DOI: https://doi.org/10.20870/oeno-one.2024.58.3.8014

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Effects of grapevine canopy leaning on grape composition and wine quality of 'Bobal'

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Associate editor: Markus Rienth

Received: 19 February 2024
Accepted: 13 August 2024
Published: 19 September 2024

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ABSTRACT

Exploring new field strategies for adapting winegrowing regions to the adverse effects of climate change becomes imperative. This study focuses on the effects of canopy architecture through the trellis systems leaning of North-South oriented vineyard's rows vertical shoot positioned (VSP) system by 30° towards either the East (ESP) or the West (WSP) as a possible adaptation technique. The trial was conducted over two seasons in a *Vitis vinifera* L. cv. Bobal vineyard located in Requena (Valencia), eastern Spain, under a temperate-warm climate. The canopy leaning was considered to affect the radiation load and its timing with potential effects on vine physiology and grape metabolism and, consequently, on grape and wine quality. Leaning treatments affected vegetative growth, likely due to slight differences in vine water status. In comparison to VSP and ESP, WSP intercepts more light in the morning, when the photosynthetic capacity of the vines is at its highest. However, this results in a more negative vine water potential, which in turn leads to a reduction in vigour with no impact on yield. Musts from the WSP showed a tendency to increase total acidity compared to VSP, while ESP tended to decrease it. This was likely due to the reduction in temperature within the cluster microclimate in WSP in comparison to the ESP and VSP treatments. In addition, musts from the WSP treatment showed higher colour intensity, anthocyanins and polyphenols, potentially attributable to its more negative potentials, likely due to its more negative potentials, which may have prompted the synthesis of phenolic compounds. Consequently, wines from the WSP treatment displayed higher total acidity and lower pH, as well as higher colour intensity, anthocyanins, and polyphenolic content. In addition, WSP wines had higher concentrations of esters and higher alcohols. The improvements in grape and wine composition are likely due to an increased cluster radiation interception in the morning and reduced heating in the afternoon when VPD is higher. Our findings demonstrate that canopy management by leaning VSP vines towards the West can be a useful technique for adapting grape and wine composition to climate change and provide insights into its physiological basis.

KEYWORDS: Aroma compounds, cluster microclimate, canopy management, polyphenolic composition, radiation load, vine physiology, water status, wine quality

INTRODUCTION

Winegrowing in regions with a Mediterranean-like climate exhibits particular sensitivity to projected climate change (IPCC, 2022) mainly due to its impact on grape and wine composition, rather than on vine performance (Sadras *et al*., 2017). Global warming results in advancements in vine phenology and potential increases in soil water deficit (Fraga *et al*., 2012). Vine water stress disrupts physiology, leading to alterations in grape development and composition (Previtali *et al*., 2022). Elevated temperatures can decouple sugar accumulation in berries from phenolic maturity, resulting in grapes being harvested at higher sugar levels to attain complete phenolic maturity (Sadras and Moran, 2012). Consequently, this leads to higher alcohol content in wines (Van Leeuwen and Destrac-Irvine, 2017). Furthermore, higher temperatures decrease organic acid content while significantly altering aromas and aroma precursors (Duchêne and Schneider, 2005; Neethling *et al*., 2012). In this context, it becomes imperative to implement adaptation strategies that mitigate the climate change effects on vine performance, berry composition, and ultimately, wine quality (Bernardo *et al*., 2018; Buesa *et al*., 2019; Poni *et al*., 2018; Sadras *et al*., 2013).

In recent years, researchers and grape growers have been exploring field strategies for adapting to the adverse effects of climate change on grape quality (Suter *et al*., 2021; Van Leeuwen and Destrac-Irvine, 2017). Potential adaptation strategies may include early harvesting, although this may not be viable as it would not allow grapes to achieve the desired phenolic maturity (Sadras *et al*., 2017), relocation of vineyards to cooler locations, either at higher altitudes or latitudes (Jones *et al*., 2005), and modification of the genetic material employed, such as grapevine varieties, clones, and rootstocks (Medrano *et al*., 2015). Other strategies involve modifications in field management techniques, including irrigation, delaying vine phenology, modulating light interception, adjustments to grapevine architecture, canopy management, etc. (Buesa *et al*., 2020; Buesa *et al*., 2021a; Caravia *et al*., 2016; Previtali *et al*., 2022; Tessarin *et al*., 2022; Torres *et al*., 2020; Xyrafis *et al*., 2023).

This study focuses on the effects of canopy management through leaning 30° the traditional vertical shoot positioned (VSP) system towards either the East (ESP) or the West (WSP). The VSP system is the most commonly used trellis system in winegrapes as it allows for mechanical harvesting and effective management of vegetation to achieve a greater number of shoots with reduced vigour, thereby enhancing yield and resulting in wines with superior sensory characteristics (Palliotti *et al*., 2014). VSP vines are typically planted in North-to-South rows, facilitating uniform light distribution in the fruiting zone on both sides of the canopy, which significantly influences berry composition (Hunter *et al*., 2017; Spayd *et al*., 2002). Under this vineyard arrangement, the radiation load on grapevines varies throughout the day, with either greater or lower light interception occurring in the early morning and afternoon, respectively, and reduced radiation interception at midday when the sun directly impacts the narrow top of the canopy (Buesa *et al*., 2021b). Moreover, alternative training systems such as the open Lyre or the Y-shape (Carbonneau *et al*., 2004; Palliotti, 2012), which aim to increase sunlight, are suitable for improving yield and grape and wine quality (Carbonneau *et al*., 2006). Particularly under warm climates, canopy shading of the clusters can benefit the microclimate conditions for grape ripening and also vine physiology (Xyrafis *et al*., 2023). In this sense, leaning the VSP canopy allows modulating the radiation interception patterns throughout the day and therefore, both vine and grape physiology. This type of canopy management was first proposed by Ing. Raúl del Monte and later adapted by Pr. Alain Carbonneau to make it adjustable: 'Espalier modulé' or 'Modulated VSP' (Carbonneau, 2009). Nevertheless, the impact of leaning the VSP canopies on grape and wine composition remains understudied (Buesa *et al*., 2020; Friedel *et al*., 2016).

