | ab | *a* | 1.98 | a | *b* | 2.00 | a | *b* | 2.12 | a | *a* | 2.08 | **ab** | ns | |
| | **SW** | 1.66 | c | *b* | 1.74 | c | *ab* | 1.77 | c | *a* | 1.77 | b | *b* | 1.87 | b | *b* | 2.00 | b | *a* | 1.89 | **c** | * | |
| | **W** | 1.82 | b | *b* | 1.88 | b | *ab* | 1.94 | b | *a* | 1.95 | a | *b* | 1.99 | a | *b* | 2.11 | a | *a* | 2.03 | **b** | * | |
| Mean of the groups | | 1.85 | | *b* | 1.90 | | *ab* | 1.96 | | *a* | 1.91 | | *b* | 1.97 | | *b* | 2.10 | | *a* | 2.03 | ***** | | |
| Total soluble solids (Brix) | **E** | 19.9 | a | *c* | 23.2 | ab | *b* | 24.3 | b | *a* | 21.0 | b | *b* | 23.5 | bc | *a* | 24.4 | b | *a* | 24.4 | **b** | ns | * |
| | **SE** | 21.2 | a | *c* | 23.7 | a | *b* | 24.4 | b | *a* | 20.6 | b | *c* | 22.9 | c | *b* | 23.8 | c | *a* | 24.1 | **b** | * | |
| | **SW** | 21.0 | a | *c* | 23.2 | ab | *b* | 24.9 | a | *a* | 22.9 | a | *b* | 24.7 | a | *a* | 25.1 | a | *a* | 25.0 | **a** | * | |
| | **W** | 20.5 | a | *c* | 22.5 | b | *b* | 24.4 | b | *a* | 22.6 | a | *b* | 24.2 | ab | *a* | 24.5 | ab | *a* | 24.5 | **ab** | * | |
| Mean of the groups | | 20.7 | | *c* | 23.2 | | *b* | 24.5 | | *a* | 21.8 | | *b* | 23.8 | | *a* | 24.5 | | *a* | 24.5 | ***** | | |
| pH | **E** | 2.94 | b | *c* | 3.10 | b | *b* | 3.23 | b | *a* | 2.95 | b | *b* | 3.06 | b | *a* | 3.04 | b | *a* | 3.14 | **b** | ns | * |
| | **SE** | 3.03 | a | *b* | 3.21 | a | *a* | 3.24 | b | *a* | 2.93 | b | *b* | 3.04 | b | *a* | 3.08 | b | *a* | 3.16 | **b** | * | |
| | **SW** | 3.06 | a | *c* | 3.17 | a | *b* | 3.32 | a | *a* | 3.06 | a | *b* | 3.11 | a | *b* | 3.22 | a | *a* | 3.27 | **a** | * | |
| | **W** | 3.00 | ab | *c* | 3.08 | b | *b* | 3.25 | b | *a* | 3.01 | a | *b* | 3.06 | b | *ab* | 3.12 | b | *a* | 3.19 | **b** | ns | |
| Mean of the groups | | 3.01 | | *c* | 3.14 | | *b* | 3.26 | | *a* | 2.99 | | *c* | 3.07 | | *b* | 3.12 | | *a* | 3.19 | ***** | | |
| Titratable acidity | **E** | 10.77 | a | *a* | 8.37 | a | *b* | 7.27 | a | *c* | 10.76 | ab | *a* | 9.43 | a | *b* | 8.56 | a | *c* | 7.92 | **a** | * | ns |

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (g $L^{-1}$ tartaric acid) | **SE** | 9.19 | b | *a* | 7.58 | ab | *b* | 6.73 | b | *c* | 10.98 | a | *a* | 9.42 | a | *b* | 8.15 | a | *c* | 7.44 | **ab** | * | |
| | **SW** | 9.17 | b | *a* | 7.43 | b | *b* | 6.07 | c | *c* | 9.71 | b | *a* | 8.16 | b | *b* | 7.18 | b | *b* | 6.63 | **b** | ns | |
| | **W** | 8.86 | b | *a* | 7.24 | b | *b* | 6.70 | b | *b* | 9.66 | b | *a* | 8.50 | b | *b* | 7.68 | ab | *b* | 7.19 | **ab** | * | |
| Mean of the groups | | 9.50 | | *a* | 7.66 | | *b* | 6.69 | | *c* | 10.28 | | *a* | 8.88 | | *b* | 7.89 | | *c* | 7.29 | * | | |
| Malic acid (g $L^{-1}$) | **E** | 3.92 | a | *a* | 2.39 | a | *b* | 1.75 | a | *c* | 3.99 | ab | *a* | 3.19 | a | *b* | 2.68 | a | *c* | 2.22 | **a** | * | ns |
| | **SE** | 2.80 | b | *a* | 1.79 | ab | *b* | 1.35 | b | *c* | 4.16 | a | *a* | 3.09 | a | *b* | 2.40 | ab | *c* | 1.88 | **a** | * | |
| | **SW** | 3.09 | ab | *a* | 2.19 | ab | *ab* | 1.46 | ab | *b* | 3.31 | bc | *a* | 2.33 | b | *b* | 2.12 | ab | *b* | 1.79 | **a** | ns | |
| | **W** | 2.42 | b | *a* | 1.58 | b | *b* | 1.18 | b | *b* | 3.07 | c | *a* | 2.37 | b | *b* | 2.04 | b | *b* | 1.62 | **a** | * | |
| Mean of the groups | | 3.06 | | *a* | 1.99 | | *b* | 1.44 | | *c* | 3.63 | | *a* | 2.75 | | *b* | 2.31 | | *b* | 1.87 | ns | | |
| Tartaric acid (g $L^{-1}$) | **E** | 8.82 | ab | *a* | 8.32 | ab | *b* | 7.39 | a | *c* | 8.62 | a | *a* | 7.82 | a | *b* | 7.03 | ab | *c* | 7.21 | **ab** | * | ns |
| | **SE** | 9.02 | a | *a* | 8.61 | a | *b* | 7.73 | a | *c* | 8.67 | a | *a* | 7.98 | a | *b* | 7.50 | a | *c* | 7.62 | **a** | * | |
| | **SW** | 8.15 | b | *a* | 7.68 | c | *b* | 6.57 | b | *c* | 8.30 | a | *a* | 7.55 | a | *b* | 6.75 | b | *c* | 6.66 | **b** | ns | |
| | **W** | 8.14 | b | *a* | 8.15 | b | *a* | 7.42 | a | *a* | 8.28 | a | *a* | 7.63 | a | *b* | 6.92 | b | *c* | 7.17 | **ab** | * | |
| Mean of the groups | | 8.63 | | *a* | 8.19 | | *b* | 7.28 | | *c* | 8.47 | | *a* | 7.75 | | *b* | 7.05 | | *c* | 7.16 | * | | |
| Total anthocyanins (mg $L^{-1}$ as malvidin-3-glucoside) | **E** | 463 | a | *b* | 517 | b | *b* | 641 | a | *a* | 667 | ab | *b* | 826 | a | *a* | 870 | a | *a* | 756 | **a** | * | * |
| | **SE** | 413 | ab | *b* | 498 | b | *a* | 544 | b | *a* | 506 | c | *b* | 651 | b | *a* | 646 | b | *a* | 595 | **b** | * | |
| | **SW** | 382 | b | *c* | 494 | b | *b* | 588 | ab | *a* | 588 | bc | *b* | 686 | b | *a* | 687 | b | *a* | 638 | **b** | * | |
| | **W** | 477 | a | *b* | 597 | a | *a* | 644 | a | *a* | 741 | a | *b* | 826 | a | *a* | 907 | a | *a* | 776 | **a** | * | |
| Mean of the groups | | 434 | | *c* | 527 | | *b* | 604 | | *a* | 626 | | *b* | 747 | | *a* | 778 | | *a* | 691 | * | | |

[1] for the same parameter and sampling date, different letters indicate significant differences between groups for $p \leq 0.05$; [2] for the same group and year, different italic letters indicate significant differences among sampling dates for $p \leq 0.05$; [3] different **bold** letters indicate significant differences among groups for $p \leq 0.05$; [4] for the same group, * indicates significant differences between years for $p \leq 0.05$, ns = not significant; [5] for each parameter, * indicate the significance of interactions between group and year, ns **=** not significant. All the differences were tested by Duncan test.

The differences between years highlighted by the ANOVA, were also confirmed by the PCA performed on the harvest values of the same variables. The first two components retained 71% of the total variance (PC1 46%; PC2 25%). The separation of the two seasons was particularly evident along PC2 (Figure 5). This separation was mainly influenced by anthocyanin concentrations (eigenvectors of 0.95) and pH (eigenvectors of -0.71). The vineyards were distributed along PC1 due to total soluble solids (eigenvector = 0.85), berry weight (eigenvector = −0.73), and malic acid concentration (eigenvector = -0.55). No clear vineyard grouping was evident in either of the two years; however, the vineyard dispersion along the first two PCs was wider in 2013 than in 2012 (Figure 5).

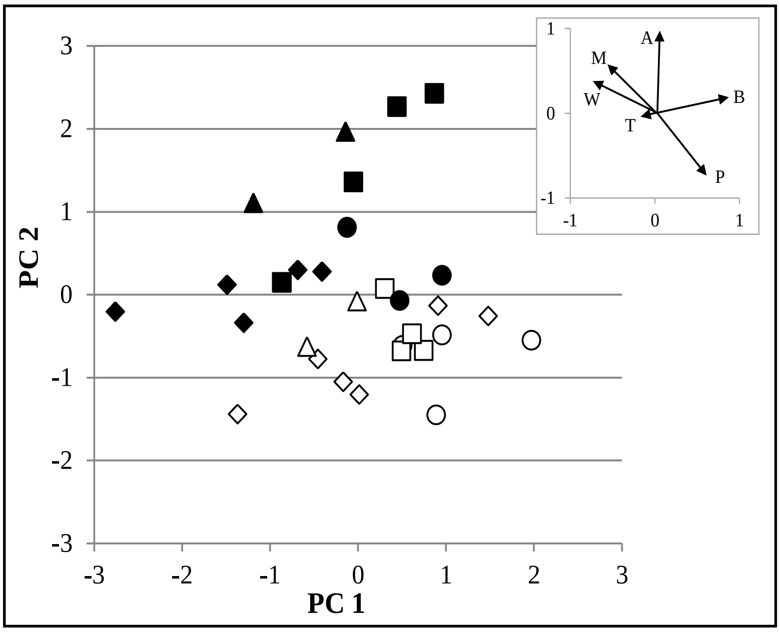


**Figure 5.** Distribution of the vineyards based on the PCA of must composition at harvest, with group discrimination by season: 2012 (Δ E ◇ SE ○ SW □ W); 2013 (▲ E ◆ SE ● SW ■ W). In the small box the values of the eigenvectors of the six variables included in the model are shown: W = berry weight, B = total soluble solids, T = titratable acidity, p = pH, M = malic acid, A = anthocyanins.

### *2.3.4. Vine Vigor and Yield*

Yield components and vine vigor were influenced by group (Table 4). On average, yield, bunch weight, and pruning weight were lower in the SW group, yield per vine was higher in the SE group due to a higher number of bunches. In 2013, the values of the yield components were higher than in 2012 but not for all vineyard groups. In 2012, the Ravaz index was similar for all groups; however, in 2013 it was highest in the more productive SE group. The interaction between group and year was only significant for yield components and Ravaz index (Table 4).

**Table 4.** Yield components assessed at harvest and vine vigor parameters assessed at winter pruning in 2012 and 2013. Data corresponds to the mean values of the vineyards belonging to each group (G): E = vineyards facing east, SE = vineyards facing south-east, SW = vineyards facing south-west, W = vineyards facing west. MU = mesoclimatic units, Y = year, G = group, GxY = interaction group x year.

| | MU | G | 2012 | | 2013 | | G | Y | GxY |
|---|---|---|---|---|---|---|---|---|---|
| Yield/vine (kg) | LM | E | 2.33 | a[1] | 2.70 | b[1] | *b*[2] | *[3] | *[3] |
| | | SE | 2.72 | a | 4.31 | a | *a* | | |
| | SB | SW | 1.74 | b | 1.50 | c | *c* | | |
| | B | W | 2.44 | a | 2.09 | bc | *b* | | |
| Bunch weight (g) | LM | E | 304 | a | 297 | b | *a* | * | * |
| | | SE | 282 | a | 399 | a | *a* | | |
| | SB | SW | 236 | b | 246 | b | *b* | | |
| | B | W | 299 | a | 322 | ab | *a* | | |
| Bunches per vine (number) | LM | E | 7.66 | b | 8.82 | b | *b* | ns | * |
| | | SE | 9.77 | a | 10.83 | a | *a* | | |
| | SB | SW | 7.51 | b | 6.28 | c | *b* | | |
| | B | W | 8.21 | b | 6.34 | c | *b* | | |
| Pruning wood per vine (g) | LM | E | 1041 | ab | 896 | a | *ab* | ns | ns |
| | | SE | 1012 | ab | 997 | a | *a* | | |
| | SB | SW | 831 | b | 694 | b | *b* | | |
| | B | W | 1142 | a | 912 | a | *a* | | |
| Shoot weight (g) | LM | E | 123 | ab | 108 | a | *a* | * | ns |
| | | SE | 105 | ab | 95 | a | *a* | | |
| | SB | SW | 102 | b | 87 | a | *a* | | |
| | B | W | 136 | a | 105 | a | *a* | | |
| Ravaz index | LM | E | 2.46 | a | 3.13 | b | *b* | * | * |
| | | SE | 2.79 | a | 4.55 | a | *a* | | |
| | SB | SW | 2.38 | a | 2.22 | b | *b* | | |
| | B | W | 2.34 | a | 2.48 | b | *b* | | |

[1] For the same parameter and year, different letters indicate significant differences among groups for $p \leq 0.05$ according to the Duncan test. [2]For the same parameter, different italic letters indicate significant differences among groups regardless of the year, for $p \leq 0.05$ according to the Duncan test; [3]significance of the differences between years (Y) and of interaction between group and year (GxY); * = significant for $p \leq 0.05$, ns = not significant.

### 2.4. Discussion

To understand the influence of topographic variability on climate at a meso scale level, a comparison was made among historical series of meteorological data recorded on five weather stations spread in the study area. Despite a wide seasonal variability of meteorological variables, the relationships among stations (and therefore among mesoclimatic units) remained constant over the years [63,64]. The current study, therefore, focused on the analysis of two bioclimatic indices (GDD and rainfall). These indices are known as main drivers of grapevine phenology and grape ripening and are expected to be impacted by climate change [65,66]. Based on historical variability of annual GDD and rainfall, three mesoclimatic units were identified: Serralunga Boscareto (SB), La Morra (LM) and Barolo (B). This partially confirmed the trend observed in a previous zoning study, although that study was done on a more limited database [52]. In the current study, the meteorological variables were not monitored in the individual vineyards. However, according to literature [13,16,21,22,67], it is possible to hypothesize differences among both mesoclimatic units and vineyard groups due to differences in slope aspect, row orientation, elevation and intercepted solar radiation (Table 1). The study was carried out in two vintages which had very different weather conditions. This gave the opportunity to test whether the vintage may act synergistically with the site topography in influencing grape ripening. Further, due to the different conditions in the study years it was possible to extrapolate a better understanding of how climate change may impact berry quality, specifically in a topographically varied viticultural region.

The SW vineyards were located at higher elevations and belonged to the warmest unit (SB) and, together with those facing SE, had the highest insolation. Contrary to expectation that temperature would decrease from an increase in elevation [8,43], but in accordance with a study on Glera cv. [41], the SW group of vineyards in this study were not the coolest ones, evidencing that a higher elevation was not enough to make vineyards cooler. The E group of vineyards had the lowest insolation and was placed in the coolest and rainiest MU (LM); the SE group, located in the same MU, was warmer than E due to both the higher insolation and a row orientation that enhanced the interception of the sun's energy [22]. The vineyards of the W group, which had the same row orientation as the E vineyards, had a similar insolation, but were located in a warmer unit (B) (Figure 1 and 2). Despite differences, and following previous findings, all vineyard row orientations were favourable to the uptake of solar energy [22]; thus, in this study vineyard topography likely prevailed over row orientation in influencing the results.

As described, all the must quality parameters were significantly influenced by the MUs and season (Table 3) and for some of them the interaction between vineyard group and year was also significant (Table 1; Table 3). Berry ripening behaved in accordance with the MUs and the related values of temperature, insolation, and precipitation which were strongly influenced by slope aspect (Table 1 and Figure 3). This has been recently confirmed by a study carried out in a mountain wine-growing region, that revealed a direct relationship between the must sugar content and an index of "solar radiation identity" calculated on the base of vineyard topography features [7]. In LM's unit, berry weight, must acidity, and anthocyanin concentration were higher, whereas TSS and pH were lower when compared with the must quality of SB's unit (SW group), in particular. With the same insolation, the cooler conditions of the E vineyard favored the accumulation of malic acid and reduced its degradation compared to the W group and also enhanced the synthesis of anthocyanins. Although groups, SE and E, were in the same MU, the malic acid and anthocyanin concentrations in SE group of vineyards were lower than in E group likely because of the greater insolation of SE vineyards and the consequent greater number of daily hot hours. The lower concentration of anthocyanins also found in the SW group, particularly in 2012, was consistent with the high temperatures of the sites and the vintage; this finding complemented studies that observed lower anthocyanin concentration in cases of high temperature or high heat accumulation [12,35,37,38,68,69,70]. The results for SW grouped vineyards were only partially in agreement with what was observed in the Douro Valley, where a negative effect of altitude and south-west aspect was observed on berry volume and yield, but not on skin total anthocyanin [43]. This was likely due to the fact that the SB MU was warm despite a higher altitude. In the current study, the observed differences among groups in both TSS and anthocyanin concentration, were not completely explained by the size of berry mass. This observation supports the hypothesis that factors other than berry mass (e.g., water availability or topo-climatic condition) may influence berry metabolism and composition [71–73]. In 2013, at harvest, musts achieved higher TSS and acidity and a higher concentration of skin anthocyanin despite greater berry mass and similar vine vigor to 2012 (Tables 3 and 4). This indicated a different evolution of the individual compounds and not a different degree of grape ripening. This evidenced that in anomalously wet and cool seasons, such as 2013 in the area of the study, excellent qualitative results can be obtained in optimal topo-climatic locations.

In addition to the influence on berry weight and must quality that were attributed to grouping and year, the year influenced the yield but not the vine vigor. Yield variation also appeared be related to the group (Table 4). This produced some differences among groups with the Ravaz index in 2013; despite it remaining at generally low values [74], the Ravax index did not negatively impact grape quality. However, the findings were not sufficient to explain the must compositional differences among groups and to support the general idea that a greater crop per vine reduces grape quality or vice versa, at least not in the range of the yield of the studied vineyards. When the most productive vineyards (SE) were compared with the less productive ones (SW), it emerged that this was due to a higher bunch number per vine and a larger berry size and that this was true both in the driest and in the wettest year. In terms of composition, TSS and pH were higher in the less productive vineyards than in the most productive ones only in the wetter year; tartaric acid was higher in the most productive vineyards in both years, while malic acid and anthocyanins concentration was similar in both the groups in both years (Table 3). Since the intercepted radiation and soil AWC of these two groups were similar, these results may reflect the wetter and fresher conditions of the LM meso-climatic unit in which the most productive vineyards were located.

The PCA confirmed the differences induced by the year, but it mostly did not group the vineyards effectively (Figure 5). Furthermore, the PCA highlighted a greater dispersion of the vineyards in 2013 compared to 2012 along both PCs. Two reasons likely contributed to this result: 1) the greater variability of the seasonal characteristics of 2013 compared to 2012, and 2) the fact that the individual compounds were influenced differently by the vintage. In the cooler and rainy season, the differences among the vineyard groups were more accentuated than in the warmer and drier season; the PCA dispersion of vineyards within the same vintage were evident or not depending on the vineyard group and therefore on topography traits (Figure 5). This suggests that in the warmer years, the grape quality would be very similar in all the vineyards, and therefore the possibility of recognizing the geographical origin of the wines would be reduced, which, on the contrary, would be enhanced in the cooler and rainy years. With the expected temperature increases associated with climate change, the occurrence of unfavourable years due to thermal excesses and prolonged periods of drought will be more and more frequent [48]. Particularly in temperate and warm climate this is not positive as some of the key berry parameters (acids, anthocyanins, and pH) can be impacted negatively in terms of quality output with increased temperatures [70].

All the results of this study confirmed the huge influence of the thermal conditions on the accumulation trend of many grape metabolites determining grape and wine quality [28,29,35,70,75–77] and, in particular, their negative impact on the anthocyanin biosynthesis as observed in pot experiments [34,35] and in field experiments [11,17,36]. Furthermore, it was also evidenced that not only the temperature itself, but also the vineyard topography together with the synergy between topo-climate and seasonal meteorological trends, plays an important role in affecting berry metabolism and grape ripening [7,17,28,69]. A highly varied topo-climate may determine different ripening pathways resulting in great differences in grape quality and in derived wines. As observed from the PCA analysis, this variation could be reduced as climate change evolves with warmer and dryer conditions being more frequent. This reduction in variation between regions within Barolo DOCG could lead to a reduction in typicality between these regions if mitigative strategies such as precision use of shade netting, leaf shading, or removal and cover cropping are not implemented by growers in an effort to preserve berry quality and typicity.

### 2.5. Conclusions

Even if a longer observation over more vintages would improve the robustness, the results of this study add elements to support the importance of the land topography in mediating the effect of the season and in influencing the quality of grape and wine production. This knowledge could be useful to help winegrowers to adapt site specific cultivation management strategies to consider, not only the weather conditions of the vintage but also the topography of their vineyards. This approach would be useful, firstly, to protect against or magnify seasonal variability and secondly, to develop management strategies for the expected long-term trends associated with climate change that affect grape ripening and may improve or decrease grape quality depending on topographic location. It would also be useful to improve pest management strategies with a more integrated and sustainable approach, especially if the microclimate were monitored at a low scale (vineyard or meso-climatic unit, for example). The use of tools typical of precision agriculture would make it easier to include the topographical characterization of a territory in zoning studies on this or other terroirs or on any farm zoning; this approach would increase the accuracy of knowledge and improve the precision of management techniques.

**Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Table S1: Main soil chemical–physical properties of each vineyard according to soil horizons.

