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Reducing the source/sink ratio of grapevine to face global warming in a semi-arid climate: Effects on volatile composition of Cabernet Sauvignon grapes and wines

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ABSTRACT

The heterogeneity of the vineyard environment caused high variability in grape metabolites and flavor profiles, and the phenomenon was more prominent in recent years of climate change. Herein, distal leaf removal was applied in semi-arid Xinjiang to adjust the source to sink ratio of grapevines for three consecutive years (2018–2020). The grape-derived volatiles showed high correlations with specific climate factors such as temperature changes in the growth period. Results showed that distal leaf removal increased the solar radiation reaching the clusters in the first few days after applying LR treatments while not affecting the temperature. The improvement in fruity and floral aroma intensity by distal leaf removal was founded not only in grape metabolites but also in wines. Moderate cluster exposure brought by distal leaf removal was beneficial for the accumulation of isoprenoids, which therefore increased the fruity and floral intensity of wines. The carry-over effect did not show in consecutively defoliated vines among vintages regarding the wine aroma profile.

1. Introduction

The volatile compounds in wines play an essential role in affecting their sensory profiles. Various volatile compounds in grapes and wines have complex changes during berries development and wine fermentation, which draw an extensive interest and attention of researchers. To improve the grape-derived aromas and wine aroma attributes through viticultural techniques is also the focus of many recent studies. In general, grape-derived compounds include norisoprenoids, terpenes, C6/C9 compounds and pyrazines, which are already present in grapes and contribute to the varietal characteristics (Wang et al., 2020). The grape-derived compounds and their precursors are formed through various pathways and are impacted in particular by climate conditions. The climate is one of the most important factors in the concept of terroir, which is known as an interactive ecosystem that characterizes a specific vineyard and impacts grape and wine quality (Anesi et al., 2015). In a certain viticultural region, the climate variations between vintages could play a dominant role in affecting most parameters related to grapes quality, followed by soil and cultivar (van Leeuwen et al., 2004). Although the climate variations among different growing seasons are usually unpredictable, dissecting the influence of vintage on wine quality can also provide helpful information for viticulture. Numerous viticultural techniques were investigated aiming at modifying microclimate in vineyards, which caused an improvement in wine flavor profiles.

In recent years, climate change has drawn the attention of many researchers and bring a profound impact on viticulture. In cool regions, the warming climate is beneficial for achieving the ideal maturity for

Abbreviations: LR1, distal leaf removal at the beginning of véraison; LR2, distal leaf removal at post-véraison; 1-LR1, distal leaf removal at the beginning of véraison in the same vines across vintages; 1-LR2, distal leaf removal at post-véraison in the same vines across vintages; 2-LR1, distal leaf removal at the beginning of véraison in new vines of each vintage; 2-LR2, distal leaf removal at post-véraison in new vines of each vintage; HS-SPME, headspace solid phase microextraction; GC–MS, gas chromatography-mass spectrometry; GDD, growing degree days; OAV, odor activity values.

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grapes, while in warm regions, the adverse effects of global warming on grape and wine composition, especially secondary metabolites, are widely reported (Gutiérrez-Gamboa et al., 2021). As a new technique that was not reported until the last decade, distal leaf removal was proved to be effective in mitigating the adverse effects of global warming on grape and wine quality and delaying ripening (Lu et al., 2022b). Distal leaf removal was a canopy management technique by removing the functional leaves in the upper canopy and altering the source-to-sink ratios of grapevines (Gutiérrez-Gamboa et al., 2021). Different from regular summer hedging, distal leaf removal did not involve the reduction of the major sink for nutrients (shoot) (Gutiérrez-Gamboa et al., 2021), which was a relatively mild technique. Thus the accumulation of total soluble solids (TSS) was slowed down and the harvest date was delayed by distal leaf removal. This could compensate for the advanced phenology of grapevines caused by global warming and avoid harvesting grapes in a warmer climate. In previous studies, most researchers focused on the influence of distal leaf removal on grape phenolic compounds (Lanari et al., 2013; Lu et al., 2022b; Palliotti et al., 2013). However, the changes in aroma profiles in response to distal removal treatment were few reported. Only Zhang et al. (2017) showed that apical leaf removal had minimal influence on the aroma composition of Shiraz wines.

In Xinjiang, viticulture had a rapid development in recent decades because of the abundant sunlight and semi-arid climate which could effectively reduce the disease occurrence. In 2020, the viticulture area of winegrapes in Xinjiang was 23,400 ha which occupied 26.6 % of the total area in China. Despite the foreseeable potential in the wine industry, there was also some weakness in the wine of this region, for example, the not outstanding aroma performance. In this region, the heatwaves frequently occurred in the grape development stage, so viticulturists usually chose a thick vine canopy to protect grapes from sunburn, which caused a high source/sink ratio and also the excessive shading of clusters. It was hypothesized that moderate exposure could improve the grape aroma performance because sunlight was beneficial for accumulating isoprenoids. Overexposure technique such as basal leaf removal has been proved to result in a decline in norisoprenoids and monoterpenes in ripening grape berries in the dry-hot climate (He et al., 2020). But the changed position of leaf removal might make a difference. Besides, the Cabernet Sauvignon grape was a principal cultivar of this region. The high sugar accumulation rate in the grape ripening stage and long-lasting harvest period required a technique to achieve the consistent maturity of harvested grapes. Thus the distal leaf removal was applied to adjust the source/sink ratio of the grapevine and delay ripening. In the present study, the effect of distal leaf removal on aroma compounds in grapes and sensory attributes of wines was investigated. Our study provides a theoretical basis for the feasibility of applying distal removal treatment in a semi-arid climate to face climate change in the future and a better understanding of the grape volatiles in response to an altered microclimate.

2. Materials and methods

2.1. Chemicals

Sodium hydroxide, perchloric acid, phosphoric acid, acetic acid, citric acid, sodium citrate, sodium acetate, ascorbic acid, sulfuric acid and potassium metabisulphite were analytical grade chemicals and were purchased from Tianjin Chemical Factory. HPLC grade solvents, including ethanol, methanol and dichloromethane, were purchased from Honeywell (Marris, Township, NJ, USA). Polyvinylpolypyrrolidone and D-gluconic acid lactone were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pure standards of volatile compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Experiment site and treatment

The experiment was performed in a commercial vineyard in the Manas region of Xinjiang (44°24′N-86°26′E, elevation 522 m) for three consecutive years (2018–2020). The experimental site had a typical semi-arid clime and the soil type was silt loam. The own-rooted Cabernet Sauvignon vines were planted in 2011 and used for the experiment. The vineyard has a northeast-southwest row orientation (52°) with vine and row spacing 1 m × 3 m. Grapevines were trained to a uniformly modified vertical shooting positioning (M-VSP) trellis system with 18–22 nodes per linear meter. Furrow irrigation was applied 750 $m^3 \cdot ha^{-1}$ when the phenological stage reached budburst, anthesis, berries pea size, véraison, and preharvest (about three weeks before harvest).

For distal leaf removal treatment, the upper canopy leaves between the second and third wire (1.2 m to 1.6 m) were manually removed at the beginning of véraison (LR1) and post-véraison (LR2), as shown in Supplementary Fig. 1. In 2018, the treatments included LR1 and LR2. In 2019 and 2020, the treatments included two schemes: leaf removal in the same vines as in 2018 (1-LR1, 1-LR2), which explored the carry-over effect; leaf removal in different vines from the former vintage (2-LR1, 2-LR2), as repeated experiments among vintages. Untreated vines were the control (C). All the vines were summer pruned twice in the whole growing season, which was performed in berry set and pre-veraison to limit the canopy height within 1.9 m above the ground. Nine blocks for each treatment were randomly distributed in three adjacent rows each year. Three blocks were selected as a replicate, including 45 vines with similar vigor.

2.3. Mesoclimate of the vineyard and microclimate of grapevines

The meteorological data of the experimental site was obtained from China Meteorological Data Service Centre (https://cdc.cma.gov.cn/), which included the average daily temperature, rainfall and sunshine duration in the whole growing season (April to September) from 2018 to 2020. The microclimate data around the bunch zone of each treatment was monitored using a HOBO micro station, including a solar radiation sensor (S-LIB-M003, Onset, Bourne, MA, USA) and a temperature sensor (S-THB-M002, Onset, Bourne, MA, USA). The data were recorded every 5 min.

2.4. Berry sampling and winemaking

At harvest, 500 berries were sampled at harvest at the same TSS level to determine volatile compounds. Besides, approximately 20 kg bunches for each replicate were manually harvested for winemaking. The bunches were crushed into 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 pectinase (Optivin, Australia) and 4 g commercial yeast (Lallemand, French) were added to the must. Alcohol fermentation was performed at a temperature-controlled workshop (24–26 °C). The skins were punched down twice a day. The alcohol fermentation was considered finished when the reducing sugar reached below 4 g/L. Then the wines were racked off with an addition of 0.02 g lactobacillus (Lalvin 31, Lallemand Inc, French). When malolactic fermentation was finished, 1.2 g SO_2 was added to wines for each container. The wines were then bottled in 750 mL bottles and stored at 10–15 °C for subsequent analysis.