Leaning canopies are particularly interesting regarding solar radiation load, the timing of canopy interception, and their effects on vine water status and cluster microclimate conditions (Carbonneau, 2009; Baeza *et al*., 2010). It is well known that the intensity of light received by berries influences their secondary metabolism, potentially leading to increased accumulation of polyphenolic compounds (Caravia *et al*., 2016). Nevertheless, other effects on plant metabolism remain largely unknown and may vary among different grapevine cultivars (Fernandes de Oliveira and Nieddu, 2016). Berries that experience high exposure to solar radiation and low temperatures (15 °C) generally exhibit higher sugar, anthocyanin, and polyphenolic compound content while displaying lower total acidity, malate content, and must pH compared to berries with less exposure or in shaded areas (Bergqvist *et al*., 2001; Fernandes de Oliveira and Nieddu, 2016). However, under warmer climates, solar radiation can have both positive and negative effects on colour formation and the content of polyphenolic compounds due to the interactive effect of solar radiation and berry overheating (Lovisolo *et al*., 2010; Mori *et al*., 2007; Tessarin *et al*., 2022; Torres *et al*., 2020; Wolf *et al*., 2003). Furthermore, excessive degradation of organic acids and aromatic precursors due to excessive solar radiation can negatively impact sensory attributes, thereby affecting wine quality (Moreno *et al*., 2015a; Sadras *et al*., 2013). Nevertheless, the available information concerning the relationship between trellis inclination and the synthesis of aromatic compounds is very limited (Vilanova *et al*., 2019). Leaning the canopy will modify the daily radiation in the vineyard, thus affecting vine water relations and cluster microclimate conditions. Hence, leaning canopies to the west (WSP) will intercept more radiation during the morning when the vapour pressure deficit (VPD) is lower and less radiation in the afternoon when the VPD is higher. The opposite is true for the ESP. How the vine regulates its water status will determine the canopy temperature. Thus, water relations and microclimate temperature will ultimately alter the concentration of various compounds in the musts and consequently the composition of the wines produced from these grapes. These compounds

include C6 compounds, higher alcohols, ethyl esters and acetates, volatile fatty acids, and α-terpineol.

The objective of this study was to investigate the effect of different canopy architectures by leaning trellis systems in North–South hedgerow vineyards on berry quality and wine composition. Our main hypothesis was that, compared to the VSP system, leaning canopies will modify the diurnal radiation interception patterns, subsequently affecting cluster microclimate conditions and plant physiology, and improving grape and wine composition. Specifically, 1) ESP vines will increase vine water status during the morning, thereby enhancing vine performance and delaying grape ripening, and 2) WSP vines will increase radiation interception by the canopy during the morning when leaves are photosynthetically more active while reducing it in the afternoon when VPD is usually higher, and protecting clusters from over sun exposure.

To this end, the effects of VSP, ESP, and WSP on grapevine water relations, yield components, vine vigour and grape and wine composition were evaluated in a 'Bobal' vineyard under Mediterranean climate conditions during two seasons. This cultivar is, after 'Tempranillo', the second largest red grape cultivar grown in Spain, accounting for 12 % of the total (54.165 has) (Ministerio de Agricultura, Pesca y Alimentación, 2020). This cultivar is considered to be drought tolerant and prone to high yields, it has compact medium-to-large sized bunches with large irregular berries and a deep black-coloured skin with high resveratrol content (Salón *et al*., 2005; Navarro *et al*., 2008). It is a cultivar suitable for producing rosé wines, which are known for their pink colour, fresh acidity, and fruity flavours, although their red wines can also age well due to its tannic structure and acidity (García-Carpintero *et al*., 2010).

MATERIALS AND METHODS

1. Location and vineyard characteristics

The trial was carried out from 2016 to 2017 in a commercial vineyard (*Vitis vinifera* L.) located in Requena, Valencia, Spain (39º30´18.10´´N, 1º13´54.30´´W; elevation 746 m a.s.l.). The trial was conducted in Bobal cultivar grafted onto 110 Richter planted in 2002 at a spacing of 2.5 x 1.5 m (2666 vines ha^{-1}). Canopy was trained to a bilateral cordon system with 12 buds per vine in a trellis system oriented North–South. Canopy management practices included manual shoot thinning before the onset of flowering. The vineyard was deficit-irrigated through 3.2 $L.h^{-1}$ drippers spaced every 1.25 m. Sustained deficit irrigation was applied at 33 % of the crop evapotranspiration (ET_c), based on crop coefficient values quantified for 'Tempranillo' (López-Urrea *et al*., 2012). Total irrigation accounted for 94.9 and 62.4 mm in 2016 and 2017, respectively. Fertigation was performed on all treatments by applying 30-20-60-16 $kg.ha^{-1}$ of N, P_2O_5, K_2O, and MgO, respectively.

The soil in the vineyard was a Typic Calciorthid according to the Soil Taxonomy, with a clay-loam to light clay texture according to the USDA, highly calcareous (37 %) and with low fertility (0.66 % in organic matter and 0.04 % in nitrogen). Soil depth was higher than 2 m with 200 $mm.m^{-1}$ of available water capacity. The climate in this area was classified as semi-arid hot-summer Mediterranean, corresponding to a temperate warm viticultural climate, with cool and moderately dry nights according to the classification system for grape-growing regions proposed by Tonietto and Carbonneau (Tonietto and Carbonneau, 2004). The 2016 and 2017 seasons were meteorologically similar (Supplementary Table 1). Mean temperature and accumulated evapotranspiration (ET_o) during the growing seasons, from April to September, were 20 and 20.4 °C and 921 and 920 mm, respectively. Rainfall in this period was 166 in 2016 and 119 mm in 2017.

2. Experimental design

The experiment consisted of three treatments, (i) Vertical Shoot Positioned (VSP); (ii) East Shoot Positioned (ESP), canopy leaned 30° towards East; (iii) West Shoot Positioned (WSP), canopy leaned 30° towards West. The canopy leaning systems were established at the beginning of the experiment by adding leaned posts, which support the catching wires and the canopy, to the existing vertical trellis system (Supplementary Figure 1). The system allows the mechanisation of the vineyard and automatic collection as the inclined posts are mobile.

The experimental design was a complete block layout with four replicates. Each experimental unit (EU), a combination of blocks per treatment, consisted of five rows with 7–9 vines each (35–45 vines). The vines in the middle of the three central rows were used for measurement and sampling, while the rest acted as buffers.

3. Field determinations

During the growing season, midday Ψ_{stem} (measurements were carried out between 11:30 and 12:30 solar time) was determined fortnightly on bag-covered leaves from 4 representative vines per EU, 16 vines per treatment, using a pressure chamber (Model 600, PMS Instruments Inc., Albany, OR, USA) (Choné *et al*., 2001).

