



**ORIGINAL RESEARCH ARTICLE**

# A comparative study on training systems and vine density in Santorini Island: Physiological, microclimate, yield and quality attributes

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## ABSTRACT

The Mediterranean basin is regarded as one of the most affected global regions by climate change. Traditionally, viticulture in this region copes with high temperatures, heat waves and drought. Such extreme conditions are expected to intensify due to climate change in the future. Our study focuses on the viticulture of Santorini Island, located in South Aegean (Greece). Local varieties trained with the traditional ‘Kouloura’ training system have been cultivated for thousands of years on the island, producing recognised high-quality PDO wines worldwide. The literature on these traditional training systems is scarce, and their investigation could aid in the adaptation of viticulture to hotter and drier future climatic conditions. The objective of this study was to compare the physiological and agronomic response of Assyrtiko grapevines to the traditional training systems ‘Kouloura’ and VSP training system over two growing seasons and to establish the factors influencing the performance of each system in the semi-arid conditions of Santorini Island. In brief, the ‘Kouloura’ training system maintained a less-stressed water status compared to VSP, while for both studied years during ‘Kouloura’ exhibited significantly higher photosynthetic rates and stomatal conductance. Regarding microclimate observations, we found that, especially during heatwaves, VSP’s grapes were more exposed to higher temperatures during midday than ‘Kouloura’ and that the ‘Kouloura’ system protected against damage from heatwaves and strong winds when compared to VSP. Investigating the mechanisms by which these traditional training systems are adapted to hot, dry climatic conditions creates applicable knowledge for developing and using alternative training systems in similar environments to adapt to climate change.

**KEYWORDS:** Assyrtiko, Kouloura, VSP, climate change, Greece

## INTRODUCTION

The viticulture of Santorini Island, in the Cyclades, is unique and has contributed to the remarkable breakthrough of Santorini's wine into the global quality wine market. The wine industry is a leading economic activity for the island, with significant value added in terms of GDP, investments and revenues. Closely related to tourism, wine is linked to Santorini's brand name as one of the top world destinations. Hence Santorini remains one of the few places in Europe where traditional viticulture is still practised. Local varieties trained with the traditional 'Kouloura' and 'Kladeftiko' training systems (Figure 1) have been cultivated for thousands of years on the island producing worldwide recognised high-quality PDO wines. The literature on these local varieties and traditional training systems is scarce. Investigations into how these unique training systems adapt viticulture to the specific climatic conditions of the island are important because they inform their possible use as alternative training systems in similar environments to adapt to climate change.

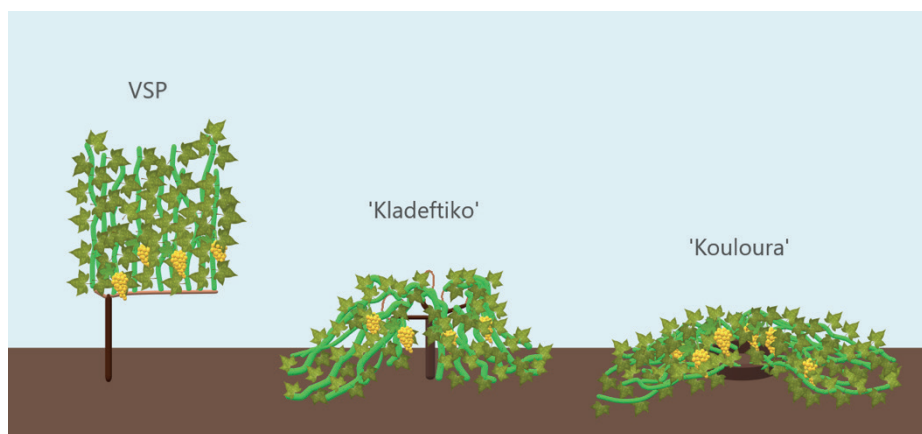
Winegrapes are one of the world's most valuable horticultural crops (Alston and Sambucci, 2019), and viticulture is facing massive challenges due to climate change, such as increased extreme precipitation events (i.e., drought and heavy rainfall), more frequent heatwaves and less frequent extreme cold temperatures and cold waves (Jones and Goodrich, 2008; Tomasi *et al.*, 2011; Xyrafis *et al.*, 2022; Jones *et al.*, 2022). Among the chief concerns are that a combination of increased temperatures and decreased rainfall will increase the frequency and/or severity of droughts (IPCC, 2022). High temperatures, in combination with decreased precipitation, can cause complete yield loss depending on the phenological stage (Venios *et al.*, 2020). Even if the yield is not affected, these conditions can lead to early technological maturation of the grape with significant sugar increases and negative impacts on wine quality (acidity, aroma, colour) (Costa *et al.*, 2016).

Adaptation measures must be planned and applied to maintain the sustainability of vineyards (Metzger and Rounsevell, 2011), and several adaptations have been reported for use in viticulture (Koundouras *et al.*, 2008; Duchêne *et al.*, 2012;

Brillante *et al.*, 2016; Petoumenou *et al.*, 2017; Fraga and Santos, 2018; Biniari *et al.*, 2023). These include a blend of strategies such as using more suitable clones/rootstocks/varieties, decreasing planting densities and/or changing training systems (Naulleau *et al.*, 2021). In particular, new training systems and different planting densities could provide complementary solutions to mitigate the effects of high temperature, radiation and water deficits. These cultural adaptations to drought may lead to yield reductions and a shift in wine aromatic profiles (Deloire *et al.*, 2022).

