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RESPONSE OF VITIS VINIFERA CULTIVARS TO MULTIPLE SUMMER STRESSES AND DEVELOPMENT OF PRECISION STRATEGIES TO MITIGATE NEGATIVE EFFECTS OF CLIMATE CHANGES ON GRAPE AND WINE

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Summary

The grapevine is a species that adapts well to challenging climatic conditions, such as water scarcity during the growing season. In a context of variable weather, characterized by the overlapping of multiple stresses such as radiative, thermal and water-related stresses, *Vitis vinifera* L. responds through adaptive mechanisms that ensure its survival but can have a detrimental effect on both yields and the quality of productions. Starting from this standpoint, the thesis aimed to develop innovative strategies to address the short to medium-term impacts of heatwaves. The principal objective was to provide grape growers with flexible and cost-effective tools to safeguard both plant vitality and production. The secondary objectives, elucidated in the three distinct experiments, vary based on the tested strategies outlined in the subsequent chapters. These objectives encompass enhancing the physiological response of vines to multiple summer stresses, mitigating sunburn damage and improving the composition of grape berries and wine.

In the first study, a multifunctional irrigation system (drip and climate conditioning) was implemented and its ability to regulate the microclimate around the fruit-zone during heatwaves was assessed. Specifically, the cooling effect on the cluster zone was evaluated concerning the physiology and production aspects of the two most cultivated red grape varieties in Italy: 'Sangiovese' and 'Montepulciano'. The selection of these grape varieties was motivated not just by their national agronomic significance but also by their distinct responses to various stress conditions. Consequently, the vines were cultivated in pots and exposed to different water regimes, including well-watered and water shortage conditions during the ripening period. In our observations, 'Montepulciano' exhibited a higher leaf assimilation rate and stomatal conductance under optimal water conditions in comparison to 'Sangiovese.' Notably, both varieties demonstrated consistent behavior even under conditions of water scarcity and in presence of misting. Additionally, vines treated with nebulized water, demonstrated increased yield without compromising technological maturity compared to water-stressed vines. In the 2023 vintage, the misting system prevented ripening blockage in 'Montepulciano' under water stress. The nebulized treated vines exhibited a significant increase in total anthocyanins concentration, indicating the microclimate's pivotal role in anthocyanin biosynthesis and reducing oxidative processes.

A field experiment was then conducted to investigate the effect of misting water on a white grape variety grown in field conditions over two years. Two distinct thermal-radiative regimes (defoliated and undefoliated) were applied in a flatland vineyard of 'Pignoletto' grapevines. The primary objective was to assess the impact of these treatments on yield attributes, berry necrosis and the concentration of secondary metabolites such as flavonols. Simultaneously, a portable and non-

destructive vis/NIR device (Da-meter© berry adapted) was utilized to monitor the level of browning of clusters subjected to thermal stress. In particular, a new index (IDAg) has been introduced to identify a relationship between this and the accumulation of flavonols in the berry. The study revealed that the use of misting consistently reduced both air and berry temperatures over two years. Vines treated with nebulized water exhibited increased yield, attributed to reduced sunburn damages and subsequent cluster weight gain. While technological maturity showed no significant differences, misting-treated vines in the scorching 2023 season demonstrated a notable trend towards increased acidity, contrasting with control and defoliated groups. Interestingly, flavonol synthesis remained unaffected by thermal reduction but was stimulated under increased light exposure, leading to exclusive overexpression in the defoliated treatment, as confirmed by the DAg index.

In conclusion, the cooling system implemented in the bunch area emerges as a valuable tool for mitigating the adverse effects of summer stresses on grapes, notably in minimizing sunburn damage on exposed clusters. Furthermore, the system demonstrated positive outcomes for both white and red grapes. In the case of the latter, it exhibited a beneficial impact on anthocyanin accumulation and berry color.

The third experiment emerges as a strategic approach for winegrowers to effectively address the impact of heatwaves in a timely manner. While *Vitis vinifera* L. is inherently drought-resistant, there is evidence suggesting a reduction in its photosynthetic capacity caused by elevated temperatures. This decline significantly influences vine growth, yield and grape composition. Specifically, one of the initial effects of climate change in viticultural areas is the accelerated progression of phenological stages, especially early berry ripening. Under these conditions, a common issue is the decoupling between technological and phenolic maturity. It is recognized that anthocyanins, crucial for phenolic maturity in red grapes, face challenges in accumulation due to high temperatures. This study aims to assess the effectiveness of a treatment with mineral compounds, such as chabasite-based zeolite, in enhancing anthocyanin accumulation and in improving physiological performance. Additionally, various parameters, including leaf temperature, vine water status, leaf photosynthetic efficiency, total soluble solids, titratable acidity, and pH were monitored over three growing seasons: 2021 in potted vines and 2022-2023 in field vines for ‘Sangiovese’. The present study demonstrates the effectiveness of foliar zeolite treatment not only in reducing canopy temperatures but also in improving anthocyanin accumulation in grapes. The latter results were confirmed by the wine obtained in the 2022 season.

Together, this research work provides a comprehensive and cohesive framework for understanding and managing multiple summer stresses in both white and red grapevines. Implementing these findings can significantly contribute to the adaptation of grapevines to the challenges posed by climate change.

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Introduction

Vitis vinifera L. is commonly cultivated in semi-arid regions where high light and temperatures often coincide with periods of drought. The complex interplay of these factors can yield both positive and negative effects on grapevines, contingent upon the timing and severity of environmental events (Palliotti and Poni, 2015). While traditionally considered drought-resistant, mounting evidence suggests a decline in the photosynthetic capacity of *Vitis vinifera* L. attributed to temperature-related stress. This decline has implications for vine growth, yield, and grape composition, particularly in the face of stresses such as excessive temperature and radiation, soil water scarcity, and high leaf-to-air Vapor Pressure Deficit (Chaves et al., 2010; Keller, 2010; de Orduňa, 2010).

This research aims to address these challenges through an investigation of short-to-medium-term strategies, including water misting in the fruit zone and the application of mineral particles. These strategies are designed to enhance physiological and biochemical responses in grapevines, potentially reducing sunburn injuries and ameliorating yield. Furthermore, recognizing that plant responses to abiotic stresses involve a combination of cultivar-specific stress avoidance and tolerance mechanisms (Tombesi et al., 2016), the study seeks to optimize resource utilization in an environment characterized by thermo-radiative excesses and water scarcity during critical vine developmental stages.

The importance of reevaluating light and water management through precise strategies is emphasized in the work. One proposed strategy involves the adoption of a multi-functional irrigation system capable of efficiently conditioning the grape cluster zone with minimal water usage. This approach is posited as a potential contribution to addressing climate change impacts and ensuring the preservation of vine physiological activities, thereby securing the survival and productivity of *Vitis vinifera* L. in challenging environmental conditions. The second approach entails the application of Italian chabasite rich-zeolite across the entire canopy during the veraison phase. This research constitutes a further exploration of the work previously initiated by the author on the 'Sangiovese' and 'Montepulciano' grape (Valentini et al., 2021; Calzarano et al., 2019).

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Chapter 1.

State of art

1.1. Climate change impacts viticulture

The last climate report of the Intergovernmental Panel on Climate Change is worrying: the surface temperature of the globe was 1.09°C higher in the period 2011-2020 than in the period 1850-1900 (IPCC, 2023). The global warming impacts viticulture and in many suitable areas maintaining grapevine production will require adaptation to climate change. As reported by Naulleau et al. (2021), an abundance of literature on the impact of climate change in viticulture has been published and three main factors have been identified: a 50% increase of biomass production in an elevated CO_2 environment, almost 3 to 4 days per decade advancement of the vegetative and reproductive cycle due to higher temperatures and a higher risk of water stress impacting yield attributes and berry composition (Bindi et al., 1996; Caffarra and Eccel, 2011; Van Leeuwen et al., 2019). In addition, Mediterranean vineyards are exposed not only to intense solar radiation, but also to water scarcity especially during the ripening period (Teslic et al., 2019; Di Carlo et al., 2019). In this multiple stress scenario, characterized by high vapor pressure deficit (VPD), the grapevine (*Vitis vinifera* L.) responds through active and passive stomatal regulation mechanisms (Schultz, 1996; Greer et al., 2010). This behavior, which is intended to reduce xylem vessel embolism, influences gas exchange depending on phenological stage and variety. In particular, some anisohydric cultivars, such as Sangiovese, maintain good stomatal conductance down to stem potential values of -1.8 MPa. Others, with less anisohydric behavior, completely close their stomata to lower negative potentials, compromising grape ripening (Tombesi et al., 2016; Palliotti et al., 2014; Valentini et al., 2022). However, when the stress worsens, vines often display leaves with chlorosis and necrosis and a significant reduction in yield parameters due to sunburn (Dinis et al., 2016). In European wine-growing regions, sunburn symptoms have shown an increasing frequency since the late 1990s in both Germany and France. In the latter case, the phenomenon has been mainly attributed to the increased frequency and intensity of heatwaves (Gambetta et al., 2021). This condition results in grapes with loss of balance between sugars and acidity, high pH and poor color (Allegro et al., 2021). More specifically, the rise in temperature is expected to lower the acidity and increase the sugar content of berries, resulting in unbalanced wines with higher alcohol content and deprived of freshness and aromatic complexity (Bernardo et al., 2018). Furthermore, black varieties suffer greatly from increased temperatures, which negatively affect anthocyanin biosynthesis (Movahed et al., 2016).

Recent studies on the molecular mechanism of reduced anthocyanin concentrations in the presence of high temperatures have confirmed that anthocyanin synthesis is inhibited and anthocyanin degradation stimulated (Pastore et al., 2017). These results are consistent with the occurrence of thermal decoupling of anthocyanin and sugar accumulation observed in Cabernet Franc and Shiraz varieties exposed to a high temperature regime (Sadras and Moran, 2012).

Due to the intensification of heatwaves and extreme events, many viticultural areas, particularly in Mediterranean climate regions, may not be suitable for growing winegrapes in the near future, unless genotypes adapted to heat stress are developed or stress-tolerant germplasm is identified and exploited. On the other hand, some agronomic techniques could alleviate the effects of climate change in the short to medium term. Some of these have been developed at the University of Bologna, such as post-veraison trimming (Filippetti et al., 2011; Valentini et al., 2019), the application of mineral particles such as chabasite-rich zeolite (Calzarano et al., 2019; Valentini et al., 2021) and the implementation of a fruit-zone cooling system to improve the microclimate conditions of the grapes during ripening (Valentini et al., 2023).

1.2. Strategies to face climate change in viticulture

Climate change adaptation is pivotal for the future of agriculture, an especially vulnerable economic sector heavily reliant on weather and climatic conditions. Broadly defined, climate change adaptation encompasses "the set of actions and processes that societies must take to limit the negative impacts of the changes and maximize their beneficial effect" (Carter, 1996). In the context of grape cultivation, the potential levers for adaptation are diverse, encompassing both the timing of technical operations along the production chain - from plantation to annual crop management - and their spatial and temporal variations (Viguie et al., 2014). The main strategies that can be employed, in both the short and long term, to counteract the advance of global warming are consolidated into five main categories. These strategies can be synergistically utilized to ameliorate the grapevine's response to a changing climate (Naulleau et al., 2021; Sun et al., 2023).
Vineyard location and vine row orientation. Selecting vineyard locations at higher altitudes emerges as a viable strategy to alleviate the impact of climate change. The orientation of vine rows significantly influences vine physiology and grape quality, particularly in warmer regions, where opting for an orientation that minimizes canopy light interception is advisable (Hannah et al., 2013).
Plant material. Selecting drought-resistant grape varieties, rootstocks and clones is crucial for

adapting to changing climate conditions (Christensen et al., 2003; Corso et al., 2014; Chrysargyris et al., 2018; Gambetta et al., 2020; Wang et al., 2021).

Training systems and canopy management. The choice of different training systems can influence temperatures within grapevine fruit zones (Valentini et al., 2022; Del Zozzo and Poni 2024). Canopy management practices, including shoot trimming or topping, offer a means to delay berry ripening and decelerate sugar accumulation (Filippetti et al., 2011; Valentini et al., 2019; Poni et al., 2023).

Water management. Irrigation stands out as a pivotal strategy to tackle water deficits induced by climate change. Employing various irrigation systems, including subsurface, drip and sprinkler irrigation, facilitates efficient water use (Williams and Baeza, 2017; Biswas 2015). The implementation of regulated deficit irrigation (RDI) enables precise control over water application, consequently enhancing the quality of grapes and wine (Caruso et al., 2023).

Soil management practices including cover cropping and soil amendments contribute to improved water retention and soil structure (Centinari et al., 2013; Gatti et al., 2022). Precision soil management, using soil moisture sensors and remote sensing, optimizes irrigation and resource use (Santesteban et al., 2017; Di Gennaro et al., 2017; Bellvert et al., 2021).

Other adaptation strategies, such as the use of mineral particles and specific yeast derivative (Pastore et al., 2020; Valentini et al., 2021) and late pruning (Allegro et al., 2020), provide additional tools to mitigate the impact of high temperatures. Late pruning, for instance, can effectively delay vine development and berry ripening, potentially counteracting the effects of global warming.

Some of these techniques have been developed in recent years at the University of Bologna. As reported below, the post-veraison trimming technique aims to re-couple technological and phenolic ripening, while the use of mineral particles distributed throughout the canopy is employed to counteract heatwaves in a climate change scenario.

Late trimming technique to couple sugar and anthocyanin accumulation

Rising temperatures have an effect on phenological times, which in general seem to become shorter. An advance is observed for grape harvest dates, which in the specific case of Burgundy, have shifted up to 13 days in even 30 years (Greer et al., 2010; Labbé et a., 2019). In particular the rise in temperature affects the time between flowering and veraison which is reduced by a little more than 1 day per °C (Duchene and Schneider, 2005). When these conditions meet other factors, such as improved vine agronomic management techniques, excess berry sugar concentration is recorded at harvest (Keller, 2010). Furthermore, a shift of wine growing regions to higher altitudes or latitudes can be expected as a consequence of global warming (Rienth et al., 2016) whereas traditional regions will not disappear (Hannah et al., 2013) but might need new approaches to better adapted to elevated temperatures (Palliotti et al., 2014). Among the available viticultural strategies, some are based on reducing carbohydrates during ripening (Filippetti et al., 2011) or treatments with antitranspirants that can reduce photosynthesis (Intrieri et al., 1983; Palliotti et al., 2013). All these strategies can be mechanized and then applied in a timely manner. The main objective is to reduce sugar accumulation to achieve wines with lower alcohol content, but also to evaluate their effectiveness in delaying the achievement of optimal sugar content combined with adequate phenolic maturity (Ribéreau-Gayon et al., 2000).

The late shoot trimming is a strategy to slowing ripening based on the competitive regrowth of laterals with subsequent reduction in total soluble solid production (TSS) as well as their accumulation in berries (Filippetti et al., 2011). Several studies have revealed that the removal of the younger and more efficient leaves around veraison may help to reduce sugar accumulation according to the leaf area/fruit ratio (Intrieri et al., 1983; Kliewer and Dokoozlian, 2005). However, the impact of this strategy on anthocyanin accumulation remains unknown. For this reason, two researches were conducted at the experimental fields of the University of Bologna in order to evaluate the effects of late trimming on the evolution of technological and phenolic maturity of berries and on the extractability of anthocyanins and tannins in wine-like solution (Filippetti et al., 2015; Valentini et al., 2019). Specifically, the two studies were carried out on 'Sangiovese' and in two different environments: the first in a hillside and non-irrigated vineyard, the second, more recent, in a flatland irrigated vineyard. In both cases, the vines were trimmed (TRIMM) leaving 8-9 nodes compared to the control plants (C) when the sugar concentration of 16 °Brix was reached.

The source limitation induced by the post-veraison trimming proved to be effective in reducing TSS in both trials. In both cases, the TRIMM vines showed a range of 50% to 67% reduction in total leaf area compared to C, resulting in a downward shift in the leaf area to fruit ratio over the respective three years of experimentation. No lateral regrowth was recorded at harvest in any vintages. In details, the technique induces reprogramming of the source-sink balance which may mitigate the excess of the sugar accumulation do to the climate change (Bobeica et al., 2015). Although there has been no recovery in terms of vegetative growth, studies have shown that procrastination over time of this technique can deplete reserve substances stored in the wood. This phenomenon is particularly evident in the hillside vineyard (Filippetti et al., 2015) where in the last year of the trial there was a decline in TRIMM production that negated the effect of late trimming on sugar concentration at harvest. Thus, we can state that the impact of leaf area on sugar accumulation is strongly influenced by yield.

In fact, in order to have a drastic reduction in soluble solids accumulation, it is necessary that the ratio between the above parameters does not fall between the values 0.7 - 1.4 m^2/kg as reported in several scientific papers (Smart and Robinson, 1991; Kliewer and Dokoozlian, 2005). But the main question centers on the relationship between sugar and anthocyanin trends since the two mechanisms are reported in the literature to be closely associated (Guidoni et al., 2008). From the results obtained in the two trials, it would seem that anthocyanin trends are more related to climatic settings than to sugar concentration (Mori et al., 2005; Pastore et al., 2017). This is probably due to the application of trimming at the end of the first ripening stage, where a strong relationship between sugars and anthocyanins occurs. Therefore, we can state that under the trial conditions the two trends appear to be uncoupled. In conclusion the post-veraison shoot trimming above the bunch area of 'Sangiovese' is a powerful tool for decrease sugar concentration at harvest without affect total and extractable phenolic compounds. Nevertheless, trimmed vines could reduce their starch reserves when the technique was performed over consecutive seasons.

Application of kaolin and chabasite-rich zeolitites

Easy-to-use techniques to cope with rising temperatures in the vineyard include the use of mineral particles distributed with an atomizer over the entire canopy at different concentration (Calzarano et al., 2019; Valentini et al., 2021). The first promising results concern the use of kaolin on apple trees and have shown the positive effects of using this mineral on plant physiology and yield (Glenn et al., 1999). In particular, kaolin is an aluminosilicate clay having the capability of reflecting a more elevated amount of potentially damaging radiation resulting in a decrease in the temperature of spray organs, such as leaves and fruits (Glenn and Puterka, 2005; Garrido et al., 2019). Due to its ability to modify the reflective properties of sprayed organs and create cooling effects, kaolin is deemed a crucial tool in minimizing sunburn damage (Dinis et al., 2016). It also positively influences phenolic concentrations, including anthocyanins (Brito et al., 2019). If under water-limiting conditions the vines treated with kaolin showed a benefit in terms of gas-exchanges, the results under water-available conditions appear to be contrasting (Brillante et al., 2016). Because of these unclear results, at the experimental station of the University of Bologna the foliar treatment of kaolin was compared to that of chabasite rich-zeolitites (Valentini et al., 2021). Zeolites are crystalline aluminosilicate of alkali and alkaline earth elements composed of tetrahedral framework of SiO_2 and AlO_4. Several earlier findings reported the effects of zeolites application on soil properties (Mondal et al., 2021) as well as zeolites 'role in plant physiology parameters, yield and quality attributes (Eroglu et al., 2017; Calzarano et al., 2019; Petoumenou, 2023). The trial was carried out in 2019 and 2020 seasons in

Bologna (44°32′ N–11°22′ E) on a seven-year experimental vineyard, cv. Sangiovese (*Vitis vinifera* L.). As reported by Valentini et al. (2021) the vines were associated with three treatments: C, untreated control vines sprayed with water; CAO, kaolin sprayed treatment and ZEO, natural Italian chabasite-rich zeolitites sprayed treatment. The minerals were distributed on the entire canopy twice each year at 3% concentration. The two applications were carried out at the beginning and at the end of veraison with the aim to evaluate the effects of minerals on fruit cooling, gas exchange, and the grape and wine composition. Although the two vintages exhibited climatic differences – with 2019 being warmer and less rainy than 2020 - both mineral particles (kaolin and zeolite) significantly influenced leaf and berry temperatures. These results are particularly intriguing because of the strong correlation between berry temperature with the content of polyphenolic compounds, such as anthocyanin. Indeed, it is known that high temperatures have a negative effect on the accumulation of anthocyanins in the skin of ripening fruits (Movahed et al., 2016; Pastore et al., 2017). This suggests that the foliar application of minerals, leading to lowering the berry temperature, promotes the biosynthesis of phenolic compounds (Conde et al., 2016) and decreases their enzymatic degradation (Mori et al., 2005). Specifically, the study showed that the application of the minerals resulted in a significant decrease in berry temperature and that this reduction is more lasting in kaolin than in zeolite as shown in Figure 1 for the warmer season.