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## 2.7. Supplementary Data

**Supplementary Table 1.** Main soil chemical-physical properties of each vineyard according to soil horizons.

| Code | MU | G | Horizons | Depth cm | Sand (%) | Silt (%) | Clay (%) | Soil reaction pH | Organic carbon (%) | Calcium carbonate (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 295 | LM | E | Ap1 | 0-60 | 22.6 | 48.5 | 28.9 | 8.0 | 2.7 | 19.1 |
| | | | Ap2 | 60-90 | 18.9 | 51.0 | 30.1 | 8.2 | 0.7 | 20.1 |
| | | | BC | 90-120 | 22.4 | 51.0 | 26.6 | 8.3 | 0.9 | 19.7 |
| 287 | LM | E | Ap1 | 0-25 | 27.0 | 50.4 | 22.6 | 8.1 | 1.5 | 21.6 |
| | | | Ap2 | 25-50 | 17.6 | 55.4 | 27.0 | 8.4 | 0.9 | 22.5 |
| 283 | LM | SE | Ap1 | 0-25 | 19.0 | 55.3 | 25.7 | 8.0 | 1.4 | 17.1 |
| | | | Ap2 | 25-45 | 13.4 | 59.8 | 26.8 | 8.2 | 0.9 | 19.1 |
| | | | Bw | 45-80 | 21.8 | 52.0 | 26.2 | 8.5 | 0.8 | 18.4 |
| | | | Bwk | 80-110 | 17.4 | 57.0 | 25.6 | 8.3 | 0.3 | 26.4 |
| | | | BCk | 110-150 | 18.9 | 49.8 | 31.3 | 8.4 | 0.0 | 22.6 |
| 292 | LM | SE | Ap | 0-40 | 28.8 | 44.8 | 26.4 | 8.1 | 1.4 | 20.5 |
| | | | Bw | 40-80 | 22.8 | 49.2 | 28.0 | 8.5 | 0.6 | 22.2 |
| | | | BC | 80-110 | 24.4 | 48.1 | 27.5 | 8.5 | 0.5 | 21.5 |
| 293 | LM | SE | Ap | 0-50 | 19.3 | 52.5 | 28.2 | 8.5 | 0.5 | 16.2 |
| | | | AB | 50-75 | 16.2 | 55.6 | 28.2 | 8.5 | 0.5 | 16.7 |
| | | | Bw | 75-105 | 17.6 | 51.3 | 31.1 | 8.5 | 0.7 | 18.1 |
| | | | Cr | 105-125 | 9.3 | 58.6 | 32.1 | 8.5 | 0.3 | 19.0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 294 | LM | SE | Ap1 | 0-30 | 29.5 | 48.2 | 22.3 | 8.6 | 0.5 | 24.7 |
| | | | Ap2 | 30-55 | 24.8 | 52.6 | 22.6 | 8.6 | 0.4 | 27.3 |
| | | | C | 55-65 | 23.8 | 53.7 | 22.5 | 8.6 | 0.3 | 19.9 |
| | | | 2Cr | 65-85 | 85.2 | 9.9 | 4.9 | 8.7 | 0.1 | 18.6 |
| 297 | LM | SE | Ap1 | 0-15 | 16.1 | 56.0 | 27.9 | 8.3 | 1.2 | 19.4 |
| | | | Ap2 | 15-45 | 10.0 | 57.0 | 33.0 | 8.4 | 1.0 | 19.5 |
| | | | AB | 45-70 | 11.2 | 58.7 | 30.1 | 8.4 | 0.7 | 19.8 |
| | | | Bt/C | 70-95 | 18.4 | 51.4 | 30.2 | 8.5 | 0.4 | 17.2 |
| 296 | LM | SE | Ap | 0-60 | 24.1 | 44.1 | 31.8 | 8.2 | 1.2 | 12.4 |
| | | | AB | 60-80 | 28.0 | 45.7 | 26.3 | 8.3 | 0.2 | 23.1 |
| | | | Bt1 | 80-105 | 25.7 | 46.2 | 28.1 | 8.3 | 0.3 | 16.1 |
| | | | Bt2 | 105-140 | 26.1 | 46.4 | 27.5 | 8.4 | 0.2 | 21.9 |
| 273 | SB | SW | Ap1 | 0-20 | 29.3 | 45.3 | 25.4 | 8.5 | 0.6 | 21.2 |
| | | | Ap2 | 20-75 | 30.1 | 46.5 | 23.4 | 8.5 | 0.7 | 21.6 |
| | | | Bw1 | 75-110 | 26.2 | 47.1 | 26.7 | 8.5 | 0.5 | 21.8 |
| | | | Bw2 | 110-130 | 28.8 | 45.0 | 26.2 | 8.3 | 0.3 | 23.7 |
| 289 | SB | SW | Ap | 0-25 | 18.7 | 56.5 | 24.8 | 8.5 | 0.4 | 26.5 |
| | | | AC | 25-50 | 19.4 | 56.5 | 24.1 | 8.5 | 0.6 | 26.7 |
| | | | C1 | 50-75 | 18.4 | 56.1 | 25.5 | 8.5 | 0.7 | 25.8 |
| | | | C2 | 75-100 | 27.5 | 49.7 | 22.8 | 8.5 | 0.4 | 24.7 |
| 291 | SB | SW | Ap | 0-30 | 36.5 | 40.5 | 23.0 | 8.5 | 0.8 | 28.1 |
| | | | Bw | 30-60 | 30.9 | 45.4 | 23.7 | 8.4 | 0.9 | 28.2 |
| | | | BC | 60-80 | 34.1 | 43.6 | 22.3 | 8.4 | 0.7 | 26.6 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | C | 80-120 | 34.3 | 42.9 | 22.8 | 8.4 | 0.7 | 27.6 |
| 274 | SB | SW | Ap | 0-25 | 20.9 | 54.6 | 24.5 | 8.1 | 0.7 | 17.4 |
| | | | AC1 | 25-55 | 28.2 | 53.7 | 18.1 | 7.8 | 0.5 | 14.0 |
| | | | AC2 | 55-75 | 18.0 | 56.9 | 25.1 | 8.0 | 0.5 | 16.8 |
| | | | C | 75-95 | 19.0 | 56.4 | 24.6 | 7.8 | 0.3 | 15.7 |
| 284 | **B** | W | Ap1 | 0-25 | 28.6 | 50.9 | 20.5 | 8.3 | 0.8 | 22.4 |
| | | | Ap2 | 25-40 | 37.8 | 44.8 | 17.4 | 8.5 | 0.4 | 21.5 |
| | | | Bw | 40-75 | 38.2 | 44.6 | 17.2 | 8.5 | 0.5 | 21.4 |
| | | | BC | 75-100 | 40.0 | 43.1 | 16.9 | 8.6 | 0.4 | 21.8 |
| | | | C | 100-125 | 35.6 | 44.5 | 19.9 | 8.5 | 0 | 25.1 |
| 285 | **B** | W | Ap | 0-25 | 25.2 | 51.5 | 23.3 | 8.2 | 1.8 | 14.6 |
| | | | AC1 | 25-40 | 17.5 | 61.7 | 20.8 | 8.6 | 0.7 | 24.9 |
| | | | AC2 | 40-75 | 16.8 | 59.9 | 23.3 | 8.5 | 0.8 | 25.2 |
| 286 | **B** | W | Ap1 | 0-25 | 19.9 | 54.6 | 25.5 | 8.5 | 1.1 | 22.8 |
| | | | Ap2 | 25-50 | 22.2 | 52.5 | 25.3 | 8.2 | 1.2 | 23.9 |
| | | | C1 | 50-100 | 27.1 | 55.2 | 17.7 | 8.6 | 0.3 | 18.4 |
| | | | C2 | 100-110 | 83.2 | 14.3 | 2.5 | 8.7 | 0.2 | 16.6 |
| 288 | **B** | W | Ap1 | 0-20 | 19.5 | 51.9 | 28.6 | 8.3 | 0.7 | 24.7 |
| | | | Ap2 | 20-40 | 18.3 | 58.4 | 23.3 | 8.5 | 0.2 | 43.8 |
| | | | Bw | 40-55 | 20.6 | 48.0 | 31.4 | 8.4 | 0.6 | 15.3 |
| | | | BwC | 55-90 | 20.6 | 49.0 | 30.4 | 8.4 | 0.6 | 18.7 |
| 290 | **B** | W | Ap | 0-30 | 19.8 | 51.8 | 28.4 | 8.4 | 0.5 | 27.4 |
| | | | AC | 30-50 | 17.7 | 55.0 | 27.3 | 8.5 | 0.3 | 28.4 |

|  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
| C | 50-90 | 24.0 | 50.8 | 25.2 | 8.4 | 0.9 | 27.8 |

# 3. The effect of temperature and UV manipulation on anthocyanins, flavonols and hydroxycinnamoyl-tartrates in cv Nebbiolo grapes (*Vitis vinifera* L.)

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**Abstract:**

This research aimed to identify the effects of increased temperature and decreased ultraviolet (UV) exposure on berry characteristics and quality parameters of cv Nebbiolo, identifying the potential risks associated with climate change for the quality of grapes and the identity of Barolo wine. This two-year research (2022 and 2023) was performed in three vineyards, at different elevations in La Morra (Piedmont, northwestern Italy), monitored from the beginning of veraison to harvest. A split-plot design was set up, applying a passive greenhouse amplifying temperature in the bunch zone ('T' = increased temperature; 'C' = control temperature), and UV blocking plastics over individual bunches ('1'= full UV exposure; '0' = UV blocked). Berry weight, skin weight, and juice total soluble solids were measured. Grape skin anthocyanins, flavonols and hydroxycinnamic acid tartaric esters were analysed by HPLC-DAD. Both treatments negatively influenced the berry weight but not the skin weight; the increased T negatively impacted the sugar per berry content. Limited UV and increased temperature negatively impacted total anthocyanins at harvest and on di-hydroxylated anthocyanins. Limited UV depressed flavonol concentration and high temperature decreased their synthesis. Increased UV promoted *cis*-*p*-coumaroyl tartaric acid and decreased *trans*-*p*-coumaroyl tartaric acid.

**Keywords:** Climate change; adaptation; fruit quality; polyphenols; terroir.

## 3.1. Introduction

The concept of terroir is complex and can include impacts from the natural environment such as soil type, climate, geomorphology, and geology on grapes and wine [1,2]. With a changing climate, the risk of loss of association of a wine to its terroir is a concern. Many of the subtle differences in the colour, flavour and texture of a cultivar and ultimately a wine produced in different terroirs are associated with polyphenols, predominantly accumulated in the berry skin. This is especially true for red wines, which undergo skin contact during fermentation to extract polyphenols and improve wine quality. Although berry polyphenols have a genetic signature, their evolution and relative abundance can largely be altered by environmental factors [3–6].

It is well established that temperatures are increasing globally as published by the Intergovernmental Panel on Climate Change (IPCC) with current (2011-2020) observed increases in average global temperatures of 1.1 °C above levels from the years between 1850-1900 [7]. Heatwaves have also been observed to increase in frequency, intensity and duration [7,8]. Effects on ultraviolet (UV) radiation from climate change are less clear. The amount of UV radiation layer reaching the earth's surface is influenced by changes in stratospheric ozone with a decrease in ozone leading to increased UV-B. UV radiation reaching the earth's surface can also be reduced by climate change from increased cloud cover, pollution, dust, smoke, and other particles [9]. The IPCC currently considers there to be a medium level of confidence that southern Europe will observe increased UV radiation while the confidence in increased UV radiation in northern Europe is low [9].

UV-B radiation may modify the quantitative and qualitative profile of grape skin flavonols and may enhance must extractable anthocyanins as was observed in cv Tempranillo [10]. UV-B can promote the accumulation of phenolic acids, stilbenes, and flavonoids in grapevine leaves, as an acclimation and protective response. High UV-B applications also increased total phenols in grape berries and, in particular, di-hydroxylated anthocyanidins and flavonols like quercetin [11].

Anthocyanins predominantly accumulate in grape skins of coloured grapes. They are the primary source of colour in red wines and they accumulate following the expression of the gene coding for UDP-glucose:flavonoid 3-O-glucosyl transferase (UFGT) at veraison [4,12,13]. The anthocyanin profile and concentration in grape skins mainly depend on the variety [5,14] but also on berry temperature, solar radiation exposure [3,15–19], and water availability [20,21]. In many grapevine varieties, malvidin 3-O-glucoside is the predominant anthocyanin [5,14]. Nebbiolo based wines are known for having a weak colour which can be associated with a generally low content of anthocyanins in the berry skins, and a prevalence of di-hydroxylated forms [3,22]. Di-hydroxylated anthocyanins are not as stable in wine as tri-hydroxylated anthocyanins which is a second reason for weak colour in Nebbiolo-based wines.

Flavonols act as a primary defense against UV exposure in vegetal tissues [5,13,16,23,24]. They accumulate in berry skin and their synthesis is stimulated by exposure to solar radiation and UV [25,26]. For this reason, agronomical practices that increase bunch exposure to solar radiation, as well as a natural low vigour of the vines, increase polyphenol concentration in the skin, particularly that of flavonols [27,28]. On the contrary, bunch shading has a detrimental effect on the synthesis of flavonols in the berry [17,29–32]. Flavonol accumulation peaks twice during berry development. The first peak occurs at flowering and the second occurs approximately 3 to 4 weeks after veraison [5]. The impact of increased temperature on flavonol concentrations is not clear with some researchers suggesting that temperature has little or no effect [33] whereas others observed a significant decrease in flavonol concentration with the application of very high temperatures (> 50 °C) for 12 hours [34]. Flavonols are perceived as a quality enhancer in wine partly due to their ability to stabilize anthocyanin colour through co-pigmentation [35,36]. Further, some flavonols are associated with a bitter flavour which is believed to enhance quality perception [37]. Flavonols are often found in higher concentrations in premium wines due to the practice of leaf removal which is used to increase airflow and to dry the grapes, to protect them against disease, and to increase exposure for treatment application in the bunch zone. Beyond the concern of loss of terroir identity, there are also new risks associated with some of these polyphenols. Specifically, the flavonol quercetin has been increasing in concentration in some regions including Tuscany [38]. Higher concentrations of quercetin can lead to deposits and increased turbidity in bottled wines, resulting in negative quality perception even if it doesn't significantly affect its flavour or aroma [39]. Further, recent research suggests that quercetin 3-O-glucuronide could be the main culprit associated with headaches from red wine consumption [40].

Hydroxycinnamic acids esterified with tartaric acid (HCTA) are non-flavonoid compounds found in grape berries and wines. HCTAs display the highest concentration of non-flavonoid compounds in berries and accumulate in berry skin and pulp [41]. HCTAs are reported as UV-B absorbing phenols in the leaves [42] where they were found to be unaffected or positively influenced by visible radiation [43]. Still, there is limited information about their accumulation in berries and the environmental factors that can influence their concentrations and profiles. In white varieties, hydroxycinnamic acids and their derivatives were negatively influenced by UV exposure [26]. In the coloured skins of Cabernet Sauvignon grapes, UV deprivation slightly reduced the concentration of HCTA [23]. The increase of cluster exposure to light, associated with leaf removal, significantly increased the concentration of HCTA in the berry skins of Tempranillo [44] and of Istrian Malvasia [45].

In red wines HCTAs are known to stabilize colour and can also impart a bitter flavour, thus they have been associated with increased quality perception [46,47]. Additionally, they have garnered attention for their potential health benefits, acting as antioxidants with possible implications in reducing the risk of Alzheimer's, Parkinson's, cardiovascular disease, and diabetes [48]. HCTAs also pose some risks to wine quality that may offset their potential benefits.

Specifically, p-coumaric and ferulic acid esters can be metabolized by *Brettanomyces* and *Dekkera bruxellensis* yeast species to ethylphenols which can significantly lower the quality of a wine aroma and flavour [49]. In previous research, it was shown that reducing the concentration of HCTAs and of the relative cinnamic acid could significantly reduce the concentration of 4-vinylguaiacol and 4-vinylphenol, notably responsible for the ‘brett’ aroma of wines [50].

The aim of this two-year research was to identify the effects of artificially altered exposure to UV radiation and temperature on total and individual anthocyanins, flavonols, and hydroxycinnamates in the skins of cv Nebbiolo grapes (*Vitis vinifera* L.) under field conditions. The artificial temperature amplification and UV limitation were intended to emulate severe conditions associated with climate change in order to determine potential risks to berry quality and associated terroir.

## 3.2. Results and Discussion

### *3.2.1. Efficacy of treatment factors*

#### 3.2.1.1 Greenhouse plastic

As expected, the passive greenhouse affected a daily increase of temperature for T treated vines compared to C treated vines, during the season, between 2 to 7 °C, with a duration of 4 to 6 hours during days with full sun. The daily average maximum temperature in T vines of all vineyards in the month of September was 5.1 °C higher than in C vines, in 2022 and 4.6 °C higher than C, in 2023 (Figure 1).

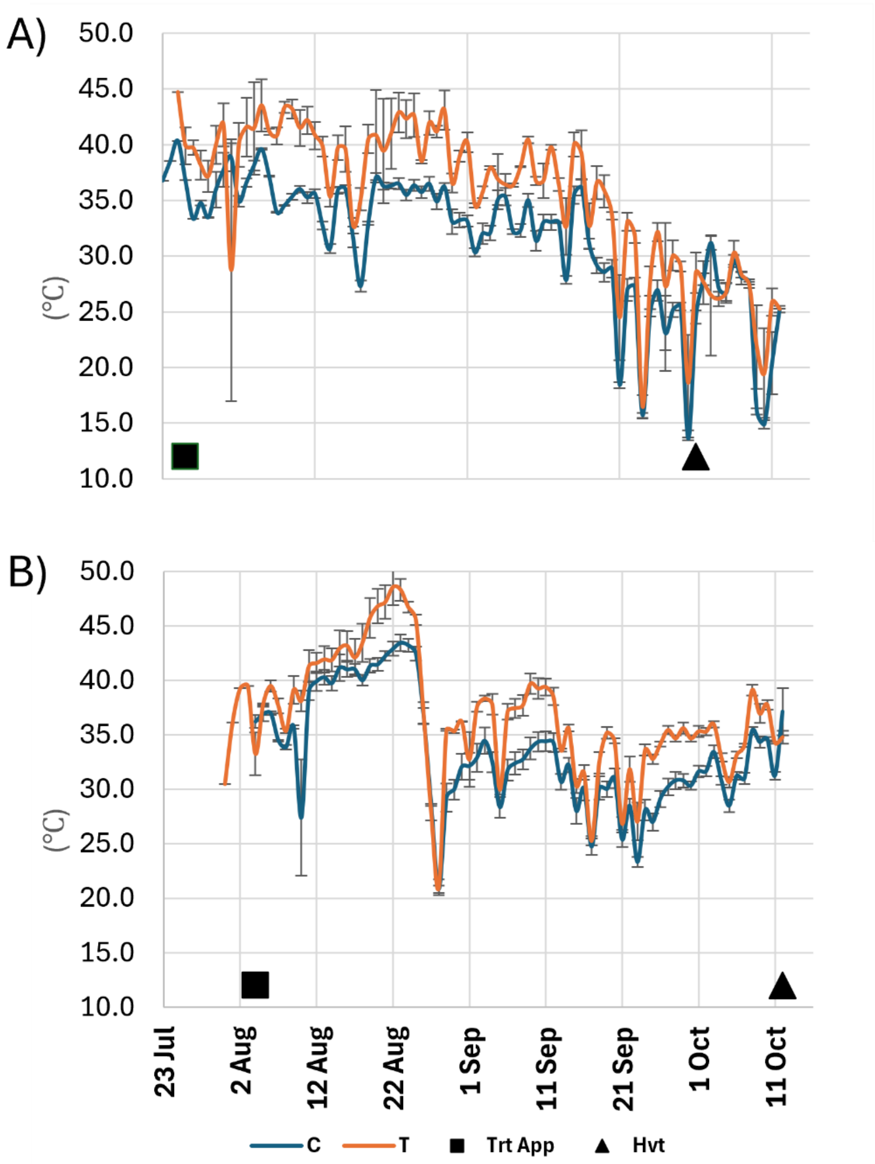

**Figure 1:** Average maximum daily temperature in vineyards of C (blue) and T treatments (orange) in 2022 (A) and in 2023 (B). Data expressed as mean values ± standard errors. Black square (■) indicates treatment application (TrtApp) date. Black triangle (▲) indicates harvest (Hvt) date.

### 3.2.1.2 UV-block

The measurements of penetrative photosynthetically active radiation (PAR), UVA, and UVB confirm the efficacy of the UV-blocking plastic in reducing UV radiation, especially UVB. It also emerged that greenhouse plastic partially reduced the UVA, UVB, and PAR by 24%, 32%, and 14% respectively (Table 1).

**Table 1:** UVA, UVB, and PAR spectral range penetrating vine canopy of treated vines compared to control (C1).

| Treatment | UVA (%) | UVB (%) | PAR (%) |
|---|---|---|---|
| C1 | 100 | 100 | 100 |
| C0 | 7 | 1 | 53 |
| T1 | 76 | 68 | 86 |
| T0 | 5 | 1 | 49 |

To confirm that UV blocking plastic treatment was not increasing temperatures, a on-way ANOVA was performed on hourly temperature measurements performed in C1 and C0 treatments between May and July of 2022. Results showed no significant difference (data not shown).

### *3.2.2. Precipitation, soil volumetric water content (SWC) and soil temperature (SoilT)*

The 2022 growing season was characterized by low precipitation. At the La Morra weather station, precipitation reached 432 mm. In contrast, 2023 experienced significantly higher precipitation, with an annual total amount of 631 mm. The most notable disparity occurred from April to June, indicating a wetter spring in 2023 compared to 2022. From the moment of the treatment application until harvest, 27 rainfall events resulted in 178 mm in 2022 with one event of 34 mm occurring between July 27 and July 29 and no events above 10 mm observed after this moment until harvest. In 2023, 14 events caused 206 mm of rain with a significant rainfall (97 mm in 11 hours) occurring on August 27-28 (Figure 2).

At the beginning of the observation in 2022, the SWC was between 0.20 and 0.22 $m^3/m^3$ in all vineyards. During the 2022 season SWC increased to 0.30 and 0.35 $m^3/m^3$ in M and H respectively, after the rainfall on June 28 (38 mm in two hours), after this it declined slowly and stabilized (Figure 2A). There was no observed increase of SWC in vineyard L associated with this rainfall. A series of rainfall events occurred from July 27 to 29 at the approximate time of treatment application, however, these events did not increase SWC in any vineyard (Figure 2A). All vineyards remained between 0.16 and 0.20m$^3$/m$^3$ SWC for the rest of the season. In the spring of 2023, several rainfall events led to a SWC of 0.3 on June 18 which declined until the rainfall on June 29 (Figure 2B). The SWC reached slightly lower values to those after the June rainfall of 2022, with a peak of 0.34 m$^3$/m$^3$ at vineyard M while in both H and L, SWC achieved lower values of 0.25 m$^3$/m$^3$ and 0.22 m$^3$/m$^3$, respectively. The second rainfall event occurred on August 27-28 and caused an increase of SWC to between 0.38 and 0.41 m$^3$/m$^3$ depending on vineyard. SWC appeared to increase significantly after each summer precipitation event greater than 15 mm. During the 2023 season, SWC remained above 0.19 m$^3$/m$^3$ in H and M while dropping to 0.168 m$^3$/m$^3$ in vineyard L at harvest (Figure 2B). In both years, vineyard L had the lowest SWC while H had the highest in 2022 and M the highest in 2023. However, at the end of the observation period, the mean SWC was quite comparable between years, especially for L and H vineyards, probably due to the different distribution of rainfall rather than their amount. Low volumes of more frequently distributed rainfall as observed in 2022 appear to maintain an SWC equal to periods receiving fewer events of higher volumes (as seen in 2023).

Soil temperature at 30 cm depth showed a peak on July 25 in 2022 immediately before the main rainfall event. This rainfall did not reflect a change in SWC but did appear to reduce SoilT (Figure 2A). Afterward, SoilT stabilized between 24.0 ℃ and 26.0 ℃ until September 18 after which, a continuous decline occurred until reaching 19.0 ℃ at harvest. The SoilT trend differed in 2023 with the maximum value of 29.7 ℃ occurring between August 24 and 27, before the major rainfall event which led to a notable decline to 23.6 ℃ on August 28. The SoilT stabilized between 21.0 and 23.0 ℃ in H and L vineyards until harvest, whereas a slight increase was observed in vineyard M until harvest. In both years, vineyard H and L trended lower in SoilT as compared to vineyard M. Observations have shown that more evenly distributed rainfall, even when less abundant (as in 2022), was more effective at maintaining soil temperature at a constant or lower level with respect to higher amounts of precipitation concentrated in specific moments (as in 2023).

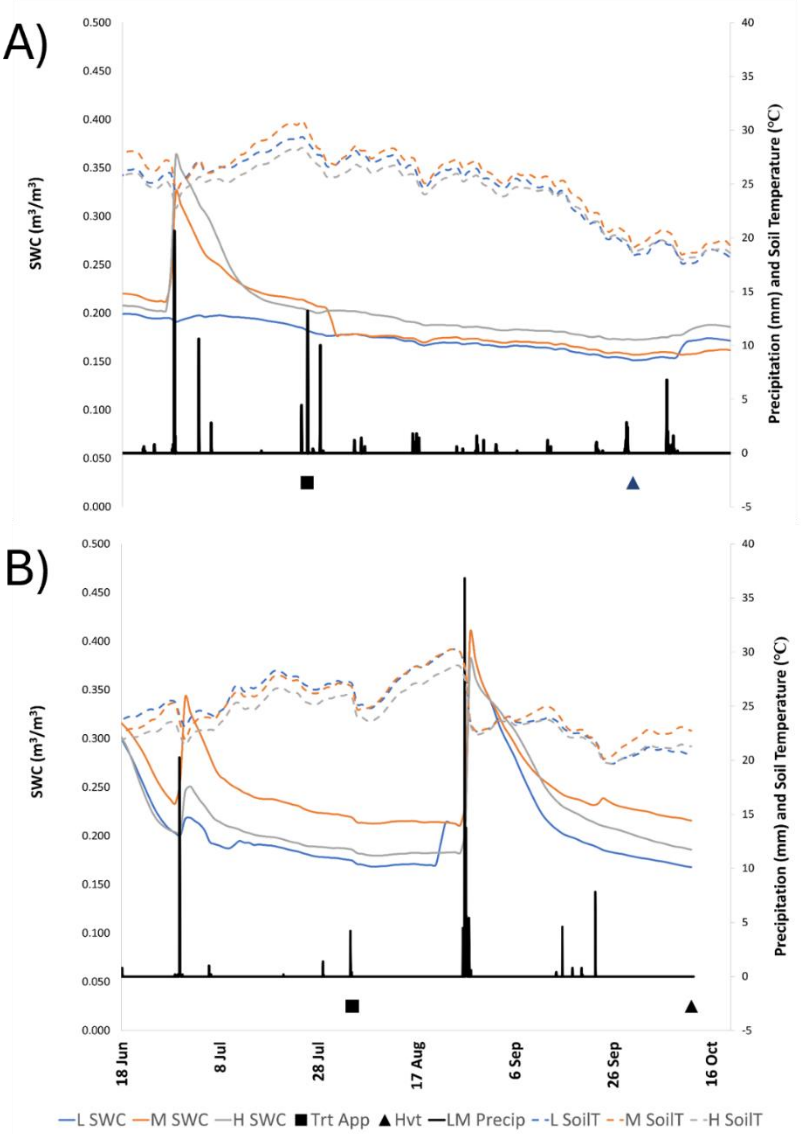

**Figure 2:** Mean daily average Soil Water Content (SWC, continuous lines) and Soil Temperature (SoilT, dashed lines) at 30 cm depth in the three studied vineyards. Precipitation was measured from La Morra weather station (black line) during berry ripening period in 2022 (A) and 2023 (B). Black square indicates the date of treatment application (Trt App). Black triangle indicates average harvest date (Hvt).