Changing training systems is particularly interesting because it has the potential of adapting the production system without changing the variety grown and, in some cases, without the need to replant and restructure the entire vineyard. A thorough training system assessment requires knowledge of vine photosynthesis, sugar and acid metabolism, micrometeorology and many other fields (Reynolds and Heuvel, 2009). Over the last decades, the main objectives of relevant experiments on training systems focused primarily on increasing the photosynthetic efficiency of the canopy by increasing the leaf area and increasing the light exposure of the grapes (Carbonneau and Casteran, 1987). However, in the context of climate change, training systems are being reconsidered with opposite objectives: on the one hand, decreasing the water demand by reducing the leaf area while maintaining an adequate sugar content in the berries and, on the other hand, leaving the grapes in the shade as much as possible (Favero *et al.*, 2010; Duchêne *et al.*, 2014).

The vine training system determines the light interception and bunch sun exposure, thus completing berry ripening. Palliotti *et al.* (2014) identified adapted training systems allowing for an optimal bunch microclimate under future climatic conditions. However, it is difficult to state which training system best adapts to drought. Over centuries, wine growers in the Mediterranean basin have developed a training system which is particularly resistant to drought and high temperatures: the so-called Mediterranean goblet or bush vine. This training system makes it possible to dry-farm vines in extremely dry environments, down to a mere 350 mm of rainfall/year (Deloire, 2012; van Leeuwen *et al.*, 2019a). We note that there is currently



**FIGURE 1.** Illustration of the traditional training systems of Santorini ('Kladeftiko' and 'Kouloura') and the VSP.

a lack of comparisons of the water use efficiency of different training systems, including traditional forms like goblet systems (Medrano *et al.*, 2015, Salvi *et al.*, 2017). In Central Europe, under relatively cool climates, pruning systems such as semi-minimal pruning are promoted as an adaptation to climate change, as they present higher yields with lower alcohol than vertical-shoot positioning systems (Clingeffer, 2010; Molitor *et al.*, 2019). However, the large water requirements of such systems would not be adapted to rainfed systems in semi-arid climates.

Santorini island (36.3932° N, 25.4615° E) is an active volcanic complex in the Cyclades (Greece), with an area of 76.19 km<sup>2</sup>, of which today 1100 ha is covered with cultivated vineyards. Santorini has a Mediterranean climate composed of high temperatures (an average of 3 days during summer with max temperatures of >35 °C was observed for the 2009–2019 period), heatwaves (which often occurred during summer) and long drought periods. These climate challenges affect yield, berry development and composition and the associated wine aromatic profiles, and are intensifying due to climate change in the Mediterranean region (Fraga *et al.*, 2017; Alba *et al.*, 2021; Xyrafis *et al.*, 2022). Recent studies reveal that in both Santorini (Greece) and in the Hérault region (South France), there is a similar increase in the annual mean temperature of 0.06 °C (Laget *et al.*, 2008; Xyrafis *et al.*, 2022). Regarding the annual precipitation of the island, an amount of 316 mm (average from 1974 through 2019) has been observed, which is even lower than in arid regions of Extremadura (Southwest Spain) and Pafos (Cyprus), where the precipitation varies from 380 to 700 mm (García-Martín *et al.*, 2022).

Own-rooted and phylloxera-free vines have been cultivated on the volcanic soil of Santorini for thousands of years. All this time, vines have been cultivated using two traditional training systems, the ‘Kouloura’ and the ‘Kladefitiko’ (Figure 1), which are well-adapted to the specific climatic conditions of the island. (Xyrafis *et al.*, 2021).

The objective of this study was to compare the physiological and agronomic response of Assyrτικο grapevines to the traditional training systems ‘Kouloura’ and VSP training system over two growing seasons and to establish the factors influencing the performance of each system in the semi-arid conditions of Santorini Island as an alternative training system to adapt viticulture in other warm, dry wine regions.

## MATERIAL AND METHODS

### 1. Experimental design

The experiment took place in the cultivation seasons 2019–2020 and 2020–2021 on vines of grape cultivar Assyrτικο (*Vitis vinifera* L.) in vineyards located in Oia, Santorini, Greece (36°28'22.5"N; 25°23'14.7"E). All vines were own-rooted. There were two vineyards: one with the traditional training system of Santorini ‘Kouloura’ where the vines are cane-pruned to 4–6 canes of 8–10 nodes at 2.3 m × 2.3 m intervals, resulting in a vine density of 1900 plants/ha; and one vineyard N-S oriented where the vines are unilateral cordon-trained (unilateral Guyot) and cane-pruned to 8–10 nodes canes at 1.9 m × 1 m intervals (double lines), resulting in a vine density of 5300 plants/ha. Importantly, the vineyards are directly adjacent to each other, and the experimental plots chosen for sampling are just meters apart, assuring homologous soil characteristics (Supplementary Figure 1). In addition, both vineyards were established in 2006 (i.e., vines are the same age) and were not irrigated. The weather condition during the studied period is mentioned in Table 1 (the climate data for the Monolithos region were obtained from the National Meteorological Service (<http://emy.gr/emy/en>)). Soil is characterised by a rocky-sandy texture, and floor management was carried out as full tillage.