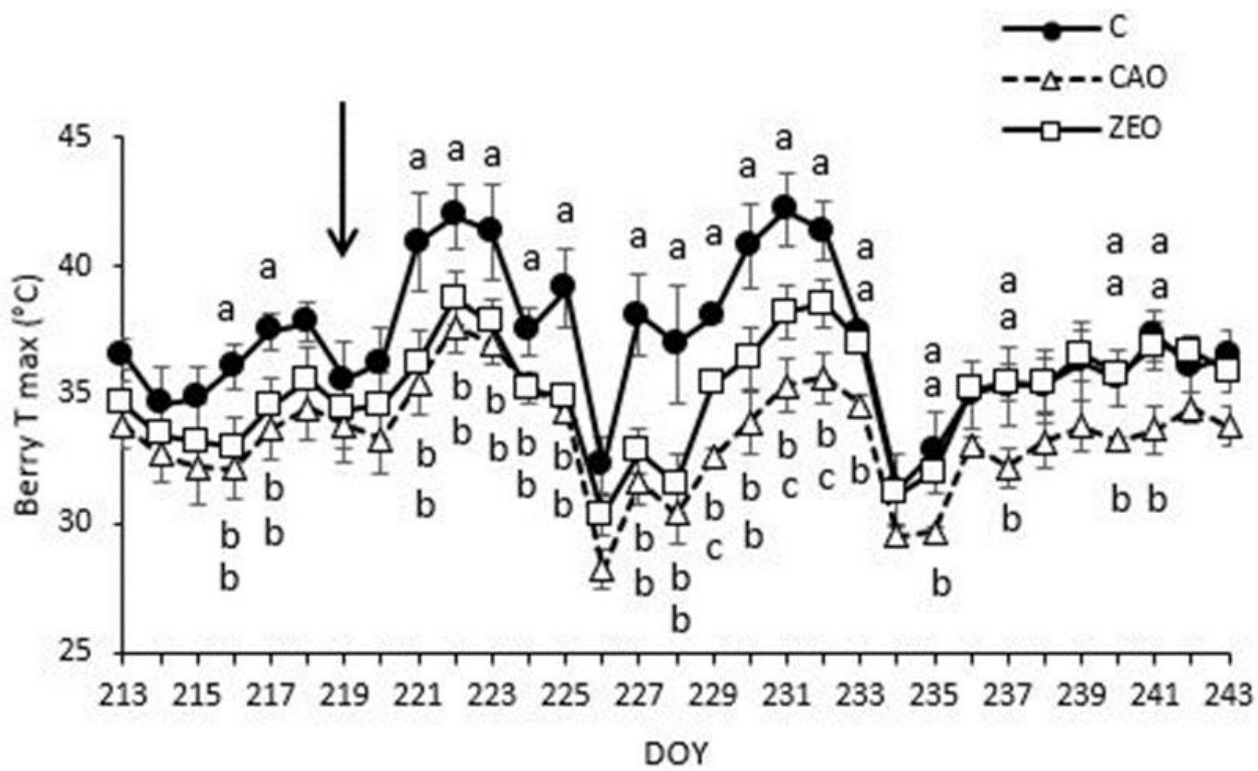

Figure 1. The trend of berry maximum temperature (°C) measured using thermo probes in August 2019. The values are presented as mean ± standard error (SE) (n = 8 per treatment). Different letters indicate significant differences between treatments according to the Tukey test (p ≤ 0.05). The arrow indicates the date of the second treatment with the mineral particles (Valentini et al., 2021).

However, the effects of the two treatments on berry yield and technological composition were similar with one exception for the kaolin thesis. Specifically, at harvest, kaolin-treated grapes showed higher organic acids than control and zeolite in both years, and it was hypothesized that this positive effect may be due to its longer-lasting sun-protective action in the hot month of August (Werblow, 1999). In fact, heatwaves during grape ripening may reduce malic acid content due to intense enzymatic activity as berry temperature increases (Ruffner et al., 1976; Sweetman et al., 2014). In conclusion, over the two-year period, the application of kaolin and zeolite increased the concentration of phenolic compounds in the berries - by up to 20% compared with the untreated control - and this result was translated into better wine color.

The mist-irrigtion system to prevent sunburn damage

The mist-irrigation was extensively studied in the 1970s, promoted as an efficient method for reducing plant water stress (Howell et al., 1971). Subsequently, overhead sprinklers were employed in various cultivations to reduce heat (Chesness and Brau 1970; Robinson, 1970; Gilbert 1970). Additionally, automated overhead sprinklers in grapevines were primarily focused on preventing leaf overheating and preserving functionality based on available knowledge of the energy balance (Pitacco et al., 1999). Moreover, the threshold for sprinkler activation varied in literature according to vineyard location and varieties: 27 °C for both Pinot Noir and Chardonnay (Pitacco et al., 1999), 30 °C for Cardinal, Carignan and Riesling (Kliewer and Schultz 1973), 32 °C in Chardonnay, Riesling and Chenin Blanc (Aljiburi et al., 1970) and 35 °C in Semillon (Greer and Weedon, 2014). The fruit-zone misting system, in line with Caravia et al. (2017), is designed to target the cluster but distinguishes itself in two key aspects. Firstly, it is positioned below the cordon, preventing direct interaction between fog and foliage while facilitating mechanical canopy management. Secondly, it imposes a thermal limit of 35 °C instead of 38 °C. This deviation is significant, considering that numerous metabolic processes, such as flavonoid synthesis, tend to decelerate or cease at temperatures around 35 °C (Jones, 2012; Pastore et al., 2017). As highlighted by other authors, the recent approaches were developed to induce evaporative cooling in grapevine canopies, reducing water consumption, minimizing leaf wetting and thereby mitigating plant diseases (Matthias and Coates, 1986; Geer and Weedon, 2014; Caravia et al., 2017). Building on these principles, an automated cooling system for the fruit-zone was implemented in the experimental vineyard of the University of Bologna over 2022 and 2023 season. The system underwent testing on potted red varieties ('Sangiovese' and 'Montepulciano') and the field-grown white variety 'Pignoletto,' as detailed in Chapters 2 and 3. These trials sought to assess the impact of misting on gas exchange, yield and berry composition.

Aims and structure of the PhD thesis

Following the state of the art, the core of the thesis is structured into three chapters focusing on the three experiments conducted during my Ph.D. research period. The thesis includes a general discussion and conclusions. The objectives of the three experiments are outlined as follows:

Experiment 1: (Chapter 2) - Introducing a novel fruit-zone cooling system aimed at improving microclimate conditions for grape ripening, especially under conditions of water, thermal, and radiative stresses. The fully automated system underwent testing on 'Sangiovese' and 'Montepulciano' grapes cultivated in pot conditions. The goal was to assess the impact of misting on gas exchanges, yield production, and berry composition for these widely cultivated red grape varieties in Italy.

Experiment 2: (Chapter 3) - The experiment aimed to evaluate the fruit-zone cooling system under field conditions, specifically investigating the effects of misting on yield production, sunburn damage, and berry attributes of 'Pignoletto' white grapes exposed to thermal-radiative stress.

Experiment 3: (Chapter 4) - This study aimed to assess the potential impact of foliar application of chabasite-rich zeolitite on fruit cooling, gas exchange, and the grape and wine composition of the 'Sangiovese' red variety. The analysis was conducted both in potted conditions and in open fields over three growing seasons characterized by frequent episodes of intense heatwaves.

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Chapter 2.

Use of an automatic fruit-zone cooling system to cope with multiple summer stresses in Sangiovese and Montepulciano grapes

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Abstract

Grapevines are frequently subjected to heatwaves and limited water availability during ripening. These conditions can have consequences for the physiological health of the vines. Moreover, the situation is often exacerbated by intense solar radiation, resulting in reduced yield due to sunburn and a decline in quality. In light of these challenges, our study aimed to develop a fruit-zone cooling system designed to mitigate grape sunburn damage and improve the microclimate conditions within the vineyard.

The system comprises a network of proximal sensors that collect microclimate data from the vineyard and an actuator that activates nebulizers when the temperature exceeds the threshold of 35 °C. The research was conducted over two years (2022 and 2023) in Bologna (Italy) using potted Sangiovese and Montepulciano vines. These two vintages were characterized by high temperatures, with varyin amounts of rainfall during the test period, significantly impacting the evaporative demand, which was notably higher in 2023. Starting from the veraison stage we compared three treatments: Irrigated control vines (WW); Control vines subjected to 50% water restriction during the month of August (WS); WS vines treated with nebulized water in the bunch area during the stress period (WS+FOG).

The application of nebulized water effectively reduced the temperature of both the air around the clusters and the clusters themselves. As we expected, Montepulciano showed better single leaf assimilation rate and stomatal conductance under non-limiting water conditions than Sangiovese while their behavior was unaffected under water-scarce conditions. Importantly, for the first time, we demonstrated that nebulized water positively affected gas exchange in both grape varieties. In

addition to this, the vines treated with the misting system exhibited higher productivity compared to WS vines without affecting technological maturity. In the 2023 vintage, the activation of the system prevented the ripening blockage that occurred in Montepulciano under water stress. Regarding the concentration of total anthocyanins, a significant increase in color was observed in WS+FOG treatment, suggesting a predominant role of microclimate on anthocyanin biosynthesis and reduction of oxidative phenomena. In conclusion, the fruit-zone cooling system proved to be an invaluable tool for mitigating the adverse effects of multiple summer stresses.

Keywords: anthocyanin, climate change, phenolic maturity, precision irrigation, viticulture, water stress

Introduction

Sangiovese and Montepulciano (*Vitis vinifera* L.) are the main red varieties grown in Italy (IOVW, 2017). Sangiovese is grown in central and northern Italy and reached 50 million hectoliters of production in 2022 (IOVW, 2022). Montepulciano, on the other hand, is a variety grown in central-southern Italy that becomes part of many red and rosé wines with appellations of origin such as Montepulciano d'Abruzzo and Cerasuolo d'Abruzzo. In the past few decades, Italian vineyards have faced heightened challenges due to pronounced exposure to intense solar radiation, elevated vapor pressure deficits (VPDs) and soaring temperatures, particularly during the crucial ripening phase in post-veraison (Jones et al., 2005; Mariani et al., 2009; Tomasi et al., 2011; Di Carlo et al., 2019). Consequently, indigenous grape varieties are experiencing increasingly harsh conditions during their reproductive stage, leading to a spatial redistribution phenomenon. This has resulted in a notable geographical shift, with areas conducive to viticulture moving towards cooler regions (Van Leeuwen, 2020; Lereboullet et al., 2013). A notable case in point is observed in Abruzzo region (Italy) where the historical harvest period for Trebbiano abruzzese extended from the second week of October during the 1800s and 1900s (Di Carlo et al., 2019). In contemporary times, however, the harvest of this variety begins in the second week of September, a strategic adjustment aimed at mitigating the adverse effects of heat on berry ripening. This alteration poses a potential risk to the quality of the grapes, as they tend to accumulate higher sugar content, potentially compromising their aromatic qualities (Palliotti et al., 2014).

According to several authors, *Vitis vinifera* L. exhibits a general anisohydric behavior in conditions of water scarcity and thermo-radiative excess (Tombesi et al., 2016; Valentini et al., 2022). In greater detail, the mentioned grape varieties showcase distinct responses to drought conditions, particularly in terms of stomatal control: Montepulciano (near-isohydric) demonstrates nearly complete stomatal closure at leaf potentials less negative than those observed in Sangiovese, which is considered anisohydric (Dal Santo et al., 2016). This mechanism aims to prevent excessive tissue dehydration and protects the plant vessels from the risk of hydraulic failure (Tyree and Sperry, 1988). Consequently, this adaptation to environmental stimuli primarily involves stomatal behavior to limit water loss while optimizing CO_2 assimilation (Schultz, 1996; Bota et al., 2001). Nevertheless, under heightened stress conditions, vines often exhibit leaves with chlorosis and necrosis, leading to a significant reduction in marketable yield due to sunburn (Dinis et al., 2016). This condition results in grapes with a low organic acid content, high pH and poor color due to anthocyanin degradation (Allegro et al., 2020).

To reduce these adverse climate effects, some short-term agronomic techniques are now available, such as foliar application of kaolin and zeolite (Calzarano et al., 2019; Valentini et al., 2021) and smart irrigation (Costa et al., 2016; Fraga et al., 2018). The mist-irrigation application was extensively studied in the 1970s, when it was promoted as an efficient method for reducing plant water stress (Howell et al., 1971). In particular, the use of overhead sprinklers in various cultivations triggered satisfactory results in heat reduction (Chesness and Brau 1970; Robinson, 1970; Gilbert 1970). Therefore, an ultra-fine misting system was developed to induce evaporative cooling in grapevine canopies, reducing both water consumption and leaf wetting, and in turn plant diseases (Matthias and Coates, 1986). Interesting results on thermal control were obtained on Cabernet Sauvignon with an evaporative cooling system placed inside the canopy activated manually based on weather forecasts (Caravia et al., 2017) and in Semillon by the Hydrocooling system (Greer et al., 2014).

Starting from these assumptions, a new fruit-zone cooling system was implemented in the experimental vineyard of the University of Bologna, in which multiple stresses often occur in summer, to improve the microclimate conditions of the grapes during the ripening. The fully automated system was tested on potted Sangiovese and Montepulciano varieties to verify the effects of misting on gas-exchanges, yield and berry composition.

Materials and Methods

Plant material and experimental design

The trials were carried out over the 2022 and 2023 seasons on potted Sangiovese and Montepulciano vines at the experimental station of the University of Bologna (Bologna, 44°32'N, 11°22'E). Plant material consisted of Sangiovese (clone TEA 10D) and Montepulciano (clone R7) vines, both grafted onto 110R rootstock, spaced at 1 m within the row and 2.5 m between rows. The vines were oriented northeast to southwest and trained to a vertical shoot positioned spur-pruned cordon. Winter pruning left 10 buds while during spring 10 shoots per vine were left. The pot experimental design included 30 vines uniformed in cluster number and randomly distributed (RCD). The vines were planted in 2019 on 30 L pots filled with a soil mixture (39% sand, 39% silt and 22% clay) with an organic matter content of 1.8% and pH of 7.8. Field capacity and wilting point were calculated after Saxton and Willey (2005) and set at 0.29 cm^3/cm^3 and 0.14 cm^3/cm^3, respectively. The pots were kept well-watered until veraison, which occurred on the day of the year (DOY) 214 in 2022 and DOY 213 in 2023, providing the amount of water lost through transpiration – approximately 4 L day^{-1} of water – distributed automatically through a dripper irrigation system. From this date and throughout berry ripening, the vines underwent different irrigation treatment depending on the following treatments: well watered vines that received 100% of the water lost by transpiration (WW); vines that received 50% of the water lost by transpiration (WS) until DOY 236 in 2022 and DOY 235 in 2023 respectively; vines that received 50% of the water lost by transpiration as WS and subjected to misting irrigation in the cluster zone (WS+FOG). In addition, each pot was entirely covered with both aluminum foil to avoid overheating and a plastic cover to avoid receiving rainwater and limiting soil evaporation. Each vine was fertilized with 10 g NPK Nitrophoska Gold (15-9-15) and the viticultural practices typical for the Emilia Romagna region were applied.

Fruit zone cooling system characteristics

The fruit-zone cooling system was composed of both a wireless sensor network (WSN) able to acquire the microclimate data within the canopy and an actuator that triggers the nebulizers when the air temperature exceeds the threshold of 35 °C. Additionally, the system could also be programmed to activate misting according to a Vapour Pressure Deficit (VPD) threshold calculated in real-time at the cluster zone thanks to its hardware part and the dedicated software. Furthermore, the system is composed of a hardware part: (a) a control unit powered by batteries recharged by a solar panel and connected to a network of sensors capable of continuously recording the relative humidity and temperature values around the fruit-zone (iFarming srl, Ravenna, RA, Italy); (b) a pipeline equipped with nebulizers which, at the operating pressure of 3.5 Bar, deliver drops of 50–55 microns in diameter. Each vine was equipped with a fogger (mod. RIVULIS F.L.F., Rivulis Irrigation, Gvat, Israel) with a flow rate of 10.4 L h^{-1}. The fruit-zone cooling system has been located under bunch area (10 cm under the cordon) to limit the direct contact between water and leaves. The software part analyzed the microclimate data collected by the nodes, and sent the impulses to a solenoid valve that automatically regulated the opening and closing of the misting system. The air temperature of 35 °C was chosen for turning on the system. Water was applied with the following cycle: on for 5 minutes, off for 15 minutes. At the end of each cycle, the system performed another air temperature check. Since the spacing both along and between rows was 1.0 m x 2.5 m, each vine received approximately 1 mm of water per hour (equivalent to 15 minutes of water spray).

Weather data acquisition

The weather conditions were recorded by a meteorological station annexed to the experimental vineyard (iFarming Srl, Ravenna, RA, IT). However, for the microclimate detail, six digital thermal probes and relative humidity sensors (iFarming, Ravenna, RA, Italy) were placed in the fruit zone within the canopy of the control and sprayed vines to detect the effect of misting on air temperature. Each sensor was then connected to a control unit to analyze in real-time, every 10 minutes, the microclimate data recorded by the sensor. Only for the year 2023, six thermocouples were inserted under the skin of WS and WS+FOG berries to continuously evaluate the effect of misting on fruit temperature. The thermocouples (iFarming srl, Ravenna, Italy) were inserted on berries of clusters distributed east, west and inside the canopy.

Physiological Measurements

The leaf stomatal conductance (gs), the net photosynthesis (Pn), the intrinsic water-use efficiency (WUEi=Pn/gs) and the substomatal CO_2 concentration (Ci) were evaluated using a portable gas exchange Li-Cor 6400 system (Li-Cor Inc., Lincoln, NE, USA) on three well-exposed main leaves per vine inserted between nodes 6 and 10. Leaf gas-exchange measurements were taken around midday on DOY 222 and 235 during 2022 year. Gas-exchanges were also assessed on DOY 219 and 223 during the 2023 season. These measurements were made with the precaution of shutting down the misting system for 45 minutes. On the same dates, the mean berry temperature (Tberry) was measured using an infrared thermometer (mod. Raynger ST, Raytek, Santa Cruz, CA, USA). Readings were taken on exposed and dry clusters (10 recordings per vine, 50 per treatment). At midday, the vine water status was evaluated by measuring the stem water potential (Ψstem) using a Scholander pressure chamber (Soilmoisture Corp., Santa Barbara, CA, USA). For each variety and dates a total of 15 leaves (five vines, one leaf per vine) per treatment were measured between 13:00 and 14:00 hours. Measurements were taken on leaves covered with aluminum foil and enclosed in a plastic bag for 90 minutes before reading.

Yield components and berry composition

At harvest, the clusters of tagged vines were counted and weighed to obtain yield. Moreover, 20 berries per vine (100 berries per treatment, 300 per each variety) were collected to analyze the following variables: total soluble solids concentration (TSS), using a self-compensating Maselli R50 refractometer (Misure Maselli, Parma, Italy); must pH and titratable acidity (TA), using a Crison titrator (Crison Instruments, Barcelona, Spain). At the same time, 20 berries for each vine were collected to evaluate total anthocyanins as reported by Mattivi et al. (2006). Anthocyanin analysis were performed with a Waters 1525 HPLC (Waters, Milford, MA) equipped with a diode array detector and a Phenomenex reversed-phase column with pre-column (Phenomenex, Castel Maggiore, Italy). Anthocyanins were quantified at 520 nm using an external calibration curve with malvidin-3-glucoside chloride as the standard (Sigma-Aldric, St. Louis, MO, USA). Sampling days were different for the two varieties under comparison: DOY 252 for Montepulciano and DOY 222, 234, 249 for Sangiovese in 2022; DOY 250 for both varieties in 2023. During winter the wood pruned from each vine was weighed (data were not reported because no difference was detected).

Statistical analysis

Data were processed for each variety by the analysis of variance over year using the mixed procedure of SAS v 9.0 (SAS Institute, Inc., Cary, NC, USA) and the treatment comparisons were analyzed using Tukey test with a cut-off at $P \leq 0.05$.

Results

Weather conditions

The 2022 season was characterized by warm and dry conditions, with a total of 260 mm of rainfall recorded from April to October. The weather data recorded during the entire trial period showed persistent heatwave during the first week of August, with air temperatures exceeding 35°C. In contrast, the third week of the month witnessed substantial rainfall as reported in Figure 1. Specifically, the experimental station documented 101 mm of rain during the stress period, as illustrated in Figure 1. In contrast, the 2023 season exhibited a different pattern, receiving 55 percent more rainfall than 2022, totaling about 560 mm during the vegetative months from April to October. Analyzing the thermo-pluviometric trend during the trial period, the first half of August was notably more humid, followed by severe heatwaves in the latter part of the month, where temperatures exceeded the established thermal threshold of 35 °C (Figure 1). Unlike the 2022 vintage, 2023 witnessed reduced rainfall, accounting for only 10% of the previous year's precipitation during the test period (10 mm versus 101.2 mm in 2023 and 2022, respectively). Notably, the difference between the seasons was the higher monthly relative humidity in the 2022 vintage, which had an impact on the vapor pressure deficit (VPD) throughout the entire test period.

Differences in 2022 and 2023 environmental conditions generated changes in system activation levels between the two observational periods. Specifically, during the 2022 season (Figure 1A), the cumulative water distribution reached an approximate value of 10 mm by the end of the trial period. This accumulation resulted from the misting system being activated on seven specific days at the beginning of August. This activation pattern was prompted by elevated air temperatures and a scarcity of rainfall events during this period. Conversely, the 2023 season exhibited a substantial increase in the cumulative water distributed, approximately 45 mm, concentrated in the latter part of the trial period spanning from DOY 222 to DOY 236 (Figure 1B). This is due to the higher frequency of system activations occurred in 2023, 15 vs the 7 days of 2022.