### *3.2.3. Air Temperature*

**Table 2:** Huglin Index (HI) from August 23 to October 8 in 2022 and 2023 at the vineyard level. Number of hours (Hrs) above 35 ℃, 40 ℃ and 45 ℃ from Aug 4 to harvest in both years. C = control treatment, T = temperature increased treatment.

| | Vineyard | 2022 | | 2023 | |
|---|---|---|---|---|---|
| | | C | T | C | T |
| HI | L | 745.8 | 896.8 | 843.5 | 961.0 |
| | M | 827.1 | 914.9 | 796.9 | 960.8 |
| | H | 763.6 | 925.3 | 854.8 | 1000.0 |
| Hrs > 35 ℃ | L | 34 | 211 | 157 | 248 |
| | M | 119 | 191 | 92 | 202 |
| | H | 61 | 172 | 163 | 200 |
| Hrs > 40 ℃ | L | 0 | 61 | 40 | 127 |
| | M | 8 | 19 | 27 | 46 |
| | H | 0 | 61 | 42 | 66 |
| Hrs > 45 ℃ | L | 0 | 11 | 0 | 37 |
| | M | 0 | 0 | 0 | 4 |
| | H | 0 | 2 | 0 | 12 |

In 2022, the HI of C treatment in vineyard M (at 360 m ASL) was higher than L (210 m ASL) and H (410 m ASL) (Table 2). However, in 2023 a reversal was observed with L and H having higher HI than M. HI was generally higher in 2023 than in 2022 but, in both seasons, the differences between HI of treatment T and that of treatment C were similar for all vineyards, with T higher than C, as expected (Table 2). However, the HI does not capture the whole picture as it does not consider short term extreme temperature conditions. For this reason, the number of hours with temperatures above 35, 40, and 45 ℃ were also calculated between August 4 and harvest date in both years. T treatment amplified the number of hours of exposure to high temperatures in all vineyards in both years. The generally higher temperatures (and HI) of 2023 were reflected in the greater number of hours with temperatures above 35 ℃ in C treatment and above 40 and 45 °C in T treatment. Notably, no vineyard had any hours with temperatures above 45 ℃ in either year in C treatment while in T treatment, L vineyard reached 11 hours in 2022 and 37 hours in 2023, M vineyard experienced 0 hours in 2022 and 4 hours in 2023 and H vineyard 2 hours above 45 °C in 2022 and 12 hours in 2023 (Table 2).

### *3.2.4. Berry Characteristics*

Berry weight at harvest was influenced by season and vineyards, being the highest in vineyard L in both years and the lowest in vineyard M in 2022 and in vineyard H in 2023, respectively (Figure 3). In vineyard M, in 2023 and in vineyard H in 2022 the berry weight, although influenced by treatments, remained at intermediate values (Figure 3). In vineyard L, no difference in berry weight at harvest was evident among treatments in either year although in 2022 at S3 berries exposed to UV (C1 and T1) reached higher berry weights than those of treatment T0; at S4, C1 had higher berry weight than T0. At harvest (S5), although C1 and T1 trended higher than C0 and T0, there was no significant difference. Berry weight in vineyard M was not different at any sample point in 2022; at harvest, in 2023, berries from C1 and T1 treatments displayed significantly higher weight than T0, while C0 was intermediate. In berries from vineyard H, a clear and significant separation among grapes exposed to ambient temperature (C1 and C0) compared to those under increased temperature (T1 and T0) started at S2 in 2022; in 2023, although there were differences at S2 and S3 between C and T treated berries, no significant difference was maintained at harvest (Figure 3). Higher thermal accumulations (vineyard L and H, in 2023, and vineyard M, in 2022) caused the berries to have a smaller mass, but with very few differences between treatments; on the contrary, berry weight displayed greater differences among treatments in the less stressing thermal conditions, such as in 2022 for vineyards L and H and in 2023 for vineyard M (Table 2, Figure 3). Despite the small and not constant differences between treatments, nor between years, it seems that the temperature increase associated with T treatment had a greater negative impact on the berry weight than the UV-block (treatment C0). This differs from other studies reporting no changes in berry weight with increased temperature [51] or reduced sun exposure ([17], or reporting an increase in berry volume and weight when solar UV-B was filtered from flowering to harvest in cv Malbec [52]. These current results agree with those found in a study on cv Nebbiolo that reported a lower berry mass in warmer seasons or vineyards [53]. The treatments slightly influenced the skin weight but season and vineyard had a higher impact on it with vineyard L having higher skin weight than vineyards M and H (Supplementary Table 1). The skin to berry ratio was significantly higher in 2023 than in 2022 at harvest, however, it showed only slight differences among treatments during both seasons (Figure 3). No differences were found at any sample point or vineyard in 2022. In 2023, in vineyard L, berries that were grown under increased temperature (T1 and T0) had a higher skin to berry ratio when compared to C1 and C0. Since the berry weight was similar among treatments, this was due to the increase of skin weight as a response to the increased temperature or, as found on cv Malbec, to the decrease of UV radiation intensity [52]. In vineyard M, berries with full exposure to UV and ambient temperature (C1) displayed a lower ratio compared to T0 with C0 being intermediate. In vineyard H, no significant difference was found at harvest in both years. However, in 2023, T1 trended higher than C1 throughout the season with significant differences at S3. The differences between years and vineyards and their interaction were significant for skin to berry ratio and skin weight (Supplementary Table 1).

TSS per berry showed variation among vineyards and between seasons but limited variations among treatments. TSS (grams per berry) reflected the berry mass and the highest content at harvest was found in the heaviest berries (vineyard L in both years) and the lowest in the smaller berries, in vineyard M in 2022 and in vineyard H in 2023. In L vineyard higher sugar content per berry in C1 berries as compared to T0 berries in 2022 at S4 and S5 was found. In 2023 no significant difference was observed at any sample point in vineyard L or H. In vineyard M, significant differences between grapes grown with full UV exposure (C1) and those with no UV exposure and increased temperature (T0) emerged at harvest in 2023 (Figure 3). In 2022, in vineyard H, berries grown under ambient temperature (C0 and C1) had a significantly higher per berry sugar content with respect to those grown under increased temperature (T1 and T0). This difference was significant from S2 to S5. The TSS was similar for all treatments when HI was highest (vineyards L and H in 2023 and vineyard M in 2022, Table 2), but it was on average lower than that achieved in the least warm conditions (Figure 3). T treatments have therefore shown the same negative impact as natural high temperatures. In all cases, extreme temperatures had a negative effect on the absolute value of TSS per berry. When significant differences were found among treatments, TSS was highest in C0 and lowest in T0, both as g/berry (Figure 3) and °Brix (Supplementary Table 1). A similar negative impact of filtered solar UV-B on soluble solids per berry was found in cv. Malbec [52].

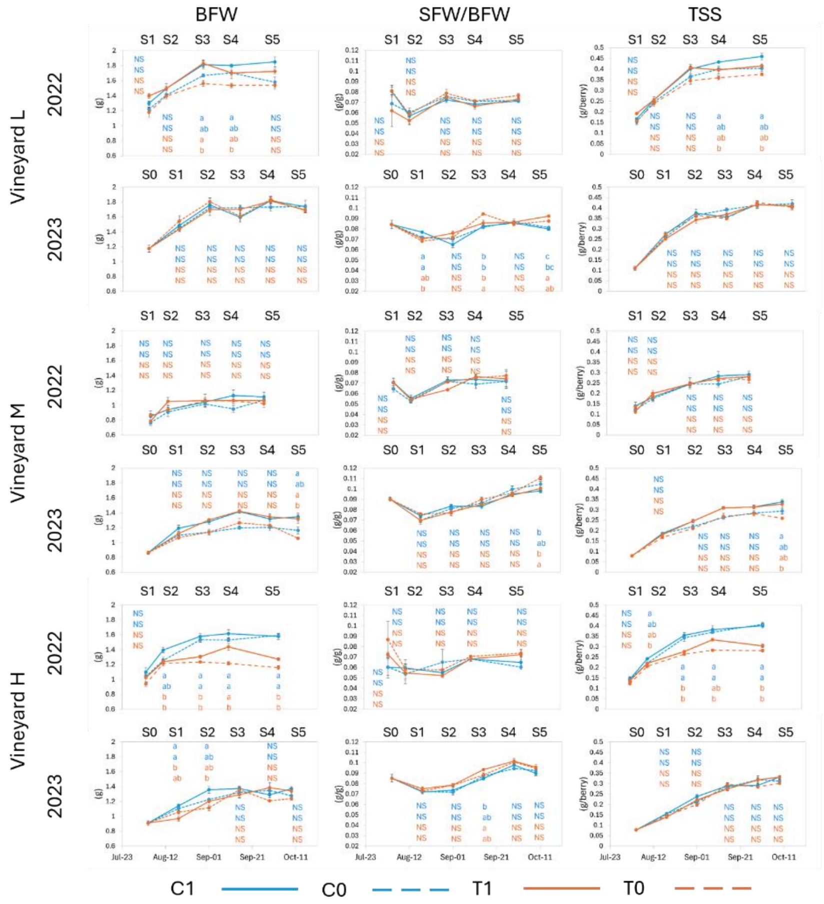

**Figure 3:** Evolution of berry fresh weight (BFW) (g), fresh skin weight/fresh berry weight ratio (SFW/BFW) (g/g) and Total Soluble Solids (TSS) (g/berry), for each vineyard from treatment application to harvest in 2022 and 2023. Error bars represent standard errors (n = 3 and n = 4 for C1 in 2023). Different letters indicate significant differences for $p \leq 0.05$. The letters are presented vertically in order: C1, C0 (blue colour), T1, T0 (orange colour). S0-S5 represent the number of the sampling.

### 3*.2.5. Anthocyanins*

When expressed as mg/kg of fresh berries, in vineyard L, the total anthocyanin concentration (TAC) at harvest was significantly influenced by the treatments in both years, with C0 berries reaching higher concentrations than both T treatments in 2022 and C1 being significantly higher than T0 in 2023. In vineyard M, although no significant differences were observed among treatments in either year at harvest, C1 berries reached the highest TAC in both years. In 2023, C0 and C1 both trended higher than T1 and T0. In vineyard H, C1 berries showed significantly higher concentration than both T treatments in both years with C0 also being significantly lower than C1 in 2023. UV treatments appeared to reduce TAC in all vineyards with respect to C1 with the exception of L vineyard in 2022 when C0 was higher (Figure 4). This reduced concentration of TAC through the removal of UV appeared to be largely due to a response from the di-hydroxylated anthocyanins which constitutes the larger proportion of TAC in cv Nebbiolo; their concentration (Supplementary Table 2) and relative proportion (Supplementary Table 3) were negatively impacted by increased temperature, but the UV removal further amplified this effect (Supplementary Table 3). The relative abundance of the tri-hydroxylated anthocyanins (Mv in particular) was positively influenced by UV removal whereas the temperature had no effects on them (Supplementary Table 3). The negative impact of high temperature on anthocyanin concentration has been shown in many studies in phytotron [19,54–56] and field conditions ([17,57]. Azuma et al., (2012) [54] found reduced anthocyanin concentration and alterations to the profile, under 35 °C, due to changes in expression of flavonoid biosynthetic pathway genes. It has also been shown that the synthesis of anthocyanins was depressed in the absence of light [54] but that high levels of radiation are not necessary for their synthesis provided the temperature was contained [58]. As previously reported [52], the important impact of UV radiation on the synthesis of anthocyanins is confirmed by their lower concentration when UV exposure was minimized in the current study.

The downregulation of F3'-5'H following the increase in temperature, in a previous study resulted in a decrease in the percentage of tri-hydroxylated anthocyanins [54]. In the current study this did not occur and when UV was limited (C0) the percentage of Mv and Pt (Supplementary Table 3) increased even when temperature was artificially increased (T0); what was observed in T0 may be due to a compensation of the negative effect of high temperatures with the positive one of lower UV intensity. On the other hand, the relative abundance and concentration of Cy and Pn decreased due to high temperatures (T1) and, to a greater extent, in the absence of UV radiation (C0 and T0) (Supplementary Table 3). High temperatures and lack of UV appear to have had different effects on the di-hydroxylated and tri-hydroxylated anthocyanins; however, the ratio di-hydroxylated:tri-hydroxylated anthocyanins decreased as a result of treatments, with some differences during the season which was affected by the vineyard and year but not by treatment and year (Supplementary Table 4).

The rate of acylation is known to increase with increased temperature [16,54,56]. Similar findings were observed consistently in each vineyard when comparing rates of acylation to TAC with T treatments trending higher than C treatments in both years. The effect of UV on anthocyanin acylation rates was less clear, with two cases (vineyards M and H in 2022) showing in C0 significantly higher values than in C1 (full light exposure). However, C0 was significantly lower than T0 suggesting that UV exposure plays less of a role in acylation rates (Supplementary Table 4). In vineyard L both T treatments had a higher ratio than C treatments (significantly higher in 2023) in both years. Vineyard M displayed significantly higher acylation rates in T0 than in C1 or C0 in 2022, while T1 was intermediate to C0 and T0. In 2023, C1 was lower than all other treatments although at harvest, differences were not significant. Finally, vineyard H also had significantly lower values for C1 treatment in 2022 compared to all other treatments while C1 and C0 trended lower than T treatments in 2023 (Figure 4 and Supplementary Table 4). In conditions characterised by high thermal accumulation in Nebbiolo berries, the synthesis of di-hydroxylated anthocyanins may decrease (especially if bunches are shaded from UV) and their acylation may increase, favouring the production of musts with a lower di/tri ratio and higher acylation rates. Considering that tri-hydroxylated and acylated anthocyanins are more stable than di-hydroxylated anthocyanins and their respective free forms, increased temperature may alter the skin anthocyanin profile at harvest, potentially increasing, in a cv like Nebbiolo, the wine colour stability over time, as previously reported [22]. In contrast to these findings, negative impacts of limited UV radiation on anthocyanin acylation have been reported [52,59] but the role of UV in this aspect would require more detailed investigations.

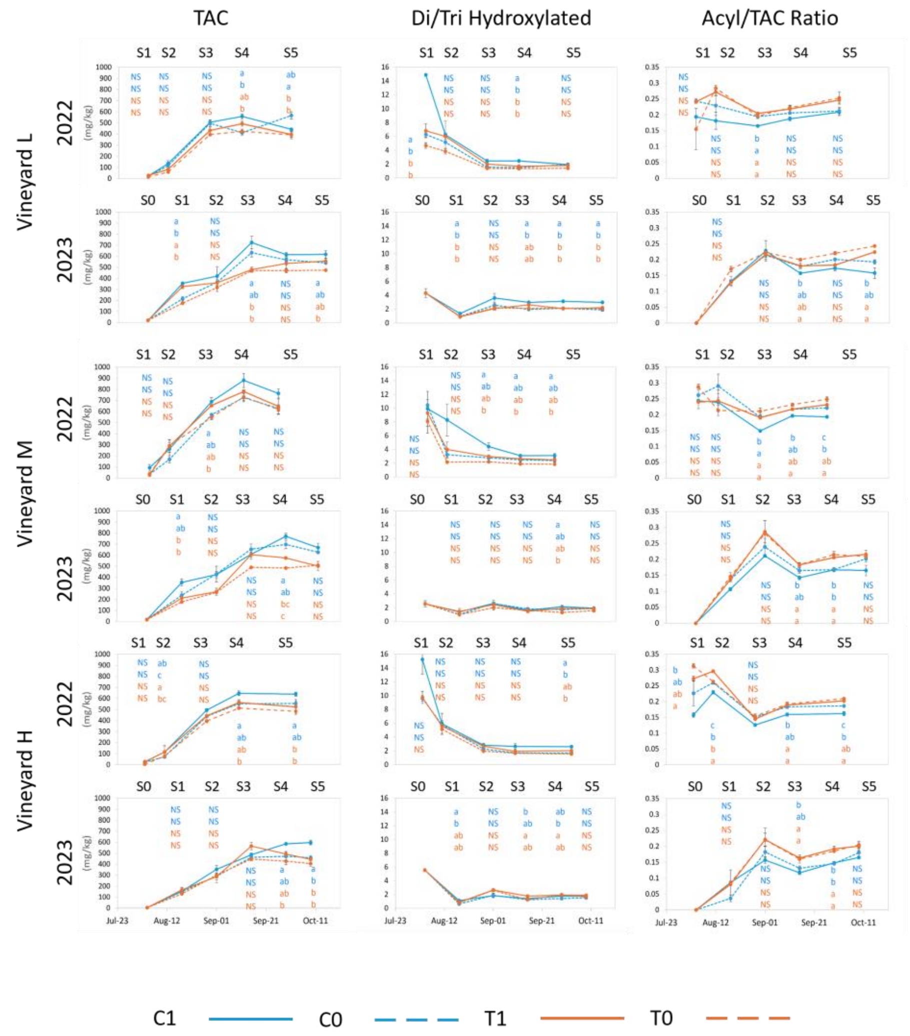

**Figure 4:** Evolution of Total anthocyanin concentration (mg/kg) (TAC), di-hydroxylated/tri-hydroxylated anthocyanin ratio [Di/Tri = (Cn+Pn)/(Df+Pt+Mv)] and acylated/TAC ratio for each vineyard from treatment application to harvest in 2022 and 2023. Error bars represent standard error (n = 3 and n = 4 for C1 in 2023). Different letters within the same column indicate significant differences between treatments. $p \leq 0.05$; NS = not significant. The letters are presented vertically in order: C1, C0 (blue colour), T1, T0 (orange colour).

Methylation of di-hydroxylated anthocyanins trended higher with limited UV exposure in all vineyards through the season (Figure 5, Supplementary Table 4). At harvest, both treatment factors (UV and T) did not significantly influence the methylation of tri-hydroxylated anthocyanins, with the only exception of vineyard H in 2023 where C0 was significantly higher than T1. For both ratios, the relationship between treatments was inconsistent and did not exhibit a general trend (Figure 5) while significant differences were observed between years and vineyards (Supplementary Table 4). At 35 °C, a decrease in the methoxylated forms (Pn in particular) was previously observed due to the downregulation of O-methyltransferase [54], and an increase of the proportion of methoxylated forms was observed in cv Merlot with higher thermal regimes (day/night temperature = 30-35/20-30 °C) [56]. The current findings in cv Nebbiolo did not support these results.

The ratio TSS/TAC in 2022 and 2023, showed some significant difference between C and T treatments at harvest in both years with C1 trending higher than both T treatments in both years and than C0 in vineyard H in 2023 (Figure 5). Until the beginning of September 2022, a linear increasing trend was observed which was similar for all treatments and vineyards; after that, TAC accumulated more slowly than TSS (ratio decreased) and the impact of treatments became more evident with different trends depending on vineyard and treatment. In 2023 the impact of UV treatments was evident earlier than in 2022 in vineyards L and M. The decrease of the ratio before harvest in 2022 for T berries occurred despite during the two weeks before harvest maximum temperatures were never above 35 °C in T treatments (Figure 1). This confirms the effect of high temperature on decoupling anthocyanin synthesis and/or accumulation and sugar accumulation as previously reported [15,60] and suggests that this can occur at temperatures lower than 35 °C. Despite the temperature that characterized the period before harvest in 2022 was lower than in 2023, the SWC in 2022 was also much lower than in 2023 (Figures 1-2) and this situation could have influenced the impact of the treatments on this ratio and its seasonal trend. The pedoclimatic conditions impacted more anthocyanins than TSS (Figures 3-4), however, in a hot year, such as 2023, increased SWC did not offset the effects of the high temperature on decoupling anthocyanin and sugar accumulation. Water deficit has previously been observed to increase the rate of anthocyanin accumulation and ratio between TAC and TSS [60,61].

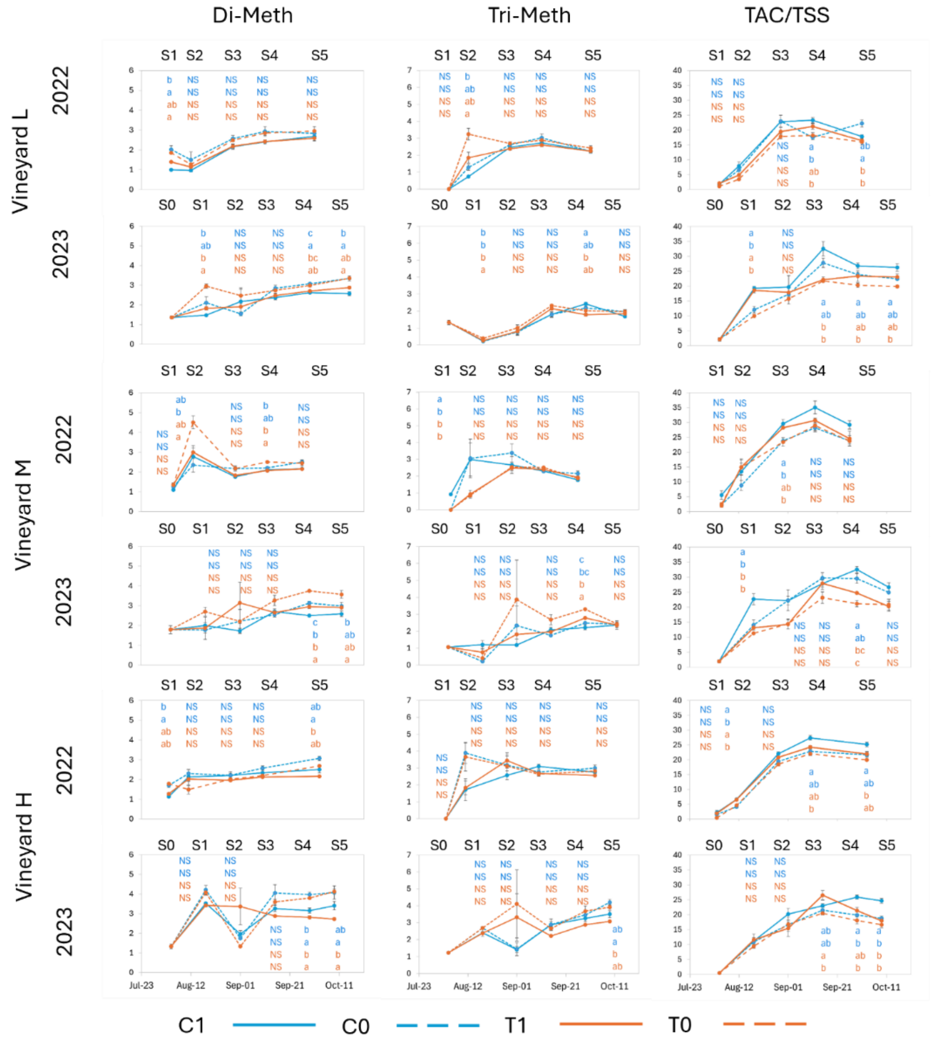

**Figure 5:** Evolution of Di-methylated ratio [Di-Meth = Pn/Cy], Tri-methylated ratio [Tri-Meth=Mv/(Df+Pt)] and TAC/TSS for each vineyard from treatment application to harvest in 2022 and 2023. Error bars represent standard error (n = 3 and n = 4 for C1 in 2023). Different letters within the same column indicate significant differences between treatments. $p \leq 0.05$; NS = not significant. The letters are presented vertically in order: C1, C0 (blue colour), T1, T0 (orange colour).

### *3.2.6. Flavonols*

Di-hydroxylated quercetin (Q) is the most abundant flavonol in Nebbiolo berries [14] where it is present as glucoside (Side) and glucuronide (Ride) forms. On average, in C1 and T1 samples the glucoside form (QSide) was about 4 times more abundant than the glucuronide form (QRide) (Supplementary

Tables 5 and 6). When UV radiation was excluded (C0 and T0), the total flavonol concentration and individual molecule concentration significantly decreased (Figure 6) as expected. QSide concentration decreased more than Qride (approximately 65% and 15%, respectively). However, kaempferol (K) glucoside and glucuronide decreased much more (about 85%) than Q forms (Supplementary Tables 5 and 6). This contributed to the increase of the QRide and MRide relative abundance (Supplementary Table 5) and to the decrease of the ratio between glucoside and glucuronide forms (Side/Ride) at harvest (Supplementary Table 6) in vines with reduced UV exposure. Side/Ride ratio was highest in C1 and T1 and lowest in C0 and T0 from the start of the sampling until harvest (Figure 6).

A positive response of flavonols to increased exposure to solar radiation has been observed multiple times in previous research [17,31,62–64]. The exclusion of UV reduced the concentration of individual flavonols [10,11,54], also modifying the relative abundance of the individual molecules. In Tempranillo, high doses of UV increased the relative abundance of the mono- and di-hydroxylated flavonols and decreased the proportion of tri-hydroxylated [10]. The concentration of total and individual flavonols decreased after reducing UV exposure in our study (Supplementary Table 5). However, the proportion of myricetin glucoside (MSide) and quercetin glucuronide increased (Supplementary Table 6) confirming that limiting UV radiation can alter the flavonol profile.

The total concentration of flavonols and individual molecules decreased with increasing temperature; the decrease did not affect MSide and QRide under natural UV exposure (T1) (Supplementary Table 6) and therefore their relative abundance increased when compared to C1, while that of the other molecules decreased (Supplementary Table 5). This suggested that the molecules have a different heat sensitivity. The increased temperature did not alter the negative influence of the lack of UV and therefore the concentrations and relative abundance of the single molecules under T0 treatments remained similar to those of C0, but always lower than those of the control (C1). This agrees with a previous study that demonstrated that temperature negatively impacted total flavonol concentration in cv Merlot, particularly when the temperature was higher than 30°C during the day and higher than 25 °C during the night [65]. In T treated vines, total flavonol concentration trended lower during both seasons although, at harvest, only in vineyard M in 2023 the difference between C1 and T1 was significant. Despite this, in 2023, the separation between C1 and T1 was more defined in all vineyards as compared to 2022 (Figure 6). This could be explained by the much higher number of hours T treated vines were exposed to temperatures above 35 ℃ in 2023 compared to 2022 (Table 2). A negative impact of temperatures higher than 35-40 °C has been observed in other studies [54,66,67]. This may suggest a non-linear relationship between temperature and flavonol synthesis or that this relation may be more influenced by prolonged temperatures above a certain threshold (35 °C in our case). Throughout the season, C1 berries contained significantly higher amounts of flavonols than T1 berries in both years and all vineyards despite only in vineyard M in 2023 differences were found at harvest (Figure 6). Otherwise, both under ambient and increased temperature, flavonol synthesis appeared to be completely depressed by the absence of UV. This is consistent with Azuma et al. (2012) [54], who found the influence of light on the expression of flavonol biosynthesis-related genes to be much more considerable than that of temperature.

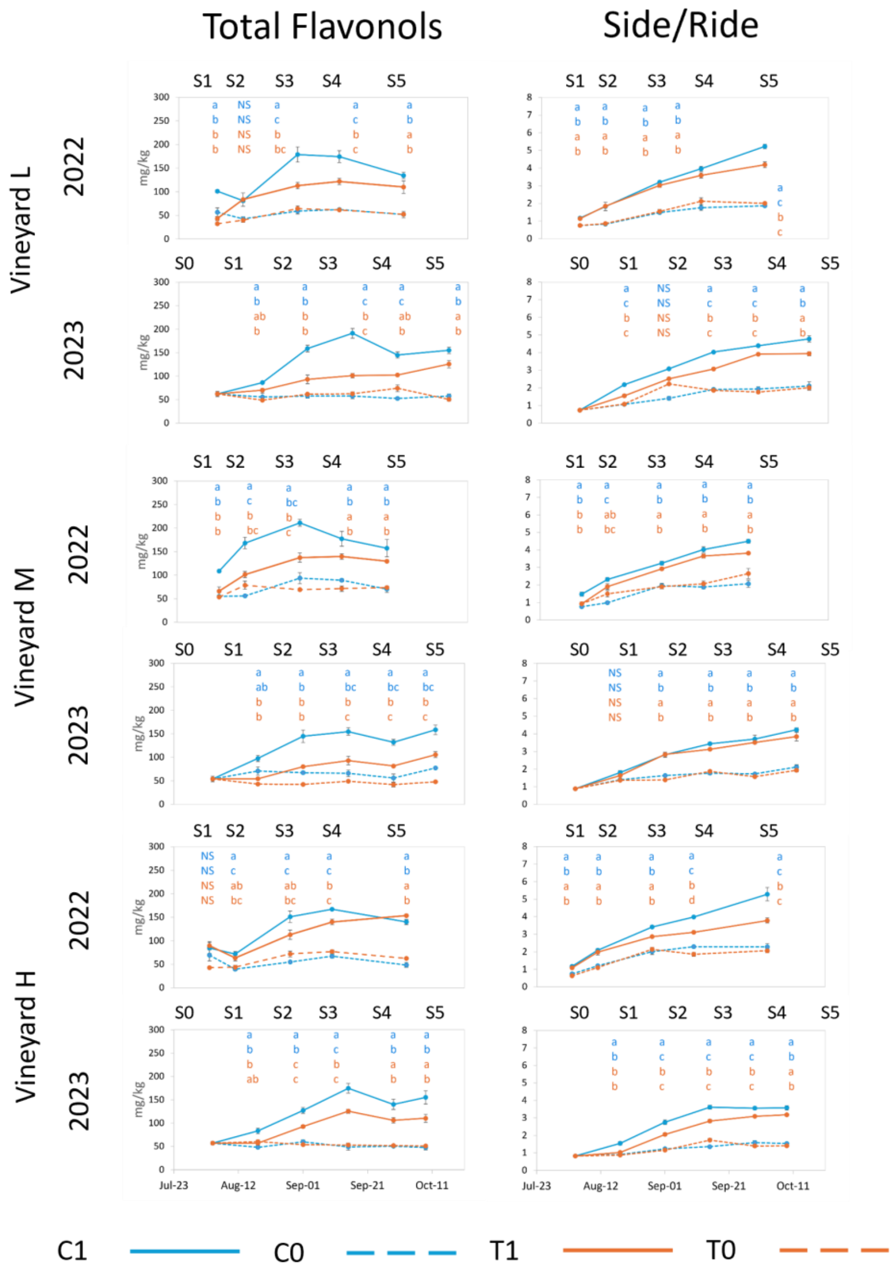

**Figure 6:** Evolution of Total Flavonols (mg/kg) and Glucoside/Glucuronide ratio for each vineyard from treatment application to harvest in 2022 and 2023. Error bars represent standard error (n = 3 and n = 4 for C1 in 2023). Different letters within the same column indicate significant differences between treatments. $p \leq 0.05$; NS = not significant. The letters are presented vertically in order: C1, C0 (blue colour), T1, T0 (orange colour).

### *3.2.7. HCTAs*

Skin HCTAs begin to accumulate early in berry development and are found in higher concentrations from bloom to veraison at which point their concentration declines during the ripening period, [68,69] as was observed in both years of this study (Figure 7).

In both years, HCTAs total concentration was higher with UV deprivation, regardless of the thermal level, in parallel with the increase of *trans*-*p*-coutaric acid concentration, the predominant form of HCTA. Conversely, UV deprivation reduced the concentration of *cis*-*p*-coutaric acid (Supplementary Table 7). UV did not affect the concentration of *trans*-caftaric acid in 2022, but its concentration reduced with increased UV exposure in 2023, resulting in a seasonal significant difference (Supplementary Table 7). The total HCTA concentration was not significantly influenced by the applied treatments at vineyard H in both years, whereas at vineyards L and M we observed significantly higher values in C0 than in T1 in 2023 and grapes from vineyard L also accumulated higher amounts of HCTA in C0 with respect to C1 in 2022 (Figure 7).

Notably, all HCTA concentrations were significantly lower in 2023 as compared to 2022, reaching average values of 489.3 mg/kg of skins in 2022 and 448.6 mg/kg of skins in 2023 (Figure 7). As with flavonols, this aspect could be explained by the longer periods that grapes were exposed to extreme temperatures in 2023, with respect to 2022, assuming that the extreme peak of temperature could have blocked the first steps of the phenylpropanoid pathway, when cinnamic acids are progressively synthesized [23]. Both the total HCTA and each individual HCTA showed significant differences between Year and Vineyard (Supplementary Table 7).

Ultraviolet radiation exposure influenced the ratio of *trans/cis* *p*-coutaric acid (Figure 7) with a decrease in *trans* isomer associated with increased UV exposure and a corresponding increase in *cis* *p*-coutaric acid isomer. *Cis*-cinnamic acid is produced through a sunlight-mediated conversion from *trans*-cinnamic acid [70] and UV exposure serves to increase levels of *cis* isomers from *trans* isomers [71]. This response was consistent during the season and at harvest in all vineyards in both years (Figure 7). Globally, this aspect was more marked in 2023, which can suggest that the higher number of heat peaks, and/or the higher SWC enhance the conversion from *trans*-coutaric to *cis*-coutaric acid, particularly when cv Nebbiolo berries receive higher UV exposure. Considering that more than 50% of HCTA composition is comprised of *trans p*-coutaric acid, the ratio between *trans* and *cis* p-coutaric acid increases by removal of UV in both years (Figure 7 and Supplementary Table 7). As *cis* isomer of *p*-coutaric acid is less stable than the *trans* isomer, the alteration of this ratio, could lead to a decreased concentration of *p*-coutaric and of *p*-coumaric acid in wines. Further investigation into the effects of altering the ratio *trans/cis*-coutaric acid through increased UV exposure, thus reducing the total concentration of HCTA in berries, is required as a potential tool for risk reduction against possible spoilage from *Brettanoymyces* yeast [50].

Caftaric acid is very oxidizable and is predominantly accumulated in pulps where it reacts with gluthathione to give origin to the GRP (grape reaction product, [72]). Considering that in red-cultivar winemaking, particularly in cv Nebbiolo grapes, the contribution of skin maceration to wine composition is important, the contribution of HCTA concentration and profiles to wine quality cannot be neglected. For this reason, the ratio between *p*-coutaric acids (*cis* + *trans* forms) and caftaric acid (*trans*) was also calculated (Figure 7). Vineyard L and M displayed no significant differences in 2022 while in 2023, grapes from L vineyard displayed at S1, S2 and at harvest higher values in treatment C0 with respect to T1 (Figure 7). Vineyard H did not show any difference during 2023, but differences were observed in 2022 from S4 to harvest with, again, C0 displaying a higher ratio than T1, suggesting that increased temperature can negatively influence the ratio, whereas lower temperature with reduced UV (C0) can increase the amount of *p*-coutaric acid compared to caftaric acid.

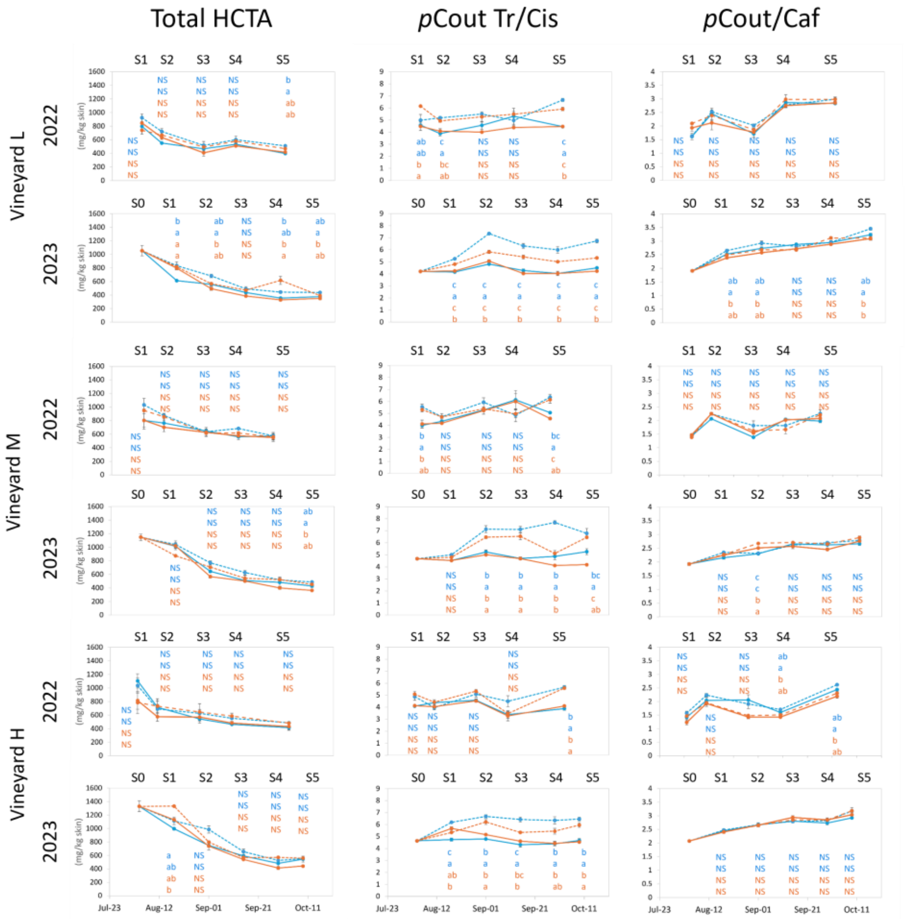

**Figure 7:** Evolution of Total HCTAs (mg/kg berry skins), ratio of trans/cis *p*-Coumarylated and *p*-Coum/Caftaric acid ratio for each vineyard from treatment application to harvest in 2022 and 2023. Error bars represent standard errors (n = 3 and n = 4 for C1 in 2023). Different letters within the same column indicate significant differences between treatments. $p \leq 0.05$; NS = not significant. The letters are presented vertically in order: C1, C0 (blue colour), T1, T0 (orange colour).

## 3.3. Materials and Methods

### *3.3.1. Experimental Site and Design*

The research was performed in 2022 and 2023 from veraison to harvest in three vineyards in the production region of Barolo wine (a wine that has a controlled and guaranteed designation of origin, DOCG) in Northwest Italy. It is a small area of approximately 80 km$^2$, characterized by steep slopes and undulating hills ranging in elevation from just below 200 m above sea level (ASL) in the valley floor to 550 m ASL at a maximum elevation.

#### 3.3.1.1. Experimental sites

The experiment was carried out in three non-irrigated vineyards at different elevations located in the municipality of La Morra (Piedmont Region, Italy). The lowest elevation vineyard (L), was located at 44°37’51.0”N 7°57’21.5”E in Bricco Rocca site at an elevation of 215 m ASL; the middle elevation vineyard (M), was located at 44°37’39.4”N 7°56’23.0”E in Brunate site, at 350 m ASL; the highest elevation vineyard (H), was at 44°37’18.6”N 7°55’48.6”E in Fossati site at an elevation of 400 m ASL. The vineyards have ESE to SSE facing slopes, and similar slope gradients ranging from 12° to 15° (Figure 8). Vineyards M and H were planted in 2002 with S04 rootstock and clones CVT 141 and CVT 71 respectively. Vineyard L was planted in 1975 onto unknown rootstock, with vines from massal selection. Vines were grown to a vertical shoot-positioned training system with single Guyot pruning (8 to 10buds/vine). The rows were positioned along the contour lines. Vineyard soils were similar with one major exception as vineyard L had much higher sand percentage than vineyards M or H (Supplementary Table 8).

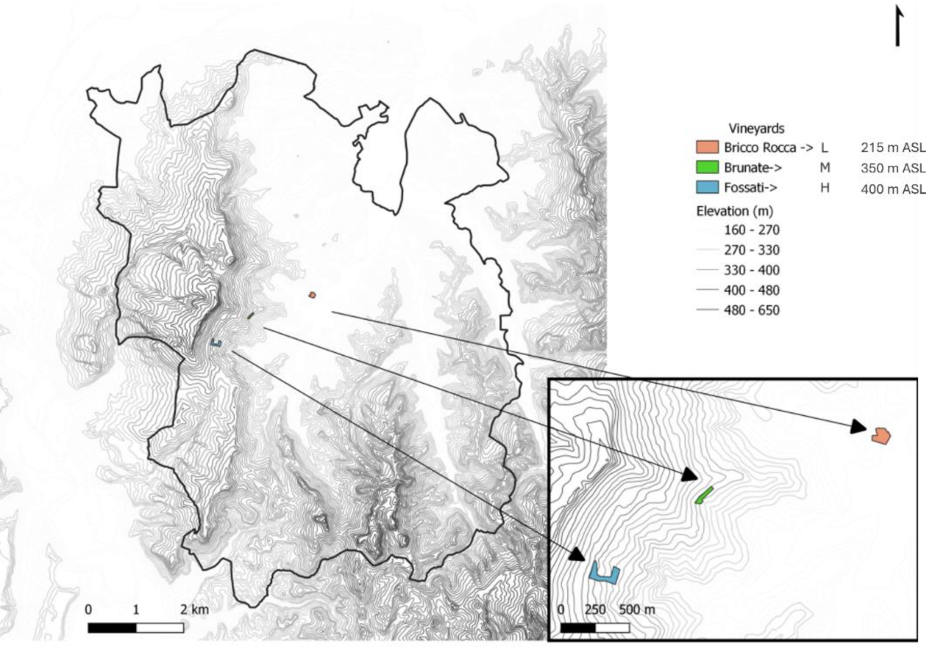

**Figure 8:** Map of Barolo DOCG production region (black border) with insert showing location of the three vineyards in study (Blue = H, Green = M, Pink = L). 10 m

resolution digital elevation model contours from TINITALY digital elevation model [73].

### 3.3.1.2. Experimental design

In each vineyard, three adjacent rows were chosen for a split-plot experimental design. The main factor “Temperature” consisted in comparing the effects of two levels of temperature: the first level being ambient (C) and the second level being increased temperature (T), obtained by placing removable transparent plastic (Serroplast®, Rutigliano, BA, Italy) inducing a passive greenhouse effect. The greenhouse was designed to cover bunches of three consecutive vines per row. The greenhouse plastic was applied from the first training wire to cover the bunch zone but not to contact the ground. Curved rods were installed on the training wire perpendicular to the row orientation, underneath the plastic to avoid direct contact between leaves and the plastic (Figure 9B). The greenhouse plastic was connected above and below bunch zone at several points to amplify temperature, without completely closing the bunch zone.

The sub-plot factor "UV" consisted in testing two levels of UV radiation. To achieve this goal, a white UV blocking plastic cover (Serroplast®) was applied (0) or not (1) over half of the grape bunches of each vine both inside the passive thermal treatment and outside (Figure 9A). Metal frames were shaped to a wide cone with the large end (bottom) approximately 40 cm in diameter and the small end (top) of 10 cm in diameter. These cones were covered in UV blocking plastic with the top open. They were hung from the first training wire and suspended over individual bunches without contacting bunches or bunch rachis. The design allowed airflow in the bunch area to ensure temperature was minimally influenced. Measurements of transmitted UVA, UVB and PAR were acquired for each treatment under midday full sun conditions (5 minutes per treatment at 10 sec/sample) to determine differences between treatments (Delta Ohm DO9847, GHM Group, Regenstauf, Germany).

Four treatments were then compared: ambient temperature and full UV exposure (C1); ambient temperature and no UV exposure (C0); amplified temperature and full UV exposure (T1) and amplified temperature and no UV exposure (T0) (Figure 9). Each vineyard had three replicates per treatment (four replicates for C1 treatment in 2023) and three vines per replicate for a total of 18 vines per vineyard. The four treatments were applied in all vineyards when berries began to develop colour, at BBCH 81 [74]: July 25 in 2022; August 4 in 2023. The experimental design was randomized with limitations based on weak production levels (this was particularly true for the "T" treatments which required multiple adjacent vines with a minimum of 4 bunches per vine for the greenhouse treatment to cover a suitable number of replicates for both T1 and T0), and disease presence in both years.

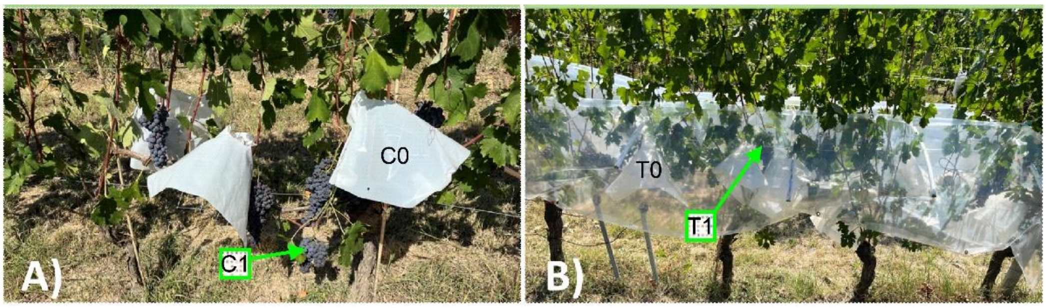

**Figure 9:** A) Bunches exposed to ambient temperature with UV exposure (C1, no plastic cover) or exposed to ambient temperature and without UV exposure (C0, white UV blocking plastic cover). B) Bunches covered by passive plastic with UV exposure (T1) or without UV exposure (T0).

### *3.3.2. Air and soil temperature, Soil Volumetric Water Content and precipitation assessment*

Air temperature was acquired in C and T treatments during the research period in both seasons. In July 2022 one temperature sensor (HOBO Datalogger MX2301A - Onset Computer Corporation, Bourne, MA, USA) was installed in the bunch zone in the centre of the middle row in each vineyard. This temperature sensor remained on site, reading ambient temperature (C treatment) until the end of harvest 2023. At the time of treatment application, a second temperature sensor was installed in the middle row under the passive greenhouse (T treatment) in each vineyard at the same height as the C temperature sensor (HOBO Datalogger MX2302 - Onset Computer Corporation) and Tinytag Plus 2 TGP 4500 (Gemini Data Loggers Ltd, Chichester, United Kingdom) which measured hourly minimum daily temperature (TMin) and maximum daily temperature (TMax). A partial Huglin Index (HI) was calculated for each treatment from August 23 to October 8 in both years. The standard HI (from April 1 to October 31) could not be calculated because sensors were installed in the vineyards in late July 2022 and some data was lost due to anomalies in data recording, particularly during the months of August and September 2023.

An assessment was also carried out inside an UV treatment cone (C0) and outside (C1) from mid-May to mid-July of 2022 to determine whether the air temperature could be amplified under the UV-blocking plastic.

Soil volumetric water content (SWC) and soil temperature were measured in each vineyard with a "5TM Soil Moisture and Temperature sensor", equipped with EM 50 Datalogger (Decagon Devices, Inc., Pullman, WA, USA). SWC sensor probes were installed in the middle row of the experimental row group into undisturbed sidewall of the borehole at 30 cm depth on June 6, 2022, and operated until October 15, 2023. A second SWC sensor was installed in each vineyard, 1 m from the first sensor, prior to the commencement of 2023 activities. The temperature and precipitation at the meso-scale were obtained from a nearby weather station (La Morra, LM at 326 m ASL) [75].

### *3.3.3. Berry sampling and berry skin preparation*

In both years, berries from each treatment were sampled randomly from both sides of all rows with 10 berries per replicate and three replicates per treatment in each vineyard and four replicates collected for C1 treatment in 2023. In 2022, treatments were applied on July 25 at the first sign of veraison. Berry sampling commenced at estimated 50% veraison (August 4 (S1)). A second sample was collected one week later at 100% veraison (August 12 (S2)), 2 intermediate samples were taken (August 29 (S3) and September 11 (S4)) prior to the final sample at harvest (September 26 (M); October 1 (H); October 3 (L) (S5)). In 2023, C1 samples were collected on the day of treatment application (August 4 (S0)). Samples were then collected at approximately 2-week intervals until harvest (August 18 (S1), September 1 (S2), September 15

(S3), September 29 (S4)), and at harvest (October 9 (M); October 12 (H); October 15 (L) (S5)). The final harvest samples were collected the day prior to commercial harvest in each vineyard in both years.

Berries were cut above the pedicel, placed in a sealed plastic bag and stored in a portable refrigerator until they could be transported to the laboratory (within 1 hr). At the laboratory, fresh berries were weighed (BFW) and then pedicels removed, with pulp separated from the berry skin. A tight sealing container with 40 ml of 3.2 pH buffer solution (120 ml/L ethanol, 5 g/L tartaric acid, 2 g/L $Na_2S_2O_5$, 22 ml/L NaOH 1 mol/L) was weighed, skins were immediately added and then the container weighed again to determine skin fresh weights (SFW); the ratio Skin weight:Berry weight was calculated. Berry skins in buffer were frozen at -20 °C. Pulps were preserved to measure Total soluble solids (TSS, Brix) by a refractometer (HI96811, Hanna Instruments, Woonsocket, RI, USA) which was then converted to grams per berry. Berry skin extracts were thawed and homogenized twice (UltraTurrax T25, IKA, Staufen, Germany) and centrifuged for 15 minutes at 2220 × g (Heraeus Primo, Thermo Fisher Scientific, Boston, USA), taking the extracts to a known final volume (50 ml). Extracts were stored into tight sealing 50 ml plastic containers and frozen prior to preparation for high performance liquid chromatography (HPLC-DAD) analysis.