The trial was conducted using a block design, with a block composed of 4 replicates with 5 vines, each replicate on each training system. Two training systems were evaluated as a) ‘Kouloura’ (KLR) and b) vertical shoot positioned (VSP).

### 2. Gas exchange, water potential, leaf-related measurements

During the season, the midday leaf water potential ( $\Psi_{\text{leaf}}$ ) and predawn water potential ( $\Psi_{\text{predawn}}$ ) were measured every two weeks by using a pressure chamber (Williams and Araujo, 2002). Measurements were taken at sun zenith for  $\Psi_{\text{leaf}}$  and at full dark for  $\Psi_{\text{predawn}}$  on five primary leaves per treatment, placed inside plastic bags and sampled from eight random vines.

Assimilation rate ( $A_n$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) and transpiration ( $E$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) were obtained by measurement of inlet and outlet  $\text{CO}_2$  and  $\text{H}_2\text{O}$  relative concentration using a portable photosynthesis system (Li-6400XT, Li-Cor, Lincoln Nebraska, USA). Single-leaf gas exchange measures were taken in the midday hours (12:30–14:30) on five primary leaves, on the same day

**TABLE 1.** Regional weather data, Santorini, Greece.

Month	Mean temperature (°C)		Min temperature (°C)		Max temperature (°C)		Rainfall period	Rainfall (mm)	
	2020	2021	2020	2021	2020	2021		2019–2020	2020–2021
June	23.0	24.0	19.7	20.9	26.2	28.0			
July	26.3	26.9	22.8	24.0	29.8	30.6	October–April	328	178
August	26.7	27.6	23.3	24.6	30.0	33.5			

and on the same vines of the water potential measurements. Five primary leaves per training system were measured among those inserted at nodes 4–6 above the distal bunch on a main shoot. Water use efficiency was calculated for both training systems.

Leaf and grape temperatures were measured with an infrared thermal camera (HT-02D KKMOON, China). For temperature and humidity under the canopy and open air for both training systems, temperature-humidity data loggers (GSP-6, Elitech, UK) were used for each training system.

### 3. Yield components and grape composition

At harvest, five clusters were randomly selected from each of the training systems. The weight of each one of the clusters was measured using a precision scale. The grape length and width were determined using callipers with 0.01 mm accuracy. Three random groups of fifty berries were collected from each cluster. Each group's weight was measured using a precision scale. It was then divided by the number of berries to calculate the mean berry weight per group. The length and width of each berry in all three groups were measured using a Vernier calliper. Last, the mean value of each group's berry length and width was calculated. The number of grapes per vine was also recorded on 5 vines per block.

Soluble solids in must, pH and total titratable acidity were determined and measured according to Stavrakaki *et al.* (2018).

For both studied vintage, damage estimation was conducted between the two training systems: in 2020, during strong wind at the flowering stage; in 2021, during a heatwave at harvest.

### 4. Statistical analysis

All statistical analyses were obtained using the JMP v.16 statistical software (SAS Institute Inc., Cary, NC, USA). The significance of the results was tested by Analysis of Variance (ANOVA) and comparisons were analysed using the Tukey test for pairwise comparison with mean separation by  $p < 0.05$ . R software (<http://www.R-project.org>) was used to format figures.

## RESULTS

### 1. Canopy structure

Any assessment of the physiological performance (photosynthesis, stomatal conductance, transpiration) of a training system needs to consider the canopy leaf surface area produced. The canopy surface area determines the majority of the system's production potential, considering the importance of external leaves with respect to total leaf area in new photosynthesis (Smart *et al.*, 1985). Individual KLR vines had larger canopy surface areas, but because of the lower planting density, the VSP system produced a larger exposed surface area per vineyard surface area (Table 2).

**TABLE 2.** Canopy surface area, canopy surface area per vineyard surface area and total canopy volume in KLR and VSP training systems.