2.5. Extraction of volatile compounds in berries

The extraction of volatile compounds in berries was according to Wang et al. (2020). For each replicate, about 60 g of frozen berries were de-seeded under liquid nitrogen. Then the de-seeded samples were grounded into powder with an addition of polyvinylpolypyrrolidone (1 g) and D-gluconic acid lactone (0.5 g). The frozen powder was transferred into 50 mL centrifuge tubes and melted at 4 °C for 8 h. The clear grape juice was obtained through centrifuging at 8000 × *g* for 15 min.

The free-form volatile compounds were extracted directly from the above clear juice. In brief, 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-form volatile compounds, 2 mL of the clear grape juice was added to Cleanert® PEP-SPE (150 mg/6 mL, Bonna-Agela Technologies, Tianjin, China) resins which had been activated with 10 mL of methanol and 10 mL of water. Then the resins were washed with 2 mL of water and 5 mL of dichloromethane to remove water-soluble compounds and free volatiles, respectively. The resins were eluted by methanol afterward and the methanolic substance was collected. The collected substance was concentrated to dryness by a rotary evaporator under vacuum at 30 °C and was redissolved in 10 mL of citrate/phosphate buffer solution (0.2 M, pH = 2.5). After hydrolyzing under 100 °C for 1 h in citric acid, 5 mL of the solution was added to a 20 mL vial containing a magnetic stirrer, 10 μL of internal standard (4-methyl-2-pentanol) and 1 g NaCl. The volatile compounds in grapes were extracted by the headspace solid-phase micro-extraction (HS-SPME), as described by Lan et al. (2016). Both free and bound samples were placed in a CTC-Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a 2-cm DVB/CAR/PDMS 50/30 μm SPME fiber (Supelco Inc., Bellefonte, PA., United States) and agitated at 500 rpm for 30 min at 40 °C. The SPME fiber was then inserted into the headspace to absorb aroma compounds at 40 °C for 30 min and was instantly desorbed into the gas chromatography (GC) injector for 8 min to desorb aroma compounds, and the injection temperature was set at 250 °C. The volatile compounds in wines followed the same procedure as the extraction of grape free volatiles.

2.6. Determination of volatile compounds in grapes and wines

The analysis of samples was performed on an Agilent 6890 gas chromatography (GC) coupled to an Agilent 5973 mass spectrometry (MS), fitted with an HP-INNOWAX capillary column (60 m × 0.25 mm, 0.25 μm, J and W Scientific, Folsom, CA, USA). The GC–MS conditions were settled according to Wang et al. (2019). The carrier gas was high purity helium with a flow rate of 1 mL/min. The oven program was set as follows: 50 °C for 1 min, increased to 220 °C at a rate of 3 °C/min, and held at 220 °C for 5 min. The temperature of the ion source and quadrupole were 250 °C and 150 °C, respectively. The full scan mode was applied to collect electron ionization mass data from m/z 30–350. The ionization voltage was set at 70 eV. The volatile compounds were identified using the Automated MassSpectral Deconvolution and Identification System (AMDIS), which could match the mass spectrum and RI with the reference standards in the NIST 11 MS database. The quantification of volatile compounds was according to the corresponding external standards, as described by Wang et al. (2019). The concentrations of those volatile compounds without corresponding standards were estimated with equations of standards having the same functional group and/or similar numbers of carbon atoms. The concentrations of volatile compounds were expressed as μg/L in wines and μg/kg of fresh berry weight in grapes.

2.7. Sensory analysis

As soon as the wines were bottled for two months, sensory evaluation was conducted. A panel of ten expert judges were selected from the local winery for sensory evaluation, with three females and seven males between the ages of 23 and 48. Ethical approval for the involvement of human subjects in this study was granted by China Agricultural University Research Ethics Committee, reference number CAUHR-20220711. For each treatment, wines for sensory evaluation were mixed from all three replicates since bottling. A blind tasting system was used to compare the wine samples with totally random order. The tasting sheet and detailed rules of scoring standards were according to China Rating System for Global Wine. Ratings were based on four elements: appearance, aroma, taste, and overall judgment, which accounted for 10 %, 30 %, 50 %, and 10 %, respectively. The wine tastings were conducted in transparent glasses at room temperature and in individual blocks. In each detailed item, points were awarded from 0 to 10 points (worst to best).

2.8. Statistical analysis

All measuring parameters had three replicates in this study. The variance analysis (ANOVA) was performed using SPSS version 22.0 at $p < 0.05$ (Duncan's multiple range test or t-test). The figures were drawn using GraphPad Prism 8.0.2 and Origin 9.0 software. Principal component analysis (PCA) and orthogonal partial least squares discrimination analysis (OPLS-DA) were carried out using SIMCA 14.1.

3. Results and discussions

3.1. Mesoclimate of the vineyard and microclimate of grapevines

Vine development, grape ripening and wine sensory attributes are highly influenced by the physical environment in which the vines grow (Ferreira, 2010). In the semi-arid Xinjiang, the grapes had rapid sugar accumulation during ripening because of the hot climate and large daily temperature ranges. After applying LR treatments, the sugar accumulation rate was slowed down and the harvest date could be delayed for 4–10 days (Supplementary Table 1). The variable harvest dates would lead to varying climate conditions that each LR treatment went through. So the meteorological data during Cabernet Sauvignon grape development was calculated in each treatment of 2018, 2019 and 2020 growing seasons, as shown in Supplementary Table 2. Compared to the three vintages, the vintage 2020 was the driest year. The average temperature in 2020 was higher than in 2018 and 2019, and the cumulative rainfall in 2020 was less than in 2018 and 2019. Fig. 1a also showed the temperature changes from budburst to harvest in three vintages. From budburst to flowering, the average temperature in 2020 was higher than in 2018 and 2019, and the variation could be up to 3 °C (Supplementary Table 2). However, in the flowering-véraison period, the temperature in 2020 was lower than the other two vintages. For sunlight duration, the vintage 2018 had higher sunshine hours than 2019 and 2020. Similar to temperature, the variations in sunshine hours among the three vintages mainly occurred in two stages: budburst-flowering and flowering-véraison. From budburst to flowering, the average daily sunlight duration in 2019 was lower than the other two vintages (Fig. 1b). However, the vintage 2020 had the lowest cumulative sunlight duration in this stage because 2020 had an advanced phenological stage and the flowering time was about two weeks earlier than 2018 and 2019. Among all climate factors, temperature played a predominant role in affecting the phenological stages of grapevines. So even though there was lower sunlight in 2020 from budburst to flowering, the high temperature in this stage could also cause a significantly advanced growth stage. Although the harvest date in 2020 was about two weeks before 2018 and 2019, the growing degree days in three vintages were similar, which indicated that GDD was a reliable indicator for predicting the phenology of grapes (Verdugo-Vásquez et al., 2017).

After applying LR treatments, the temperature around clusters was not altered, as shown in Fig. 1c. Both LR1 and LR2 had little difference in temperature changes with control in the growing season. In the first few days after applying LR treatments, the solar radiation around clusters was increased, which was found in both LR1 and LR2 (Fig. 1d). However, the difference between LR treatments and control gradually narrowed in the latter period of the growing season (20 days after véraison to harvest for LR1, 28 days after véraison to harvest for LR2) as time went by. Supplementary Fig. 2 also showed the daily solar radiation changes around the clusters. On the third day after applying LR1, the solar radiation was increased by LR treatment, especially from 16:00 to 20:00 (Supplementary Fig. 2a). Similarly, on the fourteenth day after applying LR2, the solar radiation was also increased compared to