The daily temporal evolution of the surface temperature was monitored on the days of the year (DOY) 215 and 228 (August 3 and 16, 2017, respectively). Temperature measurements were performed using a thermal infrared radiometer (Apogee MI-210, Utah, USA) at 5 to 6 moments of the day: 9:30, 11:30, 14:00, 16:30, and 18:30 local time. The canopy temperature (Tc) was measured on both sides in 8 plants per treatment. The monitored area comprised the middle zone of the canopy. Additionally, all measurements were corrected from the atmospheric and emissivity effect following the procedure recommended by Sánchez *et al*. (2008).

After veraison, once shoot growth had practically ceased, total leaf area (LA) was estimated from the allometric relations between shoot length and leaf area per shoot measured with LI-3100 Area Meter (LI-COR Inc., Lincoln, NE, USA). This relationship was established in 2016 using

10 shoots of contrasting lengths, separating the main and lateral shoots (y = 22.74x, r^2 = 0.99*** and y = 14.42x, r^2 = 0.95***, respectively). Thereafter, LA was calculated by measuring the shoot length on 3 vines per EU, 12 vines per treatment (Tregoat *et al*., 2001).

At harvest, grape yield, number of clusters, and average cluster mass were determined in every experimental vine of each EU. In winter, pruning fresh mass was weighed from samples from 4 vines per EU.

4. Grape composition determinations

Berry fresh mass was determined from two subsamples of 200 berries per EU collected from all experimental vines. Must composition was determined from 50 berries of these subsamples by hand-pressing berries. Must total soluble solids (TSS) was determined by refractometry (PR-101, Series Palette, Atago, Tokyo, Japan) and pH and titratable total acidity (TTA) by automatic titration (Metrohm, Herisau, Switzerland) with NaOH, expressed in tartaric acid equivalents. In parallel, the other 50 berries per subsample were used for determining the grape's phenolic content (see section 2.6.).

5. Laboratory scale vinifications

Grapes from each EU were separately vinified at the experimental winery of the Polytechnic University of Valencia. Thus, in each season, 12 wines were performed. Grapes were harvested in boxes (10 kg), manually destemmed, crushed, and placed in jars (2 kg). Then, 100 $mg.L^{-1}$ of potassium bisulphite (E-224, Agrovin, Alcazar de San Juan, Spain) and 200 $mg.L^{-1}$ of Saccharomyces cerevisiae Enartis Ferm Red Fruit (Sepsa-Enartis, La Rioja, Spain) were added.

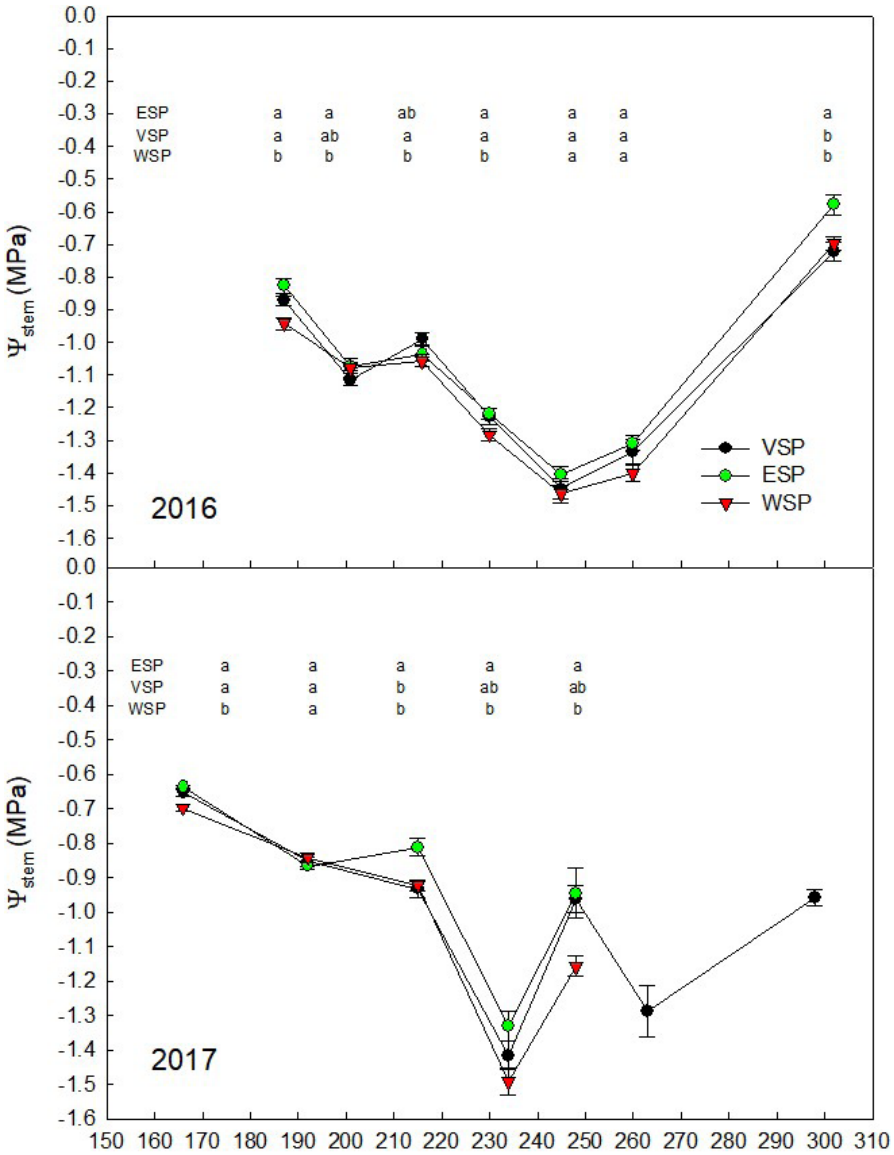


FIGURE 1. Stem water potential (Ψ_{stem}) evolution in 'Bobal' grapevines from the vertical shoot positioned (VSP), East shoot positioned (ESP), and West shoot positioned (WSP) treatments in 2016 and 2017 in Requena (Valencia), Spain (n = 16). Vertical bars indicate the standard error. Different letters denote significant differences among treatments at $p < 0.05$.

During the maceration process, the caps were punched down until homogenisation one time every day. Alcoholic fermentation (AF) was monitored by classical analytical techniques (density and temperature). Once the AF was finished (10 days), the lactic bacteria Viniferm Œ104 *Oenococcus oeni* (Agrovin, Alcazar de San Juan, Spain) were inoculated to start the malolactic fermentation (MLF). Once the MLF finished (15 days), each wine was bottled in a glass bottle and 50 $mg.L^{-1}$ of SO2 was added. Finally, wines were analysed (60 days from the end of MLF).