### *3.3.4. Anthocyanin, Flavonol and Hydroxycinnamic Tartaric Acids extract Preparation and Chromatographic Analyses*

Samples were prepared for anthocyanin and flavonol/HCTA analyses according to a method modified from Di Stefano & Cravero (1991) [76].

Anthocyanins were detected by HPLC/DAD analysis using an Agilent 1200 series equipment (Agilent Technologies, Santa Clara, CA, USA) equipped with a LiChrospher® 100 RP-18 (5 µm particle size, 25 x 0.4 cm ID) (Merck, Darmstadt, Germany) column. Formic acid:water (10:90, v/v) and formic acid:methanol:water (10:50:40, v/v/v) were used as solvent A and B, respectively. A linear gradient between 28% and 45% of solvent B over 15 min, then to 70% in 20 min, and finally to 90% in 10 min was used for the separation. The column was then washed with solvent B for 3 min, before returning to the starting condition (28% B) for 10 min. A constant flow rate of 0.8 ml/min was established. Detection was carried out at 520 nm wavelength.

Delphinidin (Df), Cyanidin (Cy), Petunidin (Pt), Peonidin (Pn), and Malvidin (Mv) 3-O-glucosides (Gluc) were detected as well as their relative acylated forms: acetated anthocyanins (Acet) and *p*-Coumaroylated anthocyanins (*p*Coum). The ratios Pn/Cy and Mv/(Dp+Pt) were calculated to estimate the degree of methoxylation of di- and tri-hydroxylated anthocyanin, respectively. The identification and quantification of the individual anthocyanins was based on the comparison of their retention time with that of pure standards, when available, and the concentration was expressed as malvidin 3-O-glucoside equivalents (Extrasynthèse, Genay, France).

Samples for flavonol and HCTA analysis were processed after dilution with 1 mol/L phosphoric acid. HPLC analysis was performed using an Agilent 1260 Infinity System (Agilent Technologies). Solvent A (phosphoric acid $10^{-3}$ mol/L) and solvent B ($CH_3OH$) were used, applying gradient elution conditions, starting with 5% B, increasing linearly to 100% B in 35 min, and keeping 100% B for 5 min, followed by a re-equilibration phase under isocratic conditions. The flow rate was 0.8 mL/min and chromatographic acquisitions were set at 360 and 320 nm. Flavonol concentrations were expressed as quercetin dehydrated equivalent per kilogram of fresh berries. Among flavonols, myricetin 3-*O*-glucoside (MSide), quercetin 3-*O*-glucoside (QSide), quercetin 3-*O*-glucuronide (QRide), kaempferol 3-*O*-glucoside (KSide) and kaempferol 3-*O*-glucuronide (KRide) were identified, based on previous published papers [14,77], and quantified as quercetin 3-O-glucosides equivalents (Extrasynthèse). The ratio of glucoside forms (Side) to glucuronides (Ride) was calculated to evaluate the relative abundance of the prevalent class of flavonol-glycosides.

HCTAs chromatograms were acquired at 320 nm and expressed as equivalents of caftaric acid per kilogram of skins. Among HCTAs, *trans* caftaric acid, and *cis* and *trans* *p*-coutaric acid were identified.

The total concentration of each class of compounds was obtained by summing the individual concentrations.

### *3.3.5. Statistical Analysis*

A generalized linear model (GLM) was used to investigate the effects of the treatments by year and vineyard including their interactions. Prior to running the GLM, normality (Shapiro-Wilk test) and homoscedasticity (Breusch-Pagan test) were assessed. Post-hoc analysis was performed using estimated marginal means (EMMs) to explore pairwise comparisons among the levels of the factors and vineyards. The contrasts were adjusted for multiple comparisons using false discovery rate correction. Statistical significance was assessed at $p \leq 0.05$.

A one-way ANOVA was performed on temperature data between C1 and C0 treatments to determine if there was a significant temperature amplification in C0 (and by extension, T0) as compared to C1 and T1.

Statistical analysis was performed with the statistical software R [78] with multcomp [79] and emmeans packages [80] using RStudio GUI [81]. Graphical representation of plots was produced with Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

## 3.4. Conclusions

At the field level, interactions between cultivar and environment are complex. Both temperature and UV exposure can impact the development of berry characteristics and polyphenols and thus the end quality and identity of a wine. In a changing climate, producers will have to consider curating management based on vineyard location, cultivar, clonal characteristics, current local risks to berry and wine quality and desired qualitative features of the wine. In the case of cv Nebbiolo, UV plays a significant role in colour development and stability due its high concentration of di-hydroxylated anthocyanins which have shown to be both temperature and UV sensitive. Increasing the percentage incidence of the tri-hydroxylated malvidin 3-O-glucoside through reduced UV exposure could increase wine colour stability; the reduction of UV exposure also leads to decreased flavonol and increased HCTA concentrations. Increased temperature has long been associated with decreased anthocyanin concentration and in this research similar observations were made. The combination of increased temperature and decreased UV exposure further amplified this decline in cv Nebbiolo grapes, suggesting that cultivars with a specific anthocyanin profile characterized by higher concentrations of di-hydroxylated anthocyanins can be manipulated through UV exposure as well as temperature. The effect of temperature on flavonol concentrations has long been debated but in this research given the passive nature of the treatments and the significant difference between the number of hours of exposure to extreme temperatures (> 40 °C), flavonols were notably lower in treatments with increased temperature, which can be explained with the general under-expression of the phenylpropanoid pathway. Although this research considered only the effects of UV exposure and temperature on some berry characteristics and flavonoids in berry skin, other factors also play a role in berry quality. Consideration of hill aspect, as well as soil water retention along with other site-specific details must also be factored into the decision-making process for appropriate vineyard management strategy to balance UV exposure and temperature for desired outcomes in berry and wine quality.

**Supplementary Materials:** Table S1: Berry characteristics at harvest: Berry Fresh Weight (BFW), Skin Fresh Weight (SFW), Skin to Berry Ratio, Total Soluble Solids (TSS) and Sugar per Berry at harvest (S5); Table 2: Anthocyanin concentration at harvest for the glucosylated forms: Delphinidin (Df), Cyanidin (Cy), Petunidin (Pt), Peonidin (Pn) and Malvidin (Mv) at harvest (S5); Table 3: Anthocyanin proportion at harvest for glucosylated forms: Delphinidin (Df), Cyanidin (Cy), Petunidin (Pt), Peonidin (Pn) and Malvidin (Mv) at harvest (S5); Table 4: Total Anthocyanin Concentration (TAC); Di-hydroxylated/Tri-hydroxylated ratio (Di/Tri); Acylated/Total Anthocyanins ratio (Acyl/TAC); Rate of methoxylation in di-hydroxylated anthocyanins {Di-Meth = Pn/Cy}); Rate of methoxylation in tri-hydroxylated anthocyanins, [Tri-Meth = Mv/(Df+Pt)]; Ratio between TAC and Total Soluble Solids (TAC/TSS) at harvest (S5); Table 5: Individual flavonol proportion (% of total) at harvest. Myricetin 3-O-glucoside (MSide); Quercetin-3-*O*-glucuronide (QRide); Quercetin-3-*O*-glucoside (QSide); Kaempferol-3-*O*-glucuronide (KRide); Kaempferol-3-*O*-glucoside (KSide) at harvest (S5); Table 6: Total and individual flavonols concentration (mg/kg) and Glucoside/Glucuronide ratio at harvest. Myricetin 3-O-glucoside (MSide); Quercetin-3-*O*-glucuronide (QRide); Quercetin-3-*O*-glucoside (QSide); Kaempferol-3-*O*-glucuronide (KRide); Kaempferol-3-*O*-glucoside (KSide); Total Glucoside/Total Glucuronide ratio (Side/Ride) at harvest (S5); Table 7:

Concentration of total and individual Hydroxycinnamic acid (HCTA), *trans* Caftaric acid (*trans* Caf*)*, *cis p*Coutaric acid (*cis p*Cou), *trans p*Coutaric acid (*trans p*Cou), Total *p*Coutaric forms (*cis + trans p*Cou), ratio *trans/cis* forms of *p*Coutaric acid (*trans/cis p*Cout) and ratio *p*Coutaric acid/Caftaric acid (*p*Cout/Caf) at harvest (S5); Table 8: Soil characteristics from a soil sample either proximate to the vineyard of research (L) or in the vineyard of research (M and H).

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## 3.6 Supplementary Data

**Supplementary Table 1:** Berry characteristics at harvest: Berry Fresh Weight (BFW), Skin Fresh Weight (SFW), Skin to Berry Ratio, Total Soluble Solids (TSS) and Sugar per Berry at harvest (S5). The significance between treatments, vineyards and years and of the interactions between factors is also reported.

| Treatment | BFW (g) | SFW (g) | Skin to Berry Ratio | TSS (Brix) | Sugar per Berry (g/berry) |
|---|---|---|---|---|---|
| C1[§] | 1.499 a[1] | 0.118 a | 0.079 b | 24.9 ab | 0.37 a |
| C0 | 1.403 b | 0.111 a | 0.080 b | 25.2 a | 0.35 ab |
| T1 | 1.402 b | 0.119 a | 0.084 ab | 24.7 b | 0.34 b |
| T0 | 1.283 c | 0.111 a | 0.087 a | 24.6 b | 0.31 c |
| Vineyard | | | | | |
| L[†] | 1.694 a | 0.134 a | 0.079 b | 24.3 b | 0.41 a |
| M | 1.144 c | 0.102 b | 0.089 a | 25.6 a | 0.29 c |
| H | 1.353 b | 0.108 b | 0.081 b | 24.6 b | 0.33 b |
| Significance | | | | | |
| Year | NS[2] | *** | *** | *** | NS |
| Vineyard | *** | *** | *** | *** | *** |
| Treatment | *** | * | * | ** | *** |
| Year*Vineyard | *** | *** | *** | * | *** |
| Year*Treatment | NS | NS | NS | ** | ** |
| Vineyard*Treatment | NS | NS | NS | NS | NS |
| Year*Vineyard*Treatment | *** | NS | NS | * | *** |

[§] The values are the average between years and vineyards or [†] between treatments and years.

[1] For each treatment and vineyard, different letters indicate significant differences with $p \leq 0.05$. [2] *** ($p < 0.0001$); ** ($p < 0.001$); * ($p < 0.05$); NS = Not Significant. Results were determined from general linear model (GLM) with post-hoc analysis based on estimated marginal means (EMMs), with false discovery rate correction.

**Supplementary Table 2:** Anthocyanin concentration at harvest for the glucosylated forms: Delphinidin (Df), Cyanidin (Cy), Petunidin (Pt), Peonidin (Pn) and Malvidin (Mv) at harvest (S5). The significance between treatments, vineyards and years and of the interactions between factors is also reported.

| Treatment | Df (mg/kg) | Cy (mg/kg) | Pt (mg/kg) | Pn (mg/kg) | Mv (mg/kg) |
|---|---|---|---|---|---|
| C1§ | 20.7 a[1] | 99.0 a | 28.4 a | 251.5 a | 112.9 a |
| C0 | 19.0 a | 71.0 b | 28.3 ab | 212.5 b | 118.9 a |
| T1 | 17.8 a | 74.1 b | 24.8 b | 185.1 c | 97.0 b |
| T0 | 18.5 a | 55.0 c | 25.1 ab | 166.7 c | 106.0 ab |
| Vineyard | | | | | |
| L† | 21.2 a | 66.0 b | 24.1 b | 188.2 b | 92.2 b |
| M | 24.0 a | 91.3 a | 30.1 a | 230.7 a | 114.2 a |
| H | 11.8 b | 67.0 b | 25.8 b | 192.9 b | 119.7 a |
| Significance | | | | | |
| Year | NS[2] | *** | NS | NS | *** |
| Vineyard | *** | *** | *** | *** | *** |
| Treatment | NS | *** | ** | *** | *** |
| Year*Vineyard | * | *** | ** | *** | NS |
| Year*Treatment | NS | NS | * | NS | NS |
| Vineyard*Treatment | NS | NS | NS | NS | NS |
| Year*Vineyard*Treatment | NS | ** | NS | * | NS |

§The values are the average between years and vineyards or †between treatments and years.

[1] For each treatment and vineyard, different letters indicate significant differences with $p \leq 0.05$; [2] *** ($p < 0.0001$); ** ($p < 0.001$); * ($p < 0.05$); NS = Not Significant. Results were determined from general linear model (GLM) with post-hoc analysis based on estimated marginal means (EMMs), with false discovery rate correction.

**Supplementary Table 3:** Anthocyanin proportion at harvest for glucosylated forms: Delphinidin (Df), Cyanidin (Cy), Petunidin (Pt), Peonidin (Pn) and Malvidin (Mv) at harvest (S5). The significance between treatments, vineyards and years and of the interactions between factors is also reported.

| Treatment | Df (%) | Cy (%) | Pt (%) | Pn (%) | Mv (%) |
|---|---|---|---|---|---|
| C1[§] | 3.3 a[1] | 15.7 a | 4.6 b | 40.4 a | 18.5 b |
| C0 | 3.3 a | 12.4 c | 5.0 a | 37.7 b | 21.6 a |
| T1 | 3.4 a | 14.3 b | 4.9 ab | 36.0 c | 19.3 b |
| T0 | 3.7 a | 11.3 c | 5.2 a | 34.7 c | 22.3 a |
| Vineyard | | | | | |
| L[†] | 4.2 a | 13.2 b | 4.9 a | 37.4 a | 18.7 b |
| M | 3.9 a | 14.4 a | 4.9 a | 36.9 a | 18.8 b |
| H | 2.3 b | 12.8 b | 5.1 a | 37.2 a | 23.7 a |
| Significance | | | | | |
| Year | **[2] | *** | *** | NS | *** |
| Vineyard | *** | ** | NS | NS | *** |
| Treatment | NS | *** | ** | *** | *** |
| Year*Vineyard | NS | *** | *** | *** | *** |
| Year*Treatment | NS | NS | * | NS | NS |
| Vineyard*Treatment | NS | * | NS | NS | NS |
| Year*Vineyard*Treatment | NS | NS | * | NS | NS |

[§] The values are the average between years and vineyards or [†] between treatments and years.

[1] For each treatment and vineyard, different letters indicate significant differences with $p \leq 0.05$; [2] *** ($p < 0.0001$); ** ($p < 0.001$); * ($p < 0.05$); NS = Not Significant. Results were determined from general linear model (GLM) with post-hoc analysis based on estimated marginal means (EMMs), with false discovery rate correction.

**Supplementary Table 4:** Total Anthocyanin Concentration (TAC); Di-hydroxylated/Tri-hydroxylated ratio (Di/Tri); Acylated/Total Anthocyanins ratio (Acyl/TAC); Rate of methoxylation in di-hydroxylated anthocyanins {Di-Meth = Pn/Cy}); Rate of methoxylation in tri-hydroxylated anthocyanins, [Tri-Meth = Mv/(Df+Pt)]; Ratio between TAC and Total Soluble Solids (TAC/TSS) at harvest (S5). The significance between treatments, vineyards and years and of the interactions between factors is also reported.

| Treatment | TAC (mg/kg) | Di/Tri | Acyl/TAC | Di-Meth (mg/kg | Tri-Meth (mg/kg) | TAC/TSS |
|---|---|---|---|---|---|---|
| C1[§] | 621.3 a[1] | 2.22 a | 0.18 c | 2.65 b | 0.45 a | 24.9 a |
| C0 | 562.7 b | 1.74 bc | 0.20 b | 3.14 a | 0.41 a | 22.3 b |
| T1 | 511.7 bc | 1.86 b | 0.22 a | 2.57 b | 0.44 a | 20.7 bc |
| T0 | 480.6 c | 1.51 c | 0.23 a | 3.19 a | 0.42 a | 19.5 c |
| Vineyard | | | | | | |
| L[†] | 498.1 b | 1.85 a | 0.22 a | 2.90 a | 0.49 a | 20.5 b |
| M | 620.6 a | 1.99 a | 0.21 a | 2.67 b | 0.48 a | 24.2 a |
| H | 513.5 b | 1.66 b | 0.19 b | 3.10 a | 0.32 b | 20.8 b |
| Significance | | | | | | |
| Year | NS[2] | *** | *** | *** | ** | NS |
| Vineyard | *** | *** | *** | *** | *** | *** |
| Treatment | *** | *** | *** | *** | * | *** |
| Year*Vineyard | *** | *** | NS | ** | *** | *** |
| Year*Treatment | NS | NS | NS | NS | NS | NS |
| Vineyard*Treatment | NS | NS | NS | * | NS | NS |
| Year*Vineyard*Treatment | NS | ** | NS | NS | NS | NS |

[§]The values are the average between years and vineyards or [†]between treatments and years.

[1]For each treatment and vineyard, different letters indicate significant differences with $p \leq 0.05$; [2]*** ($p < 0.0001$); ** ($p < 0.001$); * ($p < 0.05$); NS = Not Significant. Results were determined from general linear model (GLM) with post-hoc analysis based on estimated marginal means (EMMs), with false discovery rate correction.

**Supplementary Table 5:** Individual flavonol proportion (% of total) at harvest. Myricetin 3-O-glucoside (MSide); Quercetin-3-*O*-glucuronide (QRide); Quercetin-3-*O*-glucoside (QSide); Kaempferol-3-*O*-glucuronide (KRide); Kaempferol-3-*O*-glucoside (KSide) at harvest (S5). The significance between treatments, vineyards and years and of the interactions between factors is also reported.

| Treatment | MSide (%) | QRide (%) | QSide (%) | KRide (%) | KSide (%) |
|---|---|---|---|---|---|
| C1[§] | 3.7 c[1] | 15.3 c | 65.1 a | 2.7 a | 12.3 a |
| C0 | 7.0 a | 32.7 a | 54.2 c | 0.8 c | 4.2 c |
| T1 | 4.8 b | 18.8 b | 63.4 b | 2.1 b | 10.3 b |
| T0 | 7.5 a | 32.5 a | 53.7 c | 0.9 c | 4.1 c |
| Vineyard | | | | | |
| L[†] | 6.0 a | 23.6 b | 59.5 a | 2.1 a | 7.9 a |
| M | 5.5 a | 24.2 b | 60.4 a | 1.3 b | 7.7 a |
| H | 5.9 a | 26.6 a | 57.3 b | 1.5 b | 7.6 a |
| Significance | | | | | |
| Year | NS[2] | *** | * | *** | *** |
| Vineyard | NS | *** | *** | *** | NS |
| Treatment | *** | *** | *** | *** | *** |
| Year*Vineyard | *** | *** | *** | *** | ** |
| Year*Treatment | NS | NS | * | NS | * |
| Vineyard*Treatment | ** | NS | * | *** | NS |
| Year*Vineyard*Treatment | NS | ** | NS | *** | * |

[§] The values are the average between years and vineyards or [†] between treatments and years.

[1] For each treatments and vineyards, different letters indicate significant differences with $p \leq 0.05$; [2] *** ($p < 0.0001$); ** ($p < 0.001$); * ($p < 0.05$); NS = Not Significant. Results were determined from general linear model (GLM) with post-hoc analysis based on estimated marginal means (EMMs), with false discovery rate correction.

**Supplementary Table 6:** Total and individual flavonols concentration (mg/kg) and Glucoside/Glucuronide ratio at harvest. Myricetin 3-O-glucoside (MSide); Quercetin-3-*O*-glucuronide (QRide); Quercetin-3-*O*-glucoside (QSide); Kaempferol-3-*O*-glucuronide (KRide); Kaempferol-3-*O*-glucoside (KSide); Total Glucoside/Total Glucuronide ratio (Side/Ride) at harvest (S5). The significance between treatments, vineyards and years and of the interactions between factors is also reported.

| Treatment | Total Flavonols (mg/kg) | MSide (mg/kg) | QRide (mg/kg) | QSide (mg/kg) | KRide (mg/kg) | KSide (mg/kg) | Side/Ride |
|---|---|---|---|---|---|---|---|
| C1[§] | 150.1 a[1] | 5.5 a | 23.0 a | 97.6 a | 4.1 a | 18.5 a | 4.6 a |
| C0 | 58.9 c | 4.0 b | 19.0 b | 32.2 c | 0.5 c | 2.6 c | 2.0 c |
| T1 | 122.4 b | 5.8 a | 22.9 a | 77.7 b | 2.6 b | 12.7 b | 3.8 b |
| T0 | 56.3 c | 4.2 b | 18.0 b | 30.4 c | 0.5 c | 2.4 c | 2.0 c |
| Vineyard | | | | | | | |
| L[†] | 92.2 a | 4.7 a | 18.6 b | 57.2 a | 2.1 a | 8.8 a | 3.3 a |
| M | 102.5 a | 5.1 a | 22.1 a | 63.6 a | 1.7 a | 9.2 a | 3.1 a |
| H | 96.1 a | 4.9 a | 21.6 a | 57.6 a | 1.9 a | 9.1 a | 2.9 b |
| Significance | | | | | | | |
| Year | NS[2] | NS | NS | NS | ** | *** | *** |
| Vineyard | NS | * | *** | NS | NS | NS | *** |
| Treatment | *** | *** | *** | *** | *** | *** | *** |
| Year*Vineyard | NS | ** | NS | ** | ** | NS | *** |
| Year*Treatment | * | ** | ** | * | NS | * | * |
| Vineyard*Treatment | NS | ** | * | NS | * | NS | * |
| Year*Vineyard*Treatment | NS | * | NS | NS | NS | NS | NS |

[§]The values are the average between years and vineyards or [†]between treatments and years.

[1] For each treatment and vineyard, different letters indicate significant differences with $p \leq 0.05$; [2] *** ($p < 0.0001$); ** ($p < 0.001$); * ($p < 0.05$); NS = Not Significant. Results were determined from general linear model (GLM) with post-hoc analysis based on estimated marginal means (EMMs), with false discovery rate correction.

**Supplementary Table 7:** Concentration of total and individual Hydroxycinnamic acid (HCTA), *trans* Caftaric acid (*trans* Caf*)*, *cis p*Coutaric acid (*cis p*Cou), *trans p*Coutaric acid (*trans p*Cou), Total *p*Coutaric forms (*cis + trans p*Cou), ratio *trans/cis* forms of *p*Coutaric acid (*trans/cis p*Cout) and ratio *p*Coutaric acid/Caftaric acid (*p*Cout/Caf) at harvest (S5). The significance between treatments, vineyards and years and of the interactions between factors is also reported.

| Treatment | HCTA (mg/kg) | *Trans* Caf (mg/kg) | *cis p*Cou (mg/kg) | *trans p*Cou (mg/kg) | *cis + trans p*Cou (mg/kg) | *Trans/Cis p*Cout | *p*Cout/Caf |
|---|---|---|---|---|---|---|---|
| C1[§] | 456.3 bc[1] | 120.3 a | 55.2 a | 256.3 b | 311.6 bc | 4.6 c | 2.7 b |
| C0 | 508.5 a | 126.7 a | 48.2 b | 309.5 a | 357.7 a | 6.4 a | 2.9 a |
| T1 | 425.7 c | 113.2 a | 54.3 a | 236.9 b | 291.2 c | 4.4 c | 2.7 b |
| T0 | 485.3 ab | 124.7 a | 49.1 b | 290.0 a | 339.0 ab | 5.9 b | 2.8 ab |
| Vineyard | | | | | | | |
| L[†] | 419.0 b | 97.8 c | 47.8 b | 250.8 b | 298.6 b | 5.3 b | 3.1 a |
| M | 488.5 a | 140.3 a | 51.0 b | 282.8 a | 333.8 a | 5.6 a | 2.5 c |
| H | 499.3 a | 125.5 b | 56.3 a | 286.0 a | 342.3 a | 5.1 b | 2.7 b |
| Significance | | | | | | | |
| Year | **[2] | *** | * | NS | NS | * | *** |
| Vineyard | *** | *** | *** | *** | *** | *** | *** |
| Treatment | *** | NS | *** | *** | *** | *** | *** |
| Year*Vineyard | *** | *** | *** | *** | *** | ** | *** |
| Year*Treatment | NS | NS | NS | NS | NS | NS | NS |
| Vineyard*Treatment | NS | NS | NS | NS | NS | ** | NS |
| Year*Vineyard*Treatment | NS | NS | NS | NS | NS | NS | * |

[§] The values are the average between years and vineyards or [†] between treatments and years.