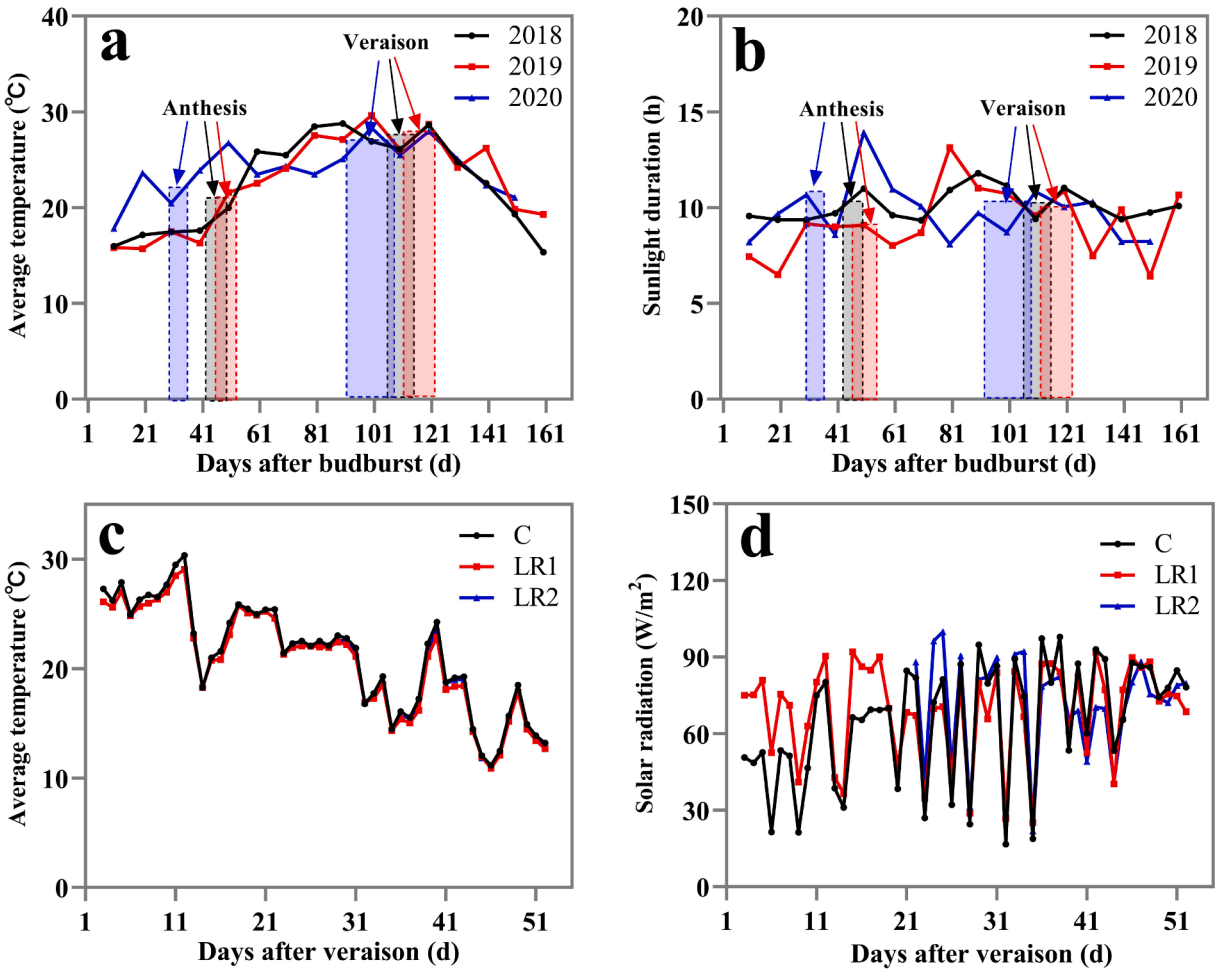

Fig. 1. Mesoclimate (a, average temperature; b, sunlight duration) of the vineyard in 2018, 2019 and 2020 growing seasons and microclimate (c, average temperature in fruit zones; d, solar radiation in fruit zones) of each treatment in 2018. Each point in Fig. 1a or Fig. 1b was calculated by the average data of ten days. Each point in Fig. 1c or Fig. 1d was calculated by the average data of each day.

control, while the LR1 did not show a difference between control (Supplementary Fig. 2b). At preharvest, the three treatments had similar cumulative solar radiation throughout the day, with no obvious increase found in LR treatments (Supplementary Fig. 2c). This phenomenon showed that the removed upper leaves would increase the transmittance of the canopy, which increased the solar radiation reaching the clusters to a certain extent in the first few days after applying LR treatments. However, as described in a previous study (Lu et al., 2022b), the vines in the experimental site had high vigor with the vigorous growth of lateral shoots. So the increased transmittance of the canopy might be covered by the growth of lateral shoots, which caused little difference between LR treatments and control in the latter period of the growing season. The increased solar radiation around clusters did not cause an augment in temperature. The same result was also found by Young et al. (2016) that leaf and lateral shoot removal in the bunch zones altered the microclimate by increasing the exposure of the berries but did not affect the temperatures in the bunch zones. Although exposure to sunlight invariably results in increased temperatures. The air was circulating around clusters in field conditions rather than in a confined environment, which would lead to little change in temperatures with the increased solar radiation in our study.

3.2. Dissecting the influence of climate factors among vintages on volatile compounds of berries

The identified volatile compounds by GC–MS and their concentrations in berries were shown in Supplementary Table 3. According to structures, the volatile compounds could be sorted into the following categories: C6/C9 compounds, benzenes, higher alcohols, norisoprenoids, aldehydes/ketones, terpenes, acids and esters. Among them, the C6/C9 compounds, terpenes and norisoprenoids were the main grape-derived aromas that could maintain the variety characteristic in wines (González-Barreiro et al., 2015). So principal component analysis was used to identify the aroma profile variations in all treatments based on C6/C9 compounds, terpenes and norisoprenoids, as shown in Supplementary Fig. 3. The first two principal components explained 69.9 % of the total variance. PC1 (R2X[1]) accounted for 40 % of the total variance and could separate the samples from 2018 and 2020. It showed that berries in 2018 had more abundant C6/C9 compounds than in 2020. PC2 (R2X[2]) accounted for 29.9 % of the total variance and could separate the samples from 2019 to other two vintages. It showed that berries in 2018 had more scarce aroma profiles than other two vintages. To further investigate how climate factors among vintages affected the volatile compounds in berries, the Pearson correlation analysis was used to select highly correlated compounds ($|r^2| > 0.7$) with each climate factor, as shown in Supplementary Table 4.

For C6/C9 compounds, the average max and min temperature in budburst-harvest, véraison-harvest, and budburst-flowering stages were all negatively correlated with two C6/C9 compounds: hexanal and (*E*)-2-hexenal. While the average max and min temperature from flowering to harvest were positively correlated with hexanal and (*E*)-2-hexenal in our study. C6/C9 compounds were abundant in various aromatically neutral

varieties and (*E*)-2-hexenal and hexanal were the most abundant C6 compounds in mature berries of Cabernet Sauvignon (González-Barreiro et al., 2015). Regarding the effects of temperatures on C6 compounds, the same result was also found by Wang et al. (2020) that total C6/C9 compounds were negatively correlated with the average daily temperature of the vineyard from flowering to harvest. Similarly, Ji and Dami (2008) reported that concentrations of 6-carbon aldehydes were higher in Traminette grapes grown on the cool site than those grown on the hot site. The daily temperature range from budburst-harvest and véraison-harvest was positively correlated with 1-hexanol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol and 2-nonenal. Although the effect of diurnal temperature differences on volatile compounds was little reported, Xu et al. (2015) showed that diurnal temperature differences were positively correlated with (*Z*)-3-hexen-1-ol in berries, which was in agreement with our study.

For norisoprenoids, the average, max and min temperature in budburst-harvest, véraison-harvest, and budburst-flowering stages were negatively correlated with some free form norisoprenoids such as (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB), (*Z*)-*β*-damascenone and (*E*)-*β*-ionone. However, the temperature was positively correlated with mang bound form norisoprenoids such as (*E*)-*β*-damascenone (B), (*Z*)-*β*-damascenone (B), TPB (B). Regarding the effects of elevated temperatures on norisoprenoids, there were still inconsistent results in previous studies. Scherzinger and Al-Babili (2008) found that both cold (20 °C) and heat stress (38 °C) allowed to increase the expression of gene CCD, which played an essential role in the generation of norisoprenoids. However, Meng et al. (2020) found that the high temperature (37 °C) repressed the activity of the *VvCCD4b* promoter. In a previous study reported by our research team (Lu et al., 2022a), the Muscat Hamburg and Victoria grapes had a lower norisoprenoid concentration in the summer season characterized by high temperature under the double cropping system. However, the opposite result was found in the Cabernet Sauvignon grapes that the winter season berries had a higher norisoprenoid concentration than the summer season berries. In the present study, the high temperature days in budburst-harvest, véraison-harvest, and flowering-véraison stages were all negatively correlated with the concentration of (*Z*)-*β*-damascenone (B). For other climate factors, the cumulative sunlight duration from budburst to harvest was positively correlated with 6-methyl-5-hepten-2-one, geranylacetone, (*E*)-*β*-ionone and *β*-ionene (B), which indicated that the promotion of light exposure to norisoprenoid accumulation (Feng et al., 2015; Wang et al., 2020).

For terpenes, the average, max and min temperature in budburst-harvest, véraison-harvest, and budburst-flowering stages were negatively correlated with three free form terpenes such as endo-borneol, hotrienol and eucalyptol. Only the bound form citronellol was positively correlated with the average, max and min temperature in budburst-harvest, véraison-harvest, and budburst-flowering stages. It is generally considered that elevated temperature could inhibit the accumulation of terpenoids due to the increased loss by volatilization (Scafidi et al., 2013). Similar to norisoprenoids, mang terpenes were positively correlated with the cumulative sunlight duration from budburst to harvest.

