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Effects of sunlight exclusion on leaf gas exchange, berry composition, and wine flavour profile of Cabernet-Sauvignon from the foot of the north side of Mount Tianshan and a semi-arid continental climate

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ABSTRACT

Canopy shading is a widely used viticultural strategy for mitigating early grape berry ripening caused by global warming. In this study, we covered half of the canopy with a black shade cloth from the pea-size stage to harvest. In the fruit zone, canopy shade treatment (ST) reduced daily average solar radiation by about 74.6 % compared to the untreated control (UC), and significantly reduced daily average temperature. ST leaves were found to have lower net assimilation rates and higher internal CO_2 concentration than in UC, which resulted in reduced yield, bunch weight, cane starch and berry total soluble solids. A delayed development stage was found in ST berries which had lower pH and higher titratable acid than UC. ST increased both berry and wine anthocyanin concentration while significantly decreasing flavonol concentration. ST wines had higher concentrations of a number of ester compounds and β-damascenone than UC wines, thus significantly enhancing the floral and fruity aroma of ST wines. Higher C_6/C_9 and fatty acids concentrations in ST berries may have caused higher ester concentrations in ST wines. The results showed significant effects on metabolites in berries and wines caused by canopy shade.

KEYWORDS

grape; wine; canopy shade; leaf gas exchange; flavour composition

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/4545

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INTRODUCTION

Global warming has profoundly impacted many crops all over the world (Lobell *et al.,* 2008). In various regions, elevated temperatures have resulted in earlier plant phenological events, and drought and other abiotic or biotic stresses have challenged cultivated plant species (Cleland *et al.,* 2007; Root *et al.,* 2003). Grapevine (*Vitis vinifera* L.) is cultivated worldwide and it has high economic value. The warmer climate has resulted in earlier vine phenological events with increased sugar at harvest. Moreover, reductions in anthocyanins, higher pH and lower titratable acid in berries and wines caused by global warming have also been reported (Parra *et al.,* 2010; Sadras and Petrie, 2012; Spayd *et al.,* 2002; Jiang *et al.,* 2017; Rienth *et al.,* 2016; Sadras and Petrie, 2011; Yamane *et al.,* 2006; Mori *et al.,* 2005; Mori *et al.,* 2007). Unsynchronised sugar and phenolic compound accumulation makes it hard for winemakers to set a harvest date (Palliotti *et al.,* 2014). When sugar and titratable acid reach a suitable level, the seed coat is still not mature enough, which can result in wine with harsh tannins and stalky sensory attributes. Most winemakers delay the harvest date to ensure phenolic maturity and to avoid a herbaceous aroma, which results in higher alcohol levels and pH in the wines. Nowadays, consumers tend to pay more attention to the concept of healthy drinking and they prefer wines with moderate alcohol content (Palliotti *et al.,* 2014), which is in conflict with the change in wine alcohol content caused by a delayed harvest. To reduce the alcohol content of wines, wineries around the world have tried strategies such as membrane technology, supercritical extraction, or vacuum distillation. However, these technologies are relatively costly (Palliotti *et al.,* 2014).

Canopy shading, a widely studied and easily applied technique, has been used to mitigate the adverse effects of global warming. Common approaches to shading are as follows: overhead shade: a shade cloth is placed above the whole vine, and a T-shaped structure is used to support the shade cloth (Greer *et al.,* 2011; Greer and Weedon, 2012; Caravia *et al.,* 2016); canopy shade: a shade cloth is placed above each side of the canopy (Caravia *et al.,* 2016; Martínez-Lüscher *et al.,* 2016; Rojas-Lara and Morrison, 1989); and bunch shade: a shade cloth, bag or box is used to shade bunches (Caravia *et al.,* 2016; Downey *et al.,* 2004; Ristic *et al.,* 2007). Overhead shade creates shade above the whole vine which also depends on shade width and mesh size. A narrow shade cloth can expose canopy and bunches in the early morning and late afternoon. The position and width of a shade cloth will define the proportion of shaded canopy, and most canopy shade treatments should cover both leaves and bunches. Shade treatments usually lead to reductions in biomass accumulation and photosynthetic rate, the increase of titratable acid (Greer *et al.,* 2010; Greer *et al.,* 2011; Greer and Weedon, 2012), and a notable decline in canopy and berry temperature (Cartechini and Palliotti, 1995; Rana *et al.,* 2004; Chorti *et al.,* 2010). However, Rojas-Lara and Morrison (1989) found that while the canopy temperature underneath the shade was significantly higher than that of the control, there was no significant difference in berry temperature.

Shading has proved to be an effective technique for delaying ripening and reducing berry TSS and wine alcohol content (Caravia *et al.,* 2016; Joscelyne *et al.,* 2007; Bahena-Garrido *et al.,* 2018). Three consecutive years of overhead shade on Shiraz grapes showed that canopy shade can significantly reduce berry cell death and berry weight loss, and protect vines from heat stress (Caravia *et al.,* 2016). The effect of shading on the secondary metabolites of grapes or wines was apparent. Research has suggested that flavonoid biosynthesis in many plants is under the control of photoreceptors (Joscelyne *et al.,* 2007). Sunlight exposure is generally thought to be beneficial for anthocyanin biosynthesis, but high temperatures can prohibit the synthesis of anthocyanins (Yamane *et al.,* 2006). The flavonol profile of red wine grapes is a reliable indicator of solar radiation (Martínez-Lüscher *et al.,* 2019). In terms of volatile compound accumulation, several studies have shown that the synthesis of aroma compounds, such as monoterpenoids and C_{13} norisoprenoids, can be promoted by adjusting light exposure around the bunches (Bahena-Garrido *et al.,* 2018).

In the semi-arid Xinjiang province, Cabernet-Sauvignon grape ripens quickly. Partial canopy shade treatment is applied with the evaluations of vine growth, flavonoids, and aroma compounds in wines. In addition, leaf gas exchange was evaluated in both shaded canopy leaves and upper exposed canopy leaves to determine if their photosynthetic capacity was compensated for by canopy shading.

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MATERIALS AND METHODS

1. Experimental sites and treatment

Own-rooted Cabernet-Sauvignon vines which had been planted in 2011 were studied in a commercial vineyard located in the county of Manas, at the foot of the northern side of Mountain Tianshan, Xinjiang province (44°24′N-86°26′E, elevation 522 m). The soil was silt loam, and the climate was semi-arid. Rows were orientated northeast-southwest (52 °) with a vine and row spacing of 1 m and 3 m respectively. Vines were trained to a modified vertical shoot-positioned (M-VSP) spur-pruned cordon system (Cheng et al., 2014), on which 15-18 nodes per linear meter of row were retained. Vines were trimmed twice in the growing season to maintain a maximum canopy height of about 1.9 m from the ground. The vines were subjected to furrow irrigation, with 75 mm water being applied each at budburst, blossom, pea-size, veraison, and pre-harvest (approximately two weeks before harvest). The meteorological data at the experimental site was obtained from the China Meteorological Data Service Centre (http://cdc.cma.gov.cn/), which included average daily temperature and hours of sunshine throughout the whole growing season (April to September) from 2009 to 2019.

A black cloth (0.1 mm thick, 1.2 m wide, 75 % absorption; Meshel Netting Co., Ltd, China) made of polyethylene was used for the partial canopy shade treatment (ST). The control (UC) received no shade treatment. The shade net covered the canopy at about 1.3 m from the soil surface, and the top was tied to the second wire (Figure 1). There were three replicates of each treatment arranged in a randomised block design distributed in three adjacent rows. Each replicate comprised a panel of 30 vines with similar growth vigour. ST was applied at the pea-size stage (approximately four weeks after flowering) until harvest in 2018.

2. Microclimate data and berry sample collection

Microclimate data from the ST and UC bunch zone were collected from micro-weather stations with temperature and solar radiation sensors (Hobo® micro station, Onset corporation, USA). The period of data collection ranged from the pea-size stage until harvest, that is 82 days in total. Temperature and solar radiation were recorded every 5 min. Growing degree days were calculated using a base temperature of 10 °C.

Berries were sampled seven times throughout berry development for physicochemical parameters. For each replicate, 100 berries were sampled. The sampling time points were as follows: 1) pre-treatment (27 June), 2) onset of veraison (30 July), 3) the stage at which the berries had obtained 100 % colour (8 August), 4) preharvest (17 August), 5) pre-harvest (27 August), 6) control harvest (UC TSS reached around 23 °Brix; 8 September), and 7) treatment harvest (ST TSS reached around 23 °Brix; 23 September). Moreover, at harvest, 500 berries were sampled, immediately frozen in liquid nitrogen and stored at -80 °C for subsequent metabolite analysis.

3. Measurement of leaf gas exchange

Gas exchange parameters were determined using a Li-6400XT portable photosynthesis system (Li-Cor® Inc., Lincoln NE, USA), with measurements being made on fully expanded leaves for both ST and UC from the following positions: i) 6th to 8th nodes, which were inside the shade cloth for ST, and ii) 10th to 12th nodes, which were outside the shade cloth for ST. Gas exchange parameters were measured five times in total during the growing season. Each gas exchange measurement comprised net assimilation rate (A_N), transpiration rate (E), stomatal conductance (g_s), and internal CO_2 concentration (C_i) of the leaves.

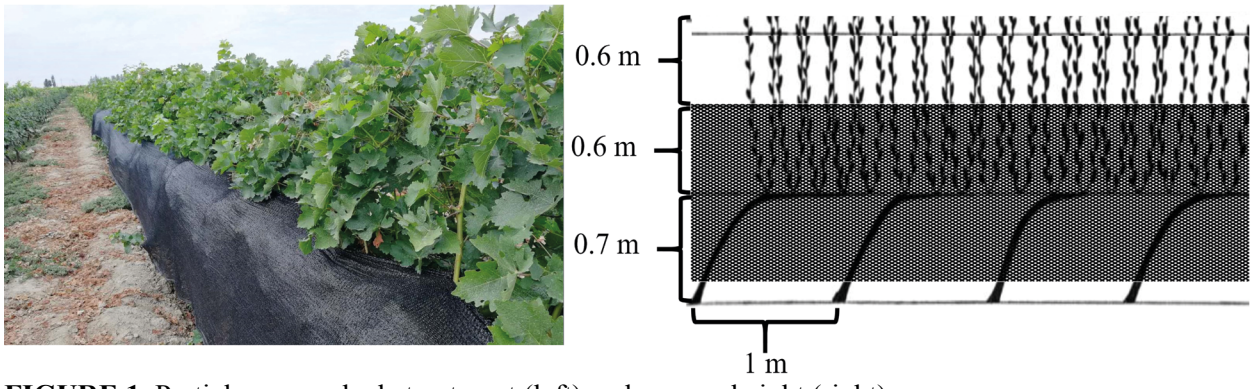


FIGURE 1. Partial canopy shade treatment (left) and canopy height (right).

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For each replicate, five leaves on the eastern canopy were measured between 09:00 and 10:00 local time. All the UC and ST replicates were measured in random order to eliminate any effects of time of measurement.

Furthermore, five leaves per replicate were measured during the day under natural light to study the diurnal variation of leaf gas exchange. The determined parameters were the same as above and recorded at the following time points: 9:00, 11:00, 13:00, 14:00, 15:00, 17:00 and 19:00.

4. Measurement of vine growth and yield parameters

One week before harvest, the area of primary and lateral leaves of ten shoots per replicate was measured using a portable leaf area meter (Yaxin-1242, Beijing, China). The length and diameter (the third internode from the base) of ten shoots per replicate were measured. The number of shoots was counted on ten vines for each replicate. SPAD value from ten leaves every replicate in the same position was measured by a chlorophyll meter (SPAD-502, Minolta Co., Ltd., Japan).

At harvest, ten randomly sampled bunches per replicate were weighed to calculate the average bunch weight, and the number of bunches was counted on ten vines for each replicate. The yield at harvest was monitored by weighing vine bunch weight. During winter pruning, canes from five vines per replicate were pruned and weighed. The third internode from the base of each of ten canes per replicate was cut off to determine soluble sugar, starch and protein content. Soluble sugar and starch were determined according to the colourimetric method (Palliotti *et al.,* 2013) using an anthrone reagent. Protein was determined according to Bradford (1976) by using Coomassie brilliant blue.

5. Small-scale fermentation

Bunches were randomly and manually harvested from 30 vines for each replicate and then transported to the laboratory. For each replicate, 20 kg of bunches were manually crushed and then transferred to 20-L stainless steel containers, and 0.8 g of SO_2 was added to the must at the same time. Then 0.4 g of pectinase (Optivin®, Australia) and 4 g of commercial yeast (Lallemand, French) were added to the must. Alcohol fermentation was conducted in a temperature-controlled room at about 24 °C, and the skins were punched down twice a day. When the reduced sugar had reached below 4 g/L, the wines were separated from the pulp, and 0.1 g lactobacillus (Lalvin 31, Lallemand Inc, France) was added. When malolactic fermentation had ended, 1.2 g of SO_2 was added to the wine, and wine samples were bottled and stored for one month at 10-15 °C before analysis.

6. Analysis of grape and wine physicochemical parameters

One hundred berries were manually squeezed, and then the must was centrifuged at 6000 × *g* for 5 min. Total soluble solids were determined using a portable refractometer (PAL-1, Atago, Japan). The juice pH was determined using a pH meter (Sartorius PB-10, Germany). Titratable acidity (TiA) was analysed by titrating the grape juice (pH 8.2) using NaOH, and it was expressed as gram tartaric acid equivalents per litre of juice. Berry skins and seeds were separated and weighed using an analytical balance (FA2004, Sunny Hengping Instrument, China; minimum of 0.001 g).

Wine pH was determined using a pH meter (Sartorius PB-10, Germany). Wine total acidity (TA) was analysed in the same way as titratable acid in grape juice. Before analysis, carbon dioxide was extracted using a degasser. The residual sugar, volatile acidity, and ethanol content of the wine were determined according to OIV (2014). CIELAB formulae were used to determine wine colour parameters: lightness (*L*), colour intensity (*C*), red hue (*a*), and yellow hue (*b*), as described in Ayala *et al.* (1997).

7. Extraction of phenolic compounds in berry skins and seeds

Grape skins were separated from the pulp when grapes were still frozen and then ground into a powder in liquid nitrogen. Afterwards, ground skin powder was freeze-dried at -40 °C. The extraction of anthocyanins and flavonols in skins was carried out according to Downey *et al.* (2007): 0.1000 ± 0.0002 g of dried skin powder was precisely weighed and put into a 2.5 mL centrifuge tube and 1 mL of a 50 % methanol aqueous solution was added. The extraction was sonicated for 20 min and centrifuged for 10 min at 8000 × *g*. The supernatant was collected, and the residue was extracted twice using the same protocols.

The extraction of flavan-3-ols in skins and seeds was carried out according to Liang *et al.* (2012). For free flavan-3-ols, 0.1000 ± 0.0002 g of dried skin or seed powder was precisely weighed and put into a 5 mL centrifuge tube with the addition of 1 mL of 70 % aqueous acetone solution (containing 0.5 % ascorbic acid).