6. Must and wine oenological parameters

The oenological basic parameters of musts and wines, such as density, ethanol, pH, TTA, and volatile acidity were determined according to the Official Methods of the European Commission (Council Regulation, 1990). The concentration of total anthocyanins was determined according to the Puissant–León method (Blouin, 1992). The total polyphenols index (TPI) and total tannins were determined according to Slinkard and Singleton (Slinkard and Singleton, 1977). Flavonoid and non-flavonoid compounds were determined by Kramling and Singleton (Kramling and Singleton, 1969). The concentration of catechins was determined following the Pompei and Peri method (Pompei and Peri, 1971) and condensed tannins according to Ribéreau-Gayon and Stonestreet (Ribéreau-Gayon and Stonestreet, 1966). The content of anthocyanins combined with tannins was calculated using the Polyvinylpyrrolidone (PVPP) Index which was measured according to Vivas *et al*. (Vivas *et al*., 1994). The Gelatin Index (tannins astringency) (Glories, 1984) and DMACH (tannin degree polymerisation) were also calculated (Vivas and Glories, 1995). The content of proanthocyanins combined with polysaccharides was determined by the Ethanol Index according to Glories (Glories, 1978).

7. Analysis of wine aroma compounds

Volatile compounds were analysed by the procedure proposed by Ortega *et al.* (2001) with slight modifications. A volume of 2.7 mL of the samples was transferred to a 10 mL screw-capped centrifuge tube that contained 4.05 g of ammonium sulphate and the following compounds were added; 6.3 mL of Milli-Q water and 20 µL of a standard internal solution consisting of 2-butanol, 4-methyl-2-pentanol and 2-octanol from Sigma Aldrich (Darmstadt, Germany) at 140 µg $m.L^{-1}$ each, in absolute ethanol from LiChrosolv-Merck (Darmstadt, Germany), and 0.25 mL of dichloromethane. The tube was shaken mechanically for 120 min and was later centrifuged at 2,900 g for 15 min. The dichloromethane phase was recovered with a 0.5 mL syringe, transferred to the autosampler and analysed. The chromatographic analysis was carried out in an HP-6890, equipped with a ZB-Wax plus column (60 m × 0.25 mm × 0.25 µm) from Phenomenex. The column temperature, initially set at 40 °C and maintained at this temperature for 5 min, was then raised to 102 °C at a rate of 4 $°C.min^{-1}$ to 112 °C at a rate of 2 $°C.min^{-1}$, to 125 °C at a rate of 3 $°C.min^{-1}$ and this temperature was maintained for 5 min and then raised to 160 °C at a rate of 3 $°C.min^{-1}$; to 200 °C at a rate of 6 °C min^{-1} and was then kept at this temperature for 30 min. The carrier gas was helium, which was fluxed at a rate of 3 $mL.min^{-1}$. The injection was done in split mode 1:20 (injection volume 2 µL) with a flame ionisation detector (FID). In addition, Kovats retention indices (KI) were calculated for the GC peaks corresponding to identifying substance by the interpolation of the retention time of normal alkane (C8–C20) by Fluka Buchs (Schwiez, Switzerland), analysed under the same chromatographic condition. The calculated KI were compared with those reported in the literature for the same stationary phase.

8. Statistical analysis

The statistical analysis used to examine the dataset was a one-way Analysis of Variance (ANOVA). When the ANOVA revealed significant differences ($p < 0.05$), a post hoc mean separation was performed using either the Duncan test (for normally distributed data) or the Kruskal–Wallis procedure from the "Statgraphics Centurion XVI" software package (version 16.0.07) (Statgraphics Technologies, The Plains, VA, USA).

RESULTS AND DISCUSSION

1. Effect of the leaning canopy on vine physiology and yield components

In general, the vineyard water status became more negative until mid-season, when sustained deficit irrigation and rainfall led to an increase (Figure 1). In both seasons, the most negative Ψ_{stem} values reached - 1.5 to - 1.3 MPa, indicating severe water stress (Poni *et al*., 2018). It should be noted that ESP showed, on most dates, less negative values than WSP (Figure 1). In addition, on some dates, the WSP showed higher vine water stress than the VSP. Nonetheless, it should be noted that the Ψ_{stem} reflects the water status at midday, and that this varies in response to the trellis leaning throughout the day (Santesteban *et al*., 2019; Buesa *et al*., 2020). Therefore, it is to be expected that the WSP will recover its water status in the afternoon compared to the VSP and, above all, to the ESP.

Regarding vegetative growth, both pruning mass and total leaf area per vine (LA) were significantly affected by the treatments (Table 1). The ESP tended to increase the pruning mass compared to WSP, with VSP showing intermediate values between the two. However, differences among treatments were statistically significant only in 2016. A similar pattern showed LA due to the highest leaf area of main and secondary shoots. WSP treatment showed in both seasons significantly lower leaf area in secondary shoots (Table 1). The differences found among treatments in vegetative growth were in good agreement with its water status evolution (Figure 1). Nevertheless, Buesa and co-workers (Buesa *et al*., 2020), reported that leaning trellises towards the West increased LA by 13 % and yield by 12 % compared to traditional VSP. In the present trial, however, there were no overall significant effects of the trellis leaning on yield components (Table 1). The only exception was in 2017 when the number of clusters per vine was significantly decreased by 14 % in the ESP compared to the other treatments.

TABLE 1. Yield components of the experiment in 'Bobal' grapevines subjected to different training systems in 2016 and 2017.

Parameter	Treatment	2016	2017	(*p*-value)
Pruning mass (kg/vine)	VSP	0.40ab	0.58a	*
	ESP	0.44b	0.61a	
	WSP	0.33a	0.53a	
Total leaf area (m^2/vine)	VSP	3.0a	4.9a	**
	ESP	3.6b	4.9a	
	WSP	2.7a	4.3a	
Main shoots leaf area (m^2/vine)	VSP	2.5a	3.5a	**
	ESP	3.0b	3.6a	
	WSP	2.3a	3.4a	
Secondary shoots leaf area (m^2/vine)	VSP	0.47ab	1.17b	**
	ESP	0.61b	1.21b	
	WSP	0.40a	0.89a	
Clusters/vine	VSP	10.8a	8.7b	ns
	ESP	10.7a	7.4a	
	WSP	10.3a	8.6b	
Cluster mass (g)	VSP	404a	384a	ns
	ESP	409a	410a	
	WSP	389a	404a	
Berry weight (g)	VSP	2.33a	3.71a	ns
	ESP	2.40a	3.56a	
	WSP	2.41a	3.44a	
Yield (kg/vine)	VSP	4.3a	3.5a	ns
	ESP	4.3a	3.1a	
	WSP	4.0a	3.5a	

VSP; vertical shoot positioned, ESP; East shoot positioned and WSP; West shoot positioned.