3.3. Effects of distal leaf removal treatments on volatile compounds in berries

3.3.1. C6/C9 compounds

The main C6/C9 compounds that showed significant differences among treatments in at least one vintage were shown in Fig. 2. Although hexanal and (*E*)-2-hexenal were the most abundant C6/C9 compounds in berries, they were not affected by LR treatments. In 2018 and 2019, there was no significant difference among treatments regarding the concentration of hexanal and (*E*)-2-hexenal. In 2020, 1-LR1 had a lower hexanal concentration than 1-LR2 and 2-LR1 while showing no significant difference with control. 1-LR2 had a higher (*E*)-2-hexenal concentration than control and 1-LR1. For 1-hexanol, (*E*)-2-hexen-1-ol, 1-nonanol and (*Z*)-3-nonen-1-ol, the promotion of their concentrations was confirmed in LR treatments. 1-LR1 (LR1) significantly increased the

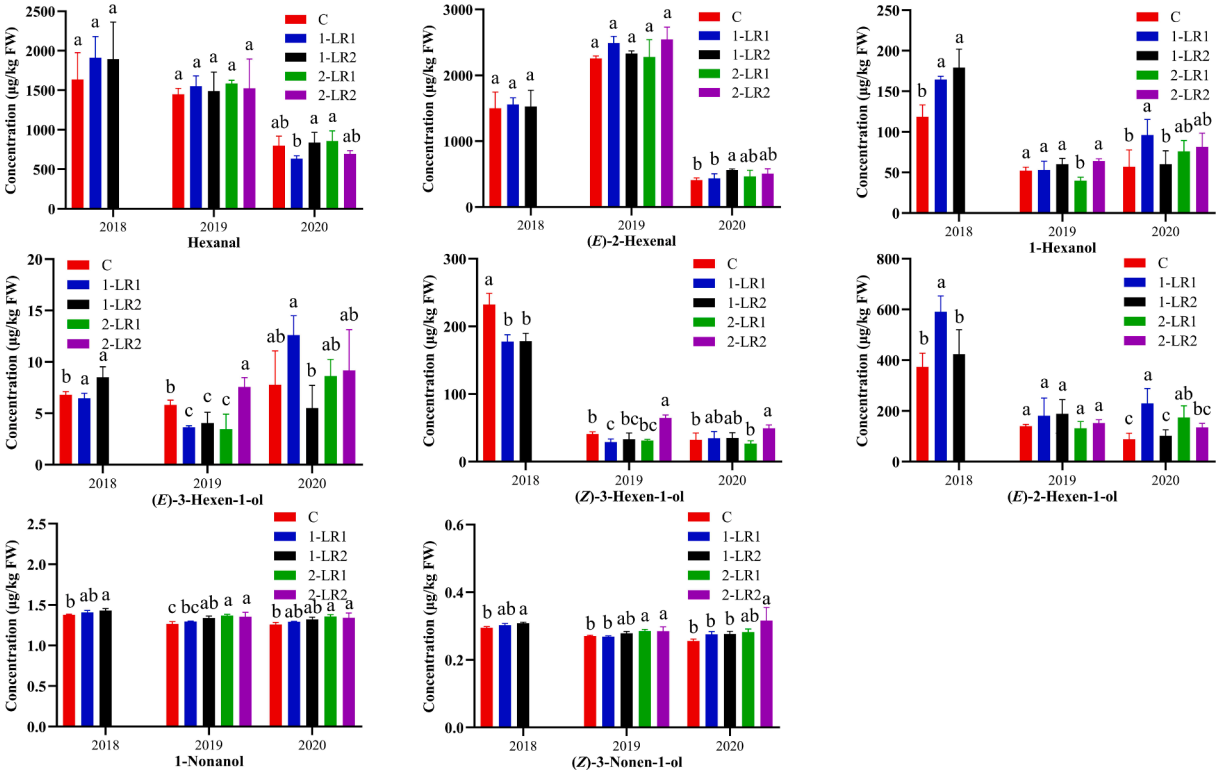

Fig. 2. Effects of distal leaf removal treatments on C6/C9 compounds of Cabernet Sauvignon grapes in 2018, 2019 and 2020 growing seasons (μg/kg FW). Different letters within a plot indicate significant differences among treatments (Duncan's multiple range test at $p < 0.05$).

concentrations of 1-hexanol and (*E*)-2-hexen-1-ol compared to control in the 2018 and 2020 growing seasons. LR2 in 2018, 2-LR1 and 2-LR2 in 2019 and 2020 all had a higher concentration of 1-nonanol than control. As for (*Z*)-3-nonen-1-ol, it had a higher concentration in LR2 in 2018, 2-LR2 in 2019 and 2020 than control. For (*E*)-3-hexen-1-ol and (*Z*)-3-hexen-1-ol, there were no consistent trends in different vintages regarding the LR influence. As reported by a previous study (Kalua & Boss, 2009), C6 volatile compounds changed from acetate esters to aldehydes and finally to alcohols during early, middle, and late berry developmental stages, respectively. So LR from véraison had limited influence on hexanal and (*E*)-2-hexenal while increasing the concentrations of 1-hexanol and (*E*)-2-hexen-1-ol in grapes. C6/C9 compounds were easily influenced by microclimate changes and light exposure at or after véraison was beneficial for accumulating C6 alcohols (He et al., 2020; Wang et al., 2020), which was also found in our study. As analyzed before, the increased solar radiation around clusters in the first few days after applying LR treatments might be the cause. However, if the light exposure around clusters was performed in the early stages of berries development, the changes in C6/C9 compounds might be insignificant (Feng et al., 2015).

3.3.2. Terpenes

Eighteen terpenes were detected by GC–MS in berries, which included eight free form compounds and ten bound form compounds, as shown in Supplementary Table 3. To better present how LR treatments affect the terpenes, the $\log_2$-fold was used to normalize the changes of each terpene concentration between LR treatments and control (LR/control), as shown in Fig. 3. The increase of many free-form terpenes such as citronellol, menthol, hotrienol was confirmed in LR treatments, all of which had higher concentrations in almost all LR treatments than in control in three vintages. Besides, the bound form *α*-terpinene also had a higher concentration in LR treatments. Only the concentration of *α*-calacorene was decreased by LR treatments. As the main compounds contributing to the floral/fruity odors of wines, terpenes were easily affected by many factors (Wen et al., 2015). The sunlight was one of the terroir factors that drew an increasing interest of researchers regarding how sunlight affected grape terpenes (Zhang et al., 2014). Skinkis et al. (2010) found that fruit from fully exposed clusters had 30 % higher concentrations of potentially volatile terpenes than shaded fruit. Similarly, Sylvie et al. (2000) showed that the artificially shaded bunches showed lower levels of monoterpenes than sun-exposed berries and berries from naturally shaded bunches. So the increase of sunlight around clusters was usually beneficial for terpene accumulation, which was also found in our study. However, excessive sun exposure could also negatively affect the concentration of terpenes (Belancic et al., 1997). In our previous study conducted in the same experimental site, various cluster exposure treatments resulted in a decline in the concentrations of monoterpenes in ripening grape berries (He et al., 2020), which showed the opposite result to the present study. This might be due to the different levels in cluster exposure caused by different leaf removal positions. As mentioned in the previous analysis, the distal leaf removal only increased the solar radiation reaching the clusters to a certain extent in the first few days after applying LR treatments. Sometimes, it was difficult to separate the environmental effects of temperature and light in a field setting (Azuma et al., 2012). In the semi-arid climate of Xinjiang, the excessive sun exposure caused by basal leaf removal might lead to the high temperature of berries, and the adverse effect of high temperature on terpene accumulation was also confirmed. But for the distal leaf removal, moderate sun exposure was more beneficial for terpene accumulation and could avoid high temperature in berries.

3.3.3. Norisoprenoids

The main norisoprenoids that showed significant differences among treatments in at least one vintage were shown in Fig. 4. For the selected free form norisoprenoids, (*Z*)-*β*-damascenone, (*E*)-*β*-ionone and TPB were affected constantly by LR treatments in different vintages. LR2 in 2018 and 2-LR2 had significantly higher concentrations of (*Z*)-*β*-damascenone, (*E*)-*β*-ionone than control. Besides, all four LR treatments in 2019 and 2-LR2 in 2020 had a higher concentration of (*E*)-*β*-ionone than control. 2-LR2 significantly increased the (*E*)-*β*-damascenone concentration in 2019, while the opposite result showed in 2020. For bound form (*E*)-*β*-damascenone, only 1-LR2 significantly decreased its concentration in 2020 than control. For the bound form *α*-ionone, only 2-LR1 and 2-LR2 significantly increased its concentration in 2019. As reported, the formation of norisoprenoids in grape berries involved carotenoid breakdown and the formation of carotenoids occurred at prevéraison and decreased afterward (Yuan & Qian, 2016). In our study, the LR treatments were performed at and post véraison, which might not affect the formation but the degradation of carotenoids. The light appeared to increase carotenoid levels in green berries and decrease major carotenoid levels during ripening (Bureau et al., 2000). After véraison, the sunshine favored the degradation of carotenoids into norisoprenoids (Baumes et al., 2002), which might be the reason of the increased free form norisoprenoids, (*Z*)-*β*-damascenone, (*E*)-*β*-ionone and TPB found in LR treatments in our study.