The extract was centrifuged for 15 min at 8000 × *g* at 4 °C. The supernatant was collected, and the residue was extracted three times following the same protocols. Then, 400 µL of the above extract was put into a 1.5 mL centrifuge tube and dried rapidly with a dry nitrogen stream. The dried extract was dissolved in 200 µL of methanol solution containing 1 % HCL and then neutralised with 200 µL of sodium acetate aqueous solution (200 mM). For various flavan-3-ol units, 0.1000 ± 0.0002 g of dried skin or seed powder was precisely weighed and put into a 10 mL centrifuge tube, mixed with 1 mL of phloroglucinol buffer (0.5 % ascorbate, 300 mM HCl, and 50 g/L phloroglucinol in methanol). After incubating at 50 °C for 20 min, 1 mL of sodium acetate (200 mM) was added to stop the reaction. The extract was centrifuged for 15 min at 8000 × *g* at 4 °C. The supernatant was collected, and the residue was extracted three times following the same protocols. Proanthocyanins were calculated by subtracting the free flavan-3-ols from the total flavan-3-ol units concentration.

8. HPLC-MS analysis of phenolic compounds in berries and wines

Phenolic compounds of berries and wines were determined by high-performance liquid chromatography/triple-quadrupole tandem mass spectrometry (HPLC-QqQ-MS/MS). An Agilent 1200 series HPLC equipped with a Poroshell 120 EC-C_{18} column (150 × 2.1 mm, 2.7 µm), coupled with an Agilent 6410 triple-quadrupole (QqQ) instrument, was used. Anthocyanins and non-anthocyanin phenols were analysed as described by Li *et al.* (2016). The methods of free flavan-3-ols and proanthocyanidins were analysed as described by Liang *et al.* (2012). The concentrations of phenolic compounds were expressed as mg/kg of fresh berry weight (FW) in grapes and as mg/L in wines.

9. Extraction of berry volatile compounds

About 70 g berries were mashed under liquid nitrogen with seeds removed and mixed with 1 g of polyvinylpolypyrrolidone and 0.5 g of D-gluconic acid lactone. After melting at 4 °C for 4 h, clear juice was obtained through centrifuging at 8000 × *g* for 15 min. For free volatile compounds, 5 mL of grape juice was added to a 20 mL vial containing a magnetic stirrer, 10 µL of internal standard (4-methyl-2-pentanol) and 1 g NaCl. For bound volatile compounds, 2 mL of the clear grape juice sample was added to Cleanert® PEP-SPE resins which had been activated with 10 mL of methanol and 10 mL of water, and the resins were eluted by methanol afterwards. The methanolic substance was placed under vacuum at 30 °C and evaporated to dryness. Ten mL of citric acid/phosphate buffer solution (0.2 M, pH = 2.5) was added to re-dissolve the dried substance. After hydrolysing under 100 °C for 1 h in citric acid, the procedure was the same as the analysis for free volatile compounds.

10. GC-MS analysis of volatile compounds in grapes and wines

Volatile compounds from grape and wine samples were extracted by headspace solid-phase microextraction (HS-SPME) and analysed by gas chromatography-mass spectrometry (GC-MS) as described by Wen *et al.* (2014). Agilent 6890 GC coupled with Agilent 5973C MS. GC was equipped with an HP-INNOWAX capillary column (60 m × 0.25 mm, 0.25 µm, J and W Scientific, Folsom, CA, USA) and used to separate volatile compounds. Qualitative and quantitative methods were used as described by Wang *et al.* (2019). The concentrations of volatile compounds were expressed as µg/L in wines and µg/kg of fresh berry weight in grapes.

11. Statistical analysis

All the data were from three biological replicates except for the microclimate data. SPSS version 22.0 for Windows was used for all significance analyses at $p < 0.05$ (*t*-test). The figures were prepared using the Origin 8.0 software and R version 3.6.1. Heatmap was prepared using the 'complexheatmap' package in R.

RESULTS

1. Weather condition and microclimate changes

As shown in Figure 2, the 2018 vintage had high temperatures and few rainfalls in the summer, which is typical of the semi-arid continental climate in Xinjiang. The monthly sunshine duration and air temperature were almost within the range of that between 2009 and 2019. In 2018, there were 20 high-temperature days (Tmax > 35 °C) from anthesis to harvest. There were three heatwaves, the longest lasting 7 days. However, there was only 38.7 mm of rainfall in total from anthesis to harvest.

After applying ST, the daily average temperature around the bunch zone decreased (Figure 3a). The growing degree days around UC and ST bunches

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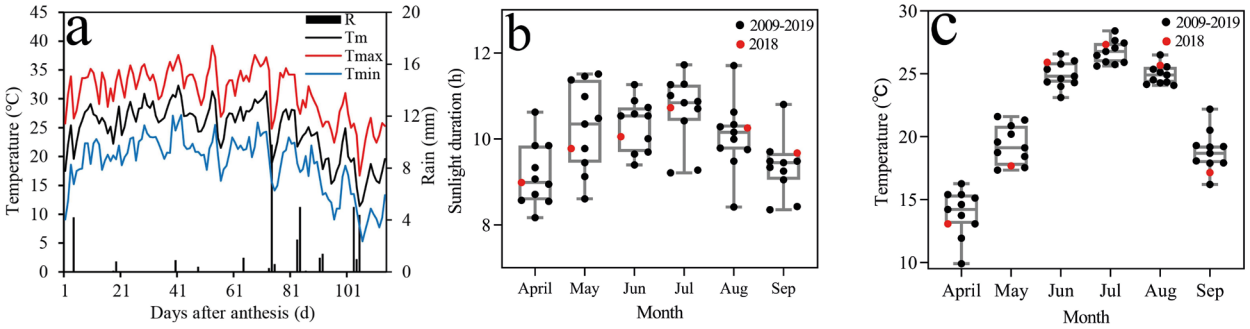


FIGURE 2. a) Daily average (Tm), maximum (Tmax) and minimum (Tmin) temperature and rainfall (R) in the 2018 growing season, b) average monthly sunlight duration, and c) temperature from Apr to Sept in experimental vineyards compared to 2009-2019.

Boxplot with data points represents the distribution of monthly sunlight duration and temperature in 2009-2019.

during the 83 data collection days were 1058.2 °C and 974.6 °C respectively. ST reduced the daily average temperature by about 1 °C and the daily average solar radiation by about 74.6 % compared to UC (Figure 3a). Figure 3b clearly shows that in one whole day during the veraison period (2 August), there was a large difference in the bunch-zone temperature (of approximately 6 °C) between ST and UC at noon. Solar radiation also decreased in ST as expected: the highest solar radiation reached about 375 W/m^2 in UC, while it reached only 100 W/m^2 in ST.

2. Effects of partial canopy shade treatment on vine gas exchange

ST reduced solar radiation significantly and led to a lower A_N of shaded leaves than the UC vines in the same position (Figure 4a). In the morning, there could be a difference of up to 9 µmol/m^2/s, which gradually decreased until at 19:00 there was no significant difference between UC and ST because of sunset. The same trend occurred for other measurements in the growing season (Figure 4b). ST had little influence on the E and g_s of the 6th-8th node leaves. It is worth noting that C_i was significantly increased by ST (Figure 4a and Figure 4b). We also determined the A_N of exposed leaves in the upper canopy; there was no significant difference in A_N between UC and ST in four out of five of the measurements, as shown in Figure 4c. This indicates that unshaded leaves do not adjust to shade stress to compensate for the loss of photosynthesis capacity of the shaded leaves. Although ST resulted in higher E and g_s than UC in two out of five of the upper canopy measurements, it cannot be confirmed whether ST vines would also adjust for these parameters.

3. Effects of partial canopy shade treatment on grapevine phenological stages and vegetative parameters

As shown in Supplementary Table 1, the onset of veraison, the end of veraison, and harvest date were delayed by 5, 9, and 15 days respectively by ST. Moreover, ST increased veraison duration by 4 days.