However, neither cluster mass nor berry mass was affected, despite the slight differences in vine water status (Figure 1).

2. Effect of the leaning canopy on the physicochemical properties of musts

Differences in the basic physicochemical parameters of 'Bobal' grapes among treatments were found (Table 2). For instance, in 2017, the TSS of musts at harvest showed a significant increase in VSP compared to the other treatments. Moreover, in both vintages, the TTA tended to be the highest in WSP, with the grapes harvested in ESP decreasing TTA. However, no significant differences were found in pH among treatments (Table 2).

Regarding grape colour intensity and phenolic characteristics, in both seasons, musts from WSP vines showed significantly the highest colour intensity (Table 3). This fact could be related to the trend observed for anthocyanins with higher values in WSP and lower values in ESP compared to VSP. Significant differences were also observed for TPI, the content of polyphenols and tannins. In general, higher values were observed in WSP and lower values in ESP, regarding control (VSP). These results agree with previous authors who reported that, across training systems, increasing cluster sun exposure was related to higher anthocyanin and polyphenol concentrations in grapes (Wolf *et al*., 2003). In this sense, the WSP had a less dense canopy due to a significant reduction in secondary shoot growth (Table 1). Moreover, WSP clusters are also more protected from exposure to direct sunlight during the afternoon than ESP and VSP. Consequently, ESP clusters may increase the microclimate temperature during the warmest and driest hours of the day to the extent of diminishing berry colour and phenolics (Bergqvist *et al*., 2001; Oliveira and Nieddu, 2016). Furthermore, mild water stress can also have a positive effect by increasing the synthesis of berry anthocyanin and polyphenols (Castellarin *et al*., 2007; Bucchetti *et al*., 2011; Berhe, 2022; Martínez-Vidaurre *et al*., 2024). This highlights the relevance that other potential factors may have on the water status of the vines such as soil type, rootstock used, crop load, soil management and irrigation strategies, etc., which may influence the effects on grape composition of canopy management practices (Ashley, 2004; Uriarte *et al*., 2016).

TABLE 2. Physicochemical properties of grapes and musts harvested from 'Bobal' vines trained with a vertical shoot positioned (VSP), East shoot positioned (ESP) and West shoot positioned (WSP) system.

Parameter	Treatment	2016	2017	*p*-value
Total Soluble Solids (°Brix)	VSP	20.66a	21.27b	*
	ESP	20.68a	19.45a	
	WSP	20.98a	19.80a	
pH	VSP	3.35a	3.41a	ns
	ESP	3.39a	3.45a	
	WSP	3.34a	3.39a	
Titratable Total Acidity (g/L)	VSP	5.66a	6.57b	*
	ESP	5.49a	6.16a	
	WSP	6.08b	6.64b	

Different letters indicate significant differences between treatments at 95 % $p < 005$ (*), ns (not significant) based on Duncan's multiple range test. Titratable total acidity expressed as g/L of tartaric acid.

3. Effect of the leaning canopy on the physicochemical properties and phenolic composition of wines

In wines, a general trend was observed regarding canopy leaning according to the basic oenological parameters of wines. Thus, lower pH and higher TTA were observed in WSP, showing significant differences among treatments in 2016 (Table 4). Nevertheless, no differences in volatile acidity were found among treatments in any vintage. In 2017, the alcoholic strength was significantly higher in VSP wines compared to ESP and WSP.

Regarding phenolic parameters and colour characteristics, a similar trend was observed in musts, accordingly higher colour intensity and anthocyanins were obtained in the case of WSP with significant differences in both vintages (Table 5). Moreover, a significantly higher content in TPI, total phenols, total flavonoids, total tannins, and catechins was observed for WSP compared to both VSP and ESP in both vintages. These results can be related to a greater amount of radiation in the morning when the photosynthetic capacity of the vines is higher, and to the fact that the bunches are protected from the afternoon sun when the air temperature is higher in the WSP system (Buesa *et al*., 2020; Intrigliolo *et al.*, 2017). Indeed, when vines lean towards the West, an early interception of light in the morning increases the rate of foliar photosynthesis (Intrigliolo *et al.*, 2017) that can lead to a greater accumulation of polyphenols in grapes (Vitrac *et al*., 2000). Moreover, the more severe vine water stress experienced by WSP (Figure 1), can accelerate grape ripening and polyphenols biosynthesis (Castellarin *et al*., 2007; Intrigliolo and Lakso, 2011; Lovisolo *et al*., 2010). On the contrary, the ESP showed no increase in the phenolic parameters of wines compared to VSP, with the exception in 2017 of total phenols, total flavonoids, and catechins (Table 5). This may be explained by the slight increases observed in vine water status in ESP (Figure 1) and its effects on the phenolic compounds in this cultivar (Salón *et al*., 2005). However, no decreases in the phenolic parameters compared to VSP were found. Therefore, increasing solar radiation exposure of the cluster may have had counteracting effects, positively influencing flavanol synthesis (Friedel *et al*., 2016; Wolf *et al*., 2003) while provoking a depletion by overheating. Differences in canopy temperature throughout the day among treatments showed that WSP reduced canopy temperature by 3–4 degrees compared to VSP and WSP (Supplementary Figure 2). These differences in canopy temperature cannot be directly translated to differences in grape temperature, but they show that these are temperatures that could lead to the degradation of phenolic compounds (Mori *et al*., 2007). In this regard, Torres and co-authors reported that higher light interception affected flavonoid accumulation, both because of its synthesis but also its degradation (Torres *et al*., 2020).

The PVPP Index increased in both vintages for WSP, showing significant differences from VSP and ESP only in 2017 (Table 5). A higher combination of anthocyanins and flavanols in WSP treatment can influence positively the wine colour stability (Escribano-Bailón *et al*., 2001). In this sense, the DMACH Index was significantly lower in WSP treatment compared to VSP and ESP, which may be caused either by precipitation of some of the proanthocyanidin molecules or by increasing their degree of polymerisation (Zanchi *et al*., 2007). Wines showed no differences in the Gelatine Index, which is commonly used to evaluate astringency. Regarding the formation of polysaccharides–tannin–protein complexes, assessed through the Ethanol Index, a higher value was found in WSP wines (no significant differences within each vintage), indicating a higher content of tannins dissolved but in turn higher formation complexes likely due to the higher content of polyphenols observed (Table 5).