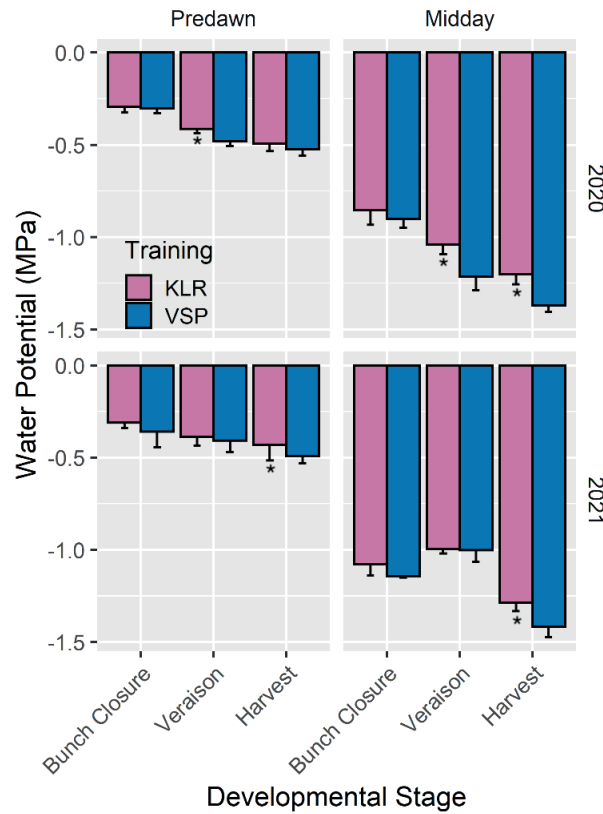
	Vine canopy surface area (m <sup>2</sup> /plant)	Canopy surface area per m <sup>2</sup> (m <sup>2</sup> /m <sup>2</sup> )	Total canopy volume (m <sup>3</sup> )
KLR	2.86	0.53	0.93
VSP	1.43	1.01	0.69

### 2. Vine water status

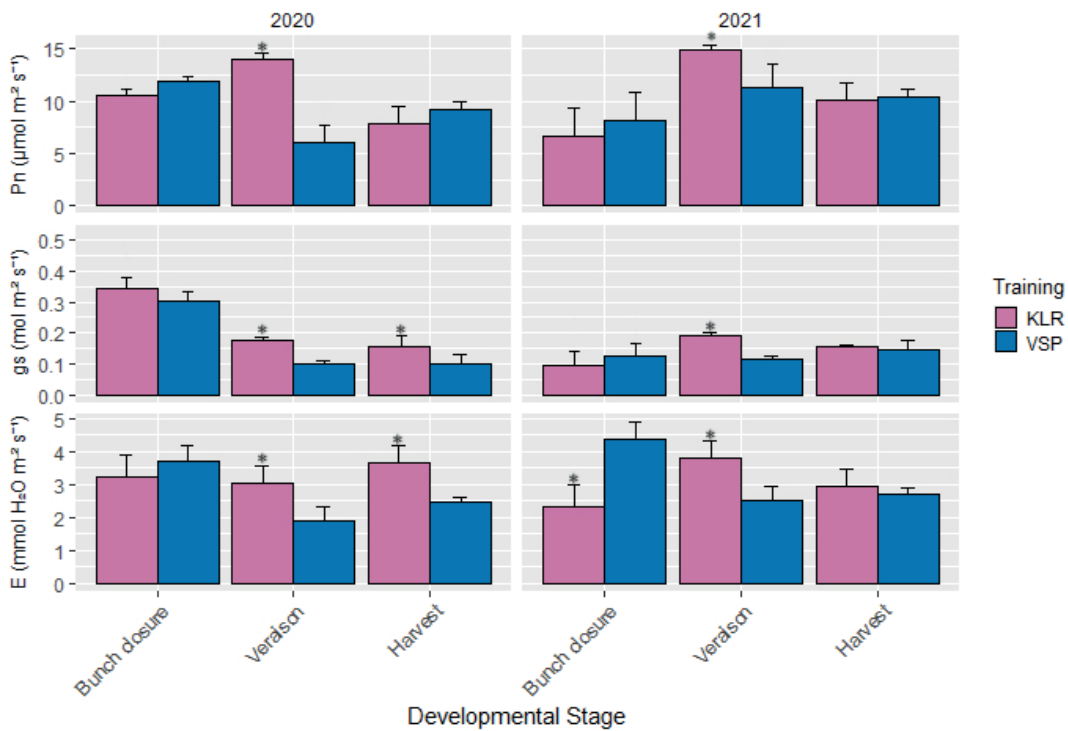
There were significant differences in vine water status between the two training systems depending on the developmental stage, and globally KLR always maintained a less stressed water status (i.e., less negative  $\Psi$ ). In 2020, VSP  $\Psi_{\text{predawn}}$  was significantly more negative than KLR (~0.1 MPa difference), and at harvest, VSP also had more negative  $\Psi_{\text{predawn}}$  than KLR, although the differences were not statistically significant. In 2021, during harvest, VSP again exhibited more negative  $\Psi_{\text{predawn}}$  than KLR (~0.1 MPa difference), while differences were not significant during bunch closure and veraison. In 2020, no significant differences in midday  $\Psi_{\text{leaf}}$  were observed at bunch closure, while at veraison and harvest, VSP was significantly more negative (~0.2 MPa difference) than KLR (Figure 2). In 2021, during harvest, VSP again exhibited more negative midday  $\Psi_{\text{leaf}}$  (~0.15 MPa difference) (Figure 2). Although KLR also maintained slightly less negative  $\Psi_{\text{leaf}}$  during bunch closure and veraison in 2021, these differences were not statistically significant.