A decrease in yield and bunch weight was observed in ST (Table 1). Compared to UC, ST resulted in an 11.1 % reduction in yield and a 10.6 % reduction in bunch weight. This indicates that biomass reduction is associated with a decrease in photosynthesis capacity caused by ST. Furthermore, starch content in canes also decreased by 16.4 % in ST compared to UC. Neither the main shoot leaf area nor the lateral shoot leaf area was affected by ST. Therefore, the leaf area/yield was significantly increased by ST. ST did not influence average shoot length, the third internode diameter or pruning weight. ST reduced crop load as a result of reduction in yield also caused by ST. ST significantly increased the SPAD value of the 6th-8th node leaves under the shade cloth, which is in agreement with previous studies (Caravia *et al.,* 2016; Cartechini and Palliotti, 1995). Grape seed number, seed weight and skin weight were not affected by ST.

4. Effects of partial canopy shade treatment on the physicochemical composition of berries

The changes in berry TSS, TiA, pH, and berry weight during the developmental stage are shown in Figure 5. A noticeable difference was observed in the ripening process between UC and ST. ST significantly decreased berry TSS and weight from the second sampling time. The maximum difference in TSS between UC and ST was 3 °Brix

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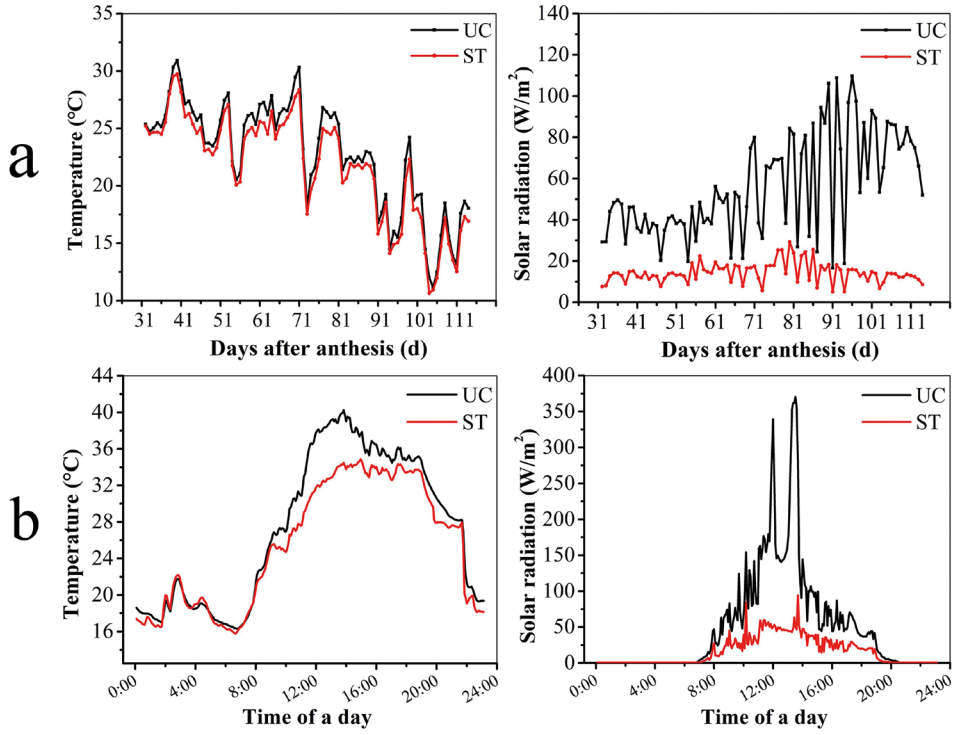


FIGURE 3. Effect of partial canopy shade on microclimate around the bunches.

a) daily average temperature and solar radiation in untreated control (UC), and canopy shade treatment (ST) of Cabernet-Sauvignon, and b) changes in temperature and solar radiation in one day in untreated control (UC) and canopy shade treatment (ST) of Cabernet-Sauvignon.

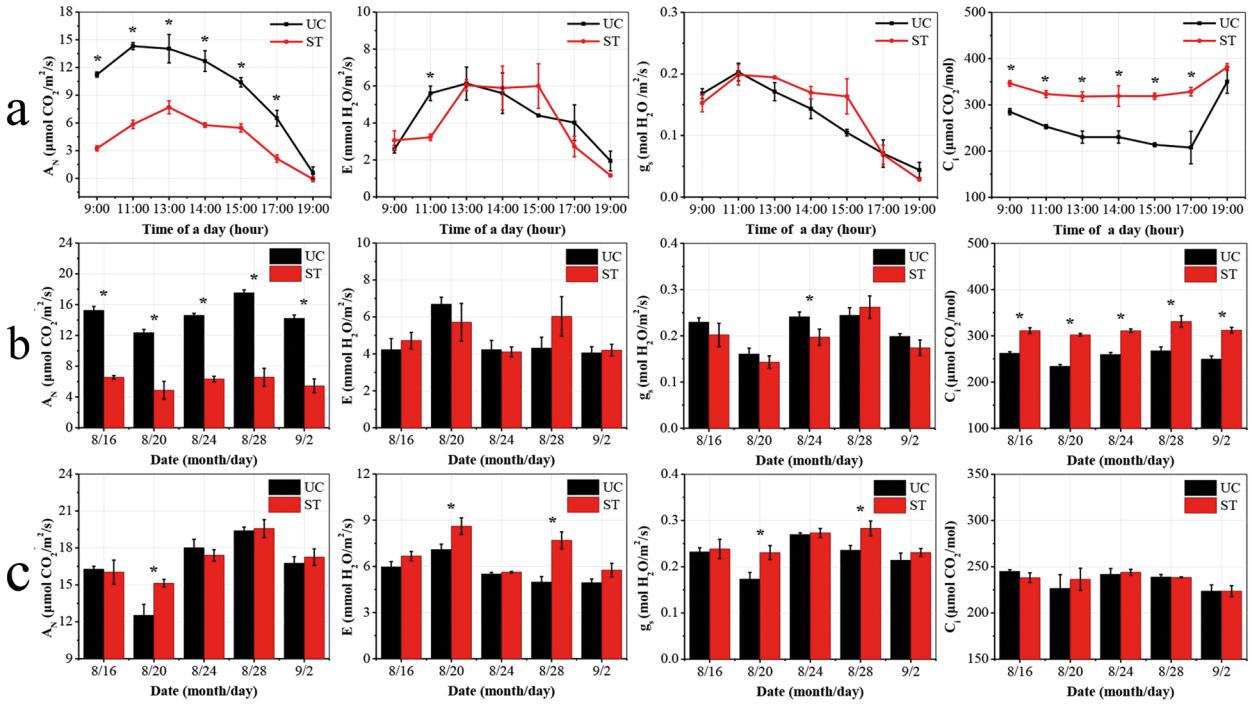


FIGURE 4. Net assimilation rate (A_N), transpiration rate (E), stomatal conductance (g_s), and internal CO_2 concentration (C_i) of leaves in untreated control (UC) and canopy shade treatment (ST) of Cabernet-Sauvignon.

a) Diurnal variation of A_N, E, g_s and C_i, b) A_N, E, g_s and C_i of 6th-8th node leaves on different days, and c) A_N, E, g_s and C_i of upper leaves outside shade film on different days. * indicates significant differences between UC and ST ($p < 0.05$, *t*-test).

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TABLE 1. Vine parameters of untreated control (UC) and shade treatment (ST) of Cabernet-Sauvignon.

Parameter	Treatment		Significance
	UC	ST	
Total shoot leaf area/shoot (m^2)	0.33 ± 0.06	0.34 ± 0.09	*NS*
Main shoot leaf area/shoot (m^2)	0.20 ± 0.04	0.20 ± 0.06	*NS*
Lateral shoot leaf area/shoot (m^2)	0.13 ± 0.01	0.14 ± 0.05	*NS*
Yield/vine (kg)	3.71 ± 0.13	3.30 ± 0.18	*
Leaf area/yield (m^2/kg)	1.85 ± 0.44	2.24 ± 0.53	*
Shoot number/m (row)	21 ± 3	22 ± 3	*NS*
Average shoot length (cm)	120.8 ± 10.1	120.5 ± 14.1	*NS*
Third internode diameter (mm)	8.63 ± 1.38	8.14 ± 1.24	*NS*
Pruning weight/vine (kg)	1.22±0.12	1.22±0.06	*NS*
Yield/pruning weight (kg/kg)	3.04 ± 0.11	2.70 ± 0.15	*
SPAD value	41.3 ± 3.9	50.9 ± 6.6	*
Cane soluble sugar (mg/g DW)	30.0 ± 0.7	30.1 ± 2.1	*NS*
Cane starch (mg/g DW)	158.3 ± 8.4	132.4 ± 7.8	*
Cane soluble protein (mg/g DW)	146.7 ± 2.3	145.2 ± 1.3	*NS*
Bunch weight (g)	110.3 ± 9.0	98.7 ± 6.0	*
Seed number/berry	1.4 ± 0.5	1.4 ± 0.0	*NS*
Seed weight/berry (g)	0.04 ± 0.00	0.04 ± 0.00	*NS*
Skin weight/berry (g)	0.14 ± 0.00	0.13 ± 0.00	*NS*

Values are reported as means ± SD of three biological replicates. * indicates there are significant differences between UC and ST ($p < 0.05$, *t*-test). NS = not significant. DW = dry weight.

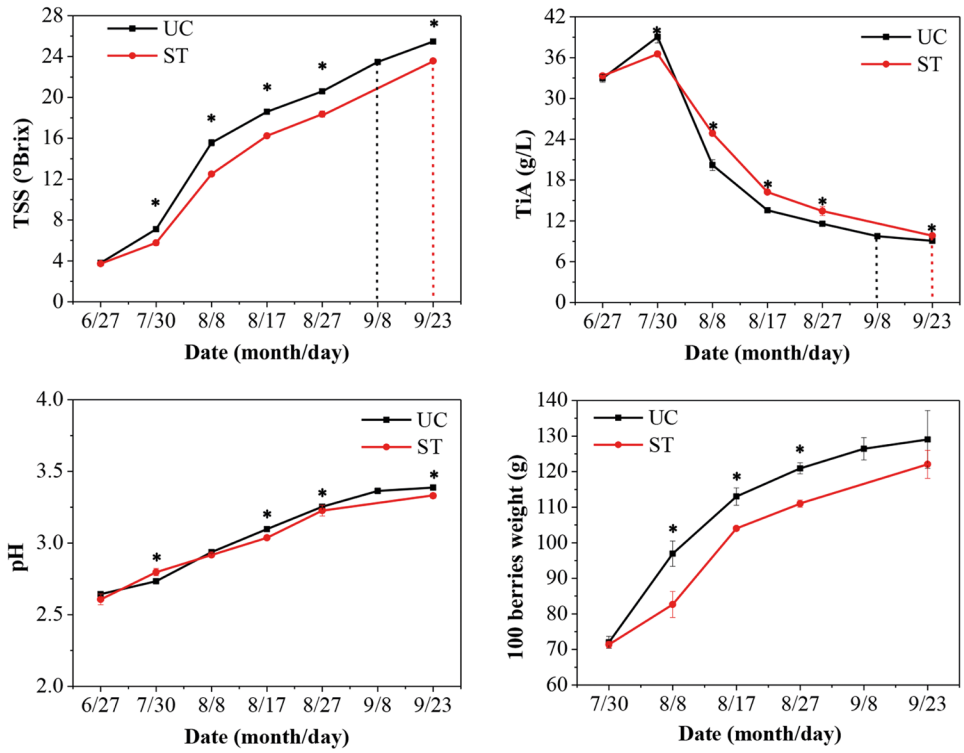


FIGURE 5. Berry physicochemical parameters of untreated control (UC) and canopy shade treatment (ST) of Cabernet-Sauvignon.

* indicates the significant difference between UC and ST ($p < 0.05$, *t*-test).

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on 8 August when UC berries had almost reached 100 % of their colour, while ST berries were at mid-veraison stage (at approximately 50 % of their colour). UC was harvested when the berry TSS had reached 23.5 °Brix, while ST berries only reached about 2 °Brix. ST was harvested 15 days later when the berry TSS had reached 23.6 °Brix, and unharvested UC berry TSS was as high as 25.5 °Brix. There was no significant difference between the two treatments in berry TSS at their respective harvest times. ST had lower berry weight than UC, although there was no significant difference in those from the last sampling stage. The maximum difference between ST and UC in terms of the weight of 100 berries on 8 August was 14.3 g. ST berries had higher TiA and lower pH than UC berries. Although there was a significant difference between the two treatments in TiA in the last sampling stage, it was mitigated compared with those in other sampling stages.

5. Effects of partial canopy shade treatment on berry flavonoids

ST influenced both anthocyanins and flavonols in grapes, but had little influence on flavan-3-ols (Table 2). ST increased the total concentration of anthocyanins in berries by 11.2 %. Five groups of anthocyanins were detected in berries, and ST increased four of them. ΣMv comprised the largest proportion of total concentration of anthocyanins, with no significant difference between UC and ST. ΣCy and ΣDp were influenced by ST with an increase of 61.4 and 33.1 % respectively. The concentrations of 3′- and 3′5′-hydroxylated anthocyanins were both increased by ST. However, due to the reduced ST berry weight and smaller ST berry volume, the anthocyanin content of each berry is also shown in Table 2. The results show that only ΣCy and ΣDp content were significantly increased by ST and there was no significant difference in other anthocyanin and total anthocyanin content between ST and UC.

TABLE 2. Concentration and content of grape phenolic compounds of untreated control (UC) and shade treatment (ST) of Cabernet-Sauvignon.

Compounds		Concentration (mg/kg FW)		Sig.	Content (µg/berry)		Sig.
		UC	ST		UC	ST	
Anthocyanins	ΣCy	15.6 ± 3.1	25.1 ± 0.3	*	19.3 ± 3.4	27.6 ± 0.8	*
	ΣDp	147.0 ± 12.3	195.7 ± 4.2	*	182.3 ± 11.8	215.3 ± 10.0	*
	ΣMv	495.3 ± 12.9	507.0 ± 11.2	*NS*	614.6 ± 3.1	557.6 ± 26.7	*NS*
	ΣPe	43.1 ± 2.3	48.0 ± 1.2	*	53.4 ± 2.0	52.8 ± 2.7	*NS*
	ΣPt	104.3 ± 6.6	119.9 ± 3.6	*	129.3 ± 5.9	131.9 ± 8.1	*NS*
	Σ3'H	58.6 ± 5.4	73.1 ± 1.4	*	72.7 ± 5.3	80.4 ± 3.5	*NS*
	Σ3'5'H	746.6 ± 30.6	822.6 ± 18.0	*	926.3 ± 19.5	904.8 ± 44.4	*NS*
	Total anthocyanins	805.3 ± 35.9	895.7 ± 19.3	*	999.0 ± 24.8	985.2 ± 47.9	*NS*
Flavonols	ΣKa	4.0 ± 0.5	0.5 ± 0.0	*	4.8 ± 0.5	0.6 ± 0.0	*
	ΣQu	27.3 ± 2.7	14.6 ± 0.7	*	33.0 ± 4.4	15.8 ± 0.4	*
	ΣMy	12.5 ± 1.3	8.1 ± 0.3	*	15.1 ± 2.2	8.8 ± 0.2	*
	ΣIs	1.7 ± 0.2	*ND*	-	2.0 ± 0.2	*ND*	-
	ΣSy	1.5 ± 0.2	0.1 ± 0.2	*	1.8 ± 0.3	0.1 ± 0.2	*
	Total flavonols	47.2 ± 4.1	23.6 ± 0.8	*	57.0 ± 6.9	25.5 ± 0.3	*
Flavan-3-ols	Terminal subunits	191.8±14.6	217.5±18.5	*NS*	237.9±13.1	238.9±16.7	*NS*
	Extension subunits	6,064.4 ± 103.9	6407.0 ± 415.9	*NS*	7,303.7±246.1	6,928.8±358.8	*NS*
	Free flavan-3-ols	229.7 ± 22.4	203.2 ± 18.9	*NS*	285.3±30.9	223.0±13.7	*
	PAs	6,256.2 ± 98.2	6,624.6 ± 433.2	*NS*	7,766.7 ± 247.2	7,275.2 ± 304.2	*NS*
	mdp	32.8 ± 2.7	30.5 ± 1.0	*NS*	32.8 ± 2.7	30.5 ± 1.0	*NS*