4. Effect of the leaning canopy on the aromatic composition of wines

A total of thirty-eight volatile compounds were quantified to determine the effect of the leaning canopy on the aromatic composition of wines (Table 6).

TABLE 3. Colour intensity and phenolic characteristics of 'Bobal' musts harvested from grapevines trained with a vertical shoot positioned (VSP), East shoot positioned, and West shoot positioned (WSP) system.

Parameter	Treatment	2016	2017	*p*-value
Colour	VSP	29.20a	24.38a	
Intensity	ESP	27.72a	24.61a	*
pH 1	WSP	34.17b	29.78b	
Colour	VSP	9.17a	8.15a	
Intensity	ESP	8.80a	8.21a	***
pH 3.2	WSP	12.20b	9.84b	
Anthocyanins	VSP	886.54ab	759.52ab	
pH1	ESP	821.88a	741.68a	*
(mg/L)	WSP	938.73b	848.23b	
Anthocyanins	VSP	321.57ab	284.57a	
pH 3.2	ESP	299.56a	282.57a	***
(mg/L)	WSP	384.24b	354.24b	
Anthocyanins	VSP	61.63a	73.59a	
Extractability	ESP	63.67a	73.40a	ns
(%)	WSP	58.44a	69.39a	
TPI pH 1	VSP	48.56a	43.72a	
	ESP	45.66a	44.77a	***
	WSP	54.14b	51.15b	
TPI pH 3.2	VSP	30.56ab	26.61a	
	ESP	27.25a	25.54a	***
	WSP	37.12b	31.07b	
Polyphenols	VSP	3221.34ab	3013.34b	
pH 1	ESP	2898.90a	2578.90a	***
(mg/L)	WSP	3515.78b	3195.78b	
Polyphenols	VSP	1831.08a	1630.92ab	
pH 3.2	ESP	1757.50a	1393.94a	***
(mg/L)	WSP	2161.91b	2017.93b	
Tannins	VSP	841.05a	791.05a	
pH 1	ESP	793.61a	729.86a	***
(mg/L)	WSP	1070.82b	889.57b	
Tannins	VSP	781.50a	682.13a	
pH 3.2	ESP	768.02a	718.02b	***
(mg/L)	WSP	982.09b	844.59c	
% Seed ripening	VSP	57.91a	56.19a	
	ESP	56.06a	55.51a	ns
	WSP	58.60a	51.50a	

Different letters indicate significant differences between treatments at $p < 005$ (*), $p < 001$ (**), $p < 0001$ (***) and ns (not significant). TPI: Total Polyphenol Index.

The total concentration of each compound is shown individually and grouped in five representative families according to their chemical structure (aldehydes, esters, alcohols, acids, and lactones).

Regarding aldehydes, the different treatments did not influence their overall concentration. In 2017, VSP wines did show a higher concentration of acetaldehyde (Table 6). This compound can provide pleasant or unpleasant notes to wines depending on their concentration. Thus, low concentrations provide positive notes such as sweet fruits, but high concentrations can lead to undesirable aromas such as baked apple, walnuts, butter or glaze (Zea *et al*., 2015). Concerning esters, in both vintages, WSP wines consistently showed significantly higher concentrations than VSP and ESP. Particularly, isobutyl acetate, ethyl isovalerate, ethyl hexanoate, ethyl lactate, hexyl acetate, diethyl succinate, and 2- phenyl-ethyl acetate obtained significant differences (Table 6). These compounds are related to fruity aromas and pleasant notes, being a high incidence in the aromatic profile of wines (Ferreira *et al*., 2000). Therefore, WSP wines could lead to aromatically fresher wines with a more marked youthful character. This can be explained by the increased sunlight exposure of the clusters in WSP due to the lower secondary shoots development (Belancic *et al*., 1997; Zhang *et al*., 2017).

The concentration of higher alcohols in WSP was significantly higher than in VSP in 2016 and significantly higher than ESP in both seasons (Table 6). This pattern showed differences mainly between WSP and ESP treatments and could indicate that the canopy leaning towards the West is most suitable to improve the level of higher alcohol in wines. In Tempranillo wines, Talaverano *et al*. (2017) reported higher levels of total alcohol in response to more negative vine water potentials, as is the case of WSP in comparison to VSP and ESP (Figure 1).

In relation to fatty acids (aromatic precursors of fruity esters), a different tendency was found depending on the vintage. In 2016, a higher content of butyric acid and hexanoic acid was found in ESP and WSP compared to the control (Table 6). Octanoic acid tended to be reduced in WSP for both vintages. Nevertheless, in 2017, isobutyric acid, butyric acid and hexanoic acid reached significantly higher levels in WSP than in control wines (VSP). Regarding lactones, as occurred in higher alcohols and fatty acids, a different pattern was observed between vintages showing significant overall differences between WSP and ESP only in 2016. Pantolactone was the only compound that consistently increased in both seasons by WSP compared to VSP and ESP (Table 6).

In general, the content of esters and higher alcohols seems to be favoured by WSP, producing a higher aromatic intensity of wines, as reported by other authors in response to the effect of the increase in vegetation height (Moreno *et al*., 2015b). These results could be related to the fact that WSP grapes, compared to VSP and ESP ones, intercepted a greater amount of radiation early in the morning and from the lower in the afternoon, when the air temperature and VPD are higher (Supplementary Figure 2). This may be due to, on the one hand, the increase in sunlight exposure of the clusters (Belancic *et al*., 1997; Zhang *et al*., 2017) and, on the other hand, a reduction in the degradation of aromatic precursors by heat (Marais *et al*., 2001), both processes leading to a higher aromatic concentration in wines.

TABLE 4. Oenological basic parameters of wines made from 'Bobal' grapes trained with a vertical shoot positioned (VSP), East shoot positioned (ESP) and West shoot positioned (WSP) system.

Parameter	Treatment	2016	2017	(*p*-value)
Alcoholic weight (g) Strength (% vol.)	VSP	11.54a	12.52b	***
	ESP	11.19a	11.68a	
	WSP	11.28a	11.66a	
pH	VSP	3.42ab	3.82a	*
	ESP	3.46b	3.83a	
	WSP	3.39a	3.79a	
TTA (g/L)	VSP	6.57b	4.67a	***
	ESP	6.16a	4.54a	
	WSP	6.65b	4.72a	
Volatile acidity (g/L)	VSP	0.30a	0.36a	ns
	ESP	0.32a	0.39a	
	WSP	0.31a	0.36a	

Different letters indicate significant differences between treatments at $p < 005$ (*) and $p < 0001$ (***). TTA (Titratable Total Acidity expressed as g/L of tartaric acid and volatile acidity in g/L of acetic acid). ns (not significant).