### 3. Photosynthesis and gas exchange

Leaf assimilation rate, leaf stomatal conductance and leaf transpiration presented significant differences at the three growth stages (bunch closure, veraison and harvest) during the 2020 and 2021 seasons (Figure 3). Specifically, photosynthesis during veraison in both years was significantly higher in the KLR training system (~8  $\mu\text{mol m}^{-2} \text{s}^{-1}$  difference in 2020 and ~4  $\mu\text{mol m}^{-2} \text{s}^{-1}$  difference in 2021). At bunch closure and harvest, there were no significant differences. Regarding the stomatal conductance, in 2020, at veraison and harvest, the VSP stomatal conductance decreased and presented lower values than KLR (~0.07  $\text{mol m}^{-2} \text{s}^{-1}$  difference). During bunch closure, there were no significant differences. Similarly, in 2021, during veraison, the VSP had lower stomatal conductance than KLR (~0.08  $\text{mol m}^{-2} \text{s}^{-1}$  difference). At bunch closure and harvest, no significant differences were found. Correspondingly, leaf transpiration values were higher in KLR during veraison in both years (~1.2  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ). At bunch closure stage in 2021, VSP was higher than KLR (~2  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ). No significant differences were observed at harvest for both training systems in 2021. No significant differences have been mentioned regarding water use efficiency between KLR and VSP training systems for both studied years (data not shown).



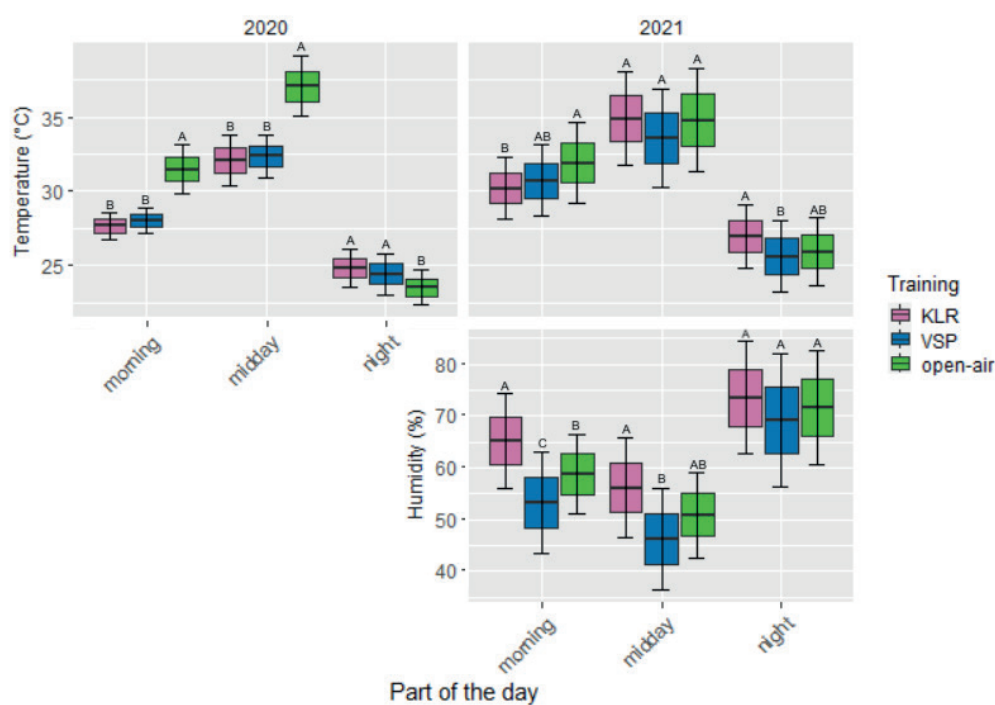
**FIGURE 2.** Water potential:  $\Psi_{\text{predawn}}$  and  $\Psi_{\text{leaf}}$  (MPa) at three growth stages (bunch closure, veraison and harvest) of KLR and VSP training systems for 2020 and 2021. The values are averages  $\pm$  SD. Averages followed by \* are different  $p < 0.05$ , Tukey's HSD,  $n = 5$ .



**FIGURE 3.** Leaf assimilation rate ( $P_n$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), leaf stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ) and the leaf transpiration ( $E$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) of KLR and VSP at three growth stages (bunch closure, veraison and harvest) during the 2020 and the 2021 season. The values are averages  $\pm$  SD. Averages followed by \* are different  $p < 0.05$ , Tukey's HSD,  $n = 5$ .

**TABLE 4.** Leaf and grape temperature during the harvest period at midday in 2021. The values are averages  $\pm$  SD. Averages followed by \* are different  $p < 0.05$ , Tukey's HSD,  $n = 10$ .

Organ measured	Temperature ( $^{\circ}\text{C}$ )		Significance
	KLR	VSP	
Leaf	$33.5 \pm 1.8$	$33.9 \pm 3.2$	n.s.
Random grapes	$32.6 \pm 0.2$	$34.3 \pm 0.6$	*
Sun-exposed side of grapes	$35.3 \pm 1$	$36.7 \pm 1.2$	*
Shaded side of grapes	$31.4 \pm 0.4$	$30.8 \pm 0.3$	n.s.
During heatwave-sun exposed side of grapes	$40.1 \pm 0.5$	$39.4 \pm 0.3$	n.s.
During heatwave-shaded side of grapes	$32 \pm 0.3$	$31.3 \pm 0.1$	n.s.