∑ = total concentration or content of different groups of anthocyanins or flavonols, Cy = cyanidin, Dp = delphinidin, Mv = malvidin, Pn = peonidin; Pt = petunidin, Ka = kaempferol, Qu = quercetin, My = myricetin, Is = isohamnetin, Sy = syringetin, mdp = mean degree of polymerisation. Values are reported as means ± SD of three biological replicates. ND = not detected. * indicates significant differences between UC and ST ($p < 0.05$, *t*-test). *NS* = not significant.

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Five flavonols molecules were detected in UC, while only four were detected in ST. ST significantly decreased all flavonol concentration and content. ST did not affect the concentration of flavan-3-ols. In addition, mean degree polymerisation (mDP) was not influenced by ST. However, ST significantly decreased the free flavan-3-ol content.

6. Effects of partial canopy shade treatment on berry volatile compounds

There were 87 aroma compounds detected in the berries from both treatments, including 58 free volatile compounds and 29 bound volatile compounds, as shown in Supplementary Tables 2 and 3. The compounds have all been grouped into nine categories according to their structure. Aroma profiles are presented as a fold change between the two treatments, and each category of UC concentration is defined as 1 (Figure 6). Only two categories of volatile compounds were significantly affected by ST. ST increased C_6/C_9 compound concentrations and fatty acids. C_6/C_9 compounds are called green leaf volatiles (GLVs) because of their fresh grass and crushed leaf aroma, leaving the sensory impression that the red wine is immature. ST significantly increased concentrations of hexanal, 1-hexanol, (*E*)-2-hexen-1-ol and (*Z*)-2-hexen-1-ol, as shown in Figure 6a. Fatty acids are critical intermediary metabolites of the flavour and aroma compounds. ST significantly increased concentrations of hexanoic acid and (*E*)-2-hexanoic acid and *n*-decanoic acid.

7. Effects of partial canopy shade treatment on wine physicochemical composition and phenol compounds

Must physicochemical parameters are shown in Table 3. In terms of TSS, pH, and TiA, there were no significant differences between UC and ST must. The wine alcohol content was lower in ST than in UC, which might be due to the lower TSS in ST must, although there was no significant difference in TSS between ST and UC. Residual sugar in UC and ST wines both reached below 4 g/L, indicating that both wines met the standards of dry red wine. ST wines had lower TA and higher pH than UC wines.

The total concentrations of anthocyanins in ST wines increased by 11.1 % compared to those in UC wines, which is similar to the must results. ST negatively influenced flavonols with almost a 50 % reduction, which was also similar to the berry results. Flavan-3-ols were significantly decreased by ST, which was different from the berry results. Phenolic acids were not affected by ST. ST wines had higher lightness (*L*), lower chromaticity (*C*), lower red colour component (*a*), and higher yellow component (*b*) than UC wines.

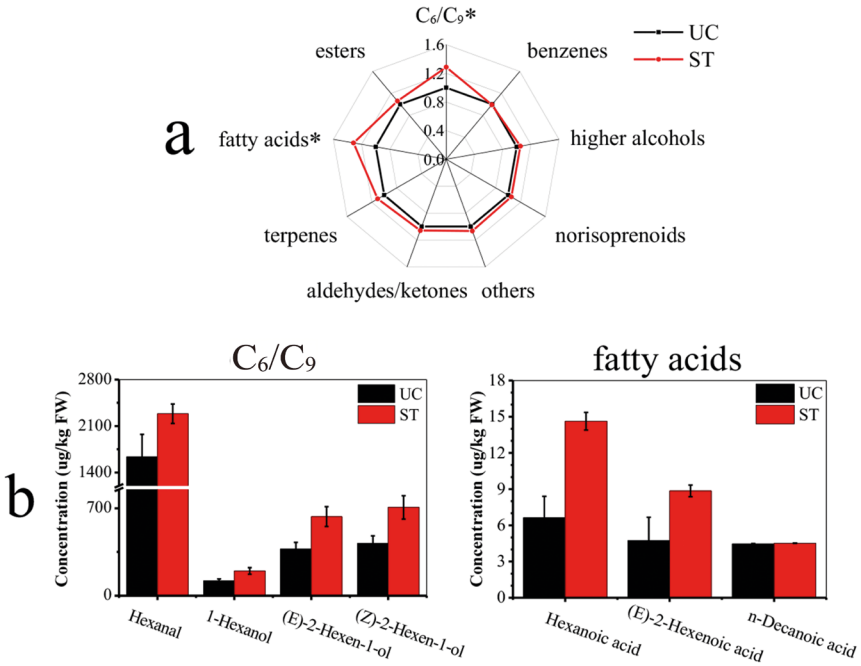


FIGURE 6. a) Aroma profiles, and b) main differential compounds of untreated control (UC) and canopy shade treatment (ST) berries.

* indicates significant differences between UC and ST ($p < 0.05$, *t*-test).

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TABLE 3. Must and wine parameters of untreated control (UC) and shade treatment (ST) of Cabernet-Sauvignon.

Fermentation stage	Parameter	Treatment		Significance
		UC	ST	
Must	TSS (°Brix)	23.3 ± 0.4	22.7 ± 0.2	*NS*
	TiA (g/L)	10.0 ± 0.4	10.4 ± 0.5	*NS*
	pH	3.33 ± 0.04	3.36 ± 0.03	*NS*
Wine	Alcohol degree (%, v/v)	12.3 ± 0.2	11.9 ± 0.1	*
	Residual sugar (g/L)	2.1 ± 0.0	1.9 ± 0.1	*
	TA (g/L)	5.2 ± 0.1	4.8 ± 0.1	*
	pH	3.89 ± 0.06	4.01 ± 0.01	*
	Volatile acid (g/L)	0.50 ± 0.05	0.48 ± 0.05	*NS*
	Anthocyanins (mg/L)	393.5 ± 16.9	437.1 ± 3.7	*
	Flavonols (mg/L)	19.8 ± 0.2	9.4 ± 0.1	*
	Flavan-3-ols (mg/L)	91.4 ± 1.0	46.6 ± 0.2	*
	Phenolic acids (mg/L)	19.0 ± 0.8	18.0 ± 0.2	*NS*
	L	63.9 ± 0.7	80.1 ± 1.3	*
	C	36.3 ± 0.4	18.9 ± 0.8	*
	a	36.0 ± 0.3	17.7 ± 0.7	*
	b	4.8 ± 0.2	6.4 ± 0.4	*

Values are reported as means ± SD of three biological replicates, * indicates significant differences between UC and ST ($p < 0.05$, *t*-test). *NS* = not significant.