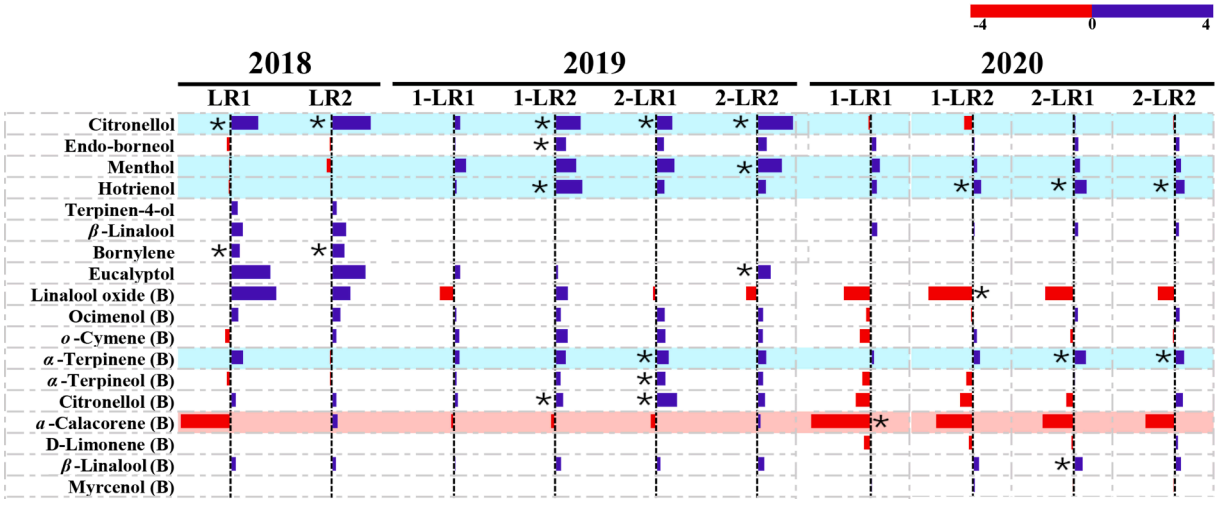

Fig. 3. Effects of distal leaf removal treatments on terpenes of Cabernet Sauvignon grapes in 2018, 2019 and 2020 growing seasons. Column plots showed the $\log_2$ fold change between the LR treatments and control (LR/control). Blue column indicated a higher concentration in the LR treatments. Red column indicated a lower concentration in the LR treatments. * indicated there were significant differences between LR treatments and control (Duncan's multiple range test at $p < 0.05$).

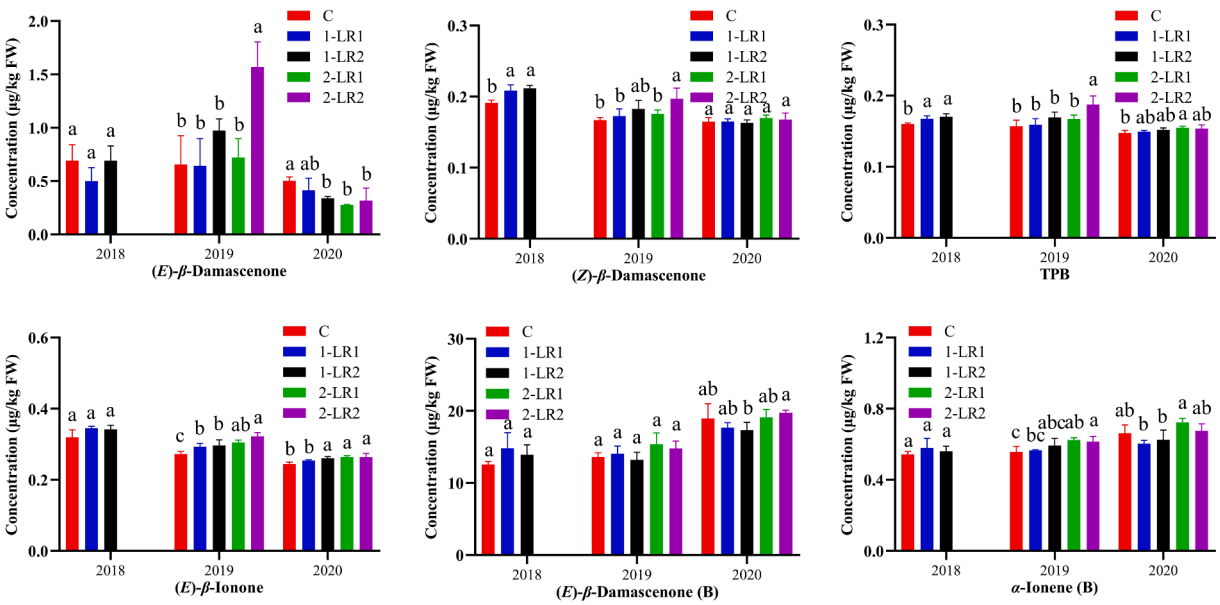

Fig. 4. Effects of distal leaf removal treatments on norisoprenoids of Cabernet Sauvignon grapes in 2018, 2019 and 2020 growing seasons. Different letters within a plot indicate significant differences among treatments (Duncan's multiple range test at $p < 0.05$).

3.4. Effects of distal leaf removal treatments on volatile compounds in wines

There were sixty-four volatile compounds identified by GC–MS in the Cabernet Sauvignon wines, including C6 alcohols, acetate esters, ethyl esters, other esters, benzenes, terpenes, norisoprenoids, higher alcohols, fatty acids, as shown in Table 1. Among all compounds, ten compounds were significantly affected by LR treatments in a consistent way in at least two vintages, including four esters, one norisoprenoid compound, two higher alcohols and three fatty acids. For esters, LR treatments increased the concentrations of isoamyl acetate, 2-phenylethyl acetate, ethyl (S)-(-)-lactate and ethyl 3-methylbutyrate. It was well known that esters were the main fermentation-derived compounds that contributed to desired fruity and floral attributes to wines. Variables that were known to affect ester production in wines included the concentration of esters or their precursors originally from grapes, fermentation conditions, and the nutrients present, especially the concentration of nitrogen compounds and must solids (Sumby et al., 2010). In our study, all LR treatments and control were strictly performed in the same fermentation conditions and procedures. So the increased esters concentration in LR treatments might not be due to variations of fermentation conditions. Four possible reasons led to the ester changes in LR treatments: (i) the increased C6/C9 concentrations were found in LR grapes in the previous analysis, which could transform into more esters during fermentation (Dennis et al., 2012); (ii) the increased fatty acids concentrations in LR wines (Table 1) provided more abundant substrates for esters which were formed based on the reaction of alcohol and carboxylic acid functional groups (Sumby et al., 2010); (iii) the probably increased amino acids in grapes, which were the main nitrogen source for yeasts and also precursors of esters. Previous studies showed that in Cabernet Sauvignon grapes, skin and pulp responded differentially to light exposure but shaded berries contained less total amino acids than the exposed berries (Pereira et al., 2006). (iv) the greater UV radiation caused by cluster exposure promoted the degradation of polyunsaturated fatty acids in grapes, as compounds that repressed the genes involved in yeast activity and the synthesis of esters during fermentation, leading to a higher concentration of esters in LR wines (Bubola et al., 2020). For norisoprenoids, only β-damascenone was detected in Cabernet Sauvignon wines. The increase of β-damascenone was shown in LR treatments with a constant result among vintages. In 2019, all four LR treatments significantly increased β-damascenone concentration in wines compared to control. In 2020, 1-LR1 and 2-LR2 both significantly increased β-damascenone concentration in wines compared to control. In the previous analysis, the LR treatments increased the free form (*Z*)-β-damascenone in grapes, which was in agreement with the results in wines. For terpenes, four compounds were detected in Cabernet Sauvignon wines in our study. However, different from the increased terpene concentrations in LR grapes, there were no significant differences between LR and control wines. Sometimes, it usually hard to associate grapes to the corresponding wines in some aspects. Wine fermentation is a complicated process during which aroma compounds experience great changes, which would result in a variation between berries and wines (Lu et al., 2021).

Values are reported as means ± SD of three biological replicates. Different letters within a row of each vintage indicate significant differences among treatments (Duncan's multiple range test at $p < 0.05$).