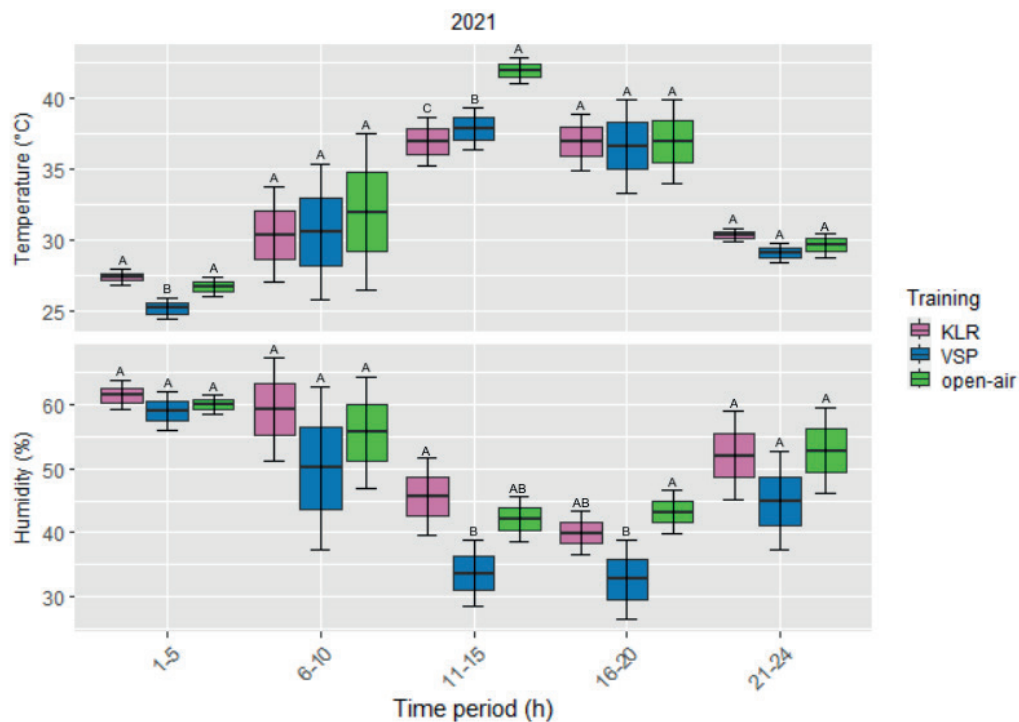
**FIGURE 4.** Open-air temperature and temperature under the canopy of KLR and VSP training systems from veraison until harvest for three periods (morning, midday and night) for the two studied years, 2021 and 2020. The values are averages  $\pm$  SD. Averages followed by different letters are different  $p < 0.05$  Tukey's HSD,  $n = 32$ .

#### 4. Microclimate and heatwave responses

There were significant differences in microclimate temperature between the two training systems at harvest. Table 3 presents measured temperatures of grapes and leaves at midday for both training systems during the harvest period and heatwave days in 2021. Leaf temperatures presented similar values with no significant difference. At midday, during harvest, the sun-exposed side of VSP grapes showed significantly higher temperatures than KLR. The shaded grapes of both training systems were not significantly different. During the heatwave, temperatures reached  $40^{\circ}\text{C}$  for KLR sun-exposed side with no significant difference from VSP. Though there

was a clear separation of temperatures between shaded and sun-exposed sides at midday and during the heatwave.

Figure 4 shows the temperature and humidity evolution during the open-air day and temperature under the canopy of KLR and VSP training systems for the period from veraison until harvest. In 2020 (Figure 4, left), during the morning, temperatures for both training systems were significantly lower ( $\sim 28^{\circ}\text{C}$ ) in comparison to open-air ( $\sim 31^{\circ}\text{C}$ ). At midday, temperatures for both training systems are significantly lower ( $\sim 33^{\circ}\text{C}$ ) than open-air temperatures ( $\sim 38^{\circ}\text{C}$ ). At night, KLR and VSP had significantly higher



**FIGURE 5.** Temperature and humidity evolution under the canopy of KLR and VSP training systems during a heatwave in 2021 at harvest. The values are averages  $\pm$  SD. Averages followed by different letters are different  $p < 0.05$ , Tukey's HSD,  $n = 5$ .

values ( $\sim 26$  °C) than open-air. In 2021 (Figure 4, right), during the morning, KLR presented significantly lower temperatures in comparison to open-air and VSP but with no significant difference. During midday, the temperatures increased, reaching values around 35 °C, while open-air, VSP and KLR exhibited similar values. At night, the VSP training system had the lowest temperatures (25 °C), and KLR had the highest temperatures (27 °C) during this period.

Regarding humidity, in 2021, during the morning, KLR had significantly higher humidity ( $\sim 66$  %) from open-air and VSP, and VSP presented the lowest values ( $\sim 55$  %). At midday, KLR showed markedly higher humidity ( $\sim 56$  %) than VSP (46 %). At night, there were no significant differences in humidity between the training systems.