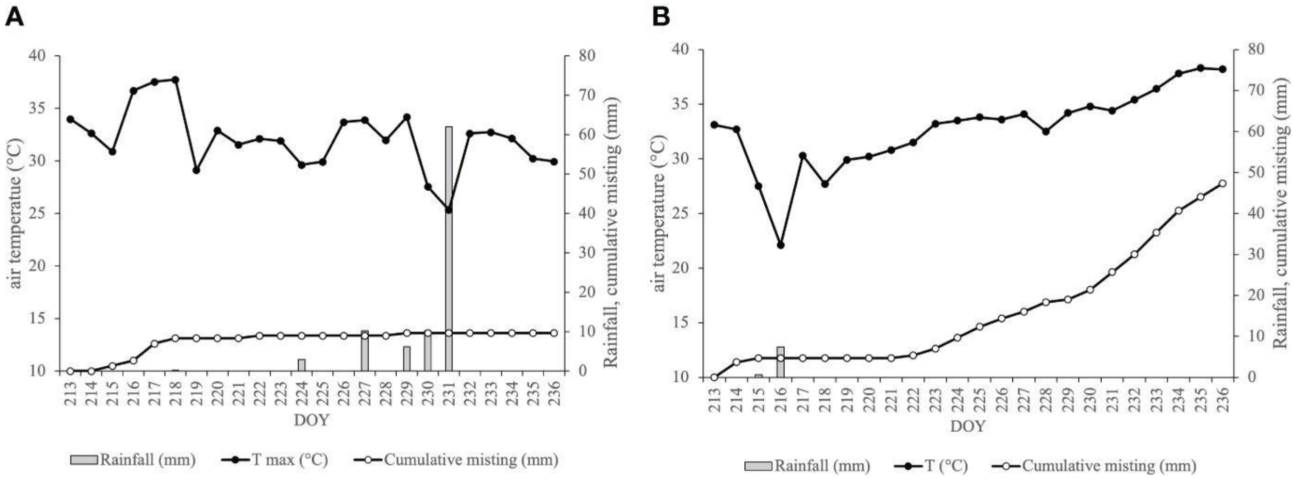

Figure 1. The cumulative misting (grey line, mm), the trend of air maximum temperature (black line, °C) and the rainfall (grey bars, mm) at a meteorological station during August (2022, A; 2023, B)

Microclimatic and Physiological Response

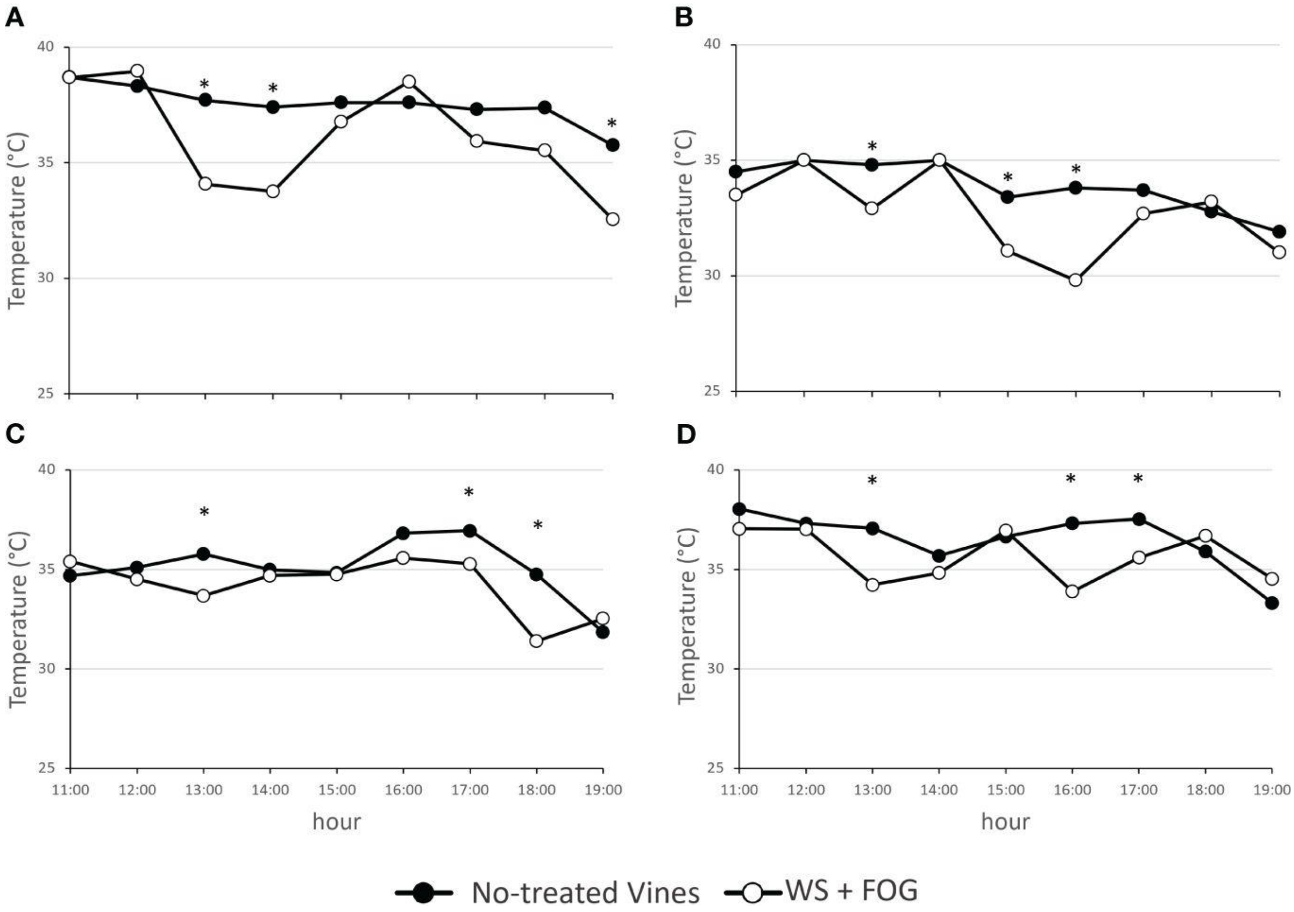

Figure 2. The trend of air temperature within the canopy on the fruit-zone during the warmest part of the day, from 11:00 to 19:00 hours, in untreated WW and WS vines (no-treated vines) and in vines treated with misting water (WS+FOG) in two representative days of 2022 (A, DOY 216; B, DOY 222) and of 2023 (C, DOY 228; D, DOY 233) seasons.

Figure 2 displays the trend of fruit-zone air temperature during the warmest part of the day, demonstrating a reduction in WS+FOG vines ranging from 1.8 to 3.6 °C in both seasons, compared to untreated vines. As described above, when the canopy air temperatures exceeded 35 °C, the system was capable of reducing the air temperature around the clusters.

In detail for 2022, the system performed 4 cooling cycles during DOY 216 and only 2 cycles for the DOY 222 (Figures 2A, 2B). In contrast, the system's response to the warm 2023 was different. In this case, 7 and 16 5-min misting water distribution for DOYs 228 and 233 were recorded (Figure 2C, 2D). In the same year, there is also evidence of a thermal rise in the afternoon starting at 15:00 h and a prompt response of the system to bring the temperature back below the thermal limit.

The fruit-zone cooling system was also effective on preserving leaves efficiency, shielding them from the adverse effects of high temperatures and maintaining their fundamental physiological functions. Specifically, the measurements, in two representative days during the trial for each year, showed that when the system was activated, WS+FOG exhibited higher values of Pn and gs compared to WS alone (Table 1 and Table 2), achieving good levels, despite in some cases significantly lower as compared to the irrigated control (WW). Moreover, especially during the first year of the trial, activating the misting system resulted in a positive response in terms of gas exchange for WS+FOG, almost in line with irrigated control (WW). The WS vines instead exhibited a 50% reduction in net photosynthesis in both varieties, accompanied by a significant decline in stomatal conductance (Table 1, Table 2). The contrasting behavior is highlighted by water use efficiency (WUEi). Specifically, WUEi demonstrated a notable rise in vines subjected to water stress (WS) compared to well-watered (WW) and WS combined with fog (WS+FOG) treatments in both cultivars. Particularly noteworthy is the doubling of WUEi under WS conditions in the Sangiovese cultivar, as illustrated in Table 1. Regarding Ci, the substomatal concentration of CO_2, it consistently decreased under WS conditions compared to the other treatments in both varieties. Checking the response of the varieties it becomes clear that after nearly 10 days of water restriction (DOY 222, Table 1), gas exchanges did not vary among Sangiovese and Montepulciano. However, this pattern changed after 20 days (DOY 235, Table 2) when WW-Sangiovese, exhibited lower values of Pn and gs compared to WW-Montepulciano, and the same trend was confirmed for the WS+FOG treatment (Table 2). The values of the WS vines remained significantly lower than the other treatments for both varieties under investigation, highlighting the strong multiple and synergistic effect of heat and water stress in vine physiology. This trend resulted in improved water use efficiency for water-stressed (WS) conditions ($p=0.07$) compared to the other treatments. Additionally, on DOY 235, the Sangiovese cultivar demonstrated significantly higher WUEi than Montepulciano (Table 2). Notably, on this day of physiological measurements, the substomatal concentration of CO_2 was significantly lower compared to the other treatments in both varieties (Table 2).

Treatment	Sangiovese			Montepulciano			Significance		
	WW	WS	WS + FOG	WW	WS	WS + FOG	var	trt	interactions
Pn	19.4 a	6.7 c	15.5 b	21.8 a	10.9 c	17.0 b	*ns*	***	*ns*
g_s	0.217 a	0.039 c	0.191 b	0.242 a	0.082 c	0.190 b	*ns*	***	*ns*
WUEi	89 b	170 a	82 b	90 b	139 a	94 b	*ns*	***	*ns*
Ci	223 a	106 b	236 a	219 a	152 b	216 a	*ns*	***	*ns*
Ψ_{stem}	-4.8 a	-8.0 c	-7.2 b	-4.1 a	-10.0 c	-6.4 b	*ns*	***	*ns*
T_{berry}	34.2 b	39.2 a	32.8 c	33.7 b	36.2 a	32.6 c	*ns*	*ns*	*var x trt* *

Table 1. Effect of mist cooling system on net photosynthesis (Pn, µmolm-2s-1), stomatal conductance (gs, molm-2s-1), intrinsic water-use efficiency (WUEi, µmolCO_2mol-1H_2O), substomatal CO_2 concentration (Ci, µmolCO_2mol-1air), stem water potential (Ψstem, Bar) and berry temperatures (Tberry, °C) recorded at midday period on potted Sangiovese and Montepulciano vines. Year 2022, August 10th (DOY 222). Means within rows designated by different letters indicate significant differences among treatments.

In the presence of significant variations among multiple fixed factors and the occurrence of interactions, differences between varieties (var) and treatments (trt) are denoted by asterisks according to ANOVA test (, P < 0.05; **, P < 0.01; ***, P < 0.001, ns=not significant). Pairwise separation was performed by Tukey test.*

Treatment	Sangiovese			Montepulciano			Significance		
	WW	WS	WS + FOG	WW	WS	WS + FOG	var	trt	interactions
Pn	15.8 a	7.5 c	9.1 b	19.6 a	7.8 c	14.7 b	*ns*	*	*ns*
g_s	0.141 a	0.037 b	0.100 a	0.290 a	0.036b	0.223 a	*ns*	*ns*	*var x trt* **
WUEi	89	172	81	90	133	89	*	*ns*	*ns*
Ci	239 a	61 b	236 a	284 a	30 b	292 a	*ns*	***	*ns*
Ψ_{stem}	-5.8 a	-16.5 b	-7.0 a	-6.5 a	-16.5 b	-7.9 a	*ns*	*	*ns*
T_{berry}	34.1 b	36.6 a	29.5 d	34.6 b	35.4 a	31.3 c	*ns*	*ns*	*var x trt* *

Table 2. Effect of mist cooling system on net photosynthesis (Pn, µmolm-2s-1), stomatal conductance (gs, molm-2s-1), intrinsic water-use efficiency (WUEi, µmolCO_2mol-1H_2O), substomatal CO_2 concentration (Ci, µmolCO_2mol-1air), stem water potential (Ψstem, Bar) and berry temperatures (Tberry, °C) recorded at midday period on potted Sangiovese and Montepulciano vines. Year 2022, August 23th (DOY 235). Means within rows designated by different letters indicate significant differences among treatments. In the presence of significant variations among multiple fixed factors and the occurrence of interactions, differences between varieties (var) and treatments (trt) are denoted by asterisks according to ANOVA test (, P < 0.05; **, P < 0.01; ***, P < 0.001, ns=not significant). Pairwise separation was performed by Tukey test.*

Table 1 and Table 2 also show the effect of the treatments on the vine water status (Ψstem) and the temperature of the berries. The value of stem water potential follows the same trend as stomatal conductance: the least negative values are recorded in WW treatments, the most negative in WS theses, while WS+FOG reaches an intermediate value, indicating a significant effect of misting on this parameter. The thermal response of berries to treatments differs. Specifically, WS+FOG reduced the berry temperature below the thermal threshold of 35 °C on both the two survey days. This thermal difference averaged 6-7 °C for Sangiovese and 4 °C for Montepulciano compared to WS. WW took an intermediate value for the two varieties but it was still significantly lower than WS (Table 1 and Table 2).

The same variables investigated in 2022 were evaluated in two representative days (DOY 219 and 223) of 2023, picked within a period characterized by severe heatwaves and very low rainfall (Table 3 and Table 4). In contrast to the previous year, one week after setting irrigation restriction, net assimilation did not appear to differ between treatments. Instead, a significant difference between the compared varieties was recorded, with Montepulciano showing better photosynthetic efficiency than Sangiovese (Table 3). Specifically, the reduction in irrigation (-50% of water lost through transpiration in WS and WS+FOG compared to WW) led to a notable decrease in both stomatal conductance and stem water potential. Simultaneously, there was an observed increase in water use efficiency and a decrease in substomatal CO_2 concentration (Table 3). No significant differences were found between treatments in berry temperature because - at the time of the measurement - the system had not been activated since the air temperature was below the threshold, 35° C (Table 3, Figure 1B). Vine responses changed with the increasing number of days of water stress (DOY 223), as highlighted in Table 4. In detail, the occurrence of multiple summer stress factors (thermal and radiative) associated with water scarcity, negatively affects the efficiency of photosystems and stomatal activity in WS and WS+FOG. Both treatments indeed exhibit near-zero assimilation and stomatal conductance values below 0.05 mol m^{-2} s^{-1}. Moreover, there was no discernible differences in water use efficiency. Surprisingly, the Ci for these treatments under irrigation restriction exceeded the values observed in well-watered conditions (WW) for both varieties (Table 4). In particular, the vines subjected to water restriction exhibited a decrease in stem water potential, reaching values almost doubled compared to the irrigated control (Table 4). In terms of berry temperature, the activation of the misting system has allowed lowering the temperature of the berries by about 3 °C compared to WS and 1 °C compared to WW for both cultivars (Table 4).

Treatment	Sangiovese			Montepulciano			Significance		
	WW	WS	WS + FOG	WW	WS	WS + FOG	var	trt	interactions
Pn	10.5 b	7.8 b	8.8 b	10.7 a	10.1a	10.5 a	*	*ns*	*ns*
g_s	0.202 a	0.101 b	0.127 b	0.207 a	0.136 b	0.162 b	*ns*	***	*ns*
WUEi	51 b	77 a	70 a	52 b	74 a	64 a	*ns*	***	*ns*
Ci	288 a	247 b	266 b	295 a	249 b	260 b	*ns*	***	*ns*
Ψ_{stem}	-6.2 a	-10.0 b	-8.3 b	-4.1 a	-9.5 b	-8.1 b	*ns*	*	*ns*
T_{berry}	28.8	28.3	28.0	27.0	27.0	26.5	*ns*	*ns*	*ns*

Table 3. Effect of mist cooling system on net photosynthesis (Pn, µmolm-2s-1), stomatal conductance (gs, molm-2s-1), intrinsic water-use efficiency (WUEi, µmolCO_2mol-1H_2O), substomatal CO_2 concentration (Ci, µmolCO_2mol-1air), stem water potential (Ψstem, Bar) and berry temperatures (Tberry, °C) recorded at midday period on potted Sangiovese and Montepulciano vines. Year 2023, August 7th (DOY 219). Means within rows designated by different letters indicate significant differences among treatments. In the presence of significant variations among multiple fixed factors and the occurrence of interactions, differences between varieties (var) and treatments (trt) are denoted by asterisks according to ANOVA test (, P < 0.05; **, P < 0.01; ***, P < 0.001, ns=not significant). Pairwise separation was performed by Tukey test.*

Treatment	Sangiovese			Montepulciano			Significance		
	WW	WS	WS + FOG	WW	WS	WS + FOG	var	trt	interactions
Pn	11.8 a	1.0 b	1.5 b	12.4 a	1.5 b	3.7 b	***	***	*ns*
g_s	0.230 a	0.027 b	0.049 b	0.203 a	0.022 b	0.047 b	*ns*	***	*ns*
WUEi	60	40	61	62	72	75	*ns*	*ns*	*ns*
Ci	275 b	461 a	322 b	272 b	343 a	294 b	*ns*	***	*ns*
Ψ_{stem}	-10.3 a	-18.5 c	-14.1 b	-9.6 a	-18.6 c	-12.3 b	*ns*	**	*ns*
T_{berry}	34.8 b	36.4 a	33.7 c	34.9 b	37.3 a	33.7 c	*ns*	***	*ns*

Table 4.. Effect of mist cooling system on net photosynthesis (Pn, µmolm-2s-1), stomatal conductance (gs, molm-2s-1), intrinsic water-use efficiency (WUEi, µmolCO_2mol-1H_2O), substomatal CO_2 concentration (Ci, µmolCO_2mol-1air), stem water potential (Ψstem, Bar) and berry temperatures (Tberry, °C) recorded at midday period on potted Sangiovese and Montepulciano vines. Year 2023, August 11th (DOY 223). Means within rows designated by different letters indicate significant differences among treatments. In the presence of significant variations among multiple fixed factors and the occurrence of interactions, differences between varieties (var) and treatments (trt) are denoted by asterisks according to ANOVA test (, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, ns=not significant). Pairwise separation was performed by Tukey test.*

To gain a deeper insight into the impact of the fruit-zone cooling system on cluster temperature, in 2023, thermocouples were placed on various berries associated with both WS and WS+FOG theses at different points within the canopy. Figure 3 illustrates the temperature fluctuations over eleven-days in August, representative of the heatwaves period. Notably, it is evident that WS exhibited a distinct temperature trend compared to WS+FOG from solar noon to 19:00 hours. This divergence reached its peak on DOY 232, with a temperature difference of 6 °C between the two treatments. It is noteworthy that berries subjected to misting consistently maintain temperatures below the critical limit of 35 °C, as initially identified through a timely infrared thermometer survey (Figure 3).

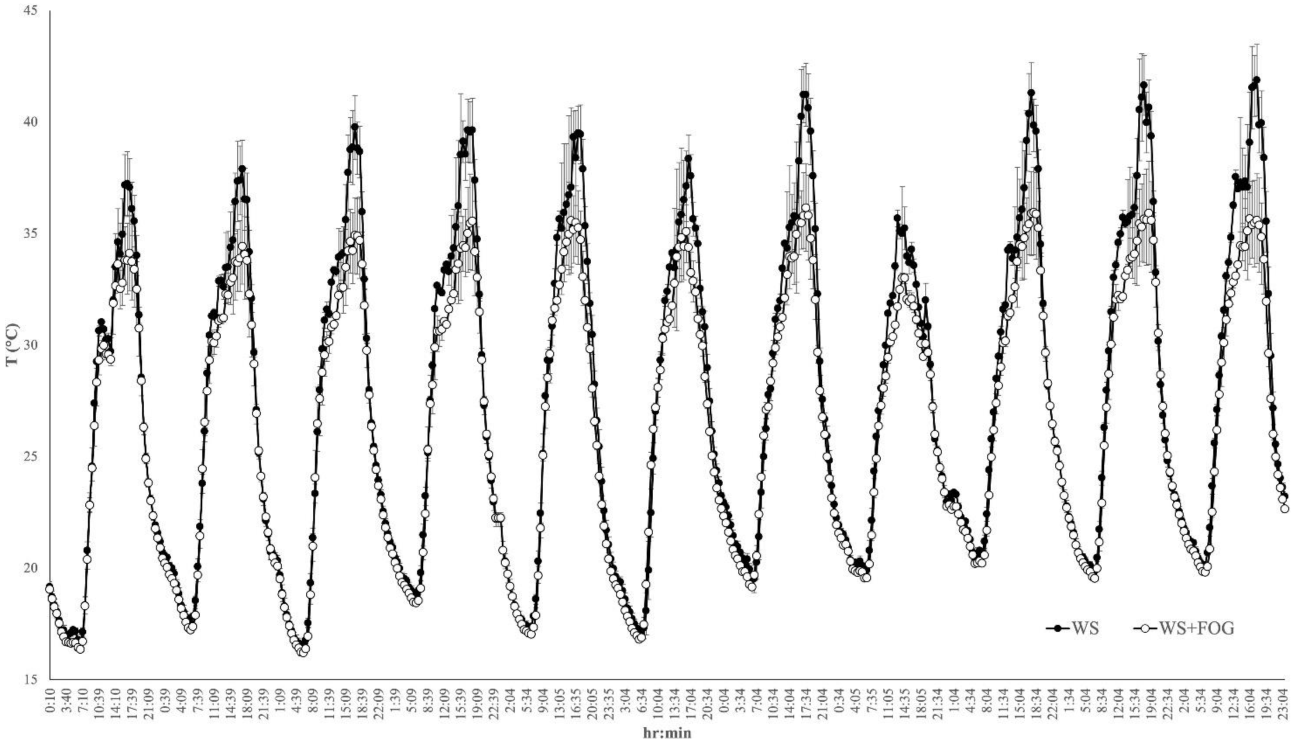

Figure 3. Trend of WS and WS+FOG berry temperatures during eleven days of August 2023 (DOY 222-DOY 232). Each data point represents the average of three thermocouples inserted into the berries facing east, west, and in the middle of the vegetative wall. Error bars indicate the standard error.

Yield components and berry composition

Despite a uniform number of clusters per vine, significant differences between treatments were reported on yield components (Table 5). Throughout the two-year trial period, a substantial 15% decrease of yield was observed in the WS thesis compared to WW in both varieties. This decline can be primarily attributed to the reduction of bunch weight, with the most pronounced effect evident in 2023 in both varieties (Table 5). The misting system emerged as a pivotal factor influencing yield. Specifically, WS+FOG demonstrated heavier clusters, aligning closely with the irrigated control, resulting in a significant yield increase compared to WS (Table 5). Conversely, when considering berry mass, a significant difference is observed between the two vintages, with 2023 exhibiting greater weights than 2022. However, the impact of treatments on the two varieties is less straightforward. Notably, WW consistently produces the largest berries, while WS yields the smallest. WS+FOG aligns with WW in berry size, particularly for Montepulciano. Conversely, it appears that the misting system does not significantly influence berry mass for Sangiovese.