3.5. Effects of distal leaf removal treatments on odor activity values in wines

The odor activity values (OAV) were usually used to estimate the contribution of aroma compounds to the wine sensory profile by dividing their concentration to their odor threshold values (Bouzas-Cid et al., 2018). The identified volatile compounds and their thresholds and aroma series in Cabernet Sauvignon wines were shown in Supplementary Table 5. The main aroma series with high OAVs were as follows: fruity, floral, caramel, herbaceous, chemical and fatty, as shown in Fig. 5. LR treatments significantly increased the intensities of fruity, floral, and caramel, indicating a significant improvement in the wine aroma caused by LR treatments. In the three aroma series, β-damascenone contributed the highest OAV among all volatile compounds. As analyzed above (Table 1), the significant increase of β-damascenone was shown in LR treatments with a constant result among vintages, which therefore caused the increased fruity, floral, and caramel intensities in LR wines (Cai et al., 2014). The increased free-form β-damascenone was also observed in grapes, which indicated that the SST treatment could improve the fruity, floral, and caramel intensity in berries and then reflected on wines. Besides the β-damascenone, the esters such as isoamyl acetate, ethyl acetate, ethyl hexanoate also had high OAVs and contributed to fruity aroma to wines. The LR treatments significantly

Table 1
Effects of upper leaf removal treatments on volatile compounds of Cabernet Sauvignon wines in 2018, 2019 and 2020 growing seasons (μg/L).

Parameters	2018			2019					2020					
	C	LR1	LR2	C	1-LR1	1-LR2	2-LR1	2-LR2	C	1-LR1	1-LR2	2-LR1	2-LR2	LR *vs* C
C6 alcohols														
1-Hexanol	2,332.66a	2,059.22b	1,996.31b	1,245.69bc	1,529.32a	1,415.38ab	1,618.09a	1,131.49c	3,068.77b	3,069.27b	3,290.23ab	2,565.91c	3,539.45a	
(*E*)-3-Hexen-1-ol	84.44a	85.22a	63.33b	39.56b	43.52ab	44.51ab	52.08a	37.68b	64.13a	63.94a	65.77a	46.69b	72.24a	
(*Z*)-3-Hexen-1-ol	64.71a	54.91a	55.72a	95.11ab	91.36b	84.36b	107.36a	81.06b	64.98a	65.13a	64.95a	44.31b	69.9a	
Acetate esters														
Ethyl acetate	69,124.51b	88,658.55a	86,626.75a	110,325.32a	82,817.78b	84,421.07b	86,949.33b	97,674.12ab	73,026.84ab	65,873.1c	75,691.37a	66,799.47bc	79,630.73a	
Isoamyl acetate	5,154.04c	7,342.56b	11,312.05a	4,953.16c	6,801.45b	7,027.8b	8,924.35a	7,518.72b	8,839.35a	8,627.79a	8,754.48a	7,987.23a	7,011.86a	↑
Hexyl acetate	241.9b	331.45b	765.64a	111.43c	156.29b	201.28a	187.24ab	105.99c	213.15a	157.83ab	184.94ab	131.76bc	77.3c	
Heptyl acetate	nd	nd	1.45	2.69b	2.86a	2.87a	2.67b	2.62b	nd	nd	nd	nd	nd	
2-Phenylethyl acetate	77.24b	72.17b	175.59a	32.25c	65.37b	57.19b	90.9a	63.92b	8.61bc	10.78a	9.16b	9.95ab	7.56c	↑
Isobutyl acetate	122.33b	244.48a	263.87a	nd	nd	nd	nd	nd	153.31a	161.88a	161.1a	128.27a	123.32a	
Ethyl esters														
Ethyl 2-methylbutyrate	nd	0.01	nd	110.95b	202.47a	205.32a	196.54a	208.15a	nd	1.31	3.21	1.67	1.75	
Ethyl butanoate	299.4c	432.85b	519.33a	497.08a	515.93a	540.59a	492.23a	540.57a	282.36ab	255.95ab	328.42a	252.14b	318.69ab	
Ethyl hexanoate	657.17b	803.45b	1,199.01a	996.23c	1,254.62ab	1,413.31a	1,169.04b	1,142.97bc	600.69a	460.98b	609.52a	413.95b	639.64a	
Ethyl (S)-(-)-lactate	32,746.78c	37,995.26b	46,334.63a	41,669.96bc	66,579.28ab	35,237.26c	49,635.36bc	84,896.24a	45,694.41c	54,412.37b	53,781.07b	51,515.76bc	61,494.85a	↑
Ethyl nonanoate	4.23a	3.89a	4.46a	4.38c	4.72bc	4.89ab	5.24a	5.13a	3.71a	3.83a	4.03a	3.7a	3.84a	
Ethyl octanoate	763.83b	817.59b	1,303.79a	1,026.06b	1,232.27a	1,370.39a	1,258.57a	1,274.99a	408.04a	337.81ab	409.17a	277.39b	439.05a	
Ethyl decanoate	299.09b	272.71b	425.59a	617.29b	766.83a	868.73a	795.72a	842.34a	233.11a	173.22b	232.74a	151.86b	246.9a	
Ethyl 9-decenoate	82.79b	83.39ab	84.95a	108.26a	108.86a	111.26a	107.02a	108.94a	82.86a	63.11b	73.8b	62.97b	79.07a	
Ethyl dodecanoate	69.97b	54.46c	94.06a	63.57a	60.26a	60.47a	56.35a	58.44a	77.7a	55.81b	75.97a	55.27b	81.68a	
Ethy hexadecanoate	25.75ab	14.34b	27.58a	31.58a	33.76a	43.43a	37.33a	38.8a	120.9ab	112.94abc	101.87bc	108.53c	125.78a	
Ethyl benzoate	nd	nd	nd	nd	nd	nd	nd	nd	20.96a	20.56a	21.45a	19.97a	21.45a	
Ethyl (*Z*)-4-decenoate	80.09b	83.92ab	91.72a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
Ethyl (*E*)-4-decenoate	47.62b	53.3b	67.94a	126.94a	126.53a	130.74a	124.19a	127.77a	nd	nd	nd	nd	nd	
Ethyl benzeneacetate	5.34a	2.66ab	3.52b	1.92c	3.2ab	2.63b	3.38a	2.97ab	0.97b	1.09a	1.06a	1.05a	1.05a	
Ethyl 3-methylbutyrate	2.29a	4.31a	3.37a	20.24b	42.67ab	44.99ab	49.33a	53.97a	12.19b	12.07b	18.28a	14.69ab	15.83ab	↑
Ethyl salicylate	5.77	0.47	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
Other esters														
Isopropyl butyrate	250.45c	338.89b	389.12a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
Methyl octanoate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
Isopentyl hexanoate	nd	nd	nd	nd	nd	nd	nd	nd	2.69ab	2.77ab	3.24a	1.77b	3.15a	
Isoamyl lactate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
Methyl decanoate	nd	nd	nd	6.73b	6.96ab	7.04a	6.97ab	6.78ab	nd	nd	nd	nd	nd	
Diethyl butanedioate	4,696.42a	2,880.86b	3,337.82ab	5,047.29a	1,841.55bc	976.12bc	654.48c	3,175.84ab	3,288.83a	3,298.2a	5,754.24a	2,978.87a	3,015.47a	
Isoamyl octanoate	53.41b	44.73b	68.06a	71.49c	85.78bc	95.48ab	101.5a	105.2a	31.89a	30.09ab	32.88a	26.29b	34.26a	
Benzenes														
Styrene	13.44a	15.82a	17.8a	27.15b	34.27ab	35.73ab	36.72ab	46.49a	nd	nd	nd	nd	nd	
Phenylethyl Alcohol	70,234.68a	43,142.1b	66,879.45a	29,100.1b	46,880.28a	47,138.01a	54,640.32a	55,334.97a	16,343.59c	32,267.71a	28,160.47ab	26,344.35ab	18,953.62bc	
Benzyl alcohol	952.72a	855.5a	928.08a	1,593.52ab	1,419.98ab	996.34b	1,557.06ab	1,659.82a	627.89a	726.31a	735.4a	505.53a	568.41a	
Benzyl acetate	14.22b	30.8b	77.69a	32.25c	65.37b	57.19b	90.9a	63.92b	nd	nd	nd	nd	nd	
Terpenes														
β-Linalool	3.78a	2.54b	4.07a	3.5b	5.72a	5.85a	5.86a	5.23a	1.35a	1.24a	1.43a	1.25a	1.5a	
Citronellol	6.26a	5.48a	6.16a	5.44a	6.02a	5.5a	5.94a	5.86a	2.52b	3.69a	3.47a	3.21a	3.23a	
α-Terpineol	3.58a	3.09a	4.2a	2.69a	3.12a	3.12a	3.32a	3.42a	3.34a	3.43a	3.7a	3.56a	4.05a	
Limonene	nd	nd	nd	nd	nd	nd	nd	nd	0.1a	0.18a	0.15a	0.11a	0.12a	
Norisoprenoids														
β-Damascenone	7.51a	7.69a	9.41a	6.64c	11.77a	9.18b	10.48ab	9.17b	2.41c	3.11ab	2.74abc	2.62bc	3.26a	↑
Higher alcohols														
1-Propanol	9,316.87a	10,095.14a	9,939.45a	8,630.92a	7,418.54bc	7,119.65c	7,036.04c	8,037.67ab	11,977.85a	8,941.34b	10,829.04a	11,321.95a	10,801.69a	↓
Isobutanol	43,577.12b	50,674.13a	42,863.63b	47,063.21a	43,167.12ab	39,041.58b	47,195.13a	46,784.99a	49,910.43b	54,552.76ab	55,348.77a	55,159.83a	52,608.88ab	
1-Penten-3-ol	nd	nd	nd	nd	nd	nd	nd	nd	10.43a	23.54a	24.66a	9.51a	9.78a	