TABLE 5. Colour characteristics and phenolic parameters of wines made from 'Bobal' grapes harvested in vines trained with a vertical shoot positioned (VSP), East shoot positioned (ESP), and West shoot positioned (WSP) system.

Parameter	Treatment	2016	2017	(*p*-value)
Colorant intensity	VSP	10.73a	9.53a	***
	ESP	9.70a	9.37a	
	WSP	13.03b	10.96b	
Hue (%)	VSP	52.76a	78.17a	ns
	ESP	53.54a	76.49a	
	WSP	51.76a	78.39a	
Anthocyanins (mg/L)	VSP	289.62a	279.46a	***
	ESP	299.18a	297.83a	
	WSP	342.65b	321.11b	
TPI	VSP	34.29a	30.43a	***
	ESP	36.11a	30.15a	
	WSP	42.57b	33.73b	
Total Phenols (mg/L)	VSP	2752.29a	2515.88a	***
	ESP	2777.08a	2801.98b	
	WSP	3006.24b	3238.23c	
Total NO Flavonoids (mg/L)	VSP	578.71a	496.75a	ns
	ESP	587.53a	494.62a	
	WSP	584.98a	508.14a	
Total Flavonoids (mg/L)	VSP	2173.58a	2019.13a	***
	ESP	2189.54a	2447.06b	
	WSP	2421.26b	2730.09c	
Total Tannins (mg/L)	VSP	1622.89a	1651.12a	***
	ESP	1576.82a	1676.07a	
	WSP	1739.25b	1837.96b	
Condensed Tannins (mg/L)	VSP	1322.82a	1182.27a	***
	ESP	1401.26a	1288.56ab	
	WSP	1651.83b	1343.85b	
Catechins (mg/L)	VSP	158.20a	91.61a	***
	ESP	180.20b	110.56b	
	WSP	204.82c	153.22c	
PVPP Index (%)	VSP	67.90a	65.44a	***
	ESP	69.09a	64.59a	
	WSP	74.33b	67.40a	
DMACH Index (%)	VSP	27.45b	34.63b	***
	ESP	31.24c	34.15b	
	WSP	23.72a	31.59a	
Ethanol Index (%)	VSP	77.46a	74.18a	***
	ESP	77.23a	78.51a	
	WSP	79.98a	79.06a	
Gelatine Index (%)	VSP	24.18a	35.35a	ns
	ESP	23.91a	35.26a	
	WSP	29.99a	37.95a	

Different letters indicate significant differences between treatments at p < 005 (*), p < 001 (**), p < 0001 (***) and ns (not significant). TPI (Total Polyphenol Index).

TABLE 6. Aromatic composition (mg/L) of wines elaborated from 'Bobal' grapes trained with a vertical shoot positioned (VSP), East shoot positioned (ESP), and West shoot positioned (WSP) system. (Part 1/3)

Compound/FAMILIES	Treatment	2016	2017	*p*-value
Acetaldehyde	VSP	0.403a	0.427b	***
	ESP	0.352a	0.354a	
	WSP	0.346a	0.332a	
Benzaldehyde	VSP	0.370a	0.456a	ns
	ESP	0.428a	0.498a	
	WSP	0.429a	0.531a	
Diacetal	VSP	0.033a	0.023a	*
	ESP	0.041a	0.050a	
	WSP	0.049a	0.058a	
ALDEHYDES	VSP	0.806a	0.966a	ns
	ESP	0.821a	0.902a	
	WSP	0.823a	0.921a	
Methyl acetate	VSP	1.514b	0.401a	ns
	ESP	0.815a	0.376a	
	WSP	1.096a	0.485a	
Ethyl acetate	VSP	0.146a	0.390b	*
	ESP	0.083a	0.291a	
	WSP	0.141a	0.392b	
Isobuthyl acetate	VSP	0.893a	1.018a	*
	ESP	1.105a	1.291b	
	WSP	1.505b	1.536b	
Ethyl isovalerate	VSP	0.453a	0.296a	ns
	ESP	0.417a	0.467b	
	WSP	0.445a	0.396ab	
Ethyl hexanoate	VSP	0.421a	0.192a	***
	ESP	0.434ab	0.208a	
	WSP	0.465b	0.316b	
Hexyl acetate	VSP	0.390a	0.516a	*
	ESP	0.365a	0.683b	
	WSP	0.576b	0.857c	
Ethyl lactate	VSP	3.215a	1.880a	**
	ESP	4.622b	3.389b	
	WSP	8.534c	7.918c	
Ethyl octanoate	VSP	1.020a	0.481a	ns
	ESP	0.854a	0.657b	
	WSP	1.293a	0.455a	
Ethyl decanoate	VSP	0.989a	0.853a	ns
	ESP	0.967a	0.770a	
	WSP	0.839a	0.801a	
Diethyl succinate	VSP	0.913ab	1.342b	***
	ESP	0.831a	1.273a	
	WSP	0.942b	1.402b	

Different letters indicate significant differences between treatments at p < 005 (*), p < 001 (**), p < 0001 (***) and ns (not significant).

TABLE 6. Aromatic composition (mg/L) of wines elaborated from 'Bobal' grapes trained with a vertical shoot positioned (VSP), East shoot positioned (ESP), and West shoot positioned (WSP) system. (Part 2/3)

Compound/FAMILIES	Treatment	2016	2017	*p*-value
Diethyl glutarate	VSP	0.550a	0.556a	ns
	ESP	0.558a	0.520a	
	WSP	0.626b	0.514a	
2-phenyl ethyl acetate	VSP	0.069a	0.025a	***
	ESP	0.064a	0.026a	
	WSP	0.092b	0.107b	
ESTERS	VSP	10.571a	7.951a	***
	ESP	11.115a	9.949b	
	WSP	16.554b	15.180c	
1-propanol	VSP	0.530a	0.422a	ns
	ESP	0.517a	0.453a	
	WSP	0.569a	0.422a	
1-butanol	VSP	0.669a	0.542c	**
	ESP	0.807ab	0.343a	
	WSP	0.965b	0.487b	
Isoamylic alcohol	VSP	245.27a	240.179b	**
	ESP	241.92a	178.901a	
	WSP	254.38a	219.075b	
Cis 3-hexenol	VSP	0.782b	0.122b	*
	ESP	0.448a	0.100a	
	WSP	0.419a	0.179b	
1-heptanol	VSP	0.060a	0.067a	ns
	ESP	0.050a	0.040a	
	WSP	0.071a	0.081b	
2.3 butanediol	VSP	5.491a	3.644a	***
	ESP	5.291a	7.046b	
	WSP	7.006b	8.878c	
Benzyl alcohol	VSP	0.560a	0.419b	ns
	ESP	0.559a	0.401b	
	WSP	0.626a	0.284a	
2-phenylethanol	VSP	58.463a	43.603b	*
	ESP	61.182a	39.438ab	
	WSP	58.103a	34.853a	
HIGHER ALCOHOLS	VSP	311.825a	288.999b	**
	ESP	306.822a	226.722a	
	WSP	322.138b	264.259b	
Isobutyric acid	VSP	0.345a	0.249b	**
	ESP	0.345a	0.173a	
	WSP	0.358a	0.319c	
Decanoic acid	VSP	0.171a	0.298b	***
	ESP	0.185a	0.139a	
	WSP	0.170a	0.273b	

Different letters indicate significant differences between treatments at p < 005 (*), p < 001 (**), p < 0001 (***) and ns (not significant).