Treatment	Sangiovese			Montepulciano			Significance			
	WW	WS	WS+FOG	WW	WS	WS+FOG	year	var	trt	interactions
Yield (kg vine^{-1})	1.47 a	1.27 b	1.49 a	1.56 a	1.37 b	1.64 a	*ns*	*ns*	*	*ns*
2022	1.39	1.42	1.60	1.48	1.28	1.52				
2023	1.54	1.12	1.37	1.63	1.45	1.76				
Cluster weight (g)	128.7 a	109.8 b	125.1 a	123.3 a	107.3 b	126.3 a	***	*ns*	*	*ns*
2022	109.8	108.5	117.5	110.0	103.2	113.7				
2023	147.7	110.8	132.7	136.7	111.3	138.9				
Berry mass (g)	1.74 a	1.68 b	1.66 b	1.98 a	1.51 b	1.91 a	***	*ns*	*ns*	*var x trt* *
2022	1.55	1.54	1.44	1.72	1.30	1.60				
2023	1.93	1.83	1.88	2.24	1.73	2.23				
TSS (°Brix)	22.2	22.4	22.5	23.5	22.0	22.7	*ns*	*ns*	*ns*	*year x var x trt* *
2022	20.9	21.8	21.9	23.6.	23.9	23.0				
2023	23.5	23.0	23.0	23.4	20.2	22.3				
pH	3.56 b	3.60 ab	3.67 a	3.47 a	3.44 a	3.49 a	***	*ns*	*ns*	*var x trt* *
2022	3.58	3.64	3.79	3.48	3.49	3.54				
2023	3.47	3.54	3.55	3.46	3.39	3.44				
TA (g L^{-1})	6.77	6.89	6.20	6.13	6.54	6.04	***	**	*ns*	*ns*
2022	6.59	6.29	5.32	5.57	5.95	5.15				
2023	6.94	7.48	7.07	6.68	7.52	6.93				
Total anthocyanins (mg kg^{-1})	638.8 b	580.5 c	759.2 a	1420.3 b	1308.7 c	1547.4 a	*	*	*	*ns*
2022	519.9	462.5	735.4	1353.6	1298.3	1578.8				
2023	847.7	698.4	783.0	1487.0	1319.0	1516.0				

Table 5. Yield attributes and berry composition parameters measured at harvest in Sangiovese and Montepulciano vines over 2022-2023 seasons (TSS = total soluble solids, TA = total acidity). Means within rows designated by different letters indicate significant differences among treatments (n=5). In the presence of significant variations among multiple fixed factors and the occurrence of interactions, differences between varieties (var), treatments (trt), and years (year) are denoted by asterisks according to ANOVA test (, P < 0.05; **, P < 0.01; ***, P < 0.001, ns=not significant). The table presents pairwise comparisons conducted with Tukey's test for further insights into the observed distinctions.*

A year x variety x treatment interaction was observed concerning soluble solids at harvest (Table 5). To provide a clearer understanding of the results, the values for each treatment in the two test years are presented in Figure 4. The analysis of the bar chart offers several insights: in 2022, Montepulciano exhibited, on average, a higher Total Soluble Solids (TSS) value than Sangiovese, with no discernible differences between treatments. In 2023, a year marked by exceptionally high pre-harvest temperatures, notable differences in the response of the WS treatment for the two varieties became apparent. Specifically, the accumulation of TSS in Montepulciano was significantly lower than in Sangiovese, by approximately 2 °Brix (Table 5). Interestingly, this pattern did not extend to

WS+FOG, where equivalent sugar levels were reinstated for each variety, as observed in WW (Figure 4).

With regard to the pH and the acidity profile of the grapes, it is widely recognized that various factors play a role, including the vegetative development of the plants, climatic conditions before the veraison stage, berry volume and abiotic factors such as temperature. A thorough examination of the total acidity values recorded in harvested grapes reveals a notable distinction between the two years: 2022 exhibited a higher pH and consequently lower acidity level compared to 2023 (Table 5). Additionally, significant variations among grape varieties are evident, with Montepulciano displaying lower organic acid content than Sangiovese. However, treatments do not appear to exert any discernible influence on the berry acidity levels (Table 5). Microclimate conditions could have also a great impact on phenolic accumulation and composition during berry ripening (Table 5). Concerning the concentration of total anthocyanins, a significant enhancement was observed in the WS+FOG treatment compared to both WS and WW. Despite anthocyanin accumulation capacity is different between the two varieties due to genetic factors, both of them exhibited a positive response to the application of misting in the cluster zone. Specifically, WS showed a 16 to 22 percent reduction of anthocyanin concentration compared to WS+FOG in Montepulciano and Sangiovese, respectively (Table 5).

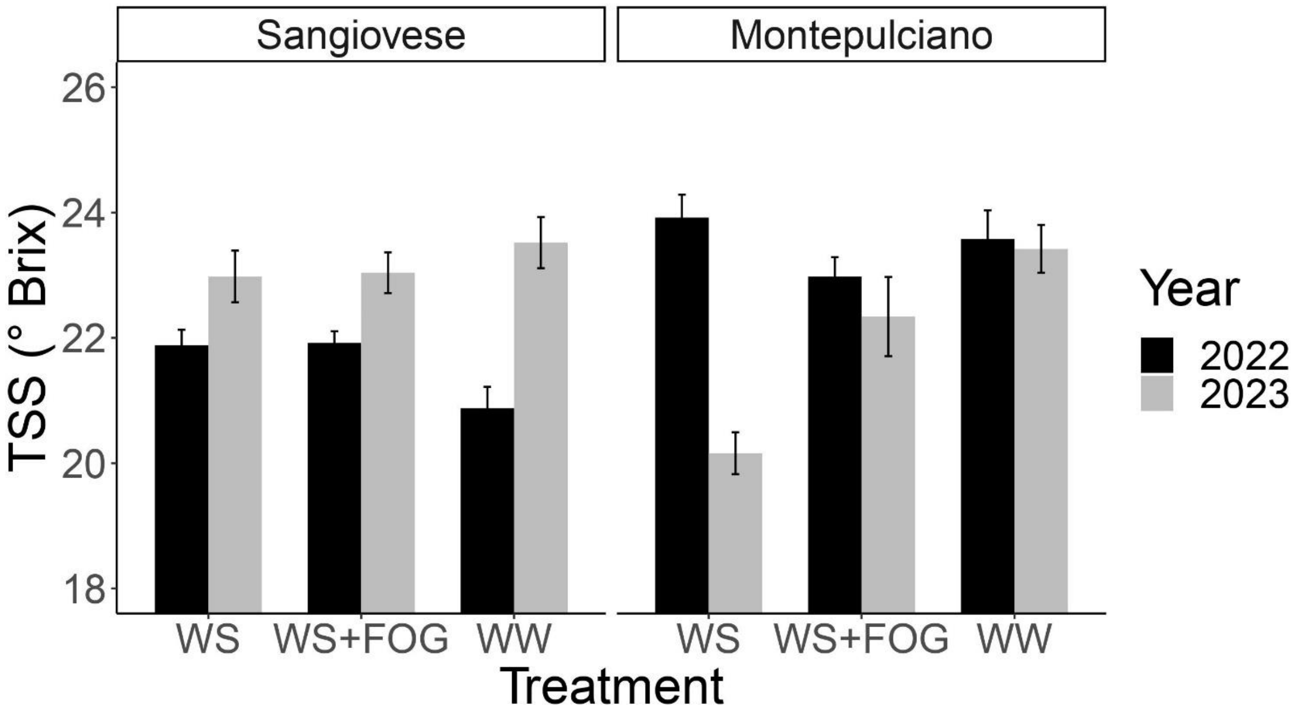

Figure 4. Bar chart illustrating the sugar concentration (TSS) at harvest for the years 2022 and 2023. The interaction among different factors - varieties, years and treatments - is evident. The two bar charts correspond to the two varieties under examination: Sangiovese and Montepulciano (n=5, error bars depict the standard error).

Discussion

In light of the proven capacity of water misting to lower the surface temperature of vine organs (Howell et al., 1971; Kliewer and Schultz, 1973), the aim of the two-year trial was to investigate the impact of an innovative fruit-zone cooling system on two grape varieties: Sangiovese and Montepulciano. As well known, these varieties are recognized for exhibiting distinct responses under conditions of both limited and unlimited water availability, particularly in the context of climate change (Palliotti et al., 2010 and 2014).

The system, originally configured to activate on the base of predetermined thresholds of vapor pressure deficit (VPD), was specifically adapted in 2022 and 2023 seasons to respond exclusively to thermal limits (T≥35 °C). This decision was thoughtfully made, taking into consideration the climatic conditions of the vineyard (Bologna, Italy) which are distinguished by almost constant humidity during summer, coupled with elevated temperatures (Teslic et al., 2019). In detail, the Mediterranean basin has been facing increasingly harsh years, consequently, vines are exposed to intense solar radiation, elevating the risk of excessive temperature rise around the fruit-zone (Poni et al., 1994; Valentini et al., 2022). This can result in reduced yields and a decline of grape quality, including anthocyanin accumulation (Orduna et al., 2010) which may be exacerbated when coupled with a substantial reduction of soil water content (Carvalho et al., 2016).

Given these challenges, the misting system was implemented at the experimental station of the University of Bologna to evaluate its impact on potted vines subjected to water restriction starting from veraison (WS+FOG) compared to both water-stressed (WS) vines and irrigated control (WW). Various parameters were meticulously assessed during this phase, encompassing not only the temperature of the fruit-zone and clusters but also gas-exchange, yield attributes and berry composition.

The innovative misting system effectively cools both the air surrounding the clusters and the clusters themselves, using minimal amount of water. Specifically, in the warm conditions of the 2022 season, the system was employed for 7 days, distributing approximately 10 mm of water cumulatively by the trial's conclusion. However, during the challenging 2023 season, elevated temperatures were recorded, leading to more frequent activations of the fruit zone cooling system and a greater volume of water distributed, especially in the latter part of August. Despite the elevated temperatures, the system performed effectively by not only cooling the air near the clusters but also maintaining the temperature of the clusters below the 35°C thermal threshold during heatwaves. These results are in line with those obtained by other similar devices designed to preserve water resources (Greer and Weedon, 2014; Caravia et al., 2016; Bianchi et al., 2022). In detail, the fruit-zone cooling system showed some innovative features: it minimized water usage through real-time thermo-hygrometric data readings (approximately 1 mm of water per hour), operated automatically and employed an advanced Wireless Sensor Network (WSN) for precise misting. In this way, two fundamental requirements for water resource conservation were met: the quantity of water used and the distribution method.

The fruit-zone cooling system demonstrated significant effects on leaf photosynthetic efficiency, alleviating the adverse impacts of high temperatures and preserving crucial physiological functions. As indicated by Zheng et al. (2021), the reduction of temperature during berry ripening positively influenced leaf gas exchanges, such as net photosynthesis. Consequently, activating the misting system to water-stressed vines yielded higher levels of photosynthesis and stomatal conductance compared to vines experiencing water stress alone. We hypothesized that the system strengthened this response by directly influencing the vapor pressure deficit (VPD) in the treated fruit zone, as reported in a previous study where the VPD of misted vines was halved compared to untreated control (Pagay et al., 2018). In our conditions, air temperature control through the fruit-zone cooling system was the main factor determining the reduction of vapor pressure deficit, with humidity generally remaining constant. The positive response of gas-exchange was particularly pronounced in the first year, almost aligning WS+FOG with the irrigated control (WW), while WS exhibited a 50 percent reduction in net photosynthesis accompanied by a decline of stomatal conductance. This phenomenon is associated with the varying microclimatic conditions within the canopy and the increased evaporative demand, impacting stomatal response in the short term. As widely recognized, stomata typically close to minimize water loss, resulting in decreased CO_2 assimilation (Flexas et al., 2002; Escalona et al., 2003). This trend was observed in WS vines, which showed an inclination towards heightened water use efficiency. Furthermore, during the 2022 season, this trend seemed correlated with the Sangiovese variety, exhibiting higher WUEi compared to Montepulciano. However, as water stress conditions intensified, exacerbated by exceptionally high temperatures experienced during the 2023 season, a notable decrease in both photosynthesis and stomatal conductance was evident (Cifre et al., 2005; Gomez del Campo et al., 2002; Souza et al., 2005; Chaves et al., 2007). While mild water stress typically depresses grapevine photosynthesis predominantly through stomatal closure (as evidenced by the improved WUEi), more severe water stress scenarios are known to involve non-stomatal inhibition of photosynthesis (Chaves, 1991; Flexas et al., 2002). During this phase, a decrease in WUEi and an increase in Ci indicate the dominance of non-stomatal limitations on photosynthesis (Cifre et al., 2005).

Additionally, the Montepulciano vines confirmed the greater physiological and productive performance compared to those of Sangiovese, under conditions of adequate irrigation (Palliotti et al., 2011). Specifically, during the 2022 season, after 20 days of water restriction, it was observed that stomatal conductance values of WS+FOG and WW were higher in Montepulciano while the response profile to WS was similar for both varieties. These results align with findings reported by Greer and Weedon (2014), emphasizing the impact of high temperatures on the Semillon variety and their depressive effect on gas-exchange. The enhanced performance of Montepulciano in comparison to Sangiovese was also evident in 2023. However, during this year, distinguishing differences between treatments subjected to water restriction became challenging due to the heightened presence of multiple stresses affecting both the WS and WS+FOG treatments (Martinez-Luscher et al., 2020).

It is widely acknowledged that grapevines are generally classified as anisohydric (Schultz, 2003), and they typically exercise precise control over stomatal opening that adapt to preserve their water status under drought conditions. This regulatory mechanism leads to a reduction of the leaf and stem water potential under conditions of water stress (Tardieu et al., 1998). Across the two years of the trial,

Ψ_{stem} exhibited a trend similar to that of stomatal conductance, with the least negative values in WW, the most negative in WS, and an intermediate value in WS+FOG, underscoring the significant impact of misting on canopy microclimate. As reported in literature, Ψ_{stem} is considered a reliable index of water status in *Vitis vinifera* L., and its values are the combination of different factors, such as VPD, soil water availability, stomatal regulation and plant hydraulic conductivity (Patakas et al., 2005). Manipulating the microclimate in the cluster zone sets off a chain reaction, influencing the plant's gas exchange processes and vine water status without directly supplying water to the soil. In our experiment, the pots were covered with plastic foil, and the water distributed by the system did not reach the ground. These findings align with research by Pagay et al. (2018) and studies on the Hydrocooling technique (Greer and Weedon, 2014), where fine mist, in direct contact with the vegetative surface, evaporates rapidly without reaching the soil. In our case, the berry temperature seems to show significant differences over time between varieties and treatments. As observed by Pagay et al. (2018), treated leaves dry faster during heatwaves (in less than one minute), unlike clusters due to their different thermal masses.

Reducing cluster temperature proved advantageous for both yield and berry composition. Notably, non-misted vines experienced a significant decrease in yield attributed to the reduction of cluster weight. This decrease was linked to an elevated occurrence of dehydration and/or necrosis phenomena, directly impacting berry mass, which are common in the ongoing climate change scenario (Bonada et al., 2013; Pastore et al., 2013; Caravia et al., 2016). Grapevines typically exhibit lower sensitivity to water stress during reproductive growth compared to vegetative growth (Korkutal et al., 2011; Williams and Matthews, 1990). However, this response may vary under climate change scenario. For instance, in severe water stress condition, bunch weight diminishes with a temperature increase of 4°C (Kizildeniz et al., 2015). This situation was also observed over the 2-year trial when the temperature gap between WS and WS+FOG reached peaks of 6°C. Additional research has highlighted that when temperatures peak at 40°C, impediments in berry growth and responses in terms of sugar accumulation occur (Greer and Weston, 2010). Specifically, Semillon berries at the veraison stage exhibited high sensitivity to heat, leading to a significantly restricted growth phase and disrupted sugar accumulation. Simultaneously, there was a sustained decline of leaf photosynthesis caused by heat, impacting the carbon supply available to meet the demands of the berries.

In our trial, soluble solids (TSS) exhibited varietal and vintage differences. In 2023, when the highest temperatures were recorded, WS-Montepulciano exhibited lower TSS, but WS+FOG vines restored similar sugar levels to WW-ones. This result is mostly associated with high temperatures during veraison and mid-ripening that affect the formation and translocation of sugars, resulting in a significant reduction (Greer and Weston, 2010). The process of sucrose loading into the grape berry involves multiple sugar transporters and sucrose metabolic enzymes. High temperatures have been found to inhibit these metabolic processes (Agasse et al., 2009). Moreover, elevated temperatures can lead to the inactivation of specific genes in the berries that play a role in the metabolism of berry ripening (Pillet et al., 2012). Montepulciano is particularly sensitive to the interaction between water scarcity and high temperatures (Tombesi et al., 2016; Dal Santo et al., 2016). Furthermore, significant differences in the response of the two varieties to the water stress imposed during veraison are evident. It is clear that what occurred in WS-Montepulciano did not occur in WS-Sangiovese, which did not show a significant decrease of soluble solids at harvest. This is attributed to the higher accumulation of ABA in the leaves of Montepulciano affecting the carbon assimilation at the whole canopy level (Soar et al., 2006; Dal Santo et al., 2016) and the excellent recovery capacity of Sangiovese during rehydration (Palliotti et al., 2014). If it is well known that the effect of water stress is more significant when imposed before veraison (Keller, 2005), this study demonstrates that when water stress acts synergistically with thermal stress, the response of sugar accumulation can vary depending on the grapevine genetics. Additionally, it suggests that fruit zone cooling can prevent a maturation halt. Furthermore, in a vintage characterized by severe water and heat stress, we can confirm that Sangiovese manages to maintain higher performance and ensure the complete ripening of the berries.

Acidity levels displayed variabilty across years, with no discernible impact from the treatments. As discussed by Sadras et al. (2012), the influence of high temperatures on titratable acidity and pH is intricate, influenced by factors such as cultivar, season and their interactions. In detail, the analysis of phenotypic plasticity unveiled distinct responses for Chardonnay, Cabernet Franc, Semillon and Shiraz, the latter showcasing a notable lack of plasticity by maintaining stable acidity and pH under diverse conditions. This challenges the prevailing notion that larger berry would exhibit dilution effects in both organic acids and sugars. Our findings suggest that grapes of varying masses attained similar technological maturities. These outcomes align with those observed in White Riesling by Kliewer et al. (1973), referencing data collected in 1971 and those obtained in Cabernet Sauvignon (Caravia et al., 2017). Thus, it seems that in all the different varieties, cooling the vines by sprinkling water has an important effect on berry growth, but little or no effect on the berry ripening process (Aljibury et al., 1975; Caravia et al., 2016).

On the contrary, the fruit-zone cooling system demonstrated promising outcomes concerning the accumulation of polyphenols such as anthocyanins. The treatment effect was significant in both years, indicating that under both multiple stress (WS) and only heat stress (WW) conditions, water spraying in the bunch zone leads to an enhancement of grape color. Our findings were primarily attributed to the cooling effect, fostering anthocyanin biosynthesis stimulation and reducing anthocyanin degradation phenomena (Allegro et al., 2021). Pastore et al. (2017) highlighted that under moderate temperature conditions, the interaction of light and temperature is synergistic, while in the presence of heatwaves, an antagonistic effect emerges. The mechanism involves the upregulation of oxidative enzymes, such as peroxidases (PODs), and a diminished efficiency in the expression of genes related to biosynthesis (Movhaed et al., 2016). The adverse impacts of elevated temperatures may be exacerbated when coupled with a substantial reduction of soil water content (Carvalho et al., 2016). In conclusion, if irrigating before an extreme heat event can increase leaf transpiration, generating a cooling effect in the fruit zone similar to that of overhead irrigation with sprinklers (Kliewer and Schultz, 1973; Martínez-Lüscher et al., 2020), it is demonstrated here that the effect of a misting system dedicated to the cluster zone has a direct impact on the accumulation of anthocyanins, reducing their degradation.

Conclusions

The fruit-zone cooling system, evaluated at the University of Bologna, effectively mitigated multiple summer stresses on Sangiovese and Montepulciano vines during the 2022 and 2023 seasons marked by elevated temperatures. Specifically, the system succeeded in lowering both fruit-zone and berry temperatures, thereby enhancing canopy gas exchanges, yield production and the berry composition. In particular, the system showed a significant effect on the accumulation of anthocyanins in both Sangiovese and Montepulciano varieties. However, additional studies are necessary to assess the impact of the fruit-zone cooling system at the field level.

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Chapter 3.

An automatic cooling system to cope with the thermal-radiative stresses in the Pignoletto white grape

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Abstract

In recent years characterized by hot dry summers, the implementation of innovative irrigation tools in the vineyard represents a crucial challenge to ensure optimal production and to avoid excess of water consumption. It is known that the grapevine reacts to multiple stresses - i.e., high temperatures and water shortage - through adaptive mechanisms that are detrimental to the yield. Furthermore, this condition is usually aggravated by high solar radiation, which could negatively affect the phenolic composition of the grapes. Therefore, a cooling system has been developed aiming to reduce bunches' sunburn damage. The system is composed of both a network of proximal sensors able to acquire the microclimatic data within the vineyard and an actuator that triggers the nebulizers when the air temperature exceeds the threshold of 35 °C. This innovative setup was tested at the University of Bologna's experimental vineyard over the 2022 and 2023 seasons, focusing on Pignoletto, an Italian white grape cultivar. The trial involved Pignoletto vines subjected to two treatments, removal of basal leaves in pre-veraison and fruit-zone water spraying according to the following scheme: non-defoliated control (C); non-defoliated vines treated with water nebulization of the bunch zone (C+FOG); vines subjected to defoliation (DEF); vines subject to both defoliation and sprayed with nebulized water (DEF+FOG). The primary objective was to assess the impact of these treatments on various parameters, including yield attributes, berry necrosis and the concentration of secondary metabolites such as flavonols. The application of nebulized water in the cluster zone proved effective in consistently lowering both air and berry temperatures across both years. Additionally, vines treated with nebulized water demonstrated enhanced productivity compared to both the control (C) and defoliated (DEF) vines. This increase in yield can be attributed to the mitigation of sunburn damages around the clusters and a subsequent rise in cluster weight. Although no significant differences were observed in technological maturity, a noteworthy trend towards increased acidity was noted in the misting-treated vines during the scorching 2023 season, contrasting with the C and DEF groups. Interestingly, the synthesis of flavonols appeared unaffected by thermal reduction but exhibited

stimulation under increased light exposure. Consequently, flavonols were found to be overexpressed exclusively in the defoliated treatments. In summary, the fruiting area cooling system emerges as a valuable tool in alleviating the adverse impacts of multiple summer stresses on grapes, particularly in reducing sunburn damage.