(*continued on next page*)

Table 1 (*continued*)

Parameters	2018			2019				2020					
	C	LR1	LR2	C	1-LR1	1-LR2	2-LR1	2-LR2	C	1-LR1	1-LR2	2-LR1	2-LR2	LR *vs* C
1-Butanol	1,277.34a	1,337.15a	1,063.17b	2,277.63a	1,759.02c	1,711.34c	1,897.38b	1,973.49b	2,070.26c	2,406.12ab	2,386.25ab	2,513.34a	2,174.14bc	
3-Methyl-1-butanol	287,876.92a	278,940.79a	291,027.15a	250,392.93a	249,076.31ab	23,1260.65b	260,041.71a	248,380.37ab	154,179.31c	169,697.59ab	177,062.46a	164,776.72b	165,346.64b	
2-Methyl-3-butyn-2-ol	nd	nd	nd	160.25c	190.91abc	195.5ab	212.68a	174.13bc	nd	nd	nd	nd	nd	
3-Methyl-1-pentanol	3.95	nd	2.6	nd	0.91	0.04	1.18	nd	nd	nd	nd	nd	nd	
2-Heptanol	nd	nd	nd	109.43a	115.8a	111.04a	104.27a	94.03a	84.11ab	65.84c	77.1b	66.01c	87.86a	
2-Octanol	nd	nd	nd	nd	nd	nd	nd	nd	236.85c	283.48ab	279.98b	267.96bc	321.17a	
1-Heptanol	12.74a	9.98a	14.38a	nd	0.75	nd	nd	nd	nd	nd	nd	nd	nd	
2-Propyl-1-pentanol	nd	nd	nd	2.04	0	nd	nd	nd	nd	nd	nd	nd	nd	
2-Ethyl-1-hexanol	nd	nd	nd	2.88a	1.68b	1.72b	1.72b	1.62b	2.02a	1.81a	1.89a	1.83a	2.09a	
2-Nonanol	nd	nd	nd	2.99b	4.13a	3.71ab	3.53ab	3.14b	1.53b	1.92a	1.42b	1.32b	1.61b	
1-Octanol	22.11a	19.48ab	14.49b	26.14a	27.71a	27.11a	27.82a	24.18a	8.07a	6.06ab	6.69ab	4.74b	8.07a	↓
2,3-Butanediol	1,410.68b	1,648.98ab	2,064.89a	6,261.34a	6,466.32a	7,445.17a	8,176.54a	8,888.16a	3,710.45ab	3,310.33b	4,355.31ab	4,532.74a	3,338.97b	
1-Decanol	1.35a	1.26a	1.2a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
Fatty acids														
2-Methoxypropanoic acid	1,554.77b	1,947.69a	1,752.66ab	1,984.88c	2,785.15ab	2,466.85b	3,181.87a	3,187.36a	1,106.31a	1,326.36a	1,188.5a	1,138.4a	1,148.44a	↑
Butanoic acid	nd	nd	nd	1,005.12a	951.61a	1,135.76a	1,089.89a	1,095.59a	1206.91a	1283.13a	1287.09a	1104.61a	1308.31a	
Hexanoic acid	3,738.77b	4,104.28b	5,421.71a	2,320.82b	2,589.11ab	3,049.56a	2,685.41ab	2,813.6ab	1879.39a	2126.75a	1954.19a	1351.54a	1675.51a	↑
Octanoic acid	3,512.88b	3,605.26b	5,397.76a	2941.9b	3,623.29ab	4,185.31a	3,585.88ab	3,932.12ab	1673.75a	2147.16a	1537.66a	1321.92a	1616.96a	↑
n-Decanoic acid	629.68a	449.2a	787.3a	240.69b	367.51ab	412.47a	297.46ab	310.22ab	245.43a	278.57a	215.33a	389.14a	260.71a	
3-Methylbutanoic acid	496.65a	539.26a	511.44a	551.31b	822.90a	872.34a	932.21a	948.79a	248.21a	332.64a	325.96a	312.09a	310.77a	
2-Methylbutanoic acid	349.24b	410.09a	362.82ab	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	

Values are reported as means ± SD of three biological replicates. Different letters within a row of each vintage indicate significant differences among treatments (Duncan's multiple range test at $p < 0.05$).

increased isoamyl acetate concentration in wines (Table 1), which also caused an augment in fruity aroma in wines (Cai et al., 2014). For herbaceous and chemical aromas in wines, there were no consistent results among vintages regarding the LR effect. Although the higher C6 aldehydes were found in berries in SST wines, their herbaceous note could transform into a fruity note (esters) during fermentation which contributed to an augment in fruity aroma in wines. For the fatty aroma, LR2 in 2018, all the LR treatments in 2019 and 2-LR2 in 2020 significantly increased its intensity in wines. In all identified fatty acids in wines, the hexanoic acid was in a high OAV and its concentration was significantly increased by LR treatments, which led to an increased fatty aroma in wines.

3.6. *Dissecting the influence of different leaf removal choices on volatile compounds of wines*

The previous analysis focused on dissecting the variations of volatile compounds between LR treatments and control. There were two aspects that still remained to be investigated: leaf removal at different times and the carry-over effect on volatile compounds of wines.

3.6.1. *Distal leaf removal in different times*

In the present study, two time points were chosen to apply distal removal treatments: onset of véraison and post véraison. The two-way ANOVA was used to select the marker compounds that showed significant differences between LR1 (leaf removal at the onset of véraison) and LR2 (leaf removal at post véraison), as well as affected by vintage and their interaction effect, as shown in Supplementary Table 6. Nineteen volatiles showed significant differences between LR1 and LR2, including eleven esters, three acids, three alcohols and two terpenes. Compared to LR1, LR2 significantly increased the concentrations of most ethyl esters such as ethyl octanoate, ethyl butanoate, ethyl hexanoate, ethyl decanoate, ethyl 9-decenoate and ethyl dodecanoate in vintage 2018 and 2020 while showing no significant differences in 2019. Ethyl esters were formed via condensation of fatty acid-CoA with ethanol during fermentation and mainly contributed to the fruity aroma of wines. The timing of bunch exposure and berry maturity might be both responsible for the concentration of ethyl esters in wine, but how these two factors influence ester level in wine needs to be further investigated (Wang et al., 2018). In the present study, the clusters of LR2 were exposed later than LR1 and harvested earlier than LR1. The later removed leaves in LR2 might also provide an adequate source for primary metabolites. In a previous study, the latter and moderate leaf removal treatment accumulate more abundant amino acids in grapes than early leaf removal (Yue et al., 2019), which could produce more esters in wines during fermentation. Although LR2 increased the concentration of β-Linalool in wines compared to LR1, there were no significant differences in grapes between LR1 and LR2.