TABLE 6. Aromatic composition (mg/L) of wines elaborated from 'Bobal' grapes trained with a vertical shoot positioned (VSP), East shoot positioned (ESP), and West shoot positioned (WSP) system. (Part 3/3)

Compound/FAMILIES	Treatment	2016	2017	*p*-value
Butyric acid	VSP	1.130a	1.349a	ns
	ESP	1.341b	1.402ab	
	WSP	1.352b	1.639b	
Isopentanoic acid	VSP	0.362a	0.244a	ns
	ESP	0.342a	0.360b	
	WSP	0.355a	0.321ab	
Hexanoic acid	VSP	0.265a	0.315a	ns
	ESP	0.301b	0.356ab	
	WSP	0.315b	0.419b	
Octanoic acid	VSP	2.100ab	1.089b	***
	ESP	2.276b	0.671a	
	WSP	1.913a	0.590a	
FATTY ACIDS	VSP	4.372a	3.543b	ns
	ESP	4.791a	3.101a	
	WSP	4.464a	3.561b	
γ-butirolactone	VSP	0.934a	0.624a	ns
	ESP	1.028a	0.493a	
	WSP	1.065a	0.486a	
β-damascenone	VSP	1.150b	0.319c	**
	ESP	0.819a	0.260b	
	WSP	1.012ab	0.183a	
α-ionone	VSP	0.554a	0.226a	**
	ESP	0.623a	0.458c	
	WSP	0.601a	0.331b	
Pantolactone	VSP	0.153a	0.273a	*
	ESP	0.141a	0.233a	
	WSP	0.214b	0.409b	
Decalactone	VSP	0.136a	0.205a	ns
	ESP	0.144a	0.229b	
	WSP	0.165b	0.213a	
LACTONES	VSP	2.927ab	1.647a	ns
	ESP	2.755a	1.673a	
	WSP	3.056b	1.623a	

Different letters indicate significant differences between treatments at p < 005 (*), p < 001 (**), p < 0001 (***) and ns (not significant).

5. Integrating the effects of canopy leaning on physiology, agronomic and oenological responses

Canopy architecture significantly affected vine water status, and thus vegetative vigour, mainly due to the increase and reduction in leaf area of secondary shoots of ESP and WSP, respectively, with respect to the VSP control (Table 1). This, together with the different radiation loads intercepted by the canopy due to its leaning, likely increased the irradiance and temperature of the clusters throughout the morning and afternoon in WSP and ESP, respectively. This generated microclimate conditions that in turn increased and reduced phenolic and aromatic compounds in WSP and VSP, respectively, with respect to the VSP. Note the importance of the timing of radiation interception, as it is in the afternoon, when VPD is higher, that the greatest interception seems to be negative for acidity and phenolic compounds and aromatic composition (ESP), whereas the opposite is true in the morning (WSP).

Nevertheless, our two seasons results also highlight the complex relationships between meteorological conditions and field management practices as other authors reported in other cultivars (Buesa *et al*., 2021a; Talaverano *et al*., 2017; Vilanova *et al*., 2019). For instance, the smaller differences in the total leaf area of the vines among treatments in 2017, when canopies were additionally denser compared to 2016, seem to dampen the effects of canopy leaning on cluster microclimate and thus on grape and wine composition. Consequently, canopy leaning should be adjusted to the specific environmental conditions of the vintage, as the angle of leaning and the timing can be modulated according to the grape ripening process. As an example, in the face of heat waves during grape ripening, leaning the trellis to the West might minimize the detrimental effects on grape composition.

CONCLUSIONS

Leaning canopy treatments affected canopy radiation load, causing significant differences in vine water status, and resulting in differences in vegetative growth. Thus, the WSP treatment increased vine water stress (Ψ_{stem}) resulting in a 15 % lower both pruning mass and total leaf area compared to the VSP and ESP treatments. However, no significant differences were observed in the yield components. Musts from the WSP showed a tendency to increase TTA compared to VSP, while ESP tended to reduce it. This is attributable to the temperature reduction in the cluster microclimate in the WSP compared to ESP and VSP. Furthermore, grapes from WSP treatment also exhibited heightened colour intensity, anthocyanins and TPI, likely due to its more negative potentials inducing the synthesis of phenolic compounds. Consequently, wines from the WSP treatment displayed lower pH and higher TTA, as well as higher colour intensity, anthocyanins, and polyphenolic composition than the other treatments. Aromatic analysis revealed that WSP wines had higher concentrations of esters and higher alcohols than the other treatments, which could be related to fruity aromas and a higher aromatic intensity of wines. These findings demonstrate that canopy leaning towards the West can be a useful technique for adapting must composition and wine quality to climate change through increasing radiation interception in the morning and decreasing cluster heating in the afternoon. These results encourage further research into the potential of canopy management practices to regulate radiation load and its timing under different cultivars and environmental conditions. It also provides insights into the physiological basis of its effects on vine water status and cluster microclimate.

ACKNOWLEDGEMENTS

This work was supported by the Spanish Ministry of Science and Innovation through the Spanish Research Agency (MCIN/AEI) and the European Union Next GenerationEU/PTR [grant numbers PID2021-123305OB-C31]. R. Ferrer-Gallego acknowledges the funding of MCIN/AEI/10.13039/501100011033 and the European Union NextGenerationEU/PRTR [RYC2021-031638-I] and [CNS2023-144458]. The authors express their gratitude to Felipe Sanz, Antonio Yeves, Diego Pérez, Giulio Caccavello, Maria Clara Merli and Javier Castel for their help in the fieldwork. Thanks are also due to 'Cajamar' and 'Lucio Gil de Fagoaga' for facilitating the experimental field.

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