Keywords: climate change, precision irrigation, sunburn damage, phenolic maturity.

Introduction

Pignoletto (Vitis vinifera L.) is a white Italian variety cultivated in the Bologna area (Italy) for the production of the Protected Designation of Origin (PDO) Colli Bolognesi Pignoletto wine. In recent decades, many of the most suitable vineyard areas in Italy have been frequently exposed to intense solar radiation, high vapor pressure deficits (VPDs) and high temperatures, especially during the ripening period (Di Carlo et al., 2019). As reported by several authors, in conditions of water scarcity and thermo-radiative excess (multiple summer stresses), Pignoletto cv. shows a near-isohydric behaviour (Palliotti et al., 2014; Frioni et al., 2020) which consists of the ability of the vine to maintain a constant leaf water potential despite variations in both soil water content and vapor pressure deficit (Tombesi et al., 2016; Valentini et al., 2022). This results in slightly conservative water use to environmental stimuli is mainly related to stomatal behavior aimed at limiting water loss (Schultz, 1996; Bota et al., 2001). However, the occurrence of multiple summer stress leads to leaf necrosis and photoinhibition and a significant reduction in yield due to sunburn damage that may appear as berry browning and/or necrosis (Filippetti et al., 2011; Dinis et al., 2016; Gambetta et al., 2022). This condition may result in grapes with a low organic acid content, high pH and atypical color due to polyphenols content and oxidation (Movahed et al., 2016). These effects are striking when the bunches are directly exposed to heat due to a lack of vegetative vigor or for canopy management choices such as late defoliation (Pastore et al., 2013; Pastore et al., 2017; Allegro et al., 2019) which is largely applied during the season mainly to reduce cluster rot infections (Zoecklein et al., 1992). Both cluster light exposure and temperature have been reported to affect the content of methoxypyrazines (MPs) in berry at harvest (Plank, 2019). In particular, direct sunlight causes the degradation of MPs and the consequent reduction of unpleasant vegetative aromas both in grapes and in wine (Allen et al., 1988; Arnold and Bledsoe, 1990). Moreover, cluster light exposure stimulates the production of flavonols which could contribute to the grape and wine bitter sensation as reported by Allegro and colleagues (2019). Some short-term agronomic techniques, such as foliar application of kaolin and zeolite (Calzarano et al., 2019; Valentini et al., 2021) and smart irrigation (Costa et al., 2016; Fraga et al., 2018), allow today to mitigate the negative effects of climate change on grapes.

The application of mist irrigation was extensively studied in the 1970s and proved to be an effective method for reducing water stress in plants (Howell et al., 1971). Therefore, ultra-fine misting systems have been developed to cool grapevine canopy air evaporatively, reducing both water consumption and leaf wetting and consequently plant diseases (Matthias and Coates, 1986). Starting from these assumptions, a fully automated fruit-zone cooling system was implemented in the experimental vineyard of the University of Bologna, characterized by summer heatweaves. The primary objective of this study was to optimize the microclimatic conditions surrounding grape clusters during the crucial ripening phase. The system's performance was evaluated on the 'Pignoletto' cultivar, with a specific focus on dissecting the effects of misting on yield, alleviating sunburn damage and unraveling the intricate interplay of light and temperature on the compositional attributes of grape berries. This scientific investigation aims to contribute valuable insights to the understanding of viticultural practices under challenging climatic conditions.

Materials and Methods

Plant material and experimental design

The trials were carried out in the 2022 and 2023 seasons in a four-year-old vineyard, at the experimental station of the University of Bologna (Bologna, 44°32'N, 11°22'E). Plants material consisted of *Vitis vinifera* L. Pignoletto vines, clone Cab3 grafted onto K5BB rootstock, spaced at 1,20 m within a single row (oriented North-East to South-West) and trained to Guyot system. Winter pruning left 10 buds while during spring 10 shoots per vine were left. The experimental design comprised 48 labelled vines, distributed across three blocks along the row. Within each block, 4 uniform vines per treatment were monitored (12 vines each treatment). The vines underwent a targeted intervention involving the manual removal of basal leaves and the application of water nebulization to the fruit zone, following the outlined scheme (Table 1): non-defoliated control (C); non-defoliated vines treated with water nebulization in the bunch zone (C+FOG); vines subjected to defoliation (DEF); vines subjected to both defoliation and nebulized water spray (DEF+FOG).

	NO FOG	**FOG**
NO DEF	C	C+FOG
DEF	DEF	DEF+FOG

Table 1. For clarity and improved interpretation of the upcoming figures and tables, the theses derived from the relationship between the defoliation and misting factors will be labelled as indicated below.

Defoliation was carried out at veraison - on DOY 216 and 222 for 2022 and 2023, respectively - coinciding with the simultaneous standardization of cluster numbers. It involved removing all main and lateral leaves from the six basal nodes of each shoot to expose the clusters to direct sunlight (Pastore et al., 2017). The soil management was carried out as follows: the inter-row was covered with ILMIX (S.I.S, San Lazzaro, BO, Italy), and mowed during the season to avoid excessive evapotranspiration, while in the under-row an offset-type cultivator tilled the soil periodically. The vineyard was not irrigated and the viticultural practices typical for the Emilia Romagna region were applied.

Fruit zone cooling system characteristics

The cooling system was composed of both a wireless sensor network (WSN), able to acquire the microclimatic data within the canopy and an actuator that triggers the nebulizers when the threshold of 35 °C is exceeded. In detail, the system is composed of a hardware part: (a) a control unit powered by batteries recharged by a solar panel and connected to a network of sensors capable of continuously recording the relative humidity and temperature values of the canopy (iFarming srl, Ravenna, RA, Italy); (b) a pipeline equipped with nebulizers which, at the operating pressure of 3.5 Bar, deliver drops of 50–55 microns in diameter. Each vine was equipped with a fogger (mod. RIVULIS F.L.F., Rivulis Irrigation, Gvat, Israel) with a flow rate of 10.4 L h-1. The misting system has been located under cluster area (10 cm under the cordon) to limit the direct contact between water and leaves. The software part analyzed the microclimate data collected by the nodes, and sent the impulses to a solenoid valve that automatically regulated the opening and closing of the misting system. The air temperature of 35 °C was chosen for turning on the system according to Pastore and colleagues (2017). Water was applied with the following cycle: fogger on 5 min, off 15 min. At the end of the cycle, the system performed another air temperature check. Since the spacing both along and between rows was 1.2 m x 2.7 m, each vine received approximately 0.8 mm of water per hour (equivalent to 15 minutes of water spray). The software part analyzed the microclimatic data collected by the nodes sending the impulses to a solenoid valve that automatically regulated the opening and closing of the misting system. The nebulized water was applied from DOY 216 (August 4nd) and from DOY 222 (August 10th) in 2022 and 2023 season respectively. As previously reported by Matthias and Coates (1986), misting systems have been developed to cool the canopy air evaporatively. Therefore, the nebulized water was not available for use at ground level.

Climate data acquisition

The weather conditions were recorded from veraison to harvest by a meteorological station annexed to the experimental vineyard (iFarming Srl, Ravenna, RA, IT). Moreover, for the microclimate detail, three digital thermal probes and relative humidity sensors (iFarming, Ravenna, RA, Italy) were placed in the fruit zone within the canopy (30 cm above the cordon). Each sensor was then connected to a Control Unit to analyze in real-time the microclimate data recorded by the sensor every 10 min during the seasons to activate the nebulizers. The mean berry temperature was measured around midday using an infrared thermometer (mod. Raynger ST, Raytek, Santa Cruz, CA, USA) on DOY 217 and 232 (2022 season) and on DOY 225 and 235 (2023 season).

Yield attributes and berry composition at harvest

At the time of harvest, the yield of tagged vines was weighed and the number of clusters was counted. The incidence of necrosis was determined by estimating the percentage of clusters affected by sunburn damage out of the total number of clusters per vine. The severity of necrosis was assessed based on the percentage of affected berries per cluster. Furthermore, 30 berries per vine (360 berries per treatment) were collected during the harvest to analyze the following parameters: total soluble solids concentration (TSS), using a self-compensating Maselli R50 refractometer (Misure Maselli, Parma, Italy); must pH and titratable acidity (TA), using a Crison titrator (Crison Instruments, Barcelona, Spain). An additional sample of 200 berries per treatment was measured using a portable vis/NIR device berry-adapted Da-meter© (Sinteleia, Bologna, Italy) to assess the browning intensity of the skin in each berry. This parameter, denoted as the IADg index, was calculated by determining the difference between the absorbances at 545 nm and 750 nm (Valentini et al., 2022). This computation provides an indication of the light reflection in proximity to the red-light band.

Analysis of berry Flavonols at harvest

Flavonols were extracted from the skins of 20 berries by immersing the peeled skins in 100 mL of methanol for a duration of 24 hours within a light-protected environment at a temperature of 20°C. Subsequently, 5 mL of the resulting supernatant underwent an acid hydrolytic process, leading to the cleavage of flavonol glycosides (Mattivi et al., 2006). For the analytical phase, a High-Performance Liquid Chromatography (HPLC) mod. Waters 1525 142 equipped with a diode array detector (DAD) and a reversed-phase column (RP18 250 x 4 mm, 5 143 μM) with a pre-column (Phenomenex, Castel Maggiore, Bologna, Italy) was employed. The quantification was achieved by measuring absorbance at 370 nm. The contents of flavonols were expressed in milligrams per kilogram of berries (mg kg^{-1}).

Statistical analysis

Data were processed by analysis of variance over year using the mixed procedure of SAS v 9.0 (SAS Institute, Inc., Cary, NC, USA) and the treatment comparisons were analyzed using Tukey test with a cut-off at P≤0.05.

Results

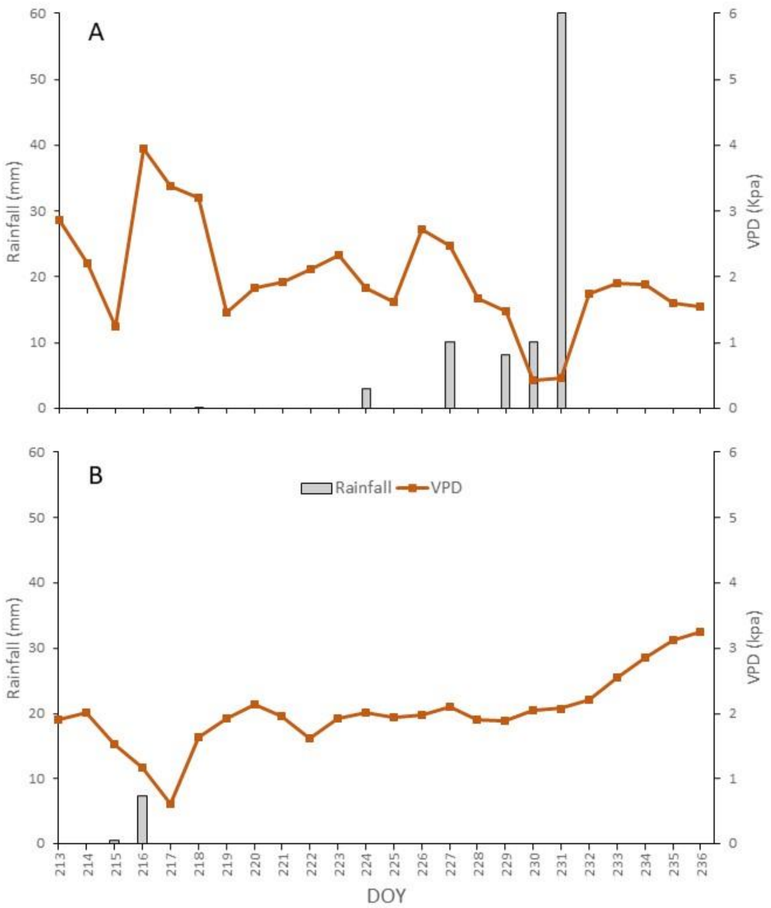

Figure 1. The trend of daily vapour pressure deficit (VPD) and the rainfall (mm) at a meteorological station during the test period (2022, A; 2023, B)

A fruit zone cooling system was employed to evaluate the effectiveness of nebulized water in mitigating sunburn damage to 'Pignoletto' throughout the 2022 and 2023 seasons, marking the first-ever field testing of the device. As detailed in Chapter 2, the 2022 season was marked by persistent heatwaves (Frioni et al., 2019) in the initial half of August, as also evidenced by elevated Vapor Pressure Deficit (VPD) values recorded at the vineyard layer by the meteorological station (Figure 1A). More precisely, in the first part of the month (from DOY 216 to 223), when the cluster where exposed to sunlight, air temperatures surpassed 35°C, prompting the activation of the cooling system (Table 2). In contrast, the latter part of the month witnessed substantial rainfall and a decline in VPD

(Figure 1A). To provide detailed insights, the experimental station recorded a cumulative rainfall of 101 mm during the period of misting system activation. Conversely, the 2023 season was characterized by intense solar radiation and persistent heatwaves, evident in the elevated daily VPD values, particularly noticeable after the DOY 230 (Table 3, Figure 1B). A closer analysis of the precipitation pattern during 2023 indicates that the initial days of August (DOY 215-DOY 2018), coinciding with the defoliation process (DOY 216), experienced relatively higher humidity. This was followed by a decrease in rainfall, with only 10 mm recorded from the pre-veraison stage to harvest (Figure 1B).

In summary, during the 2022 season, the misting system was activated promptly as the grape clusters were exposed to the sun. In contrast, in the 2023 season, system activation (DOY 224) occurred two days after the defoliation (DOY 222), reflecting a noticeable temperature disparity between the two years during veraison.

The misting system was in operation for 7 days during the 2022 season and 12 days in the 2023 season, as outlined in Table 2 and Table 3. The nebulizer cycles per day ranged from 3 to 12 in 2022 and 4 to 16 in 2023. In particular, the system maintained an average of 6 cycles per day during the 2022 season and 13 cycles per day for the year 2023. Given that each cycle dispenses water for 5 minutes, we can estimate the mist volume generated in the cluster zone over the two years. This varied from 0.8 to 3.1 mm in 2022 and 1.0 to 4.1 mm in the warmer 2023, totalling 10.0 mm and 41.2 mm across the two seasons, respectively (Table 2 and Table 3).

No direct correlation is apparent between the number of system activations and the temperature decrease around the grape cluster area in nebulized vines. However, a discernible trend emerges in FOG, indicating a temperature reduction with an increase in misting cycles compared to untreated vines. During the hottest days of 2022, with temperatures ranging between 37 and 41.6 °C and an average maximum exceeding 39 °C, the system achieved a temperature reduction ranging from 3.3 to 3.8 °C (Table 2). Comparable thermal peaks in 2023 were observed after August 20th (DOY 234 and 235). Even with 16 activations (equivalent to almost an hour and a half of misting), the air temperature dropped by just over 2 degrees, stabilizing between 36 and 37 degrees Celsius (Table 3). It is worth noting that on August 23rd (DOY 235), the average maximum air temperature in untreated vines reached 40 degrees between 11 am and 7 pm. This underscores that 2023 was indeed characterized by a warm and dry season.

DOY	Range air T (°C)	Average Maximum air T (°C)	Mist cycle per day (n)	Drop air T in FOG (°C)

216	37.0-40.6	37.7	3	-3.3
217	38.1-41.6	39.1	12	-3.8
218	39.0-41.6	38.5	6	-2.4
222	33.6-35.1	33.9	6	-1.1
223	34.1-35.6	33.7	6	-1.5
229	36.8-37.6	35.1	3	-0.4
232	33.9-35.6	33.5	3	-1.6

Table 2. Year 2022. Range Temperature (Range air T) and Average Maximum Temperature (Maximum air T) recorded on untreated vines between 11:00 and 19:00. The misting system's impact is depicted by the number of mist cycles per day and the resulting Drop in Air Temperature close to the cluster zone (Drop air T), measured in FOG-treated vines compared to the control group.

DOY	Range air T (°C)	Average Maximum air T (°C)	Mist cycle per day (n)	Drop air T in FOG (°C)
224	32.1-37.1	34.2	16	-3.7
225	33.5-37.7	34.7	14	-3.3
226	31.9-36.2	34.7	12	-1.3
227	29.8-36.4	34.9	12	-0.8
228	30.7-38.2	35.6	14	-1.2
229	29.1-35.3	34.4	4	-0.5
230	30.9-38.2	35.2	14	-1.1
231	31.3-38.7	35.9	14	-0.9
232	34.7-38.0	36.0	14	-0.9
233	32.0-39.7	37.3	14	-2.2
234	33.0-40.8	38.5	16	-2.4
235	34.8-41.9	39.9	16	-2.1

Table 3. Year 2023. Range Temperature (Range air T) and Average Maximum Temperature (Maximum air T) recorded on untreated vines between 11:00 and 19:00. The misting system's impact is depicted by the number of mist cycles per day and the resulting Drop in Air Temperature close to the cluster zone (Drop air T), measured in FOG-treated vines compared to the control group.

The temperature of the berries, whether they were exposed to direct radiation or shielded from it, consistently remained cooler for an extended duration after water delivery compared to the canopy. Analyses of berry temperature, measured with an infrared thermometer at solar noon on two representative days in each year (one being an extremely warm and dry day, DOY 217, and the other a moderately warm day, DOY 232), revealed a significant third-degree interaction among all the factors examined - FOG*DEF*DOY(YEAR) -. In 2022, the berry temperatures recorded in vines subjected to FOG consistently remained below the critical threshold of 35 °C, demonstrating a noteworthy distinction from the temperatures observed in vines without FOG treatment (Table 4), showing thermal reduction ranging from value of 1.8 to 9.5 degrees Celsius in both observed data. The two days, marked by different climatic conditions, exhibit distinct behavior of the clusters under direct exposure. In detail, there are no differences between DEF and NO DEF treatments on the 217th day of the year, while for DOY 232, the temperature value for DEF+FOG is the absolute minimum (Table 4).

		NO FOG	FOG
DOY 217	**NO DEF**	35.1 a	30.9 b
	DEF	39.0 a	29.5 b
DOY 232	**NO DEF**	33.2 a	31.4 a
	DEF	35.8 a	28.2 b

Table 4. Table 4. Berry temperatures (°C) recorded on vines with foliage and vines subjected to basal leaf defoliation, as well as on vines subjected to or without nebulization of the fruit zone during the 2022 season (DEF = defoliated; NO DEF = not defoliated; FOG =nebulized; NO FOG = untreated with misting). Different letters indicate significant differences between treatments and for each date according to the Tukey test (p ≤ 0.05)

Similar observations, as mentioned above, were also evident in 2023, where clusters subjected to misting demonstrated a significant cooling effect on DOY 225 and 235 (Table 5). As reported above, there are no differences between DEF and NO DEF treatments (Table 5).

		NO FOG	FOG
DOY 225	**NO DEF**	32.8 a	30.6 b
	DEF	35.4 a	28.1 b
DOY 235	**NO DEF**	35.9 a	33.3 b
	DEF	37.4 a	35.4 b

Table 5. Berry temperatures (°C) recorded on vines with foliage and vines subjected to basal leaf defoliation, as well as on vines subjected to or without nebulization of the fruit zone during the 2023 season (DEF = defoliated; NO DEF = not defoliated; FOG =nebulized; NO FOG = untreated with misting). Different letters indicate significant differences between treatments and for each date according to the Tukey test (p ≤ 0.05)

A comprehensive analysis of ripening curves for the two trial years reveals no significant differences between the treatments, as shown in Figure 2, except for the evolution of berry mass in the 2023 season. Remarkably, in the defoliated treatments, there was a discernible decrease in berry size leading up to harvest, as illustrated in Figure 2B. To elaborate, the berry mass displayed a reduction, corresponding with the increase in VPD at the end of August, as demonstrated in Figure 1B. Worth noting is the sudden decline of the DEF+FOG treatment, which seemed to maintain the same growth rate as the NO DEF group's berry until DOY 244, only to undergo a drastic reduction at harvest. At harvest, a marked difference is apparent between the two groups, DEF and NO DEF, as illustrated in Figure 2B, in contrast to the preceding year (Figure 2A). However, a similar trend is observed across both years concerning data related to technological maturity, including soluble solids, pH and titratable acidity. The recorded values align with the typical characteristics of the grape variety and cultivation area and no statistical difference among treatments is noted for these values in the trial seasons (Figure 2C, 2D, 2E, 2F, 2G, 2H).

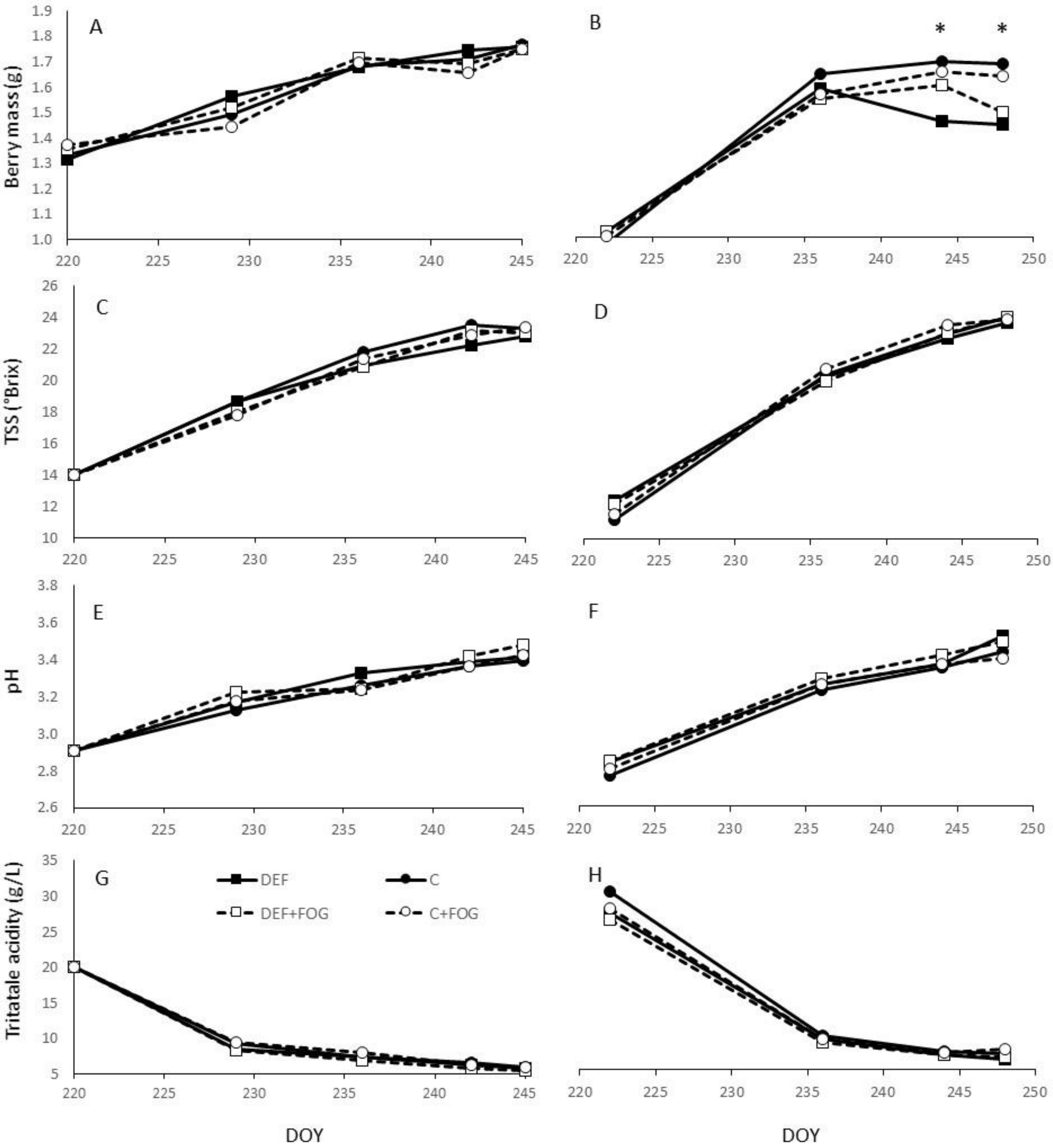

Figure 2. Trends of berry weight (A, 2022 and B, 2023) soluble solids (C, 2022 and D, 2023), pH (E, 2022 and F, 2023) and titratable acidity (G, 2022 and H, 2023) in the two test years. The asterisk indicates a significant difference between the treatments.

While no statistical differences were observed among treatments in terms of technological maturity, variations in yield at harvest were evident (Table 6). The table below highlights significant disparities in yield production between the FOG and NO FOG treatments over the two years of the trial (Table 6). The efficacy of the misting system in ensuring higher yields, even under direct solar exposure, is emphasized. Although in 2022, this outcome correlates with the average bunch mass being higher in FOG than in NO FOG, these differences do not appear significant in 2023. A more comprehensive explanation of this interaction is provided in Supplementary Material (S1) that indicates a tendency for an increase in grape cluster weight even during the warmest season. As also shown in Figure 2, berry mass at harvest differs only in season 2023 (Table 6, Supplementary Material S2), showing a sensible reduction in DEF. The analysis reveals that the warmer and drier conditions of the 2023 season had a discernible impact on various yield components, particularly influencing parameters associated with heat damage. Notably, the assessment of sunburn, including both the incidence and severity of necrosis, was conducted through direct observations of the grapes during harvest (Table 6). Regarding the influence of treatments on the incidence of necrosis, discernible disparities are apparent, particularly in the context of fruit-zone misting. The empirical findings, as delineated in Table 6, underscore a significant reduction in the prevalence of necrotized berries during the two-year observation period in FOG. In contrast, defoliated vines show a dissimilar trend over the years concerning damage incidence. Specifically, in 2022, the wettest year, defoliated vines exhibit a higher number of affected berries compared to the driest year, where the incidence of sunburn appears to be less pronounced (Supplementary Material S3). Concerning the severity of sunburn damage, the results reveal an interaction between YEAR*DEF and YEAR*FOG treatments (Table 6, Supplementary Material S4 and S5), emphasizing a higher percentage of compromised clusters in thesis exposed to direct radiation and without FOG. This outcome is specifically noticeable in the 2023 season, characterized by harsh climatic conditions.

		2022		**2023**		**Significance**
		NO FOG	FOG	NO FOG	FOG	
Cluster (n)	NO DEF	21	21	30	30	NS
	DEF	21	21	30	30	
Yield (kg)	NO DEF	2.85	3.20	3.28	3.72	FOG
	DEF	2.89	3.35	3.07	3.29	
Cluster weight (g)	NO DEF	130	150	109	124	YEAR*FOG
	DEF	140	158	104	114	
Berry mass (g)	NO DEF	1.77	1.75	1.63	1.58	YEAR*DEF
	DEF	1.76	1.75	1.42	1.45	
Necrosis incidence (%)	NO DEF	53	41	3	3	FOG YEAR*DEF
	DEF	45	35	32	17	
Necrosis severity (%)	NO DEF	3	2	6	2	YEAR*DEF YEAR*FOG
	DEF	4	4	16	6	
TSS (°Brix)	NO DEF	23.4	23.4	24.0	23.9	YEAR
	DEF	22.8	23.0	23.6	24.0	

pH	NO DEF	3.4	3.4	3.4	3.4	NS
	DEF	3.4	3.5	3.5	3.5	
Titratable acidity (g/L)	NO DEF	6.00	5.92	7.23	7.81	YEAR*DEF YEAR*FOG
	DEF	5.73	5.48	6.45	6.75	
Flavonols (mg/kg)	NO DEF	3.6	2.3	5.6	6.3	YEAR*DEF
	DEF	7.9	8.7	39.4	39.3	
IDAg	NO DEF	0.03	0.03	0.05	0.02	YEAR*DEF*FOG
	DEF	0.10	0.06	0.07	0.07	

Table 6. Yield attributes and berry composition at harvest (DEF = defoliated; NO DEF = not defoliated; FOG =nebulized; NO FOG = untreated with misting; NS = Not Significant; TSS = total soluble solids) during 2022 and 2023 seasons.

A statistical difference between the two years was observed in terms of soluble solids content (TSS) at harvest (Table 6), with a higher concentration of sugar in 2023. Although no differences were found among treatments in terms of pH, interactions emerged concerning titratable acidity (Table 6). In detail, the misting system appears to have shown no effect on grape acids in the wetter year but resulted in a significant increase in the warmer year (Supplementary Material S6). Conversely, exposed clusters exhibited a decrease in acidity compared to non-defoliated ones, with a more pronounced effect in 2023 (Supplementary Material S7). The YEAR*DEF interaction is evident also for the Flavonols parameter (Table 6, Supplementary Material S8), wherein the defoliation technique exerts an increasing influence. Notably, this effect is particularly pronounced in the warmer year. Of particular interest is the observation that, regardless of the misting treatment applied, the warmer year consistently exhibits a higher flavonoid content in the exposed clusters. The intricate interplay involving a third-degree interaction among all the factors is evident in the case of the DAg index (IDAg). Drawing from the substantial research conducted on this index, the current dataset demonstrates a pattern reminiscent of flavonols, indicating a notable and consistent increase in the exposed clusters, as delineated in Table 6. In addition, a significant interaction among all the factors involved (year, defoliation and misting) emerges, as clearly shown in Figure 3. Specifically, the effect of defoliation appears to be modulated based on the presence/absence of mist. Notably, a marked difference is observed between DEF and NO DEF when subjected to misting, with the former exhibiting the highest index. When not exposed to cooling, the vines exhibit variable behavior over the two years, while consistently maintaining a tendency toward higher IDAg in clusters exposed to direct radiation (Figure 3).

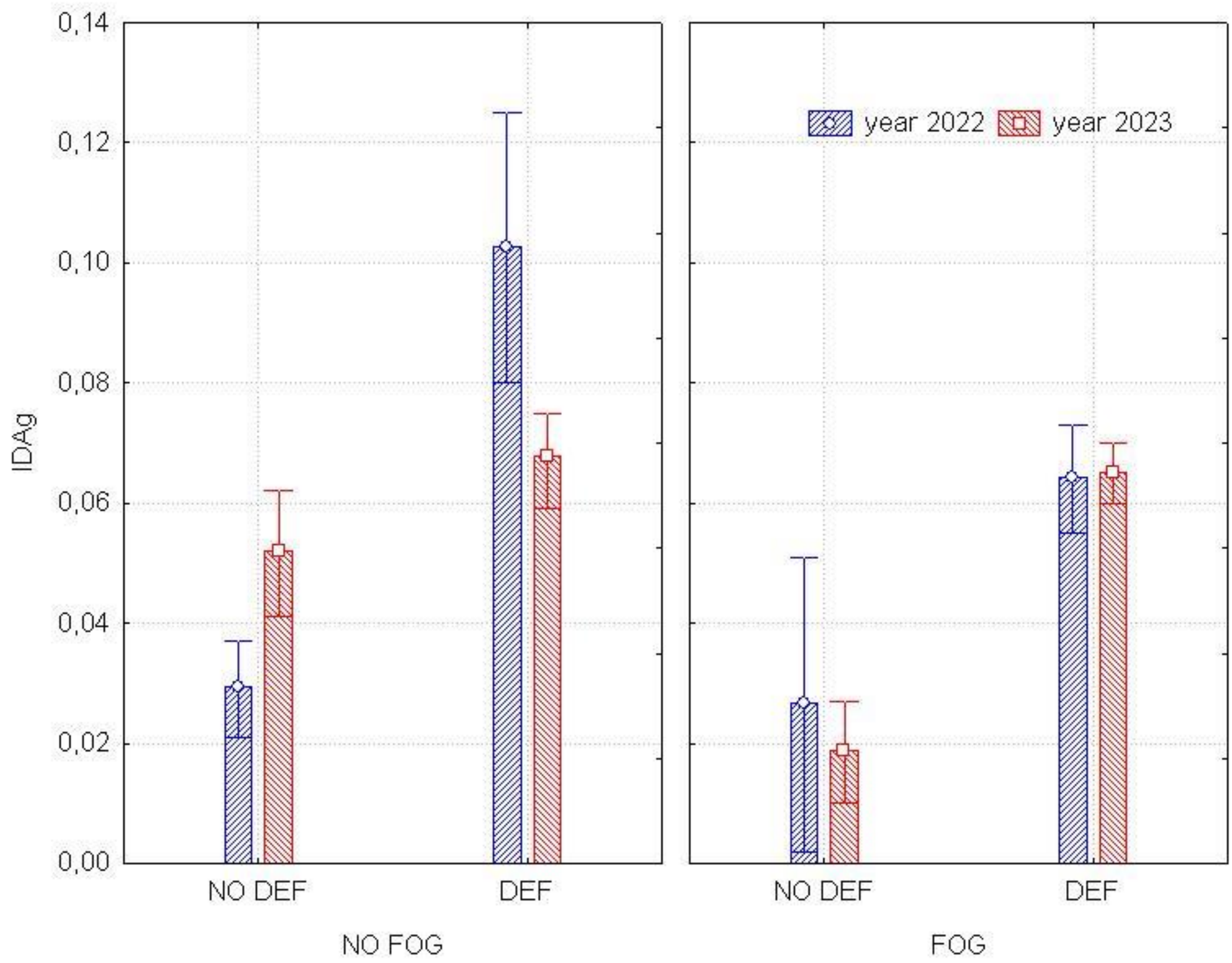

Figure 3. Bar chart illustrating the DAg index (IDAg) values at harvest for the years 2022 and 2023. The interaction among different factors - defoliation (DEF), misting (FOG) and years - is evident. Error bars depict the standard error.

Discussion

The principal aim of the study was to assess the efficacy of a water mist cooling system in mitigating sunburn damage to 'Pignoletto' white grape. Additionally, the research aimed to unravel the rule of mist in the intricate influence of light and temperature on the ripening process of grape berries. Notably, while prior studies have established the impact of cluster exposure on ‘Pignoletto’ in relation to radiation (Allegro et al., 2019), our investigation uniquely centers on unveiling the consequences of thermal modulation on vine behaviour. Subsequently, the study delves into an in-depth exploration of the effects of temperature and light on yield production and berry composition, with a specific emphasis on the roles played by bunch exposure and/or bunch cooling. This nuanced analysis aims to contribute novel insights to the existing body of knowledge (Spayd et al., 2002; Pastore et al., 2017; Movahed et al., 2016; Pastore et al., 2017).

The system was specifically designed to target the cluster, setting it apart from traditional sprinkler solutions assessed in Vitis vinifera L. and other species (Robinson, 1970; Gilbert et al., 1970; Chesness and Brau, 1970; Kliewer and Schultz, 1973). In grapevine, automate overhead sprinklers were typically focused on preventing leaf overheating and preserving their functionality based on available knowledge on energy balance (Pitacco et al., 1999). Moreover, the threshold sprinkler activation was fixed at different level according to the varieties: 27 °C in both Pinot noir and Chardonnay (Pitacco et al., 1999), 30 °C for Cardinal, Carignan and Riesling (Kliewer and Schultz, 1973), 32 °C in Chardonnay, Riesling and Chenin Blanc (Aljiburi et al., 1975), 35 °C in Semillon (Greer and Weedon, 2014). Our system, in agreement with Caravia et al. (2017), operates in the cluster zone but differs in two attributes: it is arranged below the cordon and not inside the canopy to avoid direct interference between fog and foliage; it has the thermal limit set at 35 and not 38 °C. This departure is noteworthy, considering that numerous metabolic processes tend to slow down or cease at 35 °C, including flavonoid synthesis (Jones, 2012; Pastore et al., 2017).

In summary, the 2022 and 2023 seasons were characterized by different climatic conditions. In 2022, persistent heatwaves in August prompted system activation in the first part of the month, while in 2023, intense solar radiation and heatwaves were observed during the second half of the trial period. The misting system demonstrated a discernible trend in reducing temperatures around the cluster-area during warm days, with a significant temperature reduction in both seasons. In addition, misting resulted in a sustained cooling effect on berry temperatures, maintaining cooler conditions compared to untreated vines. This effect was similar to the more recent research works obtained by misting systems aimed at water resource conservation (Greer and Weedon, 2014; Caravia et al., 2017; Bianchi et al., 2023).

Misting emerged as a pivotal factor in amplifying yields, underscoring its efficacy in enhancing grape production, even when faced with direct solar exposure. This outcome bears particular relevance for viticulturists seeking methodologies to optimize yield among climatic adversities. In detail, vines subjected to misting exhibited a substantial increase in yield, attributable to a mitigated incidence of sunburn damage and a concurrent enhancement in cluster weight. In Semillon, the increase in bunch weight was associated with the increase in berry weight in the Hydrocooling technique compared to untreated vines (Greer and Weedon, 2014), while in Riesling this gain occurred only when the overhead sprinklers worked continuously from the bloom stage (Kliewer and Schultz, 1973). An increase in berry weight was also found on Cabernet Sauvignon but no significant differences in cluster weight were evident as the system operated for an extremely short period (Caravia et al. 2017).

In contrast, no difference in yield and berry size was observed in Chardonnay treated with a high-pressure spray system, at about 70 Bar (Paciello et al., 2015). In addition to the latest research, over two trial seasons, an analysis of the positive effects exerted by the treatment on sunburn damage was shown. It is well known that in a climate change scenario, the phenomena of both berry dehydration and necrosis exert a direct influence on cluster mass, particularly when clusters are exposed directly to solar radiation (Bonada et al., 2013; Pastore et al., 2013; Caravia et al., 2017). In our trial, misting system significantly reduced the prevalence of necrotized berries over the two-year, underscoring its role in mitigating sunburn damage. To further elucidate, the most noteworthy findings emerged in the second year of the experiment, which were associated with elevated temperatures and a concurrent shortage of soil water. Indeed, the heightened evaporative demand in 2023 has been unequivocally established as a contributing factor that accentuated the severity of sunburn damage in defoliated vines. Significantly, it also underscored the decisive role of misting in alleviating injury.

Grapevine canopy management serves as a crucial tool in influencing the microclimate around berry clusters and this manipulation has far-reaching effects on berry quality traits. It’s well known that exposure to sunlight enhances the levels of sugars and flavonoids (Crippen et al., 1986) and the specific response is not only dependent on the grapevine genotype but also on the berry temperature (Coombe 1987; Guidoni et al., 2007; Downey et al., 2004; Pastore et al., 2013). Leaf removal around the clusters is a widely employed practice to regulate berry illumination and temperature, particularly in cooler region where very high temperature are not common (Jackson and Lombard, 1993) but its use in warm regions is controversial, due to the negative effect exerted by the thermal excess. As previously stated, Italy is undergoing a precipitous climate shift marked by an increase in temperatures during the grape ripening phase (Teslic et al., 2019). Consequently, defoliation practices expose clusters to high solar radiation which may exert a discernible influence on berry mass, as observed in our study, where defoliated vines exhibited a diminished berry size during the hottest season. Although the treatments did not impact sugar content and pH, a positive effect of grape cooling was observed in terms of total acidity. This finding aligns with reports from other authors (Paciello et al., 2015; Bianchi et al., 2023) and appears to be attributed to the pronounced cooling action on the cluster zone and the grapes themselves, thereby slowing down malic acid respiration (Ruffner et al., 1976).

Defoliation during veraison, a stage marked by reduced photosynthetic activity in basal leaves compared to apical ones, significantly influences light and temperature exposure, with a constrained impact on the source–sink balance (Pastore et al., 2017). Moreover, the effect of defoliation on flavonoid biosynthesis in berries is noteworthy but contingent upon the grapevine genotype (Matus et al., 2009; Pereira et al., 2006). In summary, polyphenol synthesis is strongly induced by UV radiation, activating a cascade of enzymes in the phenylpropanoid pathway, and numerous studies have corroborated an increase in these compounds in sun-exposed grapes (Gambetta et al., 2022). Flavonol synthesis is upregulated by high radiation levels, potentially serving as antioxidants and sunscreens (Kolb et al., 2003; Pastore et al., 2013). Additionally, Preys et al. (2006) noted a correlation between flavonol concentration and the bitterness of resulting wines.

Our research outcomes are consistent with those reported by Allegro et al. (2019), who observed a similar response in 'Pignoletto' cultivated in northern Italy, wherein increased cluster light exposure after leaf removal led to reduced titratable acidity and enhanced flavonol levels. While the application of nebulized water positively influenced titratable acidity, it did not exhibit a substantial impact on overall flavonol content. The noticeable increase in flavonols in defoliated vines, even in the presence of misting, suggests a significant role for light-dependent modulation, emphasizing the predominant influence of light over temperature effects. However, we postulate that the analysis of total flavonols may not capture the oxidized fraction, which could potentially contribute to berry browning. To address this, we employed a portable and non-destructive device to objectively measure red-light band reflectance, aiming to identify differences among treatments. Despite being a prototype, the berry-adapted Da-meter© yielded intriguing results. Specifically, variations in DAg index values between misted and non-misted treatments may explain the more intense grape coloration observed in the NO FOG vines, as illustrated in the supplementary materials (Figure S1 and S2).

Conclusion

Our study aimed to assess the efficacy of a water mist cooling system in mitigating sunburn damage to 'Pignoletto' white grape and to unravel the intricate influence of light and temperature on grape berry ripening. The designed misting system, specifically targeting the cluster, demonstrated a discernible trend in reducing temperatures around the cluster during warm days, significantly cooling berry temperatures in both seasons. Misting emerged as a pivotal factor in amplifying yields, showcasing its efficacy in enhancing grape production, even under direct solar exposure. This outcome is particularly relevant for viticulturists facing climatic adversities. Vines subjected to misting exhibited a substantial increase in yield, attributed to a mitigated incidence of sunburn damage and a concurrent enhancement in cluster weight. Additionally, our study explored the effects of defoliation during veraison, revealing its significant influence on light and temperature exposure, impacting flavonoid biosynthesis. The increase in flavonols in defoliated vines, even with misting, underscores the crucial role of light-dependent modulation, emphasizing its predominant influence over temperature effects. In summary, our research highlights the multifaceted impact of misting on grapevine canopy management, with positive effects on yield, sunburn damage mitigation, and berry composition. Additional research is necessary to confirm the effectiveness of the DAg index in the non-destructive evaluation of both total and oxidized fractions of flavonols.

Acknowledgements

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Supplementary material

	2022	2023
NO FOG	135 b	107 a
FOG	154 a	117 a

S1. Cluster weight interaction (YEAR X FOG). Different letters indicate significant differences between treatments according to the Tukey test ($p \leq 0.05$).

	2022	2023
NO DEF	1.76 a	1.61 a
DEF	1.75 a	1.39 b

S2. Berry mass interaction (YEAR X DEF), Different letters indicate significant differences between treatments according to the Tukey test ($p \leq 0.05$).

	2022	2023
NO DEF	46 a	3 b
DEF	40 a	11 a

S3. Necrosis incidence interaction (YEAR X DEF). Different letters indicate significant differences between treatments according to the Tukey test ($p \leq 0.05$).

	2022	2023
NO DEF	2 a	6 b
DEF	4 a	40 a

S4. Necrosis severity interaction (YEAR X DEF). Different letters indicate significant differences between treatments according to the Tukey test ($p \leq 0.05$).

	2022	2023
NO FOG	4 a	11 a
FOG	3 a	4 b

S5. Necrosis severity interaction (YEAR X FOG). Different letters indicate significant differences between treatments according to the Tukey test ($p \leq 0.05$).

	2022	2023
NO FOG	5.9 a	6.8 b
FOG	5.7 a	7.3 a

S6. Titratable acidity interaction (YEAR X FOG). Different letters indicate significant differences between treatments according to the Tukey test ($p \leq 0.05$).

	2022	2023
NO DEF	6.0 a	7.5 a
DEF	5.6 b	6.6 b

S7. Titratable acidity interaction (YEAR X DEF). Different letters indicate significant differences between treatments according to the Tukey test ($p \leq 0.05$).

	2022	2023
NO DEF	3.0 b	6.0 b
DEF	8.3 a	39.4 a

S8. Flavonols interaction (YEAR X DEF). Different letters indicate significant differences between treatments according to the Tukey test ($p \leq 0.05$).

Figure S1. 2023: FOG treatment unveils vibrant green clusters on mature Pignoletto vines, free from necrosis during harvest.

Figure S2. 2023: NO FOG treatment reveals striking yellow-bronze clusters on mature Pignoletto vines, accompanied by noticeable necrosis during harvest

Chapter 4.

Optimizing viticulture sustainability through foliar zeolite treatments: an in-depth analysis of their impact on gas-exchange, yield and the composition of Sangiovese grapes and wine

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Abstract

In the face of a changing climate increasingly characterized by hot summers, grapevines respond with adaptive mechanisms that, in some cases, ensure survival at the expense of yield and grape quality. It is known that the efficiency of the canopy - in terms of gas exchange and net assimilation - is closely related to the amount of energy it is exposed to and diminishes under conditions of water scarcity. In this perspective, resorting to emergency irrigation represents a successful mitigating strategy, but in some vineyard areas in the Mediterranean, it is impractical due to water scarcity. Among the landscape for alternative strategies, the application of minerals to mitigate the negative effects of multiple summer stresses has become a simple and effective tool available to the viticulturist. Building on this, the objective of the study was to evaluate the physiological, vegetative-productive, and qualitative response of chabasite-rich zeolite treatments on potted grapevines subjected to water stress and field-grown vines subjected to thermal-radiative stress.

The research was conducted in the three-year period 2021-2023 on Sangiovese grapevines and was divided into two distinct trials: the first on potted vines (2021 season), and the second on adult vines grown in-field (2022 and 2023 seasons). The potted trial involved 12 plants in as many lysimeters, subjected to a water restriction (50% restitution of water lost through transpiration) from veraison (DOY 200) until DOY 232 and distributed into two different treatments: water stress (WS) and water stress associated with treatment with natural zeolite (WS+ZEO). Zeolite distribution occurred foliarly on the day water stress was induced and a week after the first treatment. The field trial involved 24 non-irrigated plants distributed in three randomized blocks pertaining to two different treatments: untreated control (C) and treatment with 3% zeolite (ZEO). Zeolite distribution occurred pre and post-veraison across the entire canopy. In both trials, microclimatic conditions were monitored throughout the ripening stage using stationary proximal sensors. The effects of the mineral treatment were evaluated in terms of canopy physiology in the potted vines, while yield parameters, sunburn damage and grape composition were assessed in the field vines. In 2022, grapes harvested from the field treatments were microvinified to examine the treatment's effect on wine color.

The zeolite treatments cooled the canopies and berries of the trial vines. Temperature reductions influenced gas exchange in the pot trial and grape production and composition in the field trial. Specifically, under moderate to severe water stress, zeolite-treated potted vines showed an increase in stomatal conductance at the same stem water potential, an increase in photosynthetic activity, and parameters related to greater photosystem II efficiency (Fv'/Fm', ϕPSII, ETR). Vines from the zeolite-treated plot also showed an increase in anthocyanin concentration in both grape and wine. In conclusion, zeolite-based treatments can be a valuable tool to enhance the productive performance of Sangiovese in environments characterized by multiple summer stresses.

Keywords: climate change, temperature, mineral particles, photosynthesis, anthocyanins, wine

Introduction

In accordance with the latest OIV (International Organization of Vine and Wine) report (2019), Italy stands out as the world's leading wine producer, yielding an impressive 58.5 million hectoliters, with Sangiovese emerging as Italy's predominant grape variety, boasting cultivation exceeding 50,000 hectares (IOVW, 2017). This renowned grape variety plays a vital role in several Italian protected appellations, including Chianti, Nobile di Montepulciano and Brunello di Montalcino, enjoying global acclaim. In recent years it is imperative to acknowledge the potential detrimental effects of global warming on vine cultivation, yield and the composition of grapes and wine (Mattivi et al., 2006).

'Sangiovese' berries are characterized by a low concentration of anthocyanins in their skin, primarily composed of glycosylated anthocyanins (Santos et al., 2015). Notably, Mattivi et al. (2006) revealed a significant reduction, averaging two-thirds, in anthocyanin levels compared to the more prevalent Cabernet Sauvignon cultivar. The challenge of maintaining grape color in warm climates has prompted extensive research since the 1990s, focusing on enhancing anthocyanin content in berry skins (Mori et al., 2007; Pastore et al., 2017; Chaves et al., 2007; Lanari et al., 2014) and resultant wines (Pastore et al., 2011; Intrieri et al., 2008). This challenge intensifies amid the escalating frequency of summer days exceeding the critical temperature of 35°C (Canuti et al., 2012; Parenti et al., 2004), as reported by Movahed et al. (2016).

The Mediterranean basin is experiencing not only a substantial rise in air temperature but also a shift in precipitation distribution (Jones 2012; Teslic et al., 2019), subjecting vines to a combination of abiotic stresses impacting plant growth and fruit composition (Mittler 2006; Palliotti et al., 2015).

Dinis et al. (2020) express concerns about the synergistic effects of environmental stresses in regions grappling with water scarcity and elevated temperatures, potentially damaging photosynthetic activity and berry metabolism.

Under such stressful conditions, leaves may exhibit chlorosis and necrosis, leading to reduced growth and yield (Dinis et al., 2016). Heatwaves accelerate soluble solid accumulation, deplete organic acids, elevate pH and induce atypical aroma compounds, resulting in wines less amenable to aging, with compromised color and altered aromatic profiles (Palliotti et al., 2014).

To address these challenges, new short-term agronomic techniques have emerged, including post-veraison shoot trimming, leaf removal (Filippetti et al., 2011; Palliotti et al., 2013; Valentini et al.,2019), smart irrigation and the foliar application of particle materials such as kaolin and zeolite.

Kaolin is an aluminosilicate clay known for its ability to reflect potentially harmful radiation, thereby mitigating temperature increases on leaves and berries (Glenn 2005; Conde et al., 2018). Recent research has highlighted its positive impact on gas exchange in plants under water shortage conditions (Brito et al., 2019; Brillante et al., 2016; Frioni et al., 2019). Additionally, Kaolin's ability to alter reflective properties plays a role in reducing sunburn damage, which positively influences phenolic concentrations in plants (Dinis et al., 2018; Mohamed et al., 2019; Shellie et al., 2013; Valentini et al., 2021).

Identified by Cronstedt in 1756, zeolites are tectosilicates and encompass 54 different mineral species characterized as hydrated aluminosilicates of alkaline and alkaline earth elements (Passaglia and Sheppard, 2001). Their crystal chemistry imparts unique physical and chemical properties, including high and selective cation exchange capacity (CEC), reversible dehydration, selective molecular absorption, and catalytic behavior (Armbruster and Gunter, 2001). Thanks to these characteristics, natural zeolites find numerous applications in agriculture, as highlighted by Eroglu et al. (2017). In particular, rocks containing more than 50% of zeolites can be classified as "zeolitite" specifying the main zeolite constituent (Galli and Passaglia, 2011). Moreover, with the classification of zeolites as "non-toxic" by the International Agency for Research on Cancer (IARC, 2017) and the Food and Drug Administration's (FDA) classification as "safe" for human consumption (Rotondi et al., 2022), their use in various sectors, including agriculture, is further supported.

While the use of zeolite in soil is widespread (Perez-Caballero et al., 2008; Doni et al., 2024), its application for foliar treatments is less common. However, besides serving as a sustainable tool against pests and pathogens (Calzarano et al., 2019; De Smedt et al., 2015), it has been demonstrated that when applied to the grapevine canopy, zeolites can reduce berry temperature, thereby enhancing anthocyanin concentrations, except when applied close to harvest (Calzarano et al., 2019; Valentini et al., 2021). Acknowledging the growing interest in sustainable control of heatwaves and summer stresses in vineyards, this study evaluates the effects of foliar applications of zeolite on canopy cooling, gas exchange and grape and wine composition in Sangiovese cv.

Materials and Methods

Plant material and treatments

The trials were carried out over three years: in the 2021 the experiment was performed on two-year-old potted ‘Sangiovese’ vines and in 2022 and 2023 seasons on a seven-year ‘Sangiovese’ vineyard located at the experimental station of the University of Bologna (Bologna, 44°32'N, 11°22'E). Plant material consisted of 'Sangiovese' vines, clone TEA 10D grafted onto 110R rootstock, spaced at 1 m within a single row (oriented north-east to south-west). The young potted vines were trained with the retention of three spurs, each carrying two buds per vine. The shoots were thoughtfully arranged to form a vertical wall. In contrast, during the field trial, the mature vines were trained using the Guyot system, with 10 buds left per plant.

The *pot experiment* involved 12 vines with uniform leaf area, each placed in 30 L plastic pots filled with a soil mixture composed of 39% sand, 39% silt, and 22% clay, along with an organic matter content of 1.8% and a pH of 7.8. Field capacity and wilting point were determined following Saxton and Willey's (2005) methodology, resulting in values of 0.29 cm3/cm3 and 0.14 cm3/cm3, respectively. The 12 potted plants were arranged in corresponding lysimeters (mod. LAUMAS, ABC Bilance, Campogalliano, Italy) and were irrigated until the pre-veraison phase with an approximate daily water amount of 3 L, distributed automatically through a dripper irrigation system. Subsequently (from DOY 200 until DOY 232), the vines underwent a reduced irrigation regime, equivalent to 50% restitution of water lost through transpiration, and were divided into two treatments: water stress (WS) and water stress associated with treatment using natural zeolite chabasite-based at a concentration of 3% (WS+ZEO). The application of zeolite rich-chabasite (ZEOVER, Verdi, Reggio Emilia, Italy) was done foliarly on the day water stress was induced and repeated a week after the initial treatment. Additionally, aluminum foil covered the pots before the trial commenced to prevent overheating.

The *field trial* involved 24 non-irrigated plants distributed in three randomized blocks pertaining to two different treatments: untreated control (C) and treatment with zeolite (ZEO, Figure 1). The vineyard was located on a flat land having the clay loam and deep soil typical of the Po valley (Northern Italy). Vine spacing was 1 m x 2.8 m and, at flowering, each vine was uniformed for both number of shoots and bunches. During the growing season, the shoots were positioned by hand vertically, and slight shoot trimming was performed during the last days of June, leaving a canopy height of approximately 1.2 m. The vineyard was non-irrigated, and a standard disease control program was applied to control downy mildew, powdery mildew and Botrytis sour rot.

The treatments with Italian chabasite-rich zeolitite were carried out at a 3% concentration. The two applications were carried out at the beginning and at the end of veraison - stages 34 and 35 according to Eichorn and Lorenz (1977). The suspensions were carefully sprayed on both canopy sides using a knapsack sprayer (Model M3, Cifarelli, Pavia, Italy).

Figure 1. Example of Sangiovese vines treated with chabasite-rich zeolitite during the veraison stage. The treatment involved the entire canopy

Water status and leaf gas exchange measurements

In pot trial, midday stem water potential (Ψs) was measured on 6 vines per treatment using a Scholander-type pressure chamber (Model 3005, Soil Moisture Equipment Corp., Santa Barbara, CA, USA), at 1 pm on a mature leaf per vine on DOY 205, 211, 218, 223 and 231. Each leaf was covered with a plastic bag and aluminium foil for 2 h before the measurements (Turner and Long 1980). Leaf net Photosintesis (Pn), stomatal conductance (gs) and PSII efficiency (Fv'/Fm', ϕPSII, ETR) rates were measured using a portable gas exchange Li-Cor 6400 system (Li-Cor Inc., Lincoln Nebraska, USA) on two well-exposed mature main leaves on the same day and on the same vines monitored for the Ψstem with a broad leaf chamber under a saturated light (1500 $\mu molm^{-2}s^{-1}$) using an external lamp.

The operational efficiency of photosystem II (ΦPSII) measures the proportion of light absorbed and effectively utilized in photochemical processes (not re-emitted as fluorescence and/or dissipated as heat). It is calculated as the ratio between the photochemical extinction of maximum fluorescence and maximum fluorescence. The latter, however, is reduced compared to the maximum dark fluorescence (Fm) due to the contribution of the non-photochemical extinction fraction (NPQ). ΦPSII also provides a measure of the linear transport of electrons (Genty et al., 1989). Since ΦPSII directly measures the quantum yield of PSII, it is possible to derive the electron transport rate (ETR) from the instantaneous values of absorbed photosynthetically active radiation (PAR) and the fraction of light intercepted by the two photosystems (I-II). As quantifying the absorption by the latter is extremely challenging, a constant of 0.5 is used, while the leaf absorption coefficient is established at an average value of 0.84 ($ETR = \Phi PSII \times PPFD \times 0.5 \times 0.84$; Schultz, 1996).

In the potted trial, to account for precise vine water use, individual daily gravimetric vine water loss (Tr) was continuously measured throughout the growing season using a platform scale mod. LAUMAS (ABC Bilance, Campogalliano, MO, Italy). Pot masses were recorded every ten minutes using a CR1000 datalogger (Campbell Scientific, Inc., Logan, UT, USA). Each pot surface was covered with a plastic film to avoid interference from rainwater and to minimize losses due to soil evaporation.

Microclimate monitoring

The weather conditions were recorded by a weather station attached to the experimental station near the two experiments (iFarming srl, Ravenna, RA, IT). On the same vines, the day of leaf gas-exchange measurements (DOY 205, 211, 218, 223 and 231), the mean leaf temperature was measured at midday using an infrared thermometer (mod. Rayngert ST, Raytek, Santa Cruz, CA, USA).

Vine Growth, Yield Components and Berry Composition

In the field experiment, the vines were individually harvested on 5 September 2022 (DOY 248) and 18 September 2023 (DOY 261), and crop weight, bunch number and bunch weight were recorded. Pruning weight per vine was measured in both years during the winter (no differences were reported). At harvest, 50 berries per vine were collected and crushed. The must obtained was sieved and used for soluble solid analysis using a temperature-compensating CR50 refractometer (Maselli Misure Spa, Parma, Italy). A sample of 5 mL of the same must was then diluted seven times with bidistilled water for titration using a Crison Compact Titrator (Crison, Barcelona, Spain) with 0.25 N NaOH

(Sigma-Aldrich, St. Louis, MO, USA) to obtain pH and titratable acidity data (expressed as g L^{-1} of tartaric acid equivalents).
Total anthocyanins were analyzed by collecting 20 berries per vine and soaking the peeled skins in 100 mL methanol for 24 h (Mattivi et al., 2006). High performance liquid chromatography (HPLC) separation and quantification of anthocyanins were performed on a Waters 1525 HPLC (Waters, Milford, MA, USA) equipped with a diode array detector (DAD) and a Phenomenex (Castel Maggiore, Bologna, Italy) reversed-phase column (RP18, 250 mm × 4 mm, 5 μM). The anthocyanins were quantified at 520 nm using an external calibration curve with malvidin-3-glucoside chloride as the standard (Sigma-Aldrich, St Louis, MO, USA), following the procedure reported by Mattivi et al. (2006).
The non-destructive analysis of berry color was conducted using a berry-adapted Da-meter at harvest. The mean berry IDAr was calculated for each tagged vine through 40 measurements, sampling the basal and apical parts of the bunches, following the methodology outlined by Valentini et al. (2023).

Winemaking and analysis of wine colour

In 2022 season, grapes from all the field vines in each treatment group were harvested manually and kept separated for micro-scale vinification (three replications each for C and ZEO). For each replication, 100 kg of grapes were destemmed, crushed and transferred to fermentation tanks. Eight g hL^{-1} of potassium metabisulfite were added to the musts and they were inoculated with 20 g hL^{-1} of Saccharomyces cerevisiae. Twenty g hL^{-1} of fermentation activators (diammonium phosphate + thiamine hydro-chloride) were added at two different times during the process, at the beginning and before the end of fermentation. During the alcoholic fermentation, the mass temperature was maintained at 28°C and the skins were punched down twice a day to keep the cap wet. After 5 days of skins maceration, the solid part was separated from the liquid, and the marc was pressed at maximum 1 bar. After ten days, at the end of the fermentation process, the wines were cold stabilized, sulphated with 6 g hL^{-1} of potassium metabisulfite and stored at room temperature in 500 mL bottles closed with cork stoppers.
After 3 months, the wines were analyzed for alcohol concentration, total acidity and pH (Iland et al., 1993). Determination of the anthocyanins in the red wine samples, filtered through 0.45 μm nylon filters, was carried out according to the OIV-MA-AS 315-11 method (OIV, 2017). Thirty microliters of each sample were analyzed using HPLC and the total anthocyanins were quantified at 520 nm with malvidin-3-glucoside chloride (Sigma Aldrich, St Lou-is, MO, USA) using a calibration curve.

Statistical analysis

Data were analyzed using the R project (v 4.0.2, R Foundation for Statistical Computing, Vienna, Austria) and the treatment comparisons were analyzed using Anova (car::Anova library).

Results

Pot trial

The 2021 season was warm and dry, it was characterized by intense heatwaves with periods of prolonged drought (data not reported). During the trial period (from July to the end of August), the mean air temperature was 25 °C and the daily maximum temperature stood on average at 33 °C. Figure 1 highlights the transpiration trend of potted vines (Tr) starting from the day following the first application of zeolite. Additionally, the entire period of water restriction, affecting both treatments, is indicated, aiming to investigate the physiological response of the vines to multiple stress factors: water and thermal-radiative. In detail, the foliar zeolite treatment results in a noteworthy reduction in transpiration immediately after the application (indicated by green arrows) but, in the subsequent days, no significant differences are observed between the treatments. It is intriguing to observe an inclination toward increased transpiration in WS+ZEO, particularly during the hottest days (DOY 223-224-225). However, this trend reaches its minimum point when the maximum air temperature hits 40 °C (Figure 2).

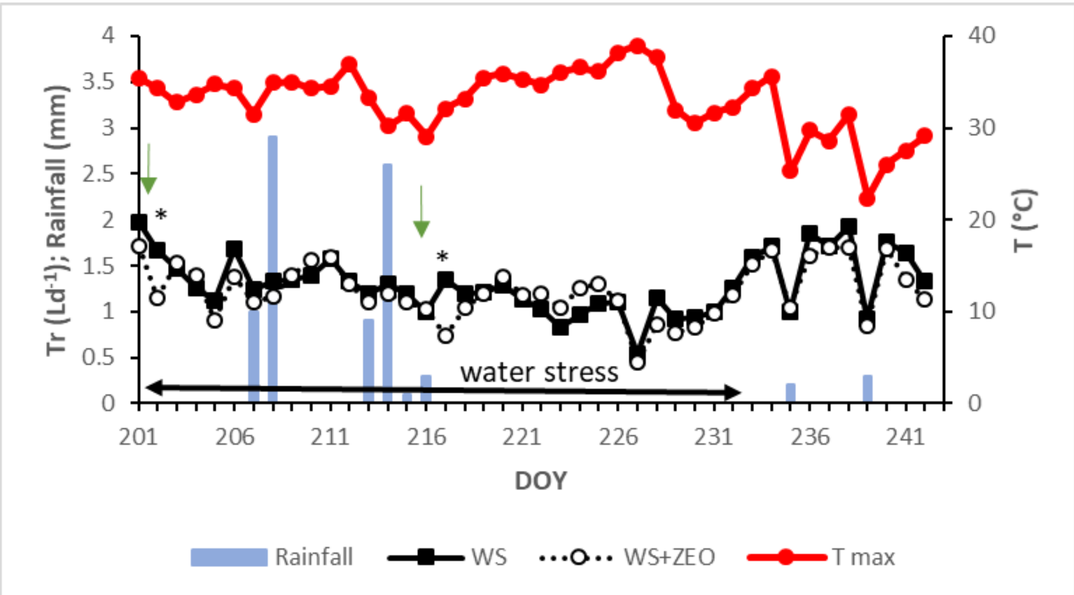

Figure 2. Trend in vines' transpiration (Tr), maximum temperatures (T max) and daily rainfall (Rainfall) at the meteorological station near the vineyard. The water restriction period is indicated by the black horizontal arrow, while the two green arrows denote the exact placement of the two zeolite treatments. Asterisks denote significant differences between the treatments.

The graph also highlights how, at the end of the water stress period, there was a temperature decrease followed by an initial increase in transpiration for both treatments. However, this transpirative increase never returned to the pre-stress level (Figure 2).

Table 1 elucidates the impact of water stress and zeolite application on the temperature of main leaves throughout the season. The temperatures are specifically recorded at solar noon, a time when the combined effects of water deficiency and thermal increase are most pronounced. The data reveal that the leaf temperature in the WS condition consistently surpasses that of WS+ZEO, with significant differences observed on three out of the four survey days (Table 1). These substantial variations persist until after August 11 (DOY 223), which marks the peak thermal disparity between the two treatments, reaching approximately 2 °C. Consequently, the discernible cooling effect induced by zeolite on water-stressed plants becomes evident (Table1).

Table 1. Leaf temperature recorded at midday on potted control (WS) and zeolite treated (WS+ZEO) ‘Sangiovese’ vines on four days respectively 3 and 10 days after the first treatment and 7 and 12 days after the second treatment. DOY 205 (24 July), DOY 211 (30 July), DOY 223 (11 August) and DOY 231 (19 August).

	205	**211**	**223**	**231**
WS	39.2 a	38.1 a	38.9 a	34.4
WS+ZEO	37.7 b	37.3 b	36.9 b	33.1

Different letters indicate significant differences between treatments according to the Anova test ($p \leq 0.05$).

Table 2 provides a comprehensive analysis of key photosynthetic parameters. These measurements were recorded during the midday period for both potted control (WS) and zeolite-treated (WS+ZEO) 'Sangiovese' vines on four days respectively 3 and 9 days after the first treatment and 7 and 15 days after the second treatment.

Table 2. PSII maximum efficiency (Fv'/Fm'), net photosynthesis (Pn, µmol $m^{-2}s^{-1}$), actual photochemical efficiency of PSII (ϕPSII), Electron Transport Rate (ETR, umol electron $m^{-2}s^{-1}$) and stomatal conductance (gs, mmol $m^{-2}s^{-1}$) recorded at midday period on potted control (WS) and zeolite treated (WS+ZEO) ‘Sangiovese’ vines on four survays data. DOY 205 (24 July), DOY 211 (30 July), DOY 223 (11 August) and DOY 231 (19 August).

	205		211		223		231	
	WS	**WS+ZEO**	**WS**	**WS+ZEO**	**WS**	**WS+ZEO**	**WS**	**WS+ZEO**
Pn	6.8 b	8.2 a	9.7 b	12.3 a	5.5 b	7.6 a	9.3	8.6
gs	0.05 b	0.12 a	0.09 b	0.13 a	0.03 b	0.05 a	0.05	0.05
Fv'/Fm'	0.33	0.37	0.32 b	0.42 a	0.38	0.37	0.43	0.40
ϕ_{PSII}	0.13 b	0.16 a	0.14 b	0.18 a	0.07	0.10	0.07	0.08
ETR	85 b	106 a	89 b	117 a	43	63	47	53

Different letters indicate significant differences between treatments according to the Anova test ($p \leq 0.05$).

The data reveal that a moderate water deficit condition corresponds to a reduction in photosynthetic activity (Table 2; Figure 3). In particular, the foliar application of zeolite significantly enhanced the assimilative process, resulting in an approximate 20% increase in Pn in July and a substantial 30% increase on August 11 (DOY 223) compared to the WS treatment. The decline in Pn observed in the WS treatment is supported by a concurrent decrease in stomatal conductance, which even halved on the hottest days (DOY 205, DOY 223).

To better understand the interaction between stomatal response and plant water status, Figure 3 relates stomatal conductance to stem water potential (Ψs). Focusing on values obtained on 6 dates, the graph highlights that, starting from the same Ψs value, WS+ZEO exhibits higher levels of stomatal conductance compared to WS. This supports the findings reported in Table 2, indicating that vines treated with zeolite demonstrate a greater ability to exchange gases at moderately negative water potentials.

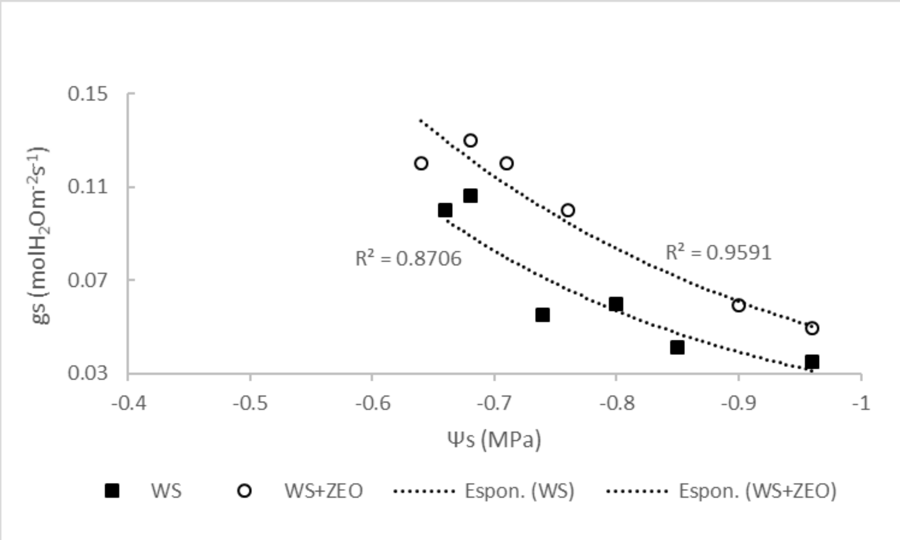

Figure 3. Relationship between stomatal conductance and stem water potential measured on the vines under trial at solar noon. Each data point represents the mean value of 6 measurements.

The maximum efficiency or quantum yield of photosystem II was assessed using a fluorimeter on well-exposed leaves (Fv'/Fm'). The values of maximum efficiency are reported in Table 2 and do not show differences among the compared treatments. However, on the warm day of July 30 (DOY 211), WS exhibits a significant reduction in Fv'/Fm' compared to WS+ZEO (Table 2).

As reported in Table 2, WS+ZEO exhibits improved photosystem II functionality under light (ϕPSII) during the water stress period compared to WS vines. The values recorded for WS+ZEO at the end of July are statistically different for the days of the year 205 and 211 (Table 2).

Table 2 underlines the trend of linear electron flow (ETR), where the ETR for the WS treatment significantly decreases on the hottest days of the season (specifically on July 24 and 30, DOY 205 and 211 respectively) compared to the zeolite treated vines (Table 2).

In-field trial

As no interaction between year and treatment was identified, Table 3 reports the average data of both yield production and grape composition for the 2022 and 2023 season of ‘Sangiovese’ grape grown in-field. The table does not reveal significant differences, both concerning yield parameters (yield per vine, average cluster weight and berry weight) and technological maturity (soluble solids, pH and titratable acidity). However, the mineral treatment's effect on anthocyanin accumulation is evident, as they are significantly higher in ZEO compared to the control at harvest (Table 3).

Table 3. Yield per vine and berry composition parameters measured at harvest in control (C) and zeolite treated (ZEO) 'Sangiovese' vines grown in field condition. The data represent the average of 2022 and 2023 seasons (TSS = total soluble solids; TA = Total anthocyanins).

	Yield per vine (kg)	Cluster weight (g)	Berry mass (g)	TSS (°Brix)	pH	Titratable cidity (g L^{-1})	TA (mg kg^{-1})	IDAr
C	3.2	213	2.4	24.5	3.50	6.14	620 b	1.85 b
ZEO	3.1	209	2.3	24.5	3.45	6.27	738 a	2.13 a

Different letters indicate significant differences between treatments according to the Anova test ($p \leq 0.05$).

Moreover, the DAr index (IDAr), obtained using the non-destructive Da-meter adapted for berries, shows a significantly higher value for the zeolite-treated group compared to the untreated control. This observation appears to have a direct relationship with the anthocyanin content, as detected earlier through wet chemical analysis (Table 3).

The key chemical findings of the 2022 wines are outlined in Table 4. Specifically, there were no discernible differences in alcohol concentration, pH and titratable acidity. Consistent with the berry composition and IDAr values, the ZEO treatment exhibited a noteworthy increase in wine anthocyanin content (Table 4).

Table 4. Chemical analysis on wines obtained from control (C) and chabasite rich-zeolitite (ZEO) treatments grapes in 2022. The wines were analyzed three months after harvest. Different letters in a column indicate significant differences after the Anova test ($p \leq 0.05$)

	Alcohol (%vol)	pH	Titratable acidity (g L^{-1})	Total anthocyanins (mg L^{-1})
C	13.9	3.5	5.50	32.4 b
ZEO	13.5	3.4	5.40	72.1 a

Different letters indicate significant differences between treatments according to the Anova test ($p \leq 0.05$).

Discussion

Over the past few decades, a significant intensification of extreme weather events, i.e. heatwaves, droughts or increases in both the frequency and intensity of rainfall has been noted (IPCC, 2013). The impact of high temperatures on viticulture will be negative for areas which already falter from being too warm to produce quality grapes. It is also well-known that when thermal excess is coupled with water scarcity, the vine responds with active and passive mechanisms that result in a reduction of photosynthetic activity and a decrease in yields due to phenomena such as photoinhibition and heat damage (Medrano et al., 2003; Tombesi et al., 2016).

In particular, several scientific reports have shown that high temperatures above 35°C resulted in a lower anthocyanin concentration in berry skins (Spayd et al., 2002; Pastore et al., 2016). 'Sangiovese' is considered a medium-colored grape when compared to the average of anthocyanin concentration observed in the phenotyping screening carried out on various European grapevine cultivars (Mattivi et al., 2006). It is therefore necessary to implement some strategies to ensure satisfactory productions avoiding the harmful effect of global warming on both the color of the grapes and the wine.

Building on the evidence indicating that the application of mineral particles can effectively reflect solar radiation, thereby reducing the surface temperature of the epigeal organs of grapevines, the objective of this experiment was to investigate the impact of chabasite-rich zeolite on 'Sangiovese'. The focus was on assessing its effects on canopy temperature, gas exchange, yield characteristics and the composition of both grapes and wine.

Over a three-year period, the application of chabasite-rich zeolite at veraison demonstrated a consistent reduction in leaf temperature and an enhancement of leaf photosynthetic efficiency in potted vines. Concurrently, when applied to adult vines during the 2022-2023 growing seasons, foliar zeolite application significantly improved the color of both grapes and wine without affecting yield and technological maturity. While the enhancement of color through the application of minerals, such as kaolin, has been well-documented (Brillante et al., 2016; Dinis et al., 2016; Shellie et al., 2005; Frioni et al., 2019), information on the use of zeolite on vines was limited (Calzarano et al., 2019; Valentini et al., 2021; Petoumenou 2023). Consequently, our research in field vines has confirmed that treating vines with leaf zeolite enables the mitigation of high temperatures that compromise the accumulation of anthocyanins in berries. This, in turn, results in a reduction in canopy temperature, thereby promoting the biosynthesis of phenolic compounds (Conde et al., 2016) and diminishing their enzymatic degradation (Mori et al., 2005; Movahed et al., 2016).

Moreover, it is well-known that 'Sangiovese' in condition of water scarcity has an anisohydric behavior (Dal Santo et al., 2016), meaning it can maximize gas-exchange even under very low water potential conditions (Tardieu and Simmoneau, 1998). Additionally, Poni and colleagues (2007) highlighted that grapevines can adjust their behavior based on the period, duration, and severity of stress. In this research, vines subjected to moderate water stress from late July to mid-August did not exhibit a shutdown of photosynthetic activity, even when the maximum air temperature exceeded 40

°C. Anyway, in the presence of zeolite treatment, we noted elevated stomatal conductance values at stem water potentials, similar to those of the untreated control. This result, concurrent with a significant improvement in photosynthetic rates, implys that zeolite could potentially exert a positive influence on enhancing CO_2 concentration at the stomatal level in grapevines, aligning with previous reports by De Smedt and colleagues (2017) in other fruit species. While several studies suggest that *Vitis vinifera* L. is particularly resistant to photoinhibition (Correia et al., 1990), this study revealed that both the maximum and operational efficiency of photosystem II under light conditions (Fv'/Fm' and ΦPSII) show damage to the reaction centers involved in CO_2 assimilation under multiple stress conditions. This effect is especially pronounced in our experiment, correlating with the previously discussed issues of stem water potential decline, partial stomatal closure, and thermal excess in the canopy.

Specifically, under conditions of severe thermal stress at solar noon, a depression of photosynthesis at the stomatal level is observed. In such limiting situations, inhibition is exacerbated by non-stomatal factors, such as decay electron transport rate and diminished Rubisco regeneration capacity (Medrano et al., 2003). It is known that any disturbance depressing photosynthetic activity, such as water deficiency, exposes leaves to a decrease in CO_2 concentration in the mesophyll and an excess of light, which, not fully utilized in the photochemical process, must be dissipated as heat. Fv'/Fm' is indicative of photoinhibition, namely the degradation of the D1 protein of PSII (Ohira et al., 2005). This serves as an indicator of the photochemical activity of the photosynthetic apparatus under light, and a reduction in this parameter may indicate damage or reduced efficiency of the PSII reaction center. This parameter is crucial, as any reduction in the ratio of variable to maximum fluorescence under multiple stress conditions is associated with an increase in NPQ (non-photochemical quenching). In some cases, this increase can be accompanied by the total inactivation of PSII reaction centers due to oxidative stress.

In a preliminary analysis, it appears that the effect of zeolite on the efficiency and functionality of photosystem II, as well as on photochemical quenching, tends to better preserve the integrity of photosynthetic mechanisms during periods of heightened stress. Indeed, this mineral possesses the ability to maximize CO_2 absorption and promote a more gradual and slow release of it (Prisa, 2020). Although zeolite trated potted vines showed differences in leaf gas-exchanges, no variation on both yield and technological parameter (soluble solids concentration, pH and tritatable acidity). was recorded between treatments in-field conditions. These data corroborate the findings previously reported by Valentini et al. (2021) in Sangiovese vines subjected to frequent heatwaves. Furthermore, wines from both treatments showed no significant variations in ethanol, pH and organic acids;

however, the ZEO treatment showed higher anthocyanin concentration, as previously shown by wet and nondestructive chemical analysis. Extracted during the fermentation process, the anthocyanins contributed to the heightened color intensity observed in wines derived from treated berries. These results align with those of other researchers who attribute the increase in anthocyanin content to temperature regulation facilitated by mineral coating (Calzarano et al., 2019; Frioni et al., 2019).
Additionally, Calzarano and collegues (2019) suggested that zeolitite might adversely affect the extractability of anthocyanins during fermentation when applied close to the harvest date. The present study underscores how the strategic application of just two treatments near veraison, a pivotal stage in anthocyanin synthesis (Pastore et al., 2013), can enhance the color of grapes and wines without altering their composition.

Conclusion

The utilization of chabasite zeolite emerges as a proactive strategy for effectively managing the increasingly frequent summer heatwaves in the Mediterranean basin. Significantly, the straightforward application of this product on canopy, resembling a conventional phytosanitary treatment, makes the method economical and available to all winegrowers. Over a three-year span of trials conducted in both pots and vineyards, compelling results have been observed. In conditions marked by moderate water stress and elevated temperatures, natural zeolite demonstrates the potential to thermally regulate the canopy, leading to an enhancement in the plants' photosynthetic efficiency. Consequently, field-grown vines exhibit a heightened production of anthocyanins, contributing to the enhanced coloration of both grapes and wine.
While these findings are promising, further investigations are warranted to explore whether, beyond its thermal effects, the zeolite treatment may also trigger an elicitor mechanism influencing the grapevine's response.

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Chapter 5.

General discussion and conclusion

More than once, I have encountered inquiries regarding the definition of *stress* concerning grapevines. This is because, by its very nature, *Vitis vinifera* L. is a heliophile species that effectively adapts to challenging conditions, such as excessive heat-radiation and water scarcity. Drawing upon Wardlaw's seminal work (1972), stress can be characterized as a potentially adverse factor for an organism, with broad acknowledgment that such factors can originate from the environment (abiotic) or result from other organisms (biotic). In the context of field conditions, grapevines seldom face singular abiotic stressors; rather, they are often affected by a combination of environmental limiting factors, encompassing excessive light and temperature, insufficient relative humidity, water scarcity, and more (Mittler, 2006). It is established that these environmental stresses set off a cascade of morpho-structural, physiological, biochemical and molecular changes that significantly impact plant growth and yield (Chaves, 2002; Wang et al., 2003). Indeed, following prevailing management practices in a significant portion of the Mediterranean region, it is anticipated that crop yields may decline by as much as 40 percent (Iglesias et al., 2007). In case of grapevine, it is anticipated that increased drought conditions will lead to a reduction in both leaf area and yield (Fraga et al., 2016), a situation further aggravated by sunburn injuries (Gambetta et al., 2021). Recent research underscores that the mechanisms enabling adaptation to summer stresses in diverse grape varieties are governed by genetically regulated stomatal sensitivity (Medrano et al., 2003; Palliotti et al., 2015). When it comes to stomatal kinetics during drought conditions, *V. vinifera* is recognized as an anisohydric species, optimizing photosynthetic gain by keeping stomata open despite a significant drop in leaf water potential (Tardieu and Simmoneau, 1998). In reality, a spectrum of responses, varying from less to more anisohydric behaviors, is evident across different grapevine cultivars, and these strategies may coexist depending on water stress intensity, duration, timing and interactions with excessive irradiance and temperature (Poni et al., 2007; Chaves et al., 2010).

Building on this foundation, the Ph.D thesis aimed to devise innovative strategies to effectively counteract the short to medium-term impacts of heatwaves, providing grape growers with adaptable and cost-effective tools to safeguard both plant vitality and yield. Over the course of the three-year trial period, two distinct approaches were examined in detail: firstly, the implementation of a fruit-zone cooling system to mitigate the effects of heatwaves on both red (Chapter 2) and white (Chapter 3) grapes; secondly, the application of sustainable chabasite-rich zeolite through foliar treatments, aimed at reducing canopy temperature and enhancing anthocyanin accumulation in ‘Sangiovese’ grapes and wine. These objectives collectively aim at improving the physiological response of vines to various summer stresses and mitigating sunburn damage.

In the first study (Chapter 2), a multifunctional irrigation system (drip and climate conditioning) was implemented and its ability to regulate the microclimate around the fruit-zone during heatwaves was assessed. Specifically, the cooling effect on the cluster zone was evaluated concerning the physiology and production aspects of the two most cultivated red grape varieties in Italy: ‘Sangiovese’ and ‘Montepulciano’. The selection of these grape varieties was motivated not just by their national agronomic significance but also by their distinct responses to various stress conditions. Consequently, the vines were cultivated in pots and exposed to different water regimes, including well-watered and water shortage conditions during the ripening period. In our observations, 'Montepulciano' exhibited a higher leaf assimilation rate and stomatal conductance under optimal water conditions in comparison to 'Sangiovese.' Notably, both varieties demonstrated consistent behavior even under conditions of water scarcity and in presence of misting. Additionally, vines treated with nebulized water, demonstrated increased yield without compromising technological maturity compared to water-stressed vines. In the 2023 vintage, the misting system prevented ripening blockage in 'Montepulciano' under water stress. The nebulized treated vines exhibited a significant increase in total anthocyanins concentration, indicating the microclimate's pivotal role in anthocyanin biosynthesis and reducing oxidative processes.

A field experiment was then conducted to investigate the effect of misting water on a white grape variety grown in field conditions over two years (Chapter 3). Two distinct thermal-radiative regimes (defoliated and undefoliated) were applied in a flatland vineyard of ‘Pignoletto’ grapevines. The primary objective was to assess the impact of these treatments on yield attributes, berry necrosis and the concentration of secondary metabolites such as flavonols. Simultaneously, a portable and non-destructive vis/NIR device (Da-meter© berry adapted) was utilized to monitor the level of browning of clusters subjected to thermal stress. In particular, a new index (IDAg) has been introduced to identify a relationship between this and the accumulation of flavonols in the berry. The study revealed that the use of misting consistently reduced both air and berry temperatures over two years. Vines treated with nebulized water exhibited increased yield, attributed to reduced sunburn damages and subsequent cluster weight gain. While technological maturity showed no significant differences, misting-treated vines in the scorching 2023 season demonstrated a notable trend towards increased acidity, contrasting with control and defoliated groups. Interestingly, flavonol synthesis remained unaffected by thermal reduction but was stimulated under increased light exposure, leading to exclusive overexpression in the defoliated treatment, as confirmed by the DAg index.

In conclusion, the cooling system implemented in the bunch area emerges as a valuable tool for mitigating the adverse effects of summer stresses on grapes, notably in minimizing sunburn damage on exposed clusters. Furthermore, the system demonstrated positive outcomes for both white and red grapes. In the case of the latter, it exhibited a beneficial impact on anthocyanin accumulation and berry color.

The third experiment emerges as a strategic approach for winegrowers to effectively address the impact of heatwaves in a timely manner (Chapter 4). While *Vitis vinifera* L. is inherently drought-resistant, there is evidence suggesting a reduction in its photosynthetic capacity caused by elevated temperatures (Medrano et al. 2003). This decline significantly influences vine growth, yield and grape composition as highlighted in various studies (Matthews and Anderson 1988; Schultz and Matthews 1988). Specifically, one of the initial effects of climate change in viticultural areas is the accelerated progression of phenological stages, especially early berry ripening. Under these conditions, a common issue is the decoupling between technological and phenolic maturity. It is recognized that anthocyanins, crucial for phenolic maturity in red grapes, face challenges in accumulation due to high temperatures. This study aims to assess the effectiveness of a treatment with mineral compounds, such as chabasite-based zeolite, in enhancing anthocyanin accumulation and in improving physiological performance. The present study demonstrates the effectiveness of foliar zeolite treatment not only in reducing canopy temperatures but also in improving anthocyanin accumulation in grapes. The latter results were confirmed by the wine obtained in the 2022 season.

Together, this research work provides a comprehensive and cohesive framework for understanding and managing multiple summer stresses in both white and red grapevines. Implementing these findings can significantly contribute to the adaptation of grapevines to the challenges posed by climate change.

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