8. Effects of partial canopy shade treatment on wine volatile compounds

Fifty-four aroma compounds were detected in the wines from both treatments (Supplementary Table 4). The compounds have all been grouped into nine categories according to the structure. Aroma profiles are presented as a fold change between the two treatments, and each category of UC concentration is defined as 1 (Figure 7a). ST significantly increased concentrations of norisoprenoids, ethyl esters, acetate esters and fatty acids, but it significantly decreased concentrations of benzenes, higher alcohols and other esters. The odour activity value of each volatile compound was calculated as the ratios of the concentration of an individual compound and its corresponding perception threshold (Cai *et al.,* 2014). The active aroma compounds were classed into seven groups according to their odour descriptors. ST significantly increased the intensities of most aroma series except the herbaceous aroma, which was mainly from C_6 alcohols (Figure 7b). Floral, fruity, caramel and roasted aroma intensities were increased by ST, which was beneficial for the wine sensory profile.

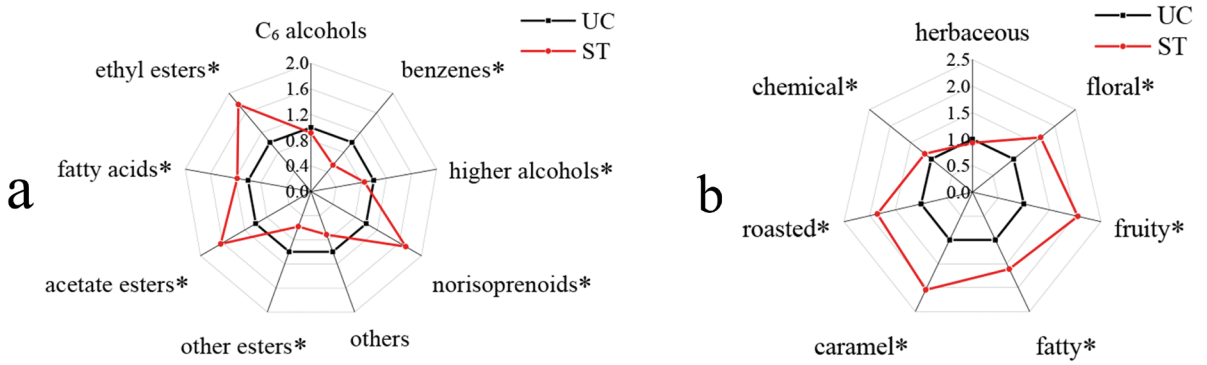


FIGURE 7. a) Aroma profiles, and b) odour activity values in wines from UC and ST.
* indicates significant differences between UC and ST ($p < 0.05$, *t*-test).

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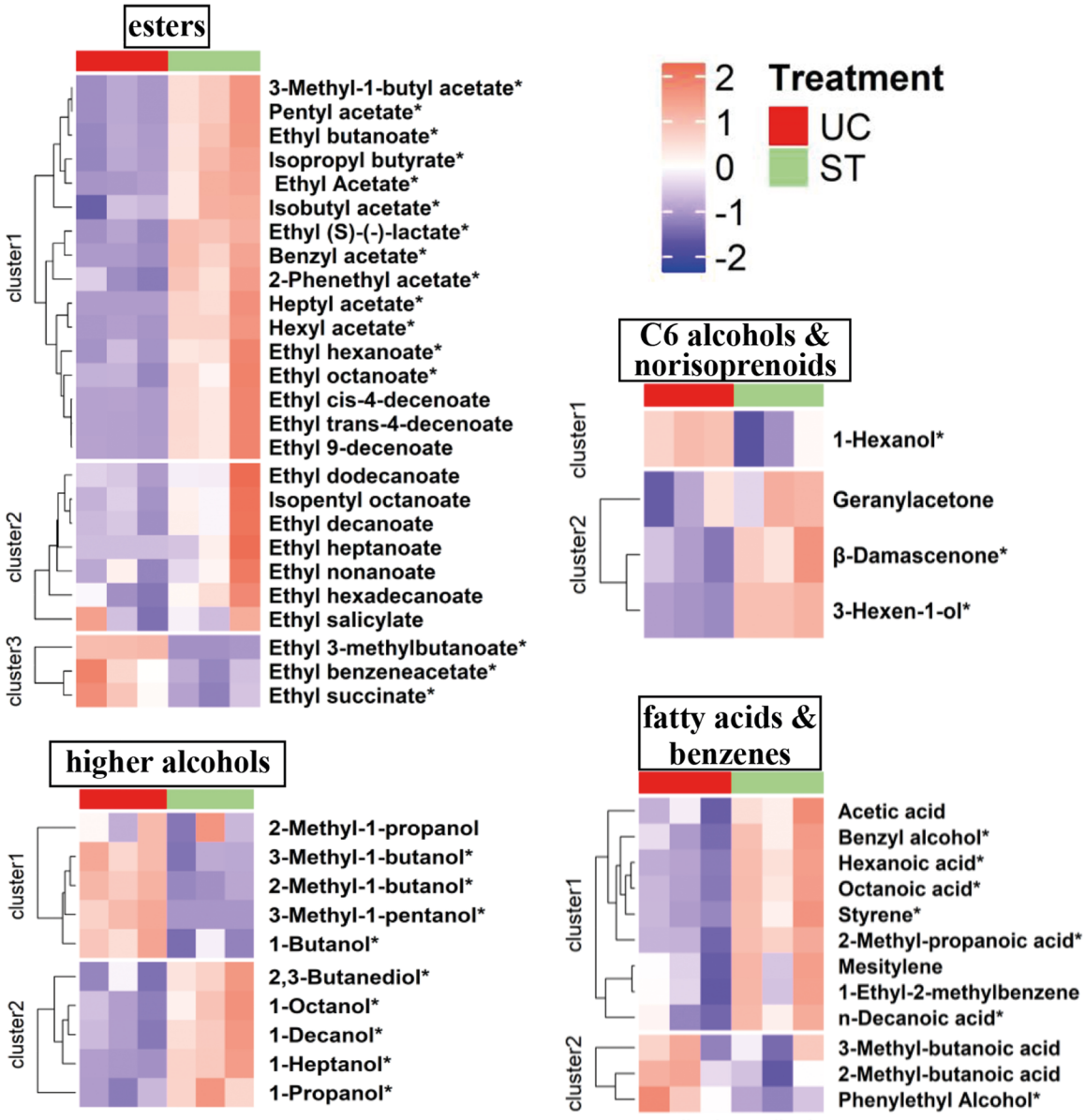


FIGURE 8. Wine aroma compounds of untreated control (UC) and canopy shade treatment (ST) of Cabernet-Sauvignon.

* indicates significant differences between UC and ST ($p < 0.05$, *t*-test).

The main aroma compounds are shown in Figure 8, in which heatmaps represent clustering results. ST increased concentrations of most ester compounds, especially in cluster 1. ST only significantly decreased ethyl succinate, ethyl benzene acetate and ethyl 3-methylbutantoate. Esters with high odour activity values, such as 3-methyl-1-butyl acetate, ethyl hexanoate, ethyl octanoate and ethyl acetate, contributed to the fruity aroma and were significantly increased by ST. Moreover, 3-methyl-1-butyl acetate, ethyl octanoate and ethyl acetate contributed to the caramel, fruity and chemical aromas respectively. For higher alcohols, canopy shading reduced the concentration of 3-methyl-1-butanol, which had a high odour activity value and contributed to the chemical, caramel and fatty aromas. Norisoprenoids were derived from grapes and greatly contributed to the "variety aroma" of wines because of their low thresholds and high odour activity values. ST significantly increased the concentration of *β*-damascenone, which contributed to the floral, fruity, and caramel aroma. Most fatty acids were increased by canopy shading, leading to a higher fatty aroma intensity in ST wines than in UC wines. Overall, there was a significant improvement in the aroma of ST wines.

