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Late-season source limitation practices to cope with climate change: delaying ripening and improving colour of Cabernet-Sauvignon grapes and wine in a hot and arid climate

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ABSTRACT

The current rise in temperatures is hastening grape ripening, resulting in unbalanced wines with high alcohol content but poor colour, aroma and phenolic composition. Late-season canopy manipulation practices aimed at reducing the size of the photosynthetic apparatus after veraison have been shown to delay ripening in cooler climates. However, these methods have not directly been compared in regions with high irradiance and temperature, where berry composition is negatively affected by late fruit exposure.

In this two-year Cabernet-Sauvignon trial, we compared the application of pinolene (P) to late shoot topping (T) and late above-bunch zone leaf removal (LR), all performed during the late stages of ripening, with an untreated control (C). Vine water status was monitored weekly from the time of treatment application until harvest. Gas exchange was monitored only in 2021, from treatment application through to harvest. Berry samples were collected to measure berry weight, total soluble solids, pH, titratable acidity, and the anthocyanin profile. At harvest, we assessed yield components, rated sun damage on clusters, and produced experimental wines. Wine colour and chemical characteristics were evaluated.

The application of P scarcely improved gas exchange and stem water potential. Conversely, LR and T increased water potential but did not affect leaf gas exchange. T was the only treatment that significantly decreased sugar accumulation, followed by P, which showed some effect although not significant. Grape yield was not altered, despite the use of P resulted in a lower berry weight in 2022. Canopy manipulation increased lighting conditions in the canopy and caused a significant presence of damaged fruits, especially in LR. Berry anthocyanins were not affected, but LR resulted in a lower concentration of total anthocyanins in the wine and altered the anthocyanin profile. P increased wine acidity, while LR and T improved wine colour.

This study suggests that late-season canopy management can effectively control ripening speeds and improve the composition of grapes and wines. However, it is essential to avoid grape overexposure by employing the appropriate techniques to ensure grape quality is not compromised.

KEYWORDS: climate change, canopy management, antitranspirants, sunburn, anthocyanins

INTRODUCTION

Viticulture is highly susceptible to significant alterations in terroir elements and grape production due to climate change (De Toda & Balda, 2015; van Leeuwen *et al.,* 2019a; Bonfante & Brillante, 2022). Particularly, high temperatures can cause ripening imbalances, such as an uncoupling of sugar accumulation and anthocyanin synthesis (colour compounds) (Rienth *et al.,* 2021). Wine regions with warm climates, such as California, are particularly prone to these changes, which are exacerbated by exposure to intense sun radiation. Prolonged exposure to the sun directly degrades quality compounds in grapes, leading to potential crop loss through sunburn, especially in severe cases (Torres *et al.,* 2020; Martínez-Lüscher *et al.,* 2017a; Martínez-Lüscher *et al.,* 2020).

Cabernet-Sauvignon, as one of the most important red wine grape varieties in California wine production, holds significant value. However, it is highly vulnerable to the ongoing climate change (Gambetta & Kurtural, 2021; Torres *et al.,* 2020; Blancquaert *et al.,* 2019). Elevated temperatures adversely affect all grape varieties, resulting in unfavourable chemical compositions characterised by higher sugar levels and reduced acidity (Keller, 2010; Jones, 2012), while potentially compromising the phenolic composition and anthocyanin concentration (Sun *et al.,* 2023). In the case of Cabernet-Sauvignon, the problem is intensified by a pronounced disparity between sugar accumulation and phenolic maturity. While sugars accumulate rapidly, the development of anthocyanins and tannins lags (Blancquaert *et al.,* 2019).

As a consequence of these issues, it is essential to find cultural practices that can improve resilience to climate change. Some of them, like late-season irrigation (Previtali *et al.,* 2022; van Leeuwen *et al.,* 2019b) or shading nets (Martínez-Lüscher *et al.,* 2020, Pallotti *et al.,* 2023), have already proven to be effective, but expensive or not adapted to the increasