

Research Article

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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Received 6 November 2024; Accepted 5 March 2025

Academic Editor: Justine Vanden Heuvel

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In response to increasingly hot and dry summers driven by climate change, grapevines (*Vitis vinifera* L.) can utilize adaptive mechanisms that often prioritize survival over yield and grape quality. The efficiency of the vine canopy, particularly in terms of gas exchange and net assimilation, declines with water scarcity, underscoring the importance of mitigating strategies such as emergency irrigation. However, in Mediterranean vineyards, water shortages often render irrigation impractical. An alternative approach is the application of minerals, such as zeolites, to mitigate the negative effects of summer stress. This study aimed to evaluate the physiological, vegetative–productive, and qualitative effects of chabazite-rich zeolite treatments on potted grapevines subjected to both water and heat stress, and on field-grown vines exposed only to heat stress. The research was conducted over a 3-year period (2021–2023) on Sangiovese grapevines, divided into two distinct trials: the first on potted vines (2021) and the second on field-grown vines (2022–2023). The potted trial involved 12 plants placed on lysimeters, subjected to water restriction (50% restitution of water lost through transpiration), and divided into two treatments: water stress vines (WS) and WS vines treated with natural zeolite (WS + ZEO). The field trial involved 24 nonirrigated plants with two treatments: untreated control (WS) and zeolite treatment (WS + ZEO). Microclimatic conditions were monitored during ripening, and the effects of zeolite were assessed in terms of canopy physiology, yield, sunburn damage, and grape composition. Grapes from the field trial vines were microvinified, and the resulting wine color was analyzed twice, after 3 months and 1 year. The results showed that zeolite treatments effectively reduced canopy temperature by two degrees Celsius, enhancing gas exchange efficiency and photosynthetic activity in potted vines. In field-grown vines, these treatments significantly improved grape composition, particularly boosting total anthocyanin levels by 19% in the berries and 10% in the resulting wine, compared to the untreated control. In conclusion, zeolite-based treatments appear to be a valuable tool for improving the productive performance of Sangiovese in environments characterized by multiple summer stresses.

1. Introduction

In accordance with the latest report from the International Organization of Vine and Wine (OIV) [1], Italy continues to rank among the world's top wine producers, with Sangiovese standing out as the most widely cultivated grape variety, covering over 50,000 ha [2]. This renowned grape variety plays a vital role in several Italian-protected appellations,

including Chianti, Nobile di Montepulciano, and Brunello di Montalcino, capturing global acclaim. As is widely known, 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 [3–6].

Sangiovese berries are characterized by a low concentration of anthocyanins in their skin, primarily composed of glycosylated anthocyanins [7, 8]. Notably, Mattivi et al. [3]

revealed for this variety 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 2000s, focusing on enhancing the anthocyanin content in berry skins [9–12] and resultant wines. This challenge intensifies amid the escalating frequency of summer days exceeding the critical temperature for anthocyanins biosynthesis and degradation of 35°C [13, 14], as reported for Sangiovese by Movahed et al. [15].

The Mediterranean basin is experiencing not only a substantial rise in air temperature but also a shift in precipitation distribution [16, 17], subjecting vines to a combination of abiotic stresses impacting plant growth and fruit composition [8, 18, 19]. Dinis et al. [20] 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 [21]. Heatwaves accelerate soluble solid accumulation, deplete organic acids, elevated pH, and induce atypical aroma compounds, resulting in wines less amenable to aging, with compromised color and altered aromatic profiles [22].

To address these challenges, new short-term agronomic techniques have emerged, including postveraison shoot trimming, leaf removal [23–25], smart irrigation and fruit-zone cooling system [26], and the foliar application of particle materials such as kaolin and zeolite [27–33].

Zeolites, particularly chabazite-rich varieties, are aluminosilicate minerals with unique open framework structures that offer numerous advantages for plant health and viticulture. Notably, zeolites exhibit high and selective cation-exchange capacity, reversible dehydration, selective molecular absorption, and catalytic behavior [34]. These properties enhance nutrient availability and improve soil structure, fostering robust root development and overall plant vigor when applied to the soil. A recent research project conducted by Cataldo et al. [35] highlighted the effects of Zeowine—a soil conditioner made with clinoptilolite and compost derived from industrial wine waste—on soil properties. The study demonstrated its potential to enhance water-holding capacity, reducing the need for vine irrigation by effectively retaining water within the zeolite structure [35, 36]. It is well established that zeolites enhance plant resilience to abiotic stresses, particularly drought, by preserving soil moisture and ensuring consistent nutrient availability, as demonstrated across various crops [37, 38].

Zeolite-rich rocks containing more than 50% zeolite can be classified as “zeolitite,” which are recognized as “non-toxic” (IARC, 2017) and “safe” for agricultural applications [39]. This safety profile makes zeolites a valuable tool for sustainable viticulture. Furthermore, in recent years zeolites have gained considerable attention for their dual role in pest and disease control [40] and their beneficial effects on plants, particularly in enhancing photosynthesis through improved CO₂ absorption [41, 42]. The application of zeolites directly to the grapevine canopy has recently been studied [42]. This

study assessed the impact of natural zeolite on fungal diseases and insect pests, marking a significant departure from conventional approaches. Subsequent studies have reinforced these findings, extending the scope beyond grapevine to other crops such as olive. For instance, treatments with zeolitic particle films have emerged as effective alternatives to chemical insecticides for controlling olive fly (*Bactrocera oleae*) infestations, while also enhancing olive oil quality [39]. Further investigations have examined the utility of zeolite under environmental stress conditions [43]. A particularly interesting study evaluated the foliar application of chabazite zeolite during the ripening period, revealing its capacity to mitigate thermal stress and manage soil water content. These applications significantly reduced sunburn damage and minimized yield loss, mirroring the benefits observed with late irrigation [33]. Similarly, it has been reported that the use of zeolite enhanced cluster weight and boosted overall yield production, further underscoring its potential in sustainable vineyard management [32]. These treatments are especially effective when applied at the onset of veraison [30, 44].

In grapevines, foliar zeolite applications have been associated with increased anthocyanin concentrations, although this effect diminishes when the applications are made close to harvest [30, 42]. Additionally, zeolitic materials have found applications in winemaking as oenological adjuvants, particularly for the removal of riboflavin and protein, functioning similarly to bentonite [45, 46].

Given the increasing interest in sustainable methods for mitigating heatwaves and summer stress in vineyards, this study aims to evaluate the effects of foliar zeolite applications on vine physiology, grape composition, and wine quality of cv. Sangiovese.

2. Materials and Methods

2.1. Plant Material and Treatments. The trials were carried out over three years: in 2021 the experiment was performed on two-year-old potted Sangiovese vines and in 2022 and 2023 seasons on a 7-year Sangiovese vineyard located at the experimental station of the University of Bologna (Bologna, 44°32'N, 11°22'E). The plant material consisted of Sangiovese vines, clone TEA 10D grafted onto 110R rootstock, spaced at 1 m within a single row (oriented northeast to southwest). 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* included 12 vines with a uniform leaf area, each planted in 30-L plastic pots containing a soil mixture of 39% sand, 39% silt, and 22% clay, with 1.8% organic matter and a pH of 7.8. The field capacity and wilting point were calculated using the methodology of Saxton and Willey (2005), as cited in Valentini et al. [8].

The 12 potted plants were arranged in corresponding lysimeters (mod. LAUMAS, ABC Bilance, Campogalliano, IT) and were irrigated until the preveraison phase with an approximate daily water amount of 3 L, distributed automatically

through a dripper irrigation system. Subsequently (from day of the year (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 WS associated with treatment using natural zeolite chabazite-based at a concentration of 3% (WS + ZEO). Zeolite-rich chabazite (ZEOVER, Verdi, Reggio Emilia, IT) was applied to the entire canopy when the WS was induced on DOY 201, with a follow-up application conducted on DOY 217. Additionally, aluminum foil was placed over the pots before the trial began to prevent overheating. The *field trial* involved 24 nonirrigated plants distributed in three randomized blocks pertaining to two different treatments: untreated control (WS) and treatment with zeolite (WS + ZEO). The vineyard was situated on a flat terrain characterized by clay soil typical of the Po Valley. The vines were arranged with a spacing of 1 m × 2.8 m, resulting in a consistent number of clusters. The shoots were meticulously positioned vertically by hand and trimmed at the end of June, maintaining a canopy height of approximately 1.2 m. The vineyard, conducted without irrigation, employs a standard program for the management of fungal diseases.

The two treatments with Italian chabazite-rich zeolite were carried out at a concentration of three percent, at the beginning and at the end of veraison—stages 34 and 35 according to Eichorn and Lorenz [47]. The suspensions (1000 L ha⁻¹ corresponding to 0.28 L vine⁻¹) were carefully sprayed on both canopy sides using a knapsack sprayer (Model M3, Cifarelli, Pavia, IT).

2.2. 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). In potted vines, midday leaf temperature was measured on two well-exposed leaves (nodes 6–10) using an infrared thermometer (model Raynger ST, Raytek, Santa Cruz, CA, USA). Measurements were taken on DOYs 205, 211, 223, and 231.

2.3. Water Status and Leaf Gas-Exchange Measurements in Potted Trial. In the potted trial, to monitor vine water use, daily gravimetric vine water loss (Tr) was measured every 10 min throughout the growing season using a platform scale mod. LAUMAS (ABC Bilance, Campogalliano, MO, IT) connected to a CR1000 datalogger (Campbell Scientific, Inc., Logan, UT, USA). To prevent disturbance from rain-water and reduce soil evaporation losses, each pot was covered with plastic film, following the method described in Valentini et al. [8].

Midday stem water potential (Ψ_s) was measured on six vines per treatment with a Scholander-type pressure chamber (Model 3005, Soil Moisture Equipment Corp., Santa Barbara, CA, USA), at one pm on a mature leaf per vine on DOYs 202, 205, 211, 218, 223, and 231. Each leaf was covered with a plastic bag and aluminum foil before the measurements [48].

A comprehensive analysis of key photosynthetic parameters was conducted. Measurements were taken at midday on both potted control (WS) and zeolite-treated

(WS + ZEO) Sangiovese vines on four occasions: 3 and 9 days after the first treatment, and 7 and 15 days after the second treatment. Specifically, leaf net photosynthesis (Pn), stomatal conductance (gs), and PSII efficiency (Fv'/Fm', ϕ PSII, ETR) were measured using a portable Li-Cor 6400 gas-exchange system (Li-Cor Inc., Lincoln, Nebraska, USA). These parameters were recorded from two well-exposed mature leaves on the main shoot (nodes 6–10), on the same day as the stem water potential measurements. The leaves were monitored using a broad leaf chamber under saturated light conditions (1500 μ mol m⁻²s⁻¹), provided by an external lamp.

2.4. Vine Growth, Yield Components, and Berry Composition in Field Trial. In the field experiment, the vines were individually harvested on September 5, 2022 (DOY 248), and September 18, 2023 (DOY 261), and the crop weight, bunch number, and bunch weight were recorded. During the winter of both years, vines were pruned and pruning weight was measured.

At harvest, 50 berries per vine were randomly sampled and analyzed for total soluble solids (TSS), pH, and titratable acidity following the methodology described by Allegro et al. [33].

Total anthocyanins (TAs) were assessed on 20 randomly collected berries using the method outlined by Pastore et al. [12]. Separation and quantification were carried out via high-performance liquid chromatography (HPLC) equipped with a diode array detector (DAD) and a reversed-phase column. TAs were quantified at 520 nm using malvidin-3-glucoside chloride as a standard [3].

Additionally, a nondestructive analysis of berry color was carried out at harvest using a vis/NIR method with a berry-adapted DA-meter. The device provides the index of absorbance (IAD_R), which correlates with TA content. For each tagged vine, IAD_R was calculated based on 40 measurements, sampling both the basal and apical parts of the bunches, following the methodology described by Valentini et al. [49].

2.5. Winemaking and Analysis of Wine Color. In 2022, grapes from field-grown vines in each treatment group were manually harvested and processed separately for microscale vinification, with three replications each for WS and WS + ZEO. For each replication, 100 kg of grapes were destemmed, crushed, and transferred to fermentation tanks following the method outlined by Sparrow and Smart [50]. Fermentation was conducted at a controlled temperature of 28°C, with the skins punched down twice daily. After 5 days of maceration, the must was pressed, and after 10 days, the wines were cold-stabilized, sulfited, and bottled in 375-mL containers sealed with cork stoppers. After 3 months, the wines were analyzed for the alcohol concentration, titratable acidity, and pH [51]. Color density and hue were determined by measuring optical density (O.D.) at 420, 520, and 620 nm (1 cm of optical path) using a Jasco 810 spectrophotometer (Tokyo, Japan) and calculated as follows: color density (CD) = O.D. 420 nm + O.D. 520 nm + O.D. 620 nm; hue

(HUE) = O.D. 420 nm/O.D. 520 nm. CIELab parameters L^* (lightness), a^* (redness), b^* (yellowness), C_{ab}^* (chroma), and H_{ab}^* (hue angle) were calculated according to OIV-MA-AS2-11 [52], while the percentage of color due to anthocyanins, copigmentation, and polymerization was determined according to Boulton [53].

The determination of the anthocyanins in the red wine samples, filtered through 0.45- μ m nylon filters, was carried out according to the OIV-MA-AS 315-11 method [54]. Thirty microliters of each sample was analyzed using HPLC, and the TAs were quantified at 520 nm with malvidin-3-glucoside chloride as the standard (Sigma-Aldrich, St Louis, MO, USA) using a calibration curve.

Phenolic acid, flavanol, and flavonol analysis of wines [55] was carried out in a HPLC instrument equipped with a quaternary gradient pump Jasco PU-2089, an autosampler Jasco AS-2057 Plus Intelligent Sampler, a Jasco UV/Vis MD-910 PDA detector, and a Jasco FP-2020 Plus Fluorescence detector (Jasco, Tokyo, Japan). Quantification was performed by means of calibration curves previously obtained by duplicate injections of pure standard solutions at known concentrations. All the analyses were carried out in triplicate.

2.6. Statistical Analysis. Data were analyzed using the R project [56], and the treatment comparisons were analyzed using Anova (car::Anova library).

3. Results

3.1. Climatic Trends in Maximum Daily Temperatures and Precipitations Over Three Years of Trials. Over the three trial years (2021, 2022, and 2023) at the University of Bologna's experimental station, maximum daily temperatures and rainfall patterns were monitored to evaluate the impact of abiotic stresses on the physiological, productive, and qualitative responses of Sangiovese grapevines (Figure 1). In particular, data from the hottest period of the year—between late July and the end of August—are reported, a time when, starting from the preveraison stage, there is a high likelihood of heatwaves and the vines are exposed to multiple stress factors (Figures 1(a), 1(b), and 1(c)).

In 2021, maximum daily temperatures remained relatively stable, ranging mostly between 30°C and 35°C throughout the observed period (DOYs 201–236). During the same year, rainfall was almost absent, with only a few insignificant rain events. The combination of consistently high temperatures and minimal rainfall suggests hot and dry climatic conditions (Figure 1(a)).

In 2022, temperatures followed a similar trend, with average values around 30°C–35°C. However, a notable drop in temperature occurred toward the end of the observation period (DOYs 230–231), coinciding with a significant rainfall event of 60 mm around DOY 231. Other smaller rain events were recorded throughout the period, indicating an overall increase in water availability compared to 2021 (Figures 1(a) and 1(b)).

In 2023, maximum temperatures showed more variability compared to the previous years, with a significant dip around DOY 216, followed by a recovery up to values close

to 35°C–40°C by the end of the period. Rainfall remained scarce, with only a couple of minor events recorded. The trend of increasing and stabilizing high temperatures toward the latter part of the period reflects particularly warm conditions (Figure 1(c)).

Upon analyzing the data, indicators of potential heatwaves can be observed across all 3 years. In 2021, the persistence of high maximum temperatures, with little fluctuation and no rainfall, suggests prolonged thermal stress. In 2022, although the temperature trend was more dynamic, the lack of significant drops in temperature before the late-season rainfall event may indicate another heatwave. In 2023, maximum temperatures stabilizing between 35°C and 40°C toward the end of the period, coupled with an absence of rainfall, strongly suggest the occurrence of a particularly intense heatwave (Figure 1).

3.2. Pot Trial. The transpiration trend of potted vines (Tr) starting from the day following the first application of zeolite is reported in Figure 2. 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 blue 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 (DOYs 223–224–225). However, this trend reaches its minimum point when the maximum air temperature hits 40°C (DOY 227) as reported in Figure 1(a).

Figure 2 also highlights at the end of the WS period, a temperature decrease trend followed by an increase in transpiration for both treatments, without achieving the prestress level.

The impact of WS and zeolite application on the temperature of main leaves throughout the season is elucidated in Table 1. The data reveal that the leaf temperature in the WS + ZEO condition consistently goes under that of WS, 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 of thermal disparity between the two treatments, reaching approximately two degrees Celsius. Consequently, the discernible cooling effect induced by zeolite on water-stressed plants becomes evident (Table 1).

The data on key photosynthetic parameters indicate that under moderate water-deficit conditions, the use of zeolite can have a positive effect on photosynthetic assimilation (Table 2). In particular, the foliar application of chabazite zeolite significantly improved the efficiency of Photosystem II, evidenced by an approximate 20% increase in net photosynthesis (P_n) observed on DOYs 205 and 211, and a substantial 30% increase on August 11 (DOY 223) compared to the WS treatment. The decrease in P_n in the WS treatment correlates with a marked reduction in stomatal conductance (g_s), which was reduced by nearly 50% on the hottest days (DOYs 205 and 223).

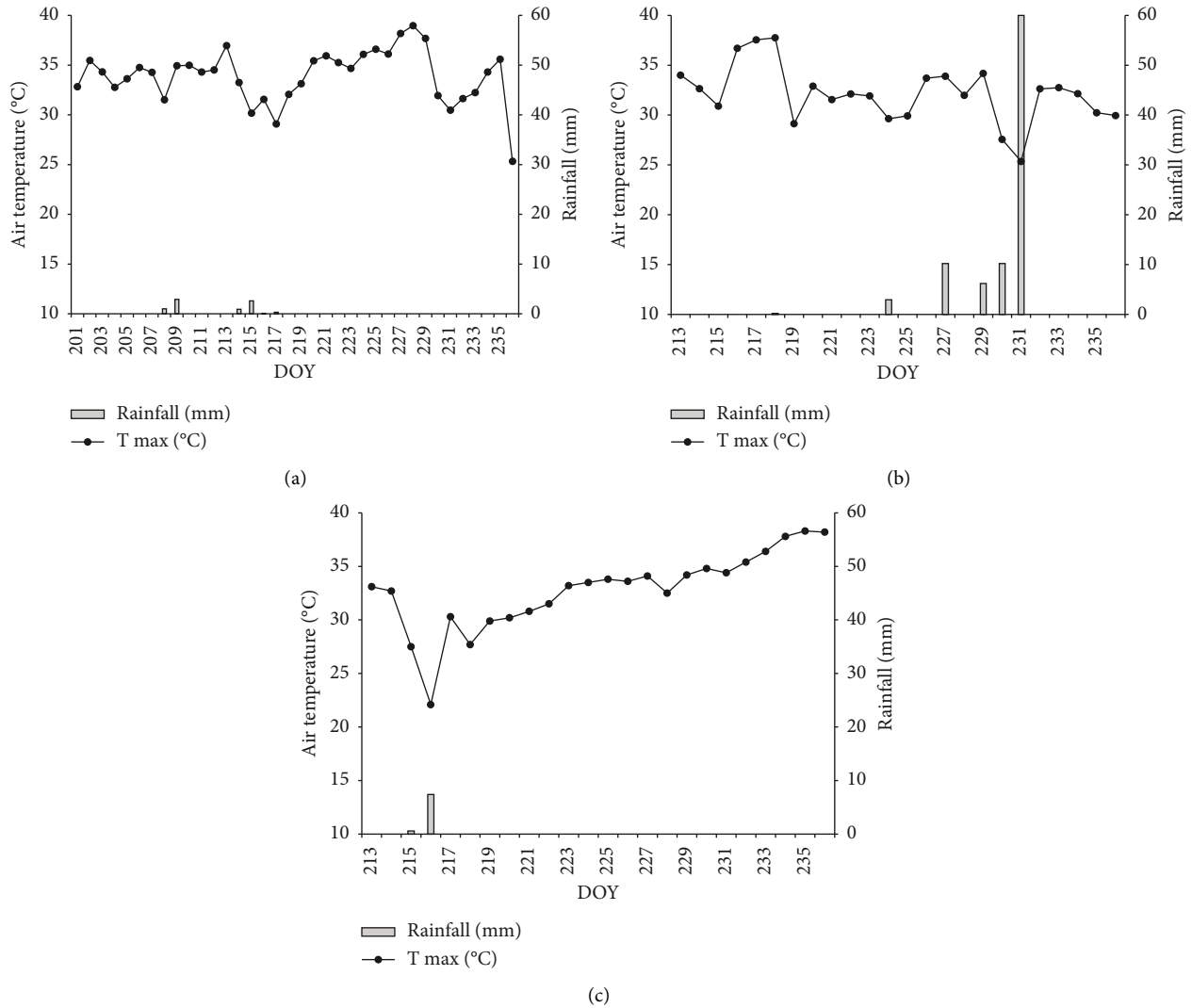


FIGURE 1: The trend of air maximum temperature (black line, °C) and the rainfall (gray bars, mm) at the meteorological station during the trial periods (2021 (a), 2022 (b), and 2023 (c)). DOY = day of the year.

To better understand the interaction between stomatal response and plant water status, Figure 3 relates stomatal conductance to stem water potential (Ψ_s). Focusing on values obtained on six dates, the graph highlights that, starting from the same Ψ_s 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, compared to the untreated vines.

The maximum efficiency or quantum yield of Photosystem II, assessed using a fluorimeter on well-exposed leaves (F_v'/F_m' , Table 2), does not show differences among the compared treatments. However, on the warm day of July 30 (DOY 211), WS exhibits a significant reduction in F_v'/F_m' compared to WS + ZEO (Table 2).

As reported in Table 2, WS + ZEO exhibits improved Photosystem II functionality under light (ϕ_{PSII}) during the WS period compared to WS vines. The values recorded for

WS + ZEO at the end of July are statistically different for the DOYs 205 and 211 (Table 2).

A similar trend is observed in the overall rate of electron transfer during the photosynthetic process (ETR). The ETR for the water-stressed treatment significantly decreases on the hottest days of the season (specifically on July 24 and 30, DOYs 205 and 211, respectively) compared to the zeolite-treated vines (Table 2).

3.3. In-Field Trial. As no interaction between year and treatment was identified, Table 3 reports the average data of both yield and grape composition for the 2022 and 2023 seasons of Sangiovese grape grown in-field. The table does not reveal significant differences, neither concerning yield parameters (yield per vine, average cluster weight, and berry weight) nor technological maturity (soluble solids, pH, and titratable acidity). However, the mineral treatment's effect on anthocyanin accumulation is evident as they are

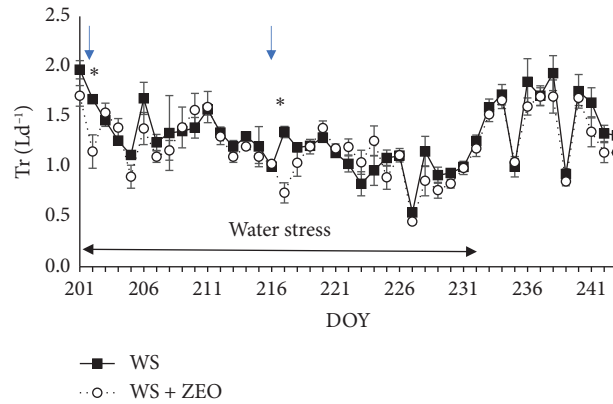


FIGURE 2: Trend in vine transpiration (Tr) measured using lysimeters under different treatments: WS (water-stressed) and WS + ZEO (water-stressed with zeolite application). DOY = day of the year. Data represent the mean of six vines \pm SE. The water restriction period is indicated by the black horizontal arrow, while the two blue arrows mark the timing of zeolite applications. Significant differences between treatments on the same day are denoted by asterisks, as determined by the ANOVA test ($*p = 0.02$ for DOY 201 and $*p = 0.01$ for DOY 217).

TABLE 1: Leaf temperature (T_{LEAF}) recorded at midday on potted control (WS) and zeolite-treated (WS + ZEO) Sangiovese vines on 4 days: 3 and 10 days after the first treatment and 7 and 12 days after the second treatment, respectively.

	T_{LEAF} ($^{\circ}C$) DOY 205	T_{LEAF} ($^{\circ}C$) DOY 211	T_{LEAF} ($^{\circ}C$) DOY 223	T_{LEAF} ($^{\circ}C$) DOY 231
WS	39.2 ± 0.27^a	38.1 ± 0.17^a	38.9 ± 0.17^a	34.4 ± 0.43
WS + ZEO	37.7 ± 0.41^b	37.3 ± 0.47^b	36.9 ± 0.47^b	33.1 ± 0.59
<i>p</i> -value	0.006	0.034	0.001	0.089

Note: DOY 205 (July 24), DOY 211 (July 30), DOY 223 (August 11), and DOY 231 (August 19). Each value represents the average temperature measured on mature well-exposed leaves ($n = 12 \pm SE$). Different letters indicate significant differences between treatments according to the ANOVA test.

TABLE 2: Maximum PSII efficiency (Fv'/Fm'), net photosynthesis (Pn), actual photochemical efficiency of PSII (ϕ_{PSII}), electron transport rate (ETR), and stomatal conductance (gs) measured at midday on potted control (WS) and zeolite-treated (WS + ZEO) Sangiovese vines across four survey dates.

	DOY 205		DOY 211		DOY 223		DOY 231	
	WS	WS + ZEO	WS	WS + ZEO	WS	WS + ZEO	WS	WS + ZEO
Pn ($\mu mol m^{-2} s^{-1}$)	6.8 ± 0.39^b	8.2 ± 0.44^a	9.7 ± 0.67^b	12.3 ± 1.39^a	5.5 ± 0.36^b	7.6 ± 0.87^a	9.3 ± 0.99	8.6 ± 1.48
<i>p</i> -value	0.016		0.049		0.023		0.727	
gs ($mol m^{-2} s^{-1}$)	0.05 ± 0.01^b	0.12 ± 0.01^a	0.09 ± 0.01^b	0.13 ± 0.02^a	0.03 ± 0.01^b	0.05 ± 0.01^a	0.05 ± 0.01	0.05 ± 0.01
<i>p</i> -value	0.001		0.049		0.012		0.783	
Fv'/Fm'	0.33 ± 0.04	0.37 ± 0.01	0.32 ± 0.01^b	0.42 ± 0.04^a	0.38 ± 0.01	0.37 ± 0.02	0.43 ± 0.02	0.40 ± 0.01
<i>p</i> -value	0.601		0.047		0.741		0.166	
ϕ_{PSII}	0.13 ± 0.01^b	0.16 ± 0.01^a	0.14 ± 0.01^b	0.18 ± 0.01^a	0.07 ± 0.01	0.10 ± 0.02	0.07 ± 0.01	0.08 ± 0.02
<i>p</i> -value	0.025		0.006		0.060		0.553	
ETR ($\mu mol electron m^{-2} s^{-1}$)	85 ± 4.50^b	106 ± 6.30^a	89 ± 7.82^b	117 ± 8.03^a	43 ± 7.90	63 ± 5.30	47 ± 7.20	53 ± 10.2
<i>p</i> -value	0.048		0.012		0.065		0.564	

Note: DOY 205 (July 24), DOY 211 (July 30), DOY 223 (August 11), and DOY 231 (August 19). Each value represents the mean of measurements taken on mature, well-exposed leaves ($n = 12 \pm SE$). Different letters denote significant differences between treatments as determined by the ANOVA test.

significantly higher in WS + ZEO compared to the control at harvest (Table 3).

Moreover, the AD_R index (IAD_R), obtained using the nondestructive 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, analyzed both after three months and one year from bottling, did not show discernible differences in the alcohol concentration,

pH, and titratable acidity (Table 4). Consistently with the berry composition and IAD_R values, the WS + ZEO treatment exhibited a noteworthy increase in the wine anthocyanin content (Table 4).

After 1 year from bottling, the analysis of wine color together with the percentage contribution of copigmentation and polymerization to the overall color was performed (Table 5). It clearly appears that the redness of WS + ZEO samples was significantly higher than that of WS (see the absorbance values at 520 nm and the a^* index in Table 5), making those wines more deeply colored (higher C_{ab}^* index)

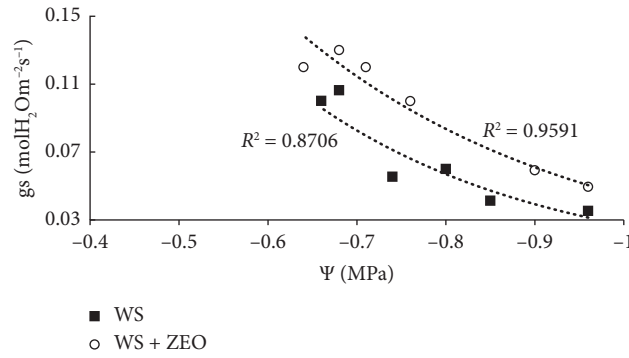


FIGURE 3: Relationship between stomatal conductance (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) and stem water potential (Ψ ; MPa) under two treatments: WS (water-stressed, black squares) and WS + ZEO (water-stressed with zeolite application, open circles). The fitted regression lines are represented as dotted lines for each treatment. Coefficients of determination (R^2) are displayed for each regression model, with p -values of 0.02 (WS) and 0.001 (WS + ZEO).

TABLE 3: Yield per vine and berry composition parameters measured at harvest in control (WS) and zeolite-treated (WS + ZEO) Sangiovese vines grown in field condition.

	Yield per vine (kg)	Cluster weight (g)	Berry mass (g)	TSS ($^\circ$ Brix)	pH	Titrateable acidity (g L^{-1})	Total anthocyanins (mg kg^{-1})	IAD _R
WS	3.18 ± 0.20	212.5 ± 12.6	2.39 ± 0.08	24.5 ± 0.18	3.50 ± 0.01	6.14 ± 0.20	620 ± 27^b	1.85 ± 0.05^b
WS + ZEO	3.11 ± 0.21	209.0 ± 14.3	2.25 ± 0.07	24.5 ± 0.19	3.45 ± 0.02	6.27 ± 0.28	738 ± 58^a	2.13 ± 0.05^a
p -value	0.71	0.80	0.06	0.85	0.06	0.61	0.012	0.001

Note: The data represent the average of 2022 and 2023 seasons \pm SE (TSS = total soluble solids; IAD_R = index of absorbance). Different letters indicate significant differences between treatments according to the ANOVA test.

and less luminous (lower L^*). The contribution to the color of copigmentation was not different between samples (equal to 7.8% of the global redness), while polymeric anthocyanins tended to be higher in WS wines ($p = 0.08$).

Flavanols, benzoic acids, cinnamic acids, flavonols, and tyrosol were also quantified (Supporting information, Table S1), but no significant differences between treatments were identified. This lack of variation may be attributed to the relatively high variability within the populations.

4. Discussion

Over the past few decades, a significant intensification of extreme weather events, i.e., heatwaves, droughts, or increases in the intensity of rainfall, has been noted [57]. The impact of high temperatures on viticulture will be negative for areas that already suffer 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 [8] and a decrease in yields due to phenomena such as photoinhibition and heat damage [58, 59].

Since Sangiovese is a cultivar characterized by a medium–low anthocyanin profile and high temperatures can reduce the accumulation of colored pigments, it is essential to adopt strategies that enhance their production, ensuring a satisfactory color in both grapes and wine [3, 12, 59].

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 chabazite-rich zeolite treatment on canopy temperature, gas exchange, yield characteristics, and the composition of both grapes and wine in cultivar Sangiovese.

The application of chabazite-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 berries and wines without affecting yield and technological maturity. While the enhancement of color through the application of minerals, such as kaolin, has been well-documented [27, 28, 60, 61], information on the use of zeolite on vines was limited [30, 32, 45]. Consequently, our research in field vines has confirmed that spraying the canopy with zeolite enables the mitigation of high temperatures that compromise the accumulation of anthocyanins in berries [33]. This, in turn, results in a reduction in the canopy and fruit temperature, thereby promoting the biosynthesis of phenolic compounds [62] and diminishing their enzymatic degradation [15, 63].

Moreover, it is well-known that Sangiovese vines under the condition of water scarcity have an anisohydric behavior [64], meaning that they can maximize gas exchange even under very low water potential conditions [8, 65]. Additionally, it has been highlighted that grapevines can adjust their behavior based on the period, duration, and severity of stress [66]. In this research, vines subjected to moderate WS

TABLE 4: Chemical analysis on wines obtained from control (WS) and chabazite-rich zeolite (WS + ZEO)-treated grapes in 2022.

	After 3-month bottling				After 1-year bottling			
	Alcohol (%vol)	pH	Titratable acidity (g L ⁻¹)	Total anthocyanins (mg L ⁻¹)	Alcohol (%vol)	pH	Titratable acidity (g L ⁻¹)	Total anthocyanins (mg L ⁻¹)
WS	13.9	3.52 ± 0.01	5.50 ± 0.21	32.4 ± 4.56 ^b	13.9	3.51 ± 0.01	5.40 ± 0.20	48.6 ± 0.51 ^b
WS + ZEO	13.5	3.44 ± 0.02	5.40 ± 0.27	72.1 ± 6.35 ^a	13.5	3.47 ± 0.02	5.40 ± 0.30	53.6 ± 1.55 ^a
<i>p</i> -value	0.85	0.06	0.61	0.01	0.85	0.77	0.60	0.04

Note: The wines were analyzed 3 months and 1 year after bottling. The mean values of alcohol concentration, pH, titratable acidity, and total anthocyanins are reported ($N = 3 \pm SE$). Different letters in a column indicate significant differences after the ANOVA test.

TABLE 5: Color analysis on wines obtained from control (WS) and chabazite-rich zeolite (WS + ZEO)-treated grapes in 2022, after one year of bottling ($N = 3 \pm SE$).

Parameter	WS	WS + ZEO	p-value
O.D. 420	0.264	0.28	0.397
O.D. 520	0.276 ^b	0.304 ^a	0.039
O.D. 620	0.069	0.074	0.271
CD	0.609	0.659	0.12
HUE	0.96	0.92	0.488
a^*	14.97 ^b	16.81 ^a	0.040
b^*	6.81	7.32	0.466
L^*	83.47 ^a	82 ^b	0.047
C_{ab}^*	16.45 ^b	18.13 ^a	0.032
H_{ab}^*	24.44	23.14	0.371
Copigmentation (%)	7.82	7.79	0.984
Polymers (%)	61.4	58.1	0.080

Note: Different letters within a row indicate significant differences based on the ANOVA test. Color density (CD) = O.D. 420 nm + O.D. 520 nm + O.D. 620 nm; hue (HUE) = O.D. 420 nm/O.D. 520 nm. CIELab parameters: L^* (lightness), a^* (redness), b^* (yellowness), C_{ab}^* (chroma), and H_{ab}^* (hue angle).

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, along with a significant improvement in photosynthetic rates, suggests that zeolite could positively influence the enhancement of CO₂ concentration at the stomatal level in grapevines, consistent with previous reports on other fruit species [41]. While several studies suggest that *Vitis vinifera* L. is particularly resistant to photoinhibition [67], this study revealed that both the maximum and operational efficiency of Photosystem II under light conditions (Fv'/Fm' and $\phi PSII$) show damage to the reaction centers involved in CO₂ 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 nonstomatal factors, such as lowered electron transport rate and diminished Rubisco regeneration capacity [58]. It is known that any disturbance depressing photosynthetic activity, such as water deficiency, exposes leaves to a decrease in the CO₂ concentration in the mesophyll and an excess of light, which, not fully utilized in the photochemical process, must be dissipated as heat. The Fv'/Fm' index decrease is indicative of photoinhibition, namely, the degradation of the D1 protein of PSII [68]. 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 nonphotochemical quenching (NPQ). 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 zeolite positively impacts the efficiency and functionality of Photosystem II, as well as photochemical quenching, helping to preserve the integrity of photosynthetic mechanisms during periods of heightened stress. This mineral enhances CO₂ absorption and allows for a more controlled and gradual release, further supporting plant resilience [69]. Although zeolite treated potted vines showed differences in leaf gas-exchanges, in field conditions no variation on both yield and berry technological parameter (soluble solids concentration, pH, and titratable acidity) was recorded between treatments. These data corroborate findings previously reported in Sangiovese vines subjected to frequent heatwaves [30]. Furthermore, wines from both treatments showed no significant variations in the ethanol content, pH, or acidity. However, the ZEO treatment resulted in a higher concentration of anthocyanins, as previously demonstrated through both wet and nondestructive chemical analyses. These anthocyanins, extracted during fermentation, contributed to the enhanced color intensity observed in wines made from the treated berries. These results align with those of other researchers who attribute the increase in the anthocyanin content to temperature regulation facilitated by mineral coating [33, 43, 61].

In addition, apart from a quantitative point of view, the treatment also appeared to qualitatively impact the color of wines to some extent. Polymeric anthocyanins, in fact, were somehow higher in C samples (Table 5). The quite high variability between samples made their respective values just above the statistical significance ($p = 0.08$), but it is worth noting that similar results were obtained in previous investigations on highly exposed or low vigor clusters [70, 71], which resulted in having an increased level of polymeric anthocyanins compared to less exposed or high vigorous treatment. This may give further confirmation that zeolite-treated grapes are affected to a lesser extent by abiotic stress.

Copigmentation appeared not to be altered by the treatment, as expected, since flavonols and phenolic acids, the main constitutive copigments in wine, were equally present in C and ZEO samples (Supporting information, Table S1).

Additionally, Calzarano and colleagues [42] suggested that zeolite 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 [12], can enhance the color of grapes and wines without altering their composition.

5. Conclusion

The foliar application of chabazite-rich zeolite demonstrates the potential to thermally regulate the canopy, leading to an enhancement in the plant photosynthetic efficiency, under conditions marked by moderate WS and elevated

temperatures. Consequently, berries of field-grown vines showed a higher anthocyanin concentration, contributing to better coloration of the resulting wine. In addition, the application of this product, resembling a conventional phytosanitary treatment, makes the method economical and available to all winegrowers.

Finally, the use of chabazite-rich zeolite resulted in a proactive strategy for effectively managing the multiple summer stresses in vineyards.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

This research did not receive any external funding and was conducted as part of the Ph.D. program at the University of Bologna.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*)

Table S1. Phenolic acid content ($\text{mg L}^{-1} \pm \text{SE}$) of wines obtained from control (WS) and chabazite-rich zeolite (WS+ZEO)-treated grapes in 2022 after one year of bottling.

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