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DISCUSSION

High quality wine is obtained from high quality and fully ripe grapes. If grapes ripen too early, they can have high sugar levels and low acidity, which will lead to excessive alcohol and lack of freshness in the resulting wines. Late ripening can result in unripe grapes and wines marked by green flavours (Parker *et al.,* 2020). We did not harvest on the same day, because the development stages of the two treatments were significantly different. When the UC grapes were ready for harvest, the ST grapes were still unripe. Therefore, ST and UC bunches were harvested for winemaking when they had reached a similar maturity, rather than on the same day. Early phenological events have been observed in a previous study in which harvest dates were found to be, on average, 17 days earlier than 50 years ago in eleven out of twelve locations in Europe (Jones *et al.,* 2005). Moreover, the harvest date of grapevines in Bordeaux was 13 days earlier in 1997 than in 1952 (Jones and Davis, 2000). In the current study, ST delayed the harvest date by 15 days; this treatment could thus compensate for early phenological events in the future.

Unlike most previous studies in which the treatment is mild, we excluded almost 75 % of solar radiation in the bunch zone and half of the canopy, resulting in a large difference in microclimate between the untreated and ST vines. The effects were significant in vine growth, gas exchanges, grape and wine metabolites changes. ST reduced the average daily temperature by about 1 °C and midday temperature by about 6 °C, which is in agreement with a previous study in which the same reduction in the temperature at midday was caused by canopy shade (Rana *et al.,* 2004). Canopy shade also reduced the number of high-temperature days compared to the control, which significantly protected vines from heat stress.

In the present study, half of the canopy leaves had lower A_N in ST than in UC. Lower carbon assimilation resulted in reduced biomass accumulation and yield, which is in agreement with previous studies (Greer *et al.,* 2011; Cartechini and Palliotti, 1995). ST significantly increased leaf C_i, which has been found to be negatively correlated with photosynthesis (Greer, 2012). Little response to the A_N loss was observed in upper exposed leaves, because no compensation occurred. However, the SPAD value, which was collinearly correlated with leaf chlorophyll content (Yadava, 1986), significantly increased in shaded leaves. When PAR decreases in leaves, chlorophyll *b* content increases and the chlorophyll *a*/*b* ratio decreases. The increase in chlorophyll *b* content under low light is due to the leaf ability to physiologically adapt to and make full use of low light (Song and Li, 2016). Starch storage in canes was significantly decreased by ST, indicating the intensity of the treatment in our study. Some relatively less intensive techniques for delaying ripening, such as leaf removal or antitranspirant application, had limited influence on soluble sugar or starch storage (Palliotti *et al.,* 2013; Palliotti *et al.,* 2014).

ST significantly decreased berry TSS due to lower A_N in ST than in UC, which is in agreement with previous studies (Greer *et al.,* 2010; Greer *et al.,* 2011; Greer and Weedon, 2012; Caravia *et al.,* 2016). Berry TiA was lower in UC compared to ST at the development stage; the same result was found by Martínez-Lüscher *et al.* (2016). UC bunches suffered more high-temperature stress than ST bunches. Many studies have reported lower TiA and malate concentrations in grapes in response to elevated temperature (Bergqvist *et al.,* 2001; Lakso and Kliewer, 1975; Martínez-Lüscher *et al.,* 2016; Sadras *et al.,* 2013). An increased breakdown and decreased replenishing of the tricarboxylic cycle intermediates was the immediate cause (Sweetman *et al.,* 2014). In our study, although the harvest date was delayed by 15 days in ST, the wine alcohol content did not increase and it was even lower than the control. ST therefore proves effective in overcoming the problem of high alcohol levels caused by global warming.

The increase in anthocyanin concentration in ST berries might be a result of decreased berry weight, because there was no significant difference between ST and UC in the anthocyanin content of each berry. Although the general view is that increased exposure will result in enhanced anthocyanin biosynthesis, there is a point at which the temperature load will begin to have a negative impact (Joscelyne *et al.,* 2007). In our study, while decreased solar radiation was an unfavourable factor for anthocyanin biosynthesis, a little high-temperature stress in ST bunches was beneficial for anthocyanin biosynthesis; the combined effects resulted in no significant difference in the anthocyanin content of each berry. However, a decrease in flavonols was observed in both berries and wines from ST. It was well known that the primary function of flavonols is to serve as a UV filter, because they absorb light in the 280-330 nm range to protect plant tissues from UV damage (Flint *et al.,* 1985; Price *et al.,* 1995).

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A high concentration of flavonols, such as quercetin-3-glucoside, has been found in bunches that received a high amount of light (Haselgrove *et al.,* 2008). In our study, as well as quercetin other flavonols were decreased by ST, especially kaempferol. ST led to a 46 % reduction in quercetin concentration in grapes compared to UC and kaempferol was reduced by 87 %. Martínez-Lüscher *et al.* (2019) found that there was a strong linear correlation between percentage of kaempferol and modelled global radiation, which is in agreement with our results. The shaded and control berries had similar proanthocyanidin levels at the harvest stage. The influence of shading on tannin levels might be cultivar dependent. No differences in tannin levels have been found between the treatments applied to Shiraz, whereas in Pinot Noir, tannin levels were found to be lower in the shaded fruit (Downey *et al.,* 2004; Cortell and Kennedy, 2006). Lower chromaticity occurred in ST wines; this might due to lower concentrations of flavonols and flavan-3-ols in ST than in UC, since these compounds are essential for the copigmentation of anthocyanins and can improve colour and stability (Ghasemifar and Saeidian, 2014; García-Marino *et al.,* 2013). Furthermore, higher pH in ST wine also negatively influenced its colour, because a rise in pH causes the concentration of anthocyanins in the flavylium state and the colour density to decline rapidly (He *et al.,* 2012).

A significant improvement in wine aromas was observed in ST, especially those linked to esters and norisoprenoids. However, in berries, C_6/C_9 and fatty acids compounds were most influenced by ST. The increase in berry C_6/C_9 and fatty acid compounds may be the cause of higher esters in ST wines; *Saccharomyces cerevisiae* may have used C_6/C_9 and fatty acids to form esters during the fermentation stage (Keyzers and Boss, 2010; Duan *et al.,* 2015), thereby improving the wine's fruity aroma. Norisoprenoids belong to grape-derived compounds and they contribute to varietal characteristics. Previous studies have demonstrated that the accumulation of norisoprenoids can be increased by light exposure (Young *et al.,* 2016; Asproudi *et al.,* 2016). Isoprene metabolic pathways have been reported to decrease in harvested bunches that have been shaded (Ma *et al.,* 2020). However, in our study, ST had no significant influence on the bound forms of norisoprenoids and terpenoids. This might be because ST vines suffered less high-temperature stress than UC vines. Lecourieux *et al.* (2017) reported that heat treatment repressed the expression of genes which encode key enzymes in carotenoid metabolism. There are no consistent results for the response of *β*-damascenone to light. Lee *et al.* (2007) founded that *β*-damascenone concentrations were highest in shaded treatments. However, Feng *et al.* (2015) founded that *β*-damascenone was positively correlated to grape bunch sunlight exposure. In our study, ST significantly increased *β*-damascenone concentration in wines, but did not affect concentrations in the berries. Wine fermentation is a complicated process during which aroma compounds experience great changes, resulting in a variation between berries and wines.

CONCLUSION

Partial canopy shade is an important horticultural technique for postponing vine phenology and protecting vines from high temperature stress. In our study, it was found to successfully delay berry ripening at the cost of yield and photosynthetic rate. Grape and wine phenolic compounds were influenced by partial canopy shade significantly with an increase in anthocyanins and a decrease in flavonols. There was a notable improvement in wine aroma in the partial canopy shade treatment, but it was accompanied by an unsatisfactory wine colour. Therefore, partial canopy shading was found to achieve its goal as a strategy for slowing down grape ripening, but it also had adverse effects. In future work, the duration of the partial shade treatment would need to be shortened to overcome these negative effects. Moreover, the shade cloth was made of polyethylene in our study; the sustainability of this material should thus be considered before applying this approach.

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