3.6.2. *Carry over effect*

In the present study, LR treatments were not only performed in the same vines in three consecutive years but also chose different vines from the former vintage in the second and third years. So the carry over effect of LR treatments could be evaluated. Regrading the volatile compounds in wines, the orthogonal partial least-squares discrimination analysis (OPLS-DA) was used to select the marker compounds that showed a difference between 1-LR (LR treatments in the same vines among vintage) and 2-LR (LR treatments in different vines of each vintage), as shown in Supplementary Fig. 4. In 2019, the 2-LR treatments had more abundant benzyl alcohol, ethyl (S)-(-)-lactate, 2-methoxypropanoic acid, 2-phenylethyl acetate, isoamyl acetate, isobutanol etc. than 1-LR, while 1-LR treatments had more abundant *n*-decanoic acid, ethyl hexanoate, 2-nonanol etc. than 2-LR. In 2020, the 2-LR treatments had more abundant α-terpineol than 1-LR, while 1-LR treatments had more abundant citronellol, isoamyl octanoate, hexyl acetate, isopentyl hexanoate than 2-LR. Among all selected compounds, only hexyl acetate

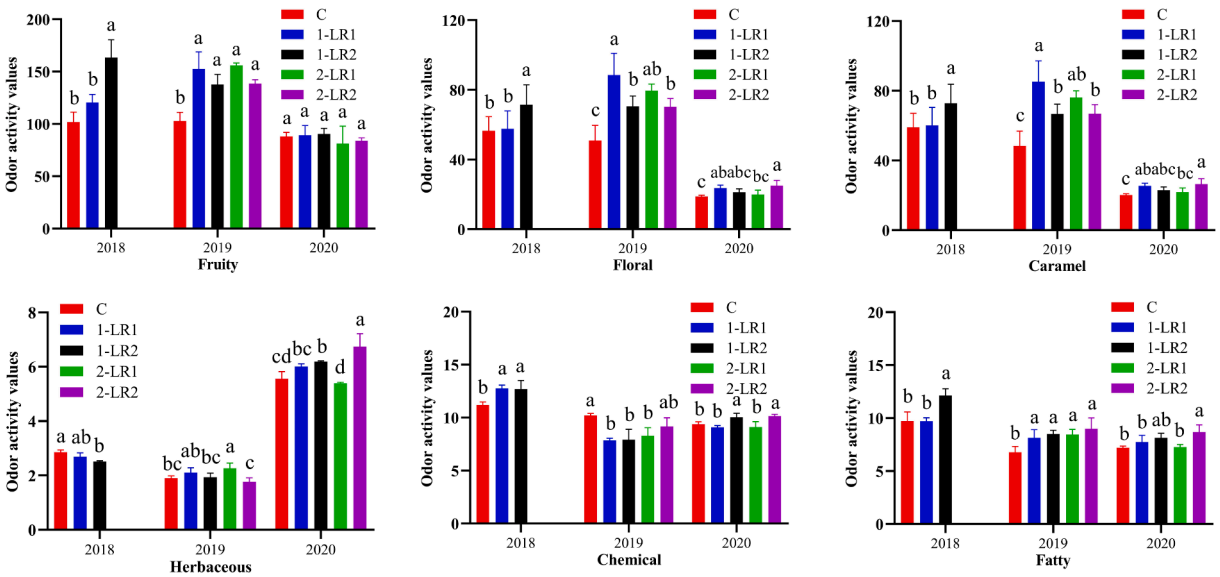

Fig. 5. Effects of distal leaf removal treatments on odor activity values in Cabernet Sauvignon wines in 2018, 2019 and 2020 growing seasons. Different letters within a plot indicate significant differences among treatments (Duncan's multiple range test at $p < 0.05$).

showed a consistent result in two years and it had a higher concentration in 1-LR treatments than 2-LR. Other compounds such as benzyl alcohol, phenylethyl alcohol and isoamyl acetate showed the opposite result in two years. So there was few carry over effect caused by LR treatments on volatile compounds in wines, which was in agreement with our previous study that whether LR in the same vines over consecutive years had limited effects on phenolic profiles and vine growth parameters (Lu et al., 2022b).

3.7. Sensory analysis of wines

Fig. 6 shows the sensory evaluation of Cabernet Sauvignon wines obtained from control (C) and LR treated grapevines in the 2018–2020 seasons. Supplementary Table 7 shows the appearance, aroma, taste, overall judgement, and total scores of each wine. The LR treatments had a higher total sensory score than control in 2018 and 2020 while showing the opposite result in 2019. In 2018, LR2 wine had a higher total score than control and LR1, especially in aroma performance (Supplementary Table 7), which was in agreement with the previous

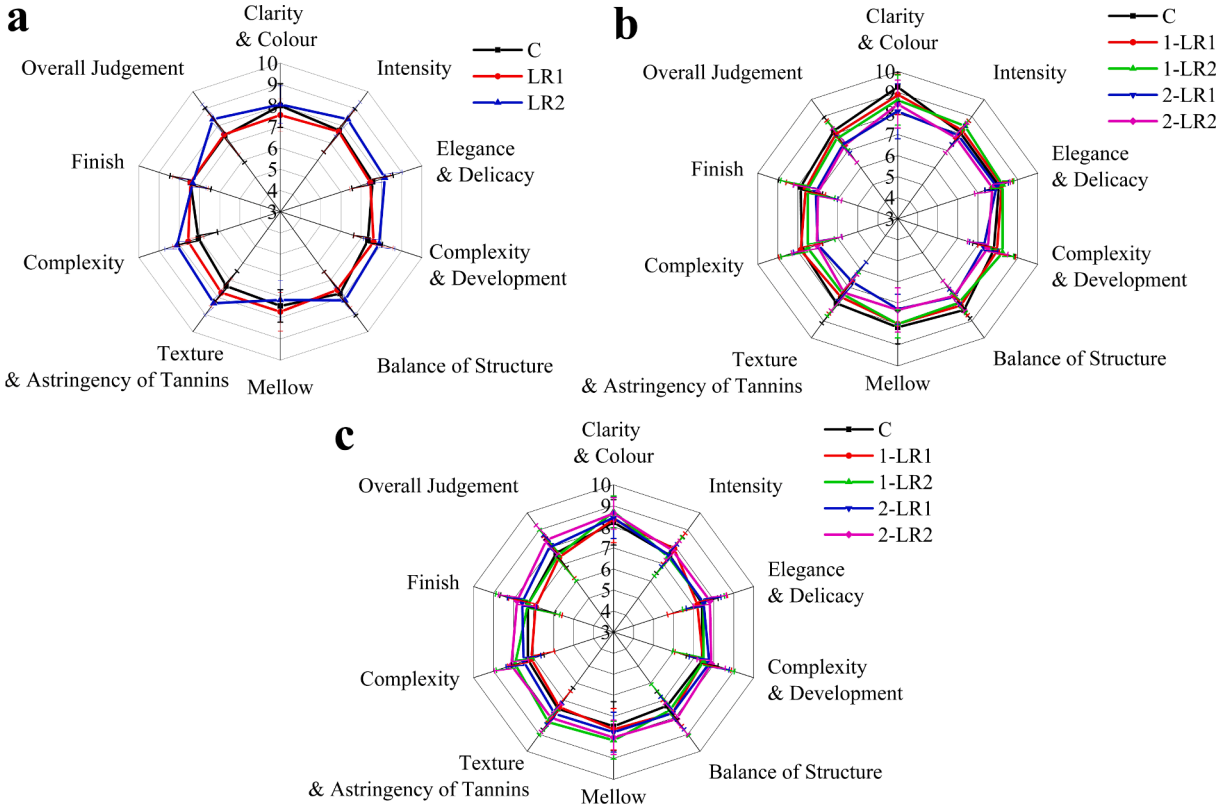

Fig. 6. Effects of distal leaf removal treatments on the sensory profile of Cabernet Sauvignon wines in 2018 (a), 2019 (b) and 2020 (c) growing seasons.

analysis that LR2 wine had the highest OAV in fruity, floral and caramel. In 2019, the LR wines had a worse appearance and taste scores than control, which led to the lower overall judgment and total score in LR wines. This was might because the LR wines had lower concentrations of anthocyanin derivatives and LR grapes had lower concentrations of free flavanols and proanthocyanidins than control in 2019, which was shown in our previous study (Lu et al., 2022b). However, in aroma performance, only 1-LR2 had a lower score than control in 2019. Although the lower total scores occurred in LR wines, the improvement of aroma by LR wines could also be confirmed, even though the lower color and taste score in LR wines might leave an unsatisfied impression to judges. In 2020, the LR wines had better performances in appearance, aroma and taste than control except for 1-LR1. Notably, the LR2 wines had higher aroma scores than LR1 in 2018 and 2020, which was in agreement with our previous study that LR2 wines had more abundant esters and β-linalool than LR1 wines in 2018 and 2020. As for the carry over effect, there was also no consistent result when comparing to 1-LR and 2- LR wines in 2019 and 2020.

4. Conclusion

In this study, distal leaf removal was found to be beneficial for the accumulation of C6 alcohols, terpenes and norisoprenoids in grapes due to the moderate exposure of clusters and more balanced source-sink vines caused by LR treatment. However, the increased C6 alcohols and terpenes in LR grapes did not show in LR wines. The increased esters and (*E*)-β-damascenone were found in LR wines which caused a higher fruity and floral aroma intensity. Compared to leaf removal at the beginning of véraison, leaf removal at post-véraison had more ethyl esters concentrations in wines. The carry-over effect did not show in LR wines which indicated that LR in consecutive years in the same vines was practical for viticulture to face global warming and delay ripening.

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CRediT authorship contribution statement

Hao-Cheng Lu: Formal analysis, Data curation, Investigation, Writing – original draft, Visualization. **Li Hu:** Software, Investigation. **Yao Liu:** Software, Investigation. **Chi-Fang Cheng:** Resources. **Wu Chen:** Resources. **Shu-De Li:** Resources. **Fei He:** Supervision. **Chang-Qing Duan:** Supervision. **Jun Wang:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100449.

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