During the heatwave of 2021, there were clear differences in fruit damage between the two training systems, where KLR exhibited 19 % damaged fruit while VSP reached 32 % (data not shown). During the heatwave, there were significant differences in the temperature and humidity evolution under the canopy between the KLR and VSP training systems and compared to the open-air temperature and humidity. Regarding temperature (Figure 5, above), the VSP training system exhibited lower values ( $\sim 25$  °C) than KLR and open-air temperature at night. During the morning, no differences were observed between the training systems and open-air temperatures. From 11:00 through 15:00, when temperatures were at their maximum, the open-air temperature reached values around 42 °C.

For the same period, VSP and KLR showed markedly lower values ( $\sim 38.5$  °C and 37 °C, respectively) than open-air temperature, while the KLR presented the lowest values. From 16:00 to 24:00, no differences were observed. No differences in humidity were observed during the night and early morning (Figure 5 below). From 11:00 until 15:00, KLR presented significantly higher humidity (47 %) than VSP ( $\sim 32$  %). Similar differences were found from 16:00 until 20:00, although they were not significant.

## 5. Yield components and grape composition

Table 4 shows the berry and bunch attributes of the Assyrτικο cultivar for the 2020 and 2021 harvest for both training systems. There were no significant differences between the two training systems for most of the analysed attributes except for bunch length, where VSP was significantly higher from KLR for both studied years and the bunch number, where KLR presented more bunches per vine compared to VSP training system.

The yield was higher in 2020 (Table 4) than the last 4 years' yield because of high rainfall rates the last year. The KLR system had a lower yield in 2020. The smaller crop produced by VSP vines in 2021 may have been caused by the low precipitation and high temperatures during summer, which may have affected the VSP training system more.

Concerning the grape composition, the only significant differences were for 2021, where VSP showed markedly higher acidity and lower sugar content than KLR.

**TABLE 4.** Yield, berry and bunch attributes of Assyrτικο cultivar for KLR and VSP training systems in 2020 and 2021. Averages followed by \* are different  $p < 0.05$ , Tukey's HSD, berry and bunch measurements  $n = 50$  and  $n = 5$ .

Year		KLR	VSP	Significance
2020	Berry length (mm)	16.8 ± 0.3	14.9 ± 0.4	n.s
	Berry width (mm)	15.6 ± 0.5	13.3 ± 0.2	n.s
	Berry weight (g)	2.7 ± 0.1	2.5 ± 0.1	n.s
	Bunch length (cm)	15.2 ± 0.7	17 ± 0.7	*
	Bunch width (cm)	7.8 ± 0.3	8.8 ± 0.8	n.s
	Bunch weight (g)	232 ± 10	210 ± 6	n.s
	Bunch number per vine	8.2 ± 3.2	4 ± 2	*
	Yield (tn ha <sup>-1</sup> )	3.6	4	
2021	Berry length (mm)	16.6 ± 0.2	15.7 ± 0.4	n.s
	Berry width (mm)	14.2 ± 0.1	14.3 ± 0.3	n.s
	Berry weight (g)	2.6 ± 0.2	2.2 ± 0.1	n.s
	Bunch length (cm)	16.6 ± 1	19.6 ± 0.5	*
	Bunch width (cm)	9.6 ± 0.4	11 ± 0.2	n.s
	Bunch weight (g)	220 ± 30	214 ± 23	n.s
	Bunch number per vine	7 ± 2.2	2.4 ± 1.4	*
	Yield (tn ha <sup>-1</sup> )	2.4	2.1	
2020	Brix	23.8 ± 0.3	23 ± 0.2	n.s
	pH	3.1 ± 0.05	3 ± 0.2	n.s
	Acidity (g tartaric acid l <sup>-1</sup> )	6.6 ± 0.3	7.1 ± 0.2	n.s
2021	Brix	23.7 ± 0.4	22.8 ± 0.4	*
	pH	3 ± 0.1	2.8 ± 0.2	n.s
	Acidity (g tartaric acid l <sup>-1</sup> )	6.9 ± 0.4	7.6 ± 0.3	*



## DISCUSSION

In this work, we analyse the traditional training system ('Kouloura') and vine density of Santorini while comparing it with the globally used VSP training system. We found significant differences in vine water status between the two training systems depending on the developmental stage, and globally, KLR always maintained a less-stressed water status (i.e., less negative  $\Psi$ ). In our study, the KLR system produced the largest exposed canopy surface area while net mean photosynthesis during veraison on fully exposed leaves was 30 % higher. KLR maintained a more beneficial microclimate, especially during heatwaves, with lower leaf, grape and under canopy temperatures during days and time periods with high temperatures than open-air and the VSP training system.

### 1. Vine water status

We showed that there were significant differences in midday vine water status for both years, where KLR appears to be more resistant to water stress than VSP. Differences in water status were also reflected in  $\Psi_{\text{predawn}}$  which suggested that KLR had more access to soil water, most likely resulting from the lower planting density (van Leeuwen *et al.*, 2019b). Our findings are consistent with published literature highlighting bush vines and low-density vineyards as more drought-resistant (Baeza *et al.*, 2005; van Leeuwen *et al.*, 2019b). Although statistically significant, the absolute differences in observed water potentials are relatively small (ranging from ~0.1–0.2 MPa), but it is important to remember that KLR maintained higher leaf gas exchange levels even with higher exposed canopy leaf areas (see below) which is important because it contributes to efficient photosynthesis and overall plant productivity (Garcia-Tejera *et al.*, 2023).

### 2. Photosynthesis and gas exchange

For both studied years during veraison, KLR exhibited significantly higher photosynthetic rates than VSP. Our findings are similar to those of Baeza *et al.*, 2005 who showed that high bush vines of cv Tempranillo in the Madrid region had higher photosynthetic rates than VSP and single curtain due to the high PAR on the fully exposed leaves.

Any assessment of the photosynthetic performance of a training system should consider the response of each leaf and the surface area it can produce, as these factors determine the majority of the system's production potential, taking into account the importance of external leaves with respect to total leaf area in new photosynthesis (Smart *et al.*, 1985). In our study, the KLR system produced the largest exposed canopy surface area (Table 2). The surface area of the VSP system was 50 % of that produced by KLR, while net mean photosynthesis during veraison on fully exposed leaves was 30 % higher in KLR vines. These observed differences are likely explained by the observed differences in water status discussed above.

These findings are consistent with differences in stomatal conductance and transpiration over time, where KLR generally maintained higher  $g_s$  and  $E$  in both years studied. Our findings are similar to those of Baeza *et al.* (2005) who found trends toward higher  $g_s$  and  $E$  in short-bush vines and lower  $g_s$  and  $E$  in VSP vines throughout the growing season. In the aforementioned study, the authors hypothesise that the lower stomatal conductance and transpiration of VSP vines resulted from their larger surface area per vineyard area and were the primary cause of the lower photosynthetic rates compared with the bush system. Archer and Strauss (1990) reported that closely spaced vines had a significantly lower stomatal conductance than more widely spaced vines because narrow spacing implied greater leaf surface area per vineyard area, increasing plant water stress, and these differences became more significant as soil water depletion advanced during the season. In this context, it is important to note again that although KLR exhibited a higher exposed canopy surface area because of the lower density, it actually exhibited a lower canopy surface area per vineyard surface area.

Similarly, two studies in dry-farmed vineyards in West Cape, South Africa, found more negative leaf water potential for closely spaced vines during the pre-veraison through the ripening period, which resulted in lower stomatal conductance and higher leaf temperatures (Archer and Strauss, 1989; Archer and Strauss, 1990). Van Zyl and Van Huyssteen (1980) obtained lower crop coefficient values in vines with larger surface area, with the highest coefficient values recorded for bush vines. They attributed their results to higher environmental temperature, more wind exposure and less ground shading in this system, factors which also apply to our experiment.

### 3. Temperature and heatwave responses

Light interception and plant microclimate within the canopy, particularly in the fruit zone, are among the most important determinants of grape berry composition (Jackson and Lombard, 1993). In general, we found that during the 2021 heatwave, KLR presented lower midday temperatures than VSP. The same was true during the 2021 harvest, where on the sun-exposed side, VSP berries presented higher temperatures than KLR.

These lower temperatures can potentially protect fruit grown with the KLR system from damage and/or decreased quality. There is an optimum temperature for vine development and grape ripening, and extreme temperature events can cause damage to leaves and grapes. High temperatures (> 35 °C) could reduce phenolic content and alter photosynthesis (Kriedemann and Smart, 1971; Spayd *et al.*, 2002; Greer and Weedon, 2013). Temperature also affects grape berry composition, particularly the type and concentration of aromatic compounds (Mira de Orduña, 2010; Wu *et al.*, 2019; Drappier *et al.*, 2019; van Leeuwen *et al.*, 2020).

Heatwaves and strong early spring winds are not uncommon on Santorini Island. These events can be extremely destructive, especially when they occur when the grapevines are in their early growth stages. After recording damages from summer heatwave and early spring winds, we found that KLR is better adapted to the climate condition of Santorini than VSP by protecting the grapes from the consequences of the extreme conditions of the island.

#### 4. Yield and quality attributes

We showed that no significant differences were observed regarding bunch and berry attributes, apart from bunch length, where VSP presented longer bunches. In addition, yields were quite similar between KLR and VSP. Thus, there does not seem to be any obvious fruit differences despite the differences in training systems and density. It is perhaps worth noting that in 2021, where there was lower precipitation and higher temperatures, the yield of KLR was higher than VSP, reinforcing our findings on KLR's adaptability. To reinforce the conclusions of the current study, similar studies could be repeated across more seasons to expand the variability of the vintage climate (Beauchet *et al.*, 2019).

## CONCLUSION

The traditional 'Kouloura' training system used in Santorini proved to be well-adapted to the extreme climatic conditions of the island. Hence, our results validated that the empirical use of 'Kouloura' persisted through time and became part of the authenticity of the landscape/terroirs of this wine region. This work details the physiological mechanisms by which this traditional training system enhances performance. Learning from traditional viticulture systems such as the one described in this study could be significant in improving global vineyard sustainability because it can highlight mechanisms that have created drought-resilient systems in the past. Our research inspires further comparative analysis of other traditional training systems and vine densities in wine-growing regions that are currently arid and those that will become so in the future.

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