[1] For each treatment and vineyard, different letters indicate significant differences with $p \leq 0.05$; [2] *** ($p < 0.0001$); ** ($p < 0.001$); * ($p < 0.05$); NS = Not Significant. Results were determined from general linear model (GLM) with post-hoc analysis based on estimated marginal means (EMMs), with false discovery rate correction.

Supplementary Table 8: Soil characteristics from a soil sample either proximate to the vineyard of research (L) or in the vineyard of research (M and H).

| | H | M | L |
|---|---|---|---|
| Sand % | 9.2 | 8.6 | 48.0 |
| Silt % | 57.9 | 62.3 | 30.0 |
| Clay % | 32.9 | 29.1 | 22.0 |
| pH | 8.2 | 8.3 | 8.2 |
| Total Carbonate | 22.6 | 23.3 | 23.0 |
| Total Nitrogen | 0.098 | 0.075 | 0.049 |
| CEC | 15.7 | 12.0 | nd |

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

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## Abstract

Changes in climate have been influencing the quality of wine grapes worldwide. The impact of extreme climate events over short periods is increasingly recognized as a serious risk to grape quality and yield quantity. In this study the mitigation effects of a pulsed water spray on vine canopy during heatwave (HW) events has been evaluated for maintaining vine condition during the growing season and grape quality. Vines of three cultivars (Malbec (ML), Bonarda (BO), and Syrah (SY)) in the UNCuyo experimental vineyard were treated with an overhead pulsed water spray (Trt). Heatwaves were defined as days with a minimum temperature of no less than 21 °C and a maximum temperature of no less than 35 °C. Two heat waves were identified during the growing season. Samples were collected at weekly intervals from veraison to harvest. During 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 collected at several intervals during the day to evaluate physiological responses. Berries were collected at each sample date as well as at harvest. Berry weights, soluble solid content, and pH were measured. LWP, SWP, $F_v/F_m$, PI, and gs were significantly higher while LT was lower in treated vines as compared to the control 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 control (Ctl), displaying reduced physiological stress in the treated vines. At harvest, anthocyanin profile, total polyphenol index (TPI), fruit yield, number of bunches and their average weight, berry weight, soluble solid content, and pH were also evaluated. Bunch weight was significantly higher in Trt vines for all cultivars. No differences were identified in total anthocyanin concentration. These findings imply that vines subjected to targeted overhead water treatment during heatwaves experienced diminished physiological stress and yielded higher grape production, without increasing the risk for potential fungus diseases in the context of Mendoza climate. Consequently, this practice could serve as a valuable mitigation strategy for alleviating adverse effects of heatwaves.

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

## 4.1. **Introduction**

Although climate change is often associated with an expected gradual increase in global average temperatures of between 1.5 and 4 °C (Solomon *et al.*, 2007), it is becoming increasingly clear that the issues of greater concern with relation to climate change are 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. It is being observed that heatwaves are increasing not only in the number of observed events but also in the duration and severity of these events (Perkins-Kirkpatrick and Lewis, 2020). Although the grapevine has a good ability to adapt to various environmental pressures, long-lasting extremely high temperatures or heatwaves may 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 by disrupting the photosynthetic apparatus, affecting electron transport rates and provoking stomatal closure to conserve water, consequently lowering the leaf water potential (Carvalho *et al.*, 2015). If stomatal closure occurs, the transpiration rate will also decrease (Carvalho *et al.*, 2015; Greer and Weedon, 2014). Extended periods of exposure to extreme heat conditions can lead to a reduction in these processes and thus a reduction in vine health and berry quality (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 inhibition of photosystem II (PSII) activity (the main driver of photosynthesis). The duration and severity of heatwaves are both important considerations for risk to plant health and production but the rate of increase in temperature can also pose a threat due to a reduced potential for the plant to acclimate (Carvalho *et al.*, 2015; Webb *et al.*, 2010). Further, a vine's response to heat stress can vary based on the specific grape cultivar and the phenological stage (Zha *et al.*, 2018).

Indicators of optimal vine health can 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 gain a picture of 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) estimates 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, the 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 observed by Millan *et al.* (2023). Vines under severe water deficits (below -16 bar SWP) can experience increased tension in the water column which leads to cavitation of the xylem, leaf shedding and possible 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). It can result in many measurements including the $F_v/F_m$ ratio and the Performance Index (PI), which quantifies the functionality of the electron flow through photosystem II (Ceusters, 2019). Chlorophyll *a* fluorescence is a recognized abiotic stress indicator in grapevine and is known to be influenced negatively by drought conditions, extreme light exposure and extreme heat (Ju *et al.*, 2018, 2021; Su *et al.*, 2015). Relative chlorophyll content measures 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 the chloroplast structure making them more globular in shape while also inducing a structural disorganization of thylakoid membranes within chloroplasts. This is identified as the main area of potential injury in 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 between 4 to 10 hours of exposure to 45 °C. However, Bensalem-Fnayou *et al.* (2011) observed changes to grapevine chloroplasts only 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, affecting their composition and ultimately wine quality (Rogiers *et al.,* 2022). In previous studies and in commercial practice, heatwaves have been known to impact yields negatively, as well as berry quality. Further, increasing temperatures have been associated with increasing total soluble solid (TSS) and a decrease in acid content, particularly malic acid 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 berry growth and increase berry dehydration, thus reducing yields as well as to delay ripening (Bindi *et al.*, 1996; Rienth *et al.*, 2021).

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

Flavonols, from a wine quality perspective, are known to stabilize anthocyanins through co-pigmentation (Asen *et al.*, 1972) and may impart bitter flavour qualities (Ferrer-Gallego *et al.,* 2016) to wine both of which can enhance wine quality. The effect of extreme temperature on flavonols is less clear. However, Gouot *et al.,* (2019b) established that at extreme temperatures (>50 °C), flavonol concentrations were significantly negatively impacted in Shiraz berries. In this same study it was established that temperatures reaching 46 °C did not impact anthocyanins content, nevertheless their profile was modified.

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 increased irrigation prior to and during heatwaves to offset evapotranspiration (ET) losses and maintain plant physiological function (Naulleau *et al.*, 2021; Previtali *et al.*, 2023; Savi *et al.*, 2018; Webb *et al.*, 2010). Irrigation strategies 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 vine $^{-1}$ day$^{-2}$ (Hellman, 2019). In the case of heatwave mitigation, often, ET is maintained above 1.00. In some regions water is a scarce resource. This can lead to limited capacity to protect vines with an irrigation strategy (Webb *et al.,* 2010).

Aerial sprinkler systems have also been used historically to mitigate both increasing average temperatures as well as extreme short-term heatwaves (Gilbert *et al.,* 1971). Kliewer and Schultz, (1973) investigated the effect sprinklers had on leaf and berry temperature with treatments applied at a threshold temperature of 30 °C, finding a notable reduction in temperature of 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 of treated vines. In both of these pieces it appears that the canopy irrigation was untargeted. Caravia *et al.* (2017) focused on heatwave mitigation with treatment application occurring at a threshold temperature of 38 °C.

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

## 4.2. Materials and methods

### *4.2.1. Experimental design*

#### 4.2.1.1. Experimental site

The trial 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: Malbec (ML) (clone N° 2), Syrah (SY) (clone N° 84), and Bonarda (BO) (clone N° 9) were used for the experiment. Vines were spaced at 1m intervals within rows and 2.2 m between rows, all oriented north-south and were managed with spur pruning, employing a fruit load adjustment tailored to the individual vigour of each plant, utilizing a bilateral cordon system with shoots vertically positioned.

Previous to budbreak, flowering, fruit set and post-veraison stages, all vines were drip irrigated for 48 h. Drip distance was 1 m and water application rate was 2 L $h^{-1}$. Air temperature was measured by two temperature sensors (iButton 1 Wire® Thermochron® Maxim Integrated USA) placed sheltered inside plastic boxes avoiding direct sun exposure and installed at either 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 recognized as a sensitive period for flavonoid development (Gouot *et al.*, 2019).

#### 4.2.1.2. Experimental design and sampling

The experimental design consisted of a randomized plot of 5 rows (3 rows for treated and 2 rows for control) with 6 or 9 plants $row^{-1}$, respectively. The experimental unit consisted of two plants of the same cultivar $row^{-1}$. Three replicates of each treatment were used (Supplementary Figure 1).

In treated rows (Trt) (n = 3), two jet nozzles were installed at the end of each row. The jets were aligned longitudinally with the canopy (Supplementary Figure 2). Each jet emitted a pulsed spray of water to the top of the canopy for 15 min (1 s on, 2 s off) during 12 h from 8 am to 8 pm. The jets were activated exclusively during heatwaves (HW). Each jet emitted an average of 200 ml $h^{-1}$. This resulted in a daily total of 2.4 L $HWday^{-1}$ $jet^{-2}$ or 0.53 L $plant^{-1}$ $HWday^{-2}$. The treatment was applied only to the canopy with minimal water contacting the soil. Leaf wetness sensors (PHYTOS 31, Meter Group) were installed in two treatment rows to monitor leaf surface wetness by measuring the dielectric constant of the sensor's upper surface and thus determining the evaporative time between treatment application. Control rows (Ctl) (n = 3) lacked the pulsed spray application.

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

#### 4.2.1.3. Physiological and berry sampling protocol

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

From S1 to S5 leaf water potential (LWP) was measured at 5 am (pre-dawn), 8 am, 11 am, 2 pm and 5 pm. Completely healthy, dry and mature leaves were randomly selected from the middle of the canopy at the first training wire outside of the direct exposure of the water droplets which were distributed in the top third of the canopy area. Each leaf was placed in a plastic bag and sealed prior to removal by cutting of the petiole with a razor blade. Leaves were immediately placed in a pressure chamber operated in the field (Model 4, Biocontrol).

Relative chlorophyll content (CC) of leaves was measured with the SPAD-502Plus, (Konica Minolta, Osaka, Japan) in duplicate on each replicate at 8 am, 11 am, 2 pm and 5 pm. Leaf (LT) and Berry temperature (BT) were measured on fully exposed leaves and clusters on each vine. These were measured in duplicate per replicate at the same four times per day as CC by 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 am to 5 pm 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,

$\Psi o$ = efficiency that a trapped exciton can move an electron to the downstream of $QA^-$ on the electron transport chain.

These two variables were measured at 11 am and 2 pm from the same leaves at each sample point using a fluorometer (Pocket PEA; Hansatech Instruments, England) to quantify plant stress.

All non-destructive leaf related measurements (CC, $F_v/F_m$, PI, LT, and gs) were measured on the same leaves (pre-marked with ribbon) at each sample point during the experiment. Stem Water Potential (SWP) was measured from 2 to 3 pm after covering intact leaves with aluminium foil for 30 min prior to leaf removal and measurement.

At each sample point two sets of twelve berries were collected at 5 pm (approximately the hottest point of the day). Berries were cut above the pedicel, stored in an insulated environment cooled with ice (with no direct berry contact) and transported directly to 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 later phenolic analysis.

Final harvest dates (S6 and S7) were determined when berries reached a median TSS of 24 °Brix. Malbec and Syrah reached maturity five days earlier than Bonarda and were harvested on February 23 (S6) while Bonarda reached 24 ºBrix on February 28 (S7). Each replicate was harvested separately. Average yield plant$^{-1}$ (kg), number of bunches plant$^{-1}$ and weight bunch$^{-1}$ (g) were measured.

### *4.2.2. Phenolic Analysis*

#### 4.2.2.1. Grape berry phenolic extraction

Frozen berries were immersed briefly in water and skins were removed immediately. Skins were dried and ground until powder using liquid nitrogen. Phenolic compounds were then extracted using a modified method according to Revilla *et al.* (1998) with minor modifications (de Rosas *et al.,* 2022). To summarize, 150 mg of skin were macerated with 1850 µl of MeOH:HCl (99:1, *v/v)* (HCl 10 N) (Sintorgan, Buenos Aires, Argentina) in darkness at 20 ºC for 24 h. This was followed by centrifugation for 20 min at 14,000 rpm at 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).

#### 4.2.2.2. Anthocyanin HPLC-DAD Analysis

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 by constructing a 5-point standard curve using malvidin hydrochloride (Sigma) as the standard. The concentration in mg $g^{-1}$ berry of 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) was determined. Anthocyanins were quantified at 520 nm using a calibration curve from a commercial standard of malvidin-3- glucoside chloride (Sigma, St. Louis, MO, USA). Anthocyanin concentration was expressed as mg $g^{-1}$ of berry skin fresh weight (mg $g^{-1}$ FW). From these concentrations, total anthocyanin content (TAC) was calculated as the sum of the 9 anthocyanins. Compositional ratios were calculated based on content compared to TAC. Variation in tri vs di-substitution, acylation, acetylated and coumaroylated ratios were calculated as follows:

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

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

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

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

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

### 4.2.2.3. Total Polyphenol Index

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

### *4.2.3. Statistical Analysis*

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. Data are presented as means of three replicates (n = 3) with 2 vines as experimental unit per replica. Mean comparisons were performed by Tukey test, considering $p \leq 0{,}05$ (*) significant, $p \leq 0.01$ (**) highly significant, $p \leq 0.001$ (***) very highly significant, and 'NS' not significant. T-tests were performed on means of maximum daily in-row temperatures between Ctl and Trt. Resulting *p* values were transformed (-10log) for graphical display.

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.

## 4.3. Results and Discussion

### *4.3.1. Heatwaves*

During the growing season two heatwaves occurred (Figure 1). The first heatwave (HW1) was shorter, lasting only two days. It occurred from January 23 to 24, 2023 with an average maximum daytime temperature measured in the canopy of control vines of 37.2 ºC. The second heatwave (HW2) commenced on February 5 and was completed on February 12, 2023 with an average maximum daytime temperature of 38.9 ºC and with two days over 40.0 ºC in the Ctl vines. During the heatwaves the water spray treatment was applied for a consecutive 2 and 8 days, respectively. Figure 1 illustrates that the onset of HW1 exhibited a more gradual incline, with a daily temperature increase of less than 1 ºC in the three days prior to the onset of the heatwave. In contrast, the three days preceding HW2 had an average daily increase of 2 ºC day$^{-1}$ (Figure 1).

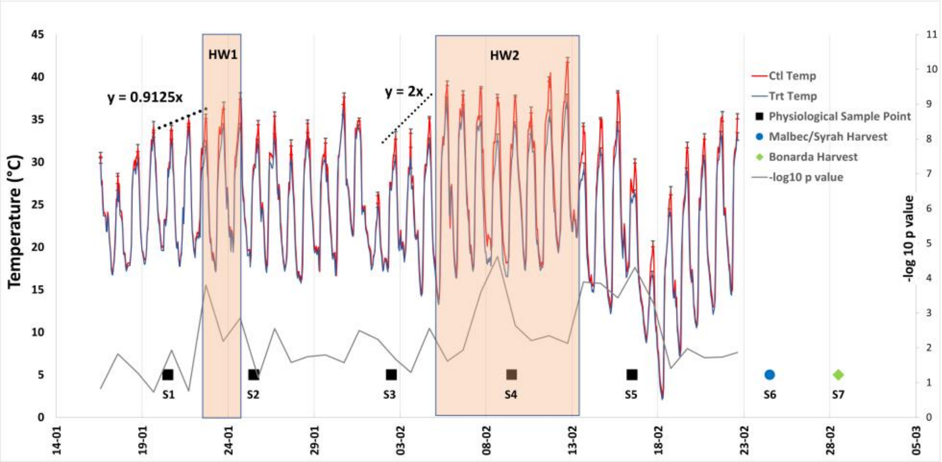

Figure 1. Average in-canopy temperature for Treated (Trt) and Control (Ctl) with standard error. HW1 and HW2 events (orange), sample dates (S1 to S7). -log10 transformation of *p* values from t-test for each daily maximum Ctl and Trt temperature (>1.3 is ≤ 0.05 *p*-value). Average temperature increase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

The average daytime canopy temperature of Trt vines was lower than Ctl vines during treatment (Figure 1). During HW1, Ctl vines averaged a daytime maximum temperature 2.8 ºC higher than Trt vines and during HW2 this average difference was 3.8 ºC higher. This trend continued after treatment application ceased (after heatwaves), particularly after HW2 with an average difference of 3.1 ºC and an increased significance of the difference as noted by the increased -log10 value. Minimum (night) temperatures were similar between Trt and Ctl throughout the experiment except for two nights during the second heatwave (February 8 and 9).

Humidity in this growing region is quite low due to the rain shadow effect caused by proximity to the Andes mountain range. Humidity values rarely rise above 50 % after 8 am and consistently dropped below 20 % in the afternoon during the month of February in Mendoza area. The leaf wetness sensor (Phytos 31/LWS) showed that water from the treatment application was completely evaporated within 30 min of the completion of each treatment cycle thus leaving vines dry for 30 min prior to the next treatment application cycle, creating non-predisposing conditions for pathogenic fungi at the canopy level

(Supplementary Figure 3). For this reason, disease pressure was not a concern in this study. This mitigative strategy is best applied in regions with lower relative humidity to reduce potential for increased pathogenic fungal infection. The quality of accessible water for this treatment must also be considered since according to Kliewer and Schultz (1973) water high in total salts can lead to salt deposits on leaves and berries as well as provoke necrosis of leaf tissue, moreover it could clog the pipes and sprinklers.

### *4.3.2. Physiological Response*

#### 4.3.2.1. Leaf and Stem Water Potential

Pre-dawn water potential, as well as 2 pm and 5 pm LWP showed a significant difference between Trt and Ctl after the first heatwave for all cultivars (S2) (Figure 2a, d and e). These differences between Trt and Ctl vines were no longer observed for all time points except 5 pm (Figure 2e) on S3 which was 8 days after the end of HW1. S4 samples were taken in the middle of HW2 and all cultivars showed significantly higher LWP in Trt vines at all sample points during the day. These significant differences were retained at S5 (three days after the end of HW2) with a greater separation between Trt and Ctl vines as compared to S4 suggesting that the longer, more severe heatwave led to extended periods of water stress with values of -15 bar at 11 am, 2 pm and 5 pm for the Ctl vines on S5. The higher water potential exhibited by plants of the 3 cultivars irrigated with overhead sprinklers may have been due to an increase in the relative humidity of the surrounding canopy environment, reducing the vapor pressure deficit and thus decreasing water loss through transpiration. Water uptake through the leaves was ruled out because the water droplets remained on the leaf surface for only 30 min before evaporating while aerial irrigation was in operation (Supplementary Figure 3).

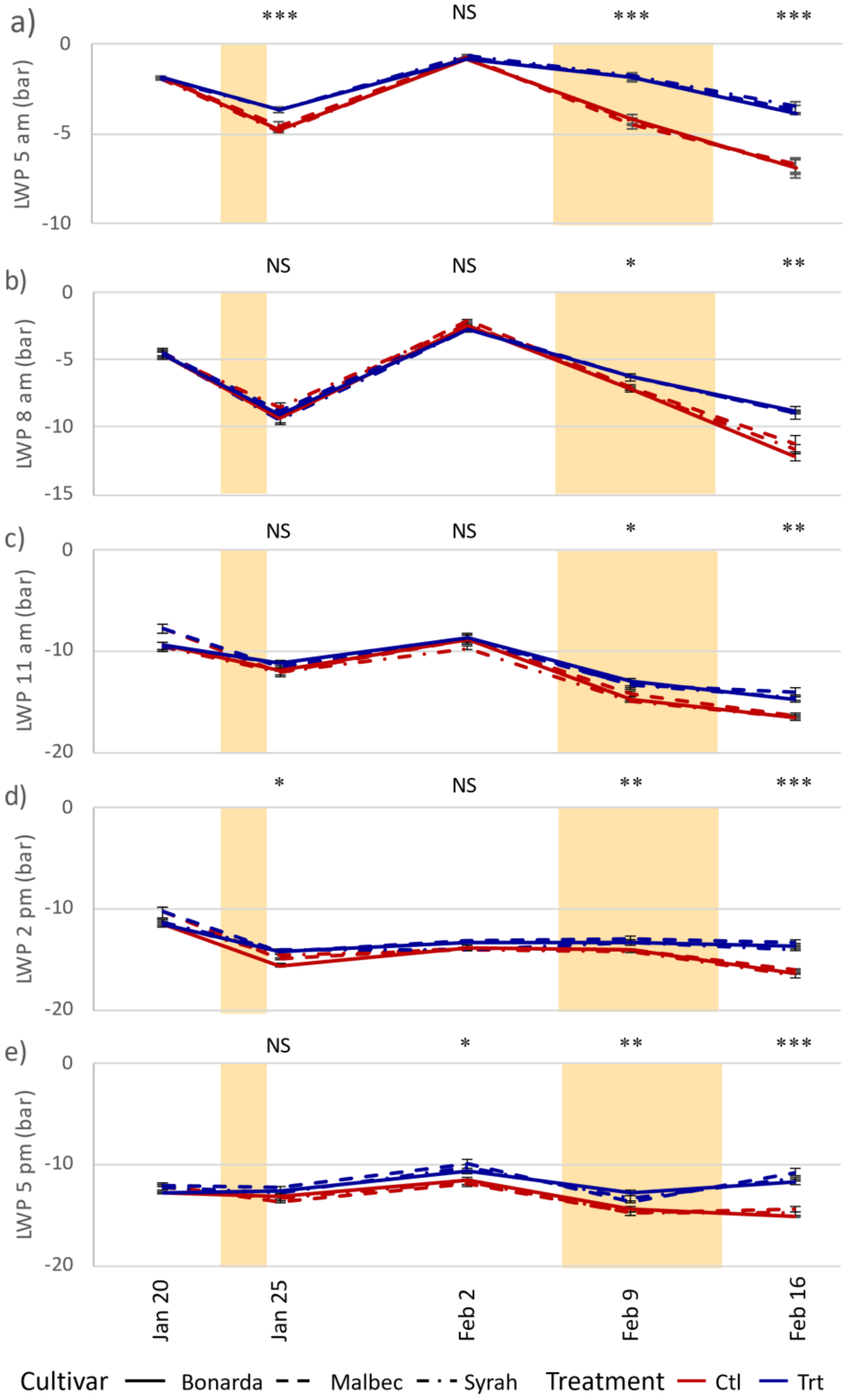

Figure 2. Leaf water potential variation for cultivars Bonarda, Malbec and Syrah

collected during maturity stage (S1-S5) at different day hours a) pre-drawn (5 am), b) early-morning (8 am), c) near midday (11 am), d) early afternoon (2 pm), e) afternoon (5 pm). Data points are means ±SE (n = 3). Statistical significance determined by two-way ANOVA with Tukey Test post-hoc. $p \leq 0.05$ '*', $p < 0.001$ '**', $p < 0.0001$ '***', NS: not significant.

Stem water potential showed no significant difference between cultivars at all sample points. After HW1 (S2) only Malbec Trt vines were significantly lower than their Ctl. At S3 there were no observable differences between Trt and Ctl vines for all cultivars (Supplementary Table 1). During HW2 (S4) all three cultivars showed an upward trend for Trt vines with Bonarda and Syrah having a significant response comparing Trt to Ctl (Figure 3a). Although Malbec did not have a significant difference at S4, the response showed a similar trend of higher values for Trt than in Ctl. After HW2 (S5) all three cultivars showed clear significantly higher SWP in Trt vines as compared to Ctl vines and all cultivars showed much lower variance in their response when compared to measurements during S4 (Figure 3b). These findings were consistent with those from other research focussed on heatwave mitigation with irrigation or sprinkler application (Cogato *et al.,* 2021; Martínez-Lüscher *et al.,* 2017). At S5 all Ctl vines had a SWP below -16 bar which suggests water deficit extreme enough to trigger xylem cavitation (Gambetta *et al.,* 2020). Water potentials below -16 bar were also observed for LWP at 11 am and 2 pm on S5 for Ctl vines while Trt vines maintained an LWP and SWP above -15 bar during and after the heatwaves.

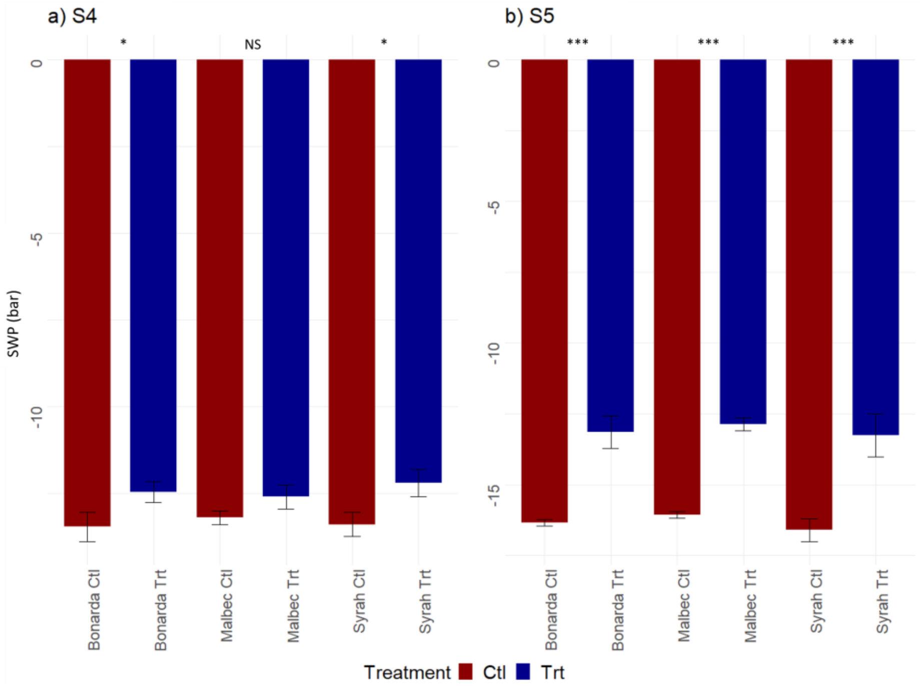

Figure 3. Stem Water Potential (SWP) (bar) at S4 (during HW2) (a) and S5 (after HW2) (b) for the cultivars Bonarda, Malbec and Syrah treated (Trt) or not (Ctl). Statistical significance determined by two-way ANOVA with Tukey Test post-hoc. '*' $p < 0.05$, '***' $p < 0.0001$; NS: Not significant.

#### 4.3.2.2. Stomatal Conductance

Results from S4 (during HW2) showed no significant differences at 8 am but an increasing significant separation between Trt and Ctl vines during the day with Trt having higher values than Ctl vines (Figure 4a). This indicates that Trt vines were better able to maintain transpiration rates with increasing heat during the heatwave than were vines with no treatment. Similar findings were made by (Cogato *et al.*, 2021) with midday gs remaining higher in vines treated with a sprinkler treatment than control vines both during and after the heatwave. As observed by Sadras *et al.* (2012), increased leaf temperature at a constant relative humidity increases VPD exponentially thus potentially inducing stomatal closure. On S5 (after HW2) at 8 am, Trt vines had a gs significantly higher than Ctl vines. Gradually the gs of Trt vines decreased until 5 pm at which point there was no significant difference between Trt and Ctl for all cultivars (Figure 4c) with Trt gs decreasing substantially compared to Ctl. The response of Trt at S5 is similar to previous research done by Sabir and Yazar, (2015) on diurnal range of gs under normal growing conditions (25 °C to 30 °C air temperature, 60 to 70 % relative humidity). This suggests that at S5 Trt vines were returning to a lower stress gs function while Ctl vines were still restricted in stomatal activity. Stomatal conductance was higher in the treatment for all three cultivars because the plants had a reduced canopy temperature which resulted in a lower water loss from transpiration and higher stomatal conductance which allowed vines to remain hydrated and sustain a higher LWP and SWP than Ctl vines. The canopy wetting of the treated plants allowed to maintain a more humid environment 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. On the other hand, Ctl significantly decreased the gs likely to diminish the vulnerability to cavitation under the demanding atmospheric conditions during the heatwave (Hochberg *et al.,* 2017; Gambetta *et al.,* 2020). The possibility of foliar absorption of the sprayed water during the treatment was highly unlikely due to the short time in which the small water droplets remained on the leaves (30 min $h^{-1}$, Supplementary Figure 3) prior to evaporating. Additionally, no surfactant was used, which diminished water from entering through hydathodes.

#### 4.3.2.3. Leaf and Berry Temperature

LT only displayed consistent significant responses for all cultivars during the second heatwave (S4) at 5 pm (Figure 4b) where temperatures were higher in Ctl vines than in Trt, which was considered as an indicator of leaf cooling due to the treatment. During S4, LT trended inversely with the response of gs (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 Trt vines from 8 am until 2 pm. This behaviour was likely due to the rehydration and recovery of the vines after the second heatwave, and the regulation of the water balance to adapt to the cooler and moister conditions during the morning. The relationship between gs and LT was no longer present at S5, since 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 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 trigger xylem cavitation in Ctl vines.

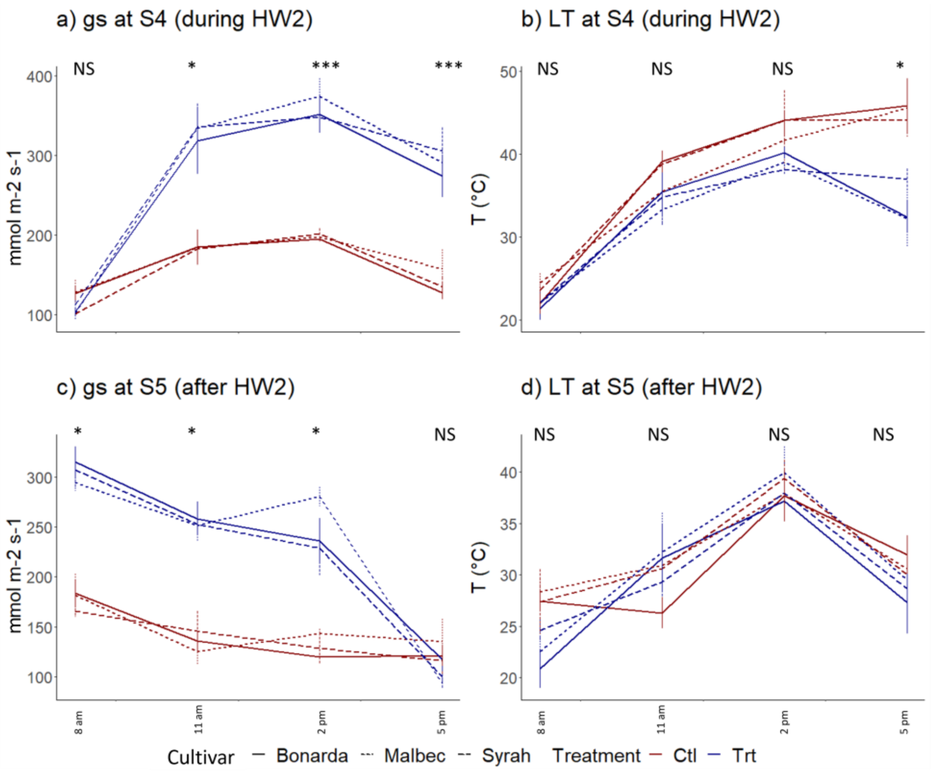

Figure 4. Stomatal Conductance (gs) (a), (c) and Leaf Temperature (LT) (b), (d) measured at 08 am, 11 am, 2 pm and 5 pm (during HW2) (S4) (a, b) and S5 (three days after HW2) (c, d) in Trt and Ctl vines of cultivars Bonarda, Malbec and Syrah. Statistical significance determined by two-way ANOVA (n = 3) with Tukey test post-hoc. ‘*’ $p \leq 0.05$, ‘***’ $p \leq 0.0001$, NS: Not Significant.

Under high air humidity, as occurs in the morning, grapevine stomata may be widely open (Sabir and Yazar, 2015). This was observed in all S5 vines (Figure 4c). In this work, the stomata closure of Ctl vines compared to 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 increased VPD. As a consequence, stomatal closure, reduction in photosynthetic rate and photosystem II efficiency occur as was observed in Ctl vines during HW2. These mechanisms help protect the plant against damage associated with high temperatures by maintaining its water balance (Gambetta *et al.*, 2020).

During the second heatwave (S4) at 5 pm , Trt vines of the three cultivars showed significantly lower BT (36.02 ºC) than the Ctl vines (45.22 ºC) ($p < 0.05$), due to evaporative cooling of Trt vines that served to dissipate the heat. During the recovery phase, on S5 at the same time, the differences between Trt and Ctl vines were significantly lower (28.92 ºC and 31.23 ºC, respectively ($p < 0.1$)). While the primary aim of the treatment during the heatwave was not to cool the berries directly, it unexpectedly led to an evaporative cooling effect of these organs at 5 pm, when the temperature was the highest. In contrast, other studies have directly applied spray to the bunch zone and observed a direct decrease in temperature at this level (Paciello *et al.*, 2017).

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

Chlorophyll content showed no significant difference between Trt and Ctl for all cultivars at all times and at all sample dates (Supplementary Figure 4).

Photosystem II (PSII) is usually suspended or destroyed before other cellular functions are disrupted (Zhang *et al.*, 2018). Chlorophyll *a* Fluorescence of the photosystem II which is considered to be the most sensitive physiological system of the grapevine to heat stress, represented here by the $F_v/F_m$ ratio, was not significantly affected after HW1 (S2 and S3) for all cultivars (Supplementary Table 2). During HW2, $F_v/F_m$ am values were not affected by the treatment for all cultivars (Figure 5a), however $F_v/F_m$ pm measurements were significantly different between Trt and Ctl vines for the three genotypes with Trt vines having higher values (Figure 5b).These values returned to non-significant differences for both am and pm measurements at S5 (Supplementary Table 2). The Performance Index (PI) was also not significant for all cultivars in both the morning and afternoon on S2, S3 and S5 (Supplementary Table 3). During HW2 (S4) although not significant, all cultivars trended higher in Trt vines. In the afternoon during HW2 the higher value in Trt vines 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 5 pm during HW2 (Figure 4b). The significantly higher values for $F_v/F_m$ and PI during HW2 for treated vines, particularly in the hottest part of the day suggests that the treatment was supporting 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 observed temporarily during HW2 in Ctl vines suggesting that the treatment application supports PSII functioning during prolonged heatwaves.

During heatwaves, it is beneficial to water vines regularly to maintain transpiration, cooling the canopy and bunches (Hayman *et al.,* 2012). Heatwaves can induce a vine “shut down,” halting photosynthesis due to stomatal closure to conserve water, resulting in slowed 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, leading to lower fruit quality overall. Further, extreme heatwaves can lead to cavitation fatigue where vines are more prone to xylem cavitation (Hochberg *et al.*, 2017) or even worse, vine mortality. Treatments aimed at reducing canopy temperatures have shown significant results, lowering temperatures by approximately 4-5 °C (Cogato *et al.*, 2021; Paciello *et al.,* 2017). Similar effects were observed in this treatment while using 5 % of the water volume applied in traditional mitigative irrigation practices. Further investigation into water volume thresholds where the treatment is still able to support vine physiological performance, as well as, investigating increased trigger temperatures for treatment activation can both help to further reduce the demand on water for this mitigative application.

The physiological results observed in this study indicate that administering a controlled dosage of water droplets onto the canopy's surface during 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 Trt vines as was observed with LWP, SWP and gs at S5, three days after the second heatwave. Although grapevine demonstrates an important resilience in adjusting to diverse environmental challenges, prolonged periods of excessively high temperatures or heatwaves can have lasting impacts on both yield attributes and vine physiology. After the second heatwave there remained significant differences between treated vines as compared to control vines, with treated vines displaying healthier physiological traits than control vines. This shows a protecting effect on the plants from stress not only during the treatment but also in the days following. Longer term positive 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 requires further investigation.

Some cultivars are more sensitive to heat stress than others. The Semillon cultivar is particularly sensitive to heat stress during flowering and ripening, experiencing reduced photosynthesis due to both stomatal and non-stomatal limitations and requiring up to two weeks to recover (Greer and Weedon, 2014). Hydrocooling, activated at a threshold temperature of 35 °C, extended the period for 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 as Greer and Weedon (2014) proposed. 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.

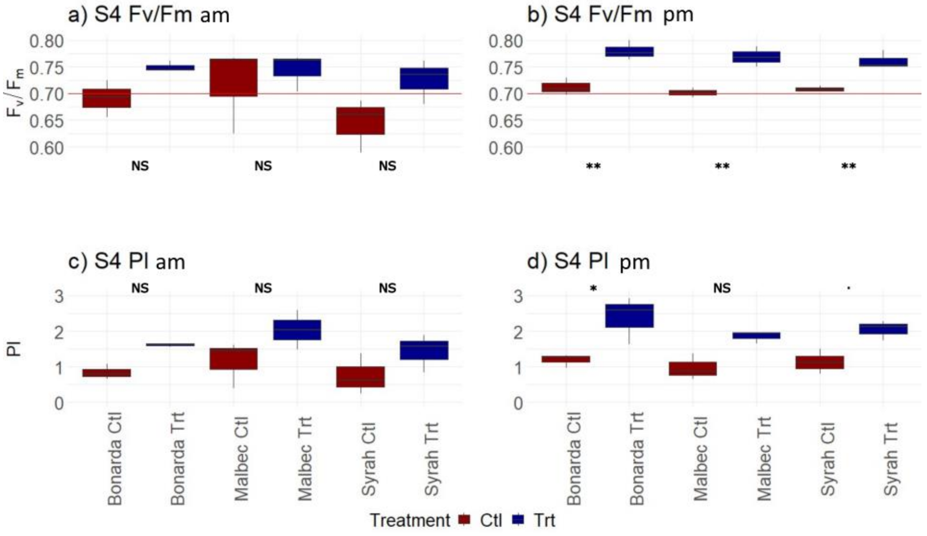

Figure 5. Chlorophyll Fluorescence ($F_v/F_m$) (a), (b) and Performance Index (PI) (c), (d) measured at S4 (during HW2) for cultivars Bonarda, Malbec and Syrah treated (Trt) and control (Ctl). Red line – $F_v/F_m$ threshold for PSII normal functioning (Bolhar-Nordenkampf *et al.*, 1994). Based on two-way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

### *4.3.3. Harvest Parameters*

#### 4.3.3.1. TSS and pH

No statistical differences were found for berry soluble solid content and pH between the Trt and Ctl vines at all sample points analysed (S1-S7), for the three cultivars (Figure 6a and 6b) as was previously reported by de Rosas *et al.* (2022), who tested Malbec, Merlot and Pinot Noir cultivars under heat temperature treatment. Moreover, the results of the present work were in agreement with the observations made by Greer and Weedon (2014) and Caravia *et al.* (2017), where TSS was not different between treatments. In both of these cases the research used a threshold temperature of 35 °C and 38 °C, respectively for sprinkler activation to mitigate impacts of heatwaves. Paciello *et al.* (2017) found a significant difference in TSS with a nebulized spray applied to the canopy and the bunch zone. In their research, grapes in treated vines had lower sugar compared to control. Importantly, the threshold temperature used to activate the nebulized spray was 30 °C which was 5 degrees below the temperature threshold commonly used to identify heatwaves. Paciello *et al.* (2017) also observed a significant difference in pH with a lower pH in treated vines than control. These results were similar to findings made by Kliewer and Schultz (1973) and Aljibury *et al.* (1975) who used threshold temperatures of 30 °C and 32 °C respectively. This suggests that a sprinkler treatment utilized as a mitigative practice for heatwave damage is unlikely to reduce TSS or pH whereas, if a sprinkler treatment is applied at a lower threshold temperature than those associated with heatwaves, berry ripening can be delayed with an associated reduction in TSS and pH. Further, reducing the temperature threshold for treatment application increases water usage due to the increased frequency of treatment application. Therefore, the temperature threshold for treatment application needs to be carefully considered to preserve berry quality and yield parameters.

#### 4.3.3.2. Berry weight, Number of Bunches plant$^{-1}$, Bunch weight and Fruit yield

Table 1 shows some berry physical traits at harvest. The post-hoc comparison done by Tukey test after two-way ANOVA analysis, showed that berry weight (g), bunch weight (g) and fruit yield (kg plant$^{-1}$) were significantly higher in Trt vines (1.89, 62.94, and 2.22, respectively), while, Ctl vines showed the lowest values (1.49, 38.44 and 1.68, respectively). The increase achieved in fruit yield by Trt plants was approximately 27 % compared to Ctl vines, indicating that the higher LWP of these 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 change significantly between Trt and Ctl vines as vines of each cultivar were pruned to equal number of buds prior to budbreak and the heat stress was not so great during the study to initiate bunch loss. This demonstrates that Trt plants mainly increased fruit yield due to berry weight and bunch weight.

Related to cultivars, Bonarda showed the higher berry weight value, compared to Syrah cultivar. Malbec also showed high berry weight, but it was not different in comparison with Bonarda and Syrah. The bunch weight was responsible for fruit yield in the case of Syrah and Malbec cultivars, which registered 2.577 and 2.522 kg plant$^{-1}$, respectively, values that were significantly higher than the average yield of Bonarda (0.751 kg plant$^{-1}$). Although this last cultivar recorded larger berry sizes, it showed the lowest number of bunches, which lead to a significantly lower yield plant$^{-1}$ compared to Syrah and Malbec (Table 1). Since the management of the different cultivars as well as the climate were the same, the variations in the number of bunches plant$^{-1}$ among 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 favorable to Trt vines with respect to Ctl vines for each cultivar. Trt *Bonarda presented higher values of berry weight than Ctl*Syrah, while Ctl*Bonarda registered lower bunch weight compared to Trt*Syrah.

Fruit yield was not significantly different for any treatment-cultivar interaction. However, the three genotypes trended to higher Trt values as compared to their respective Ctl values. These results are similar to findings in other research 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). Notably, Paciello *et al.* (2017) did not observe a significant difference between Trt and Ctl vines with a nebulized spray in the bunch zone activated with a threshold temperature of 30 ºC.

Table 1. Berry weight (g), Bunches plant$^{-1}$ (number=n), Bunch weight (g) and Fruit yield (kg plant$^{-1}$) at harvest.

| **Treatment** | **Berry weight (g)** | | **Bunches plant$^{-1}$ (n)** | | **Bunch weight (g)** | | **Fruit yield (kg plant$^{-1}$)** | |
|---|---|---|---|---|---|---|---|---|
| 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 average (n = 3). Different letters within the same column indicate significant differences among 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 NSindicates not significant.

### 4.3.3.3. Total polyphenol index

Total polyphenol index (TPI) was not significantly different for any cultivar at any sample point between Trt and Ctl (Figure 6). This is in concert with much of the literature which observes no significant effect of temperature exposure on total polyphenols concentration (Cohen *et al.,* 2008; Tarara *et al.,* 2008). Some research suggests that at extreme temperatures (>45 ºC) decreases in overall flavonol concentrations can be observed (Gouot *et al.,* 2019a). Such extreme temperatures were not observed during these two natural heatwave events where the maximum observed temperature was on February 12, 2023 at

42 ºC with only two days seeing temperatures above 40 °C during the course of the season (Figure 1).

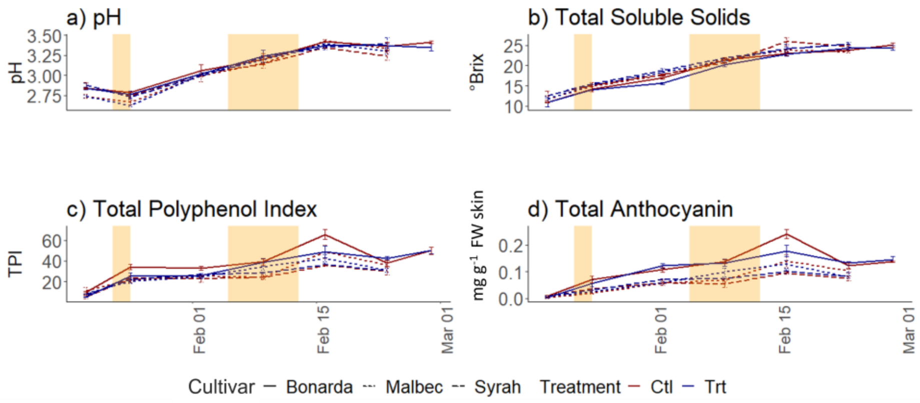

Figure 6: a) pH, b) Total Soluble Solid, c) Total Polyphenol Index and d) Total Anthocyanin evolution during growing season. No significant differences ($p < 0.05$) between Trt and Ctl were identified consistent for all cultivars.

#### 4.3.3.4. Anthocyanins

Table 2 shows the anthocyanin content at harvest time for the three tested cultivars. Df was the unique anthocyanin that presented a significantly higher content in Trt compared to Ctl. The rest of the free and conjugated anthocyanins, as well as the total anthocyanins, did not show significant differences between Trt and Ctl. When analysing the cultivars, it was found that Bonarda presented the highest content of Df, Cn, Pt, Po, Mv, MvCu, and total anthocyanin content. Moreover, total anthocyanins did not show any significant difference ($p \leq 0.05$) between Ctl and Trt at any sample point during the experiment. However, the concentration of PoAc, MvAc, and PoCu were the lowest for this genotype. Nevertheless, cultivar Bonarda showed significantly higher content ($p \leq 0.1$) at S5 than Malbec and Syrah cultivars (Figure 6d).

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

The percentage of Mv, did not show any differences although its coumaroylated version did, being higher in the Ctl with respect to Trt (Table 3). When comparing the cultivars, Bonarda presented the highest percentage of glycosylated forms (62.02 %), while Syrah and Malbec the lowest (57.42 and 55.07 %, respectively). The composition of anthocyanins in Bonarda was also altered with an increase in the percentage of glycosylated anthocyanins in Trt vines which was observable from S5 until harvest (S7). The behavior was inverse when the percentage of acylated and coumaroylated forms were analyzed, with Syrah and Malbec being the cultivars that showed the highest percentage of these forms (Table 3), while Bonarda, the lowest, beginning at S5 and continuing to harvest (S7).

In the present study, although malvidin-3-*O*-glucoside (Mv) did not show any significant differences among the tested cultivars, it was the predominant anthocyanin found, with an average content of 43.47 %, followed by malvidin-3-*O*- glucoside coumaroylated (MvCu), Pt, MvAc, Po, PoCu, Df, PoAc and Cn, having in the last 7 anthocyanins different behaviour according to the genotypes.

Some differences in the anthocyanin profile among treatment and cultivars were observed. Grapes from Trt Bonarda presented the highest content of glycosylated forms, the lowest content of MvCu and coumaroylated along with Syrah and the lowest content of acylated anthocyanins.

Finally, MvCu presented the lowest value for Trt Bonarda along with Trt and Ctl Syrah. On the contrary, Ctl Bonarda and Trt and Ctl Malbec showed the highest contents.

As previously commented for total anthocyanins, treatment appears to negatively influence the percentage of the most stable forms, namely acylated -derivatives. An associated rise in the amount of acylated and tri-hydroxylated anthocyanins in Malbec and Bonarda grapes when they were exposed to elevated temperature conditions was also observed by de Rosas *et al.* (2017). This suggests pigment acylation as a possible stress-response mechanism for attenuating heat temperature negative effects. In the present study, the acylation of anthocyanins into more stable forms was found to increase in Bonarda Ctl vines exposed to higher temperatures during heatwaves, suggesting a potential mechanism for coping with temperature stress. These results agreed also with those previously reported by de Rosas *et al.* (2022) in Malbec and other cultivars. The variation in impact of the treatment on a cultivars composition and concentration of anthocyanins suggests a variation in plasticity based on cultivar. Although Syrah, Malbec and Bonarda were exposed to the same treatment, Bonarda had a significant alteration of anthocyanin composition with less acylation and coumaroylation, while Malbec and Syrah did not. These results are in line with previous observations about cultivar plasticity and anthocyanin response to temperature (Gilbert *et al.*, 1971; de Rosas *et al.,* 2022). Thus, phenotypic plasticity, characterized 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 for some cultivars than others due to varying plasticity in terms of anthocyanin profile.

Table 2. Total Anthocyanin content (mg $g^{-1}$ 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 average (n=3). Different letters within the same column indicate significant differences among 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 average (n=3). Different letters within the same column indicate significant differences among 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' indicates not significant.

## 4.4. Conclusion

With the increase in observed number, severity, and duration of heatwaves globally, combined with reduced accessibility to water, strategies to reduce vine stress without relying on large volumes of water are needed. While it is common practice to apply supplementary irrigation before a heatwave to enhance the cooling effect of transpiration and promote evaporative cooling, the overhead pulsed micro-spray water treatment proposed in this study, administered at canopy level, required significantly less water volume—only 0.53 L vine$^{-1}$ HW day$^{-2}$—compared to an estimated minimum of 10.6 L vine$^{-1}$ day$^{-1}$ for an irrigation-based mitigation strategy.

This method applied during heatwave showed a clear and prolonged reduction in canopy temperature which led to a reduction in vine physiological stress metrics associated with extreme heat and drought events. Further, when heatwaves occurred 15 days before harvest it seems to be an appropriate way to alleviate the negative effect of heatwave stresses on yield without affecting grape anthocyanin and polyphenol content, neither pH nor TSS, independently of the cultivars used in this study. The impacts of the treatment appeared to be longer lasting after heatwaves with more sudden increases in temperature and of longer duration suggesting it’s capacity to aid vines in adapting to more extreme changes in temperature as well as extreme temperatures themselves. The integration of this technology into vineyard practices will provide viticulturists from warm and dry regions with a mitigation tool to manage heatwaves and proactively adapt to the challenges posed by climate change without increasing the risk of fungal disease development. At the same time, this technology can be used during winter to combat frosts.

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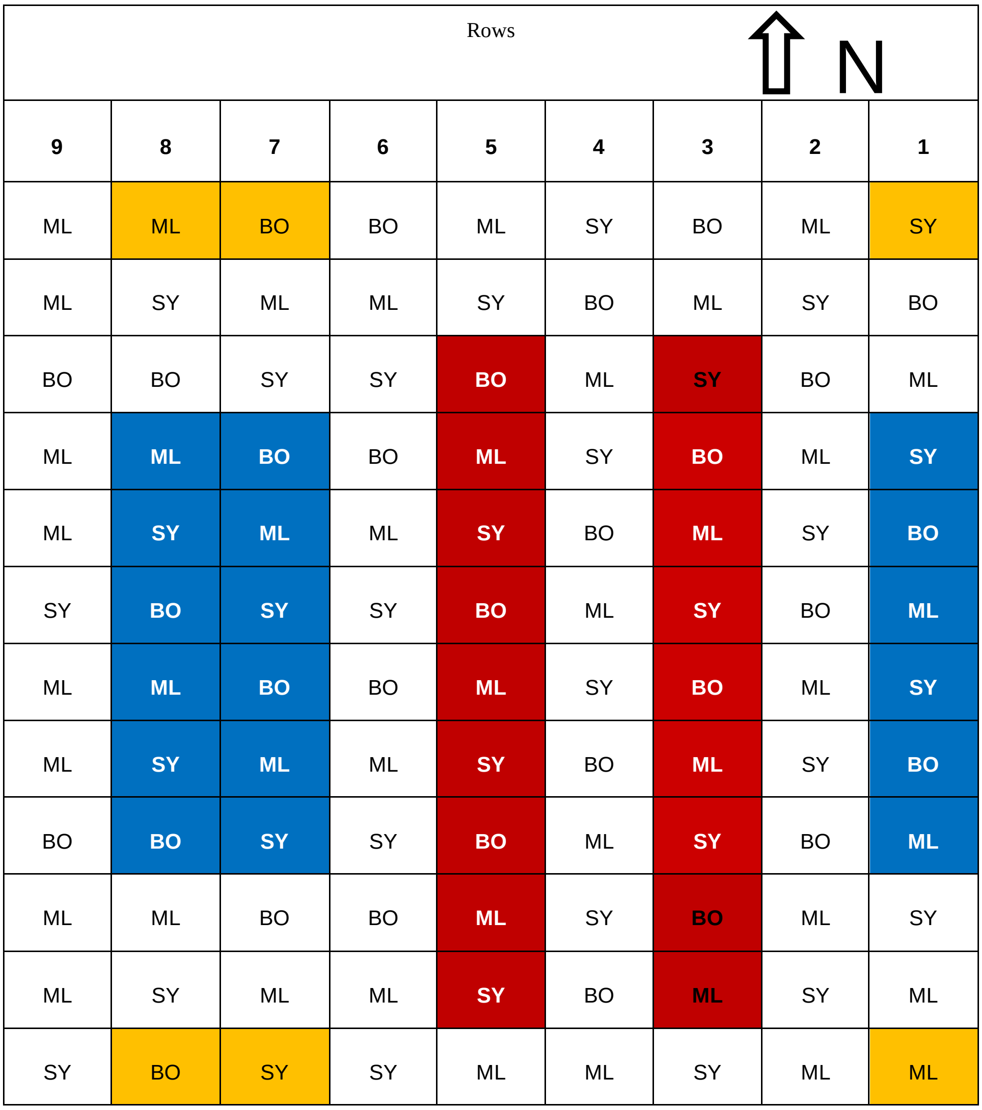
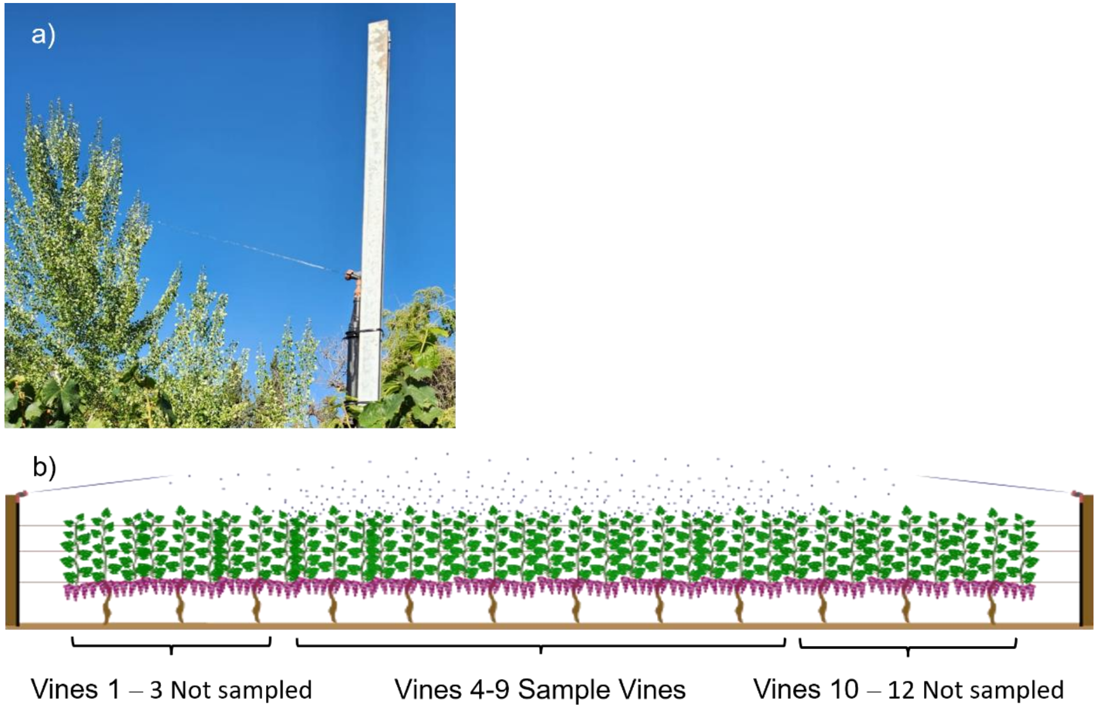
## 4.6. Supplementary Data

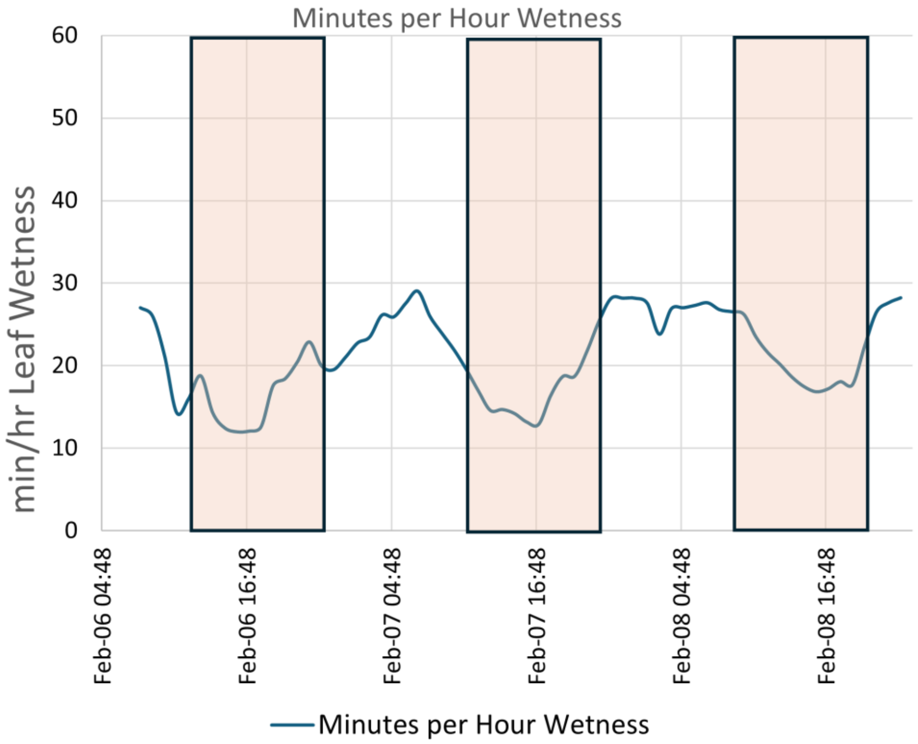


| 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|
| ML | ML | BO | BO | ML | SY | BO | ML | SY |
| ML | SY | ML | ML | SY | BO | ML | SY | BO |
| BO | BO | SY | SY | **BO** | ML | **SY** | BO | ML |
| ML | **ML** | **BO** | BO | **ML** | SY | **BO** | ML | **SY** |
| ML | **SY** | **ML** | ML | **SY** | BO | **ML** | SY | **BO** |
| SY | **BO** | **SY** | SY | **BO** | ML | **SY** | BO | **ML** |
| ML | **ML** | **BO** | BO | **ML** | SY | **BO** | ML | **SY** |
| ML | **SY** | **ML** | ML | **SY** | BO | **ML** | SY | **BO** |
| BO | **BO** | **SY** | SY | **BO** | ML | **SY** | BO | **ML** |
| ML | ML | BO | BO | **ML** | SY | **BO** | ML | SY |
| ML | SY | ML | ML | **SY** | BO | **ML** | SY | ML |
| SY | BO | SY | SY | ML | ML | SY | ML | ML |

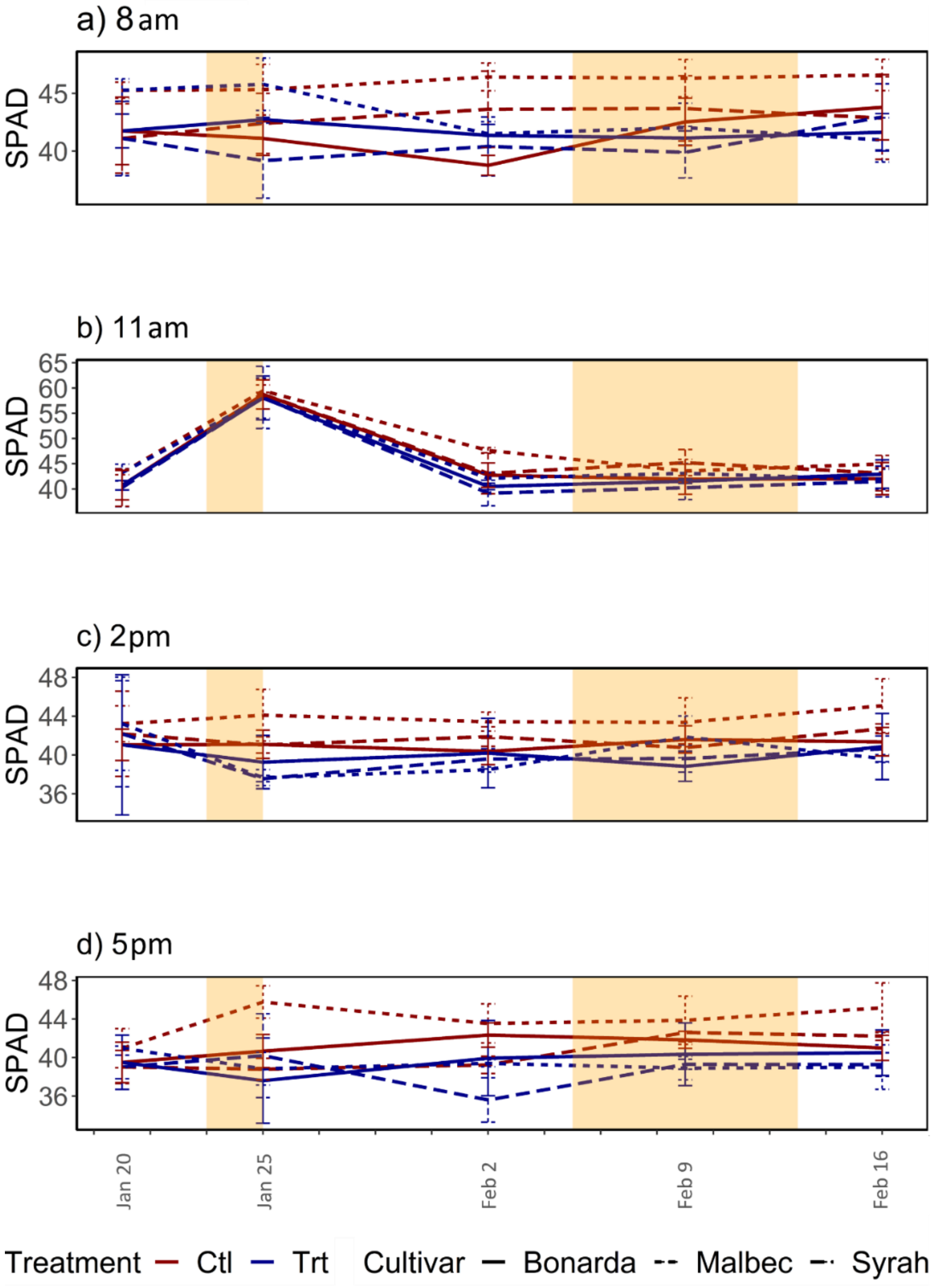
Supplementary Figure 1. Experimental design. Treated vines receiving full coverage from jets (rows: 1, 7 and 8: blue), Control vines (rows: 3 and 5: red), Borders (rows: 2, 4, 6 and 9). Location of jet nozzles (yellow).



Supplementary Figure 2: a) Image of jet nozzle during treatment application of a targeted pulse of water droplets; b) Sideview schematic of treatment application. The first and the last three vines of each Trt row were not sampled.



Supplementary Figure 3: Leaf wetness sensor showing minutes per hour wetness during HW2 from February 6 to Feb 8, 2023. Blue line indicates min of hr that leaf wetness sensor detected residual water; light orange shaded areas = treatment application occurring.



a) 8am

b) 11am

c) 2pm

d) 5pm

Supplementary Figure 4: Chlorophyll Content at each time point (8 AM, 11 AM, 2 PM, 5 PM) for physiological sample points from S1 to S5 for cultivars Bonarda, Malbec and Syrah. Heatwave days are represented by light orange blocks. Treated (Trt) vines (blue), Control (Ctl) vines (red); Data points are means ± SE (n = 3). No significant differences identified between Trt and Ctl for any cultivar.

Supplementary Table 1: Stem Water Potential from S2 to S5.

| **Treatment** | **S2** | | **S3** | | **S4** | | **S5** | |
|---|---|---|---|---|---|---|---|---|
| Trt | -10.6 | b | -11.0 | | -12.4 | a | -13.1 | a |
| Ctr | -9.86 | a | -10.6 | | -13.4 | b | -16.3 | b |
| ***p-value*** | ***0.0158*** | ***** | ***0.08*** | **NS** | ***0.0001*** | ******* | ***0.0001*** | ******* |
| **Cultivar** | | | | | | | | |
| Syrah | -10.4 | | -11.0 | | -12.8 | | -14.9 | |
| Malbec | -10.4 | | -11.0 | | -12.9 | | -14.5 | |
| Bonarda | -11.0 | | -11.3 | | -13.0 | | -14.5 | |
| ***p-value*** | ***0.1379*** | **NS** | ***0.411*** | **NS** | ***0.706*** | **NS** | ***0.22*** | **NS** |
| **Treatment x Cultivar** | | | | | | | | |
| Ctr*Bonarda | -10.8 | ab | -11.3 | | -13.5 | c | -16.3 | b |
| Trt*Bonarda | -11.3 | b | -11.2 | | -12.5 | ab | -13.1 | a |
| Ctr*Malbec | -9.6 | a | -11.2 | | -13.2 | bc | -16.1 | b |
| Trt*Malbec | -11.3 | b | -10.9 | | -12.6 | abc | -12.9 | a |
| Ctr*Syrah | -10.4 | ab | -11.3 | | -13.4 | bc | -16.6 | b |
| Trt*Syrah | -10.5 | ab | -10.7 | | -12.2 | a | -13.3 | a |
| ***p-value*** | ***0.068*** | ***** | ***0.603*** | **NS** | ***0.006*** | ****** | ***0.000*** | ******* |

Values are expressed as average (n=3). Different letters within the same column indicate significant differences among 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' indicates not significant.

Supplementary Table 2: $F_v/F_m$ measured in the morning (am) and afternoon (pm) during S2, S3 and S5.

| | $F_v/F_m$ am | | | | | | $F_v/F_m$ pm | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **S2** | | **S3** | | **S5** | | **S2** | | **S3** | | **S5** | |
| Trt | 0.700 | | 0.808 | | 0.749 | | 0.660 | | 0.787 | | 0.780 | |
| Ctr | 0.745 | | 0.812 | | 0.807 | | 0.709 | | 0.775 | | 0.701 | |
| ***p-value*** | ***0.405*** | **NS** | ***0.745*** | **NS** | ***0.0611*** | **NS** | ***0.242*** | **NS** | ***0.101*** | **NS** | ***0.204*** | **NS** |
| | | | | | | | | | | | | |
| **Cultivar** | | | | | | | | | | | | |
| Syrah | 0.740 | | 0.818 | | 0.788 | | 0.665 | | 0.786 | | 0.757 | ab |
| Malbec | 0.733 | | 0.820 | | 0.773 | | 0.685 | | 0.797 | | 0.658 | a |
| Bonarda | 0.696 | | 0.803 | | 0.802 | | 0.704 | | 0.800 | | 0.868 | b |
| ***p-value*** | ***0.764*** | **NS** | ***0.415*** | **NS** | ***0.6995*** | **NS** | ***0.734*** | **NS** | ***0.263*** | **NS** | ***0.0410*** | ***** |
| | | | | | | | | | | | | |
| **Cultivar x Treatment** | | | | | | | | | | | | |
| Ctr*Bonarda | 0.748 | | 0.789 | | 0.775 | | 0.687 | | 0.782 | | 0.775 | |
| Trt*Bonarda | 0.644 | | 0.773 | | 0.723 | | 0.721 | | 0.791 | | 0.738 | |
| Ctr*Malbec | 0.739 | | 0.793 | | 0.752 | | 0.727 | | 0.773 | | 0.391 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trt*Malbec | 0.726 | | 0.805 | | 0.686 | | 0.750 | | 0.795 | | 0.702 | |
| Ctr*Syrah | 0.748 | | 0.800 | | 0.764 | | 0.821 | | 0.770 | | 0.663 | |
| Trt*Syrah | 0.731 | | 0.793 | | 0.706 | | 0.723 | | 0.775 | | 0.628 | |
| ***p-value*** | ***0.729*** | **NS** | ***0.611*** | **NS** | ***0.978*** | ***NS*** | ***0.368*** | **NS** | ***0.587*** | **NS** | ***0.825*** | **NS** |

Values are expressed as average (n=3). Different letters within the same column indicate significant differences among Treatments (Trt and Ctl), Cultivars (Syrah, Malbec and Bonarda) and Treatment x Cultivar interaction. Tukey test p≤ 0.05 (*) and 'NS' indicates not significant.

Supplementary Table 3: Performance Index measured on S2, S3 and S5.

| | PI am | | | | | | PI pm | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **S2** | | **S3** | | **S5** | | **S2** | | **S3** | | **S5** | |
| Trt | 2.26 | | 3.12 | | 1.34 | | 1.62 | | 3.45 | | 0.97 | |
| Ctr | 2.73 | | 3.09 | | 2.59 | | 2.16 | | 3.02 | | 0.71 | |
| ***p-value*** | ***0.691*** | **NS** | ***0.96*** | **NS** | ***0.085*** | **NS** | ***0.453*** | **NS** | ***0.282*** | **NS** | ***0.310*** | **NS** |
| **Cultivar** | | | | | | | | | | | | |
| Syrah | 2.61 | | 3.25 | | 1.75 | | 2.12 | | 2.77 | | 0.84 | |
| Malbec | 2.20 | | 3.47 | | 1.67 | | 1.97 | | 3.61 | | 0.63 | |
| Bonarda | 2.67 | | 2.60 | | 2.47 | | 1.59 | | 3.33 | | 1.06 | |
| ***p-value*** | ***0.939*** | **NS** | ***0.596*** | **NS** | ***0.573*** | **NS** | ***0.816*** | **NS** | ***0.230*** | **NS** | ***0.383*** | **NS** |
| **Cultivar x Treatment** | | | | | | | | | | | | |
| Ctr*Bonarda | 3.82 | | 3.12 | | 3.35 | | 1.44 | | 3.00 | | 1.04 | |
| Trt*Bonarda | 1.52 | | 2.07 | | 1.59 | | 1.73 | | 3.67 | | 1.07 | |
| Ctr*Malbec | 2.24 | | 3.27 | | 2.01 | | 2.37 | | 3.27 | | 0.18 | |
| Trt*Malbec | 2.17 | | 3.67 | | 1.32 | | 1.57 | | 3.95 | | 1.08 | |
| Ctr*Syrah | 2.14 | | 2.87 | | 2.41 | | 2.67 | | 2.79 | | 0.92 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trt*Syrah | 3.08 | | 3.63 | | 1.10 | | 1.57 | | 2.74 | | 0.76 | |
| ***p-value*** | ***0.526*** | **NS** | ***0.563*** | **NS** | ***0.810*** | **NS** | ***0.703*** | **NS** | ***0.684*** | **NS** | ***0.208*** | **NS** |

Values are expressed as average (n=3). Different letters within the same column indicate significant differences among Treatments (Trt and Ctl), Cultivars (Syrah, Malbec and Bonarda) and Treatment x Cultivar interaction. Tukey test p≤ 0.05 (*), p≤ 0.01 (**), p≤ 0.001 (***), and 'NS' indicates not significant.

# 5. Thesis Conclusion

Considering that the impact of climate change on grape berries used for winemaking is a complex undertaking. This is amplified when considering varied natural terroir features which may exacerbate risk. Particularly, topographical changes in landscape can mean a great difference in solar insolation and temperature exposure. This thesis aimed in part to identify major features of the natural terroir which significantly impact berry quality and can be associated with variation in the style of Barolo wines. In Barolo DOCG it appears that slope aspect plays a larger role in influencing grape quality and yield parameters than does elevation as established in Chapter 2 (Mania et al., 2021). However, the research investigating elevation in Chapter 2 was not well distributed between vineyards with differing aspects. In Chapter 3, the research occurred in two years with anomalous climatic behaviour rendering it quite difficult to ascertain any real differences in berry quality associated with elevation. The reason for this increase in the heat sum index at the La Morra weather station is unknown and requires further observation to determine if this climate alteration is long lasting, what the potential mechanisms behind it are and if this significant increase could pose new risks to the terroir of SE facing slopes in Barolo DOCG.

Chapter 3 also suggests that variation in quality berry production by elevation in Barolo DOCG is likely linked more to climate variation at differing elevations and less associated with increased UV exposure at increasing elevation. Considering that total flavonol concentration was not significantly higher at the higher altitude vineyard it can be posited that elevations ranging from 200 m ASL to 550 m ASL do not observe enough of a difference in UV radiation exposure to elicit a response in flavonol production in cv Nebbiolo.

In terms of the effects of increased temperature and variation in UV, it is confirmed that increased temperature can alter anthocyanin profile and proportion of anthocyanins while in cultivars with high concentration of di-hydroxylated anthocyanins like Nebbiolo, UV can also play a role with increased UV reducing their concentration and relative amounts. Flavonols were unsurprisingly reduced by reduced UV exposure, however, higher temperature also appears to induce a reduction in flavonol concentration. Finally, HCTA's showed some response predominantly to UV exposure, particularly in total concentration and ratios between *cis/trans p*-coumarylated and caftaric/*p*-coumarylated suggesting that viticultural management techniques such as leaf removal or pruning strategies can be applied to manipulate all three types of phenols investigated in Chapter 3 by altering UV exposure. The aim of the viticultural management strategy will vary depending on specific identified risks in each vineyard or even vary within a vineyard.

Finally, considering increased temperature and heatwave events which are becoming more common and were observed in the second year of research in Chapter 3, potential mitigative strategies such as a targeted pulsed spray show promise as an alternative to resource intensive irrigation strategies for the protection of vine health and berry quality during periods of extreme heat. Although the results of this mitigative strategy were positive, the infrastructure is costly. To reduce costs, installation of the infrastructure could be limited to vineyards with slope aspects with increased solar insolation, and temperatures such as SE to SW facing slopes as identified in Chapter 2. Further, this mitigative application should be evaluated for its effectiveness in maintaining vine health in new plantings to reduce vine mortality from extreme heat. With a more limited water resource in this complex topography, a mitigative strategy with reduced water demand for vine protection during heatwaves is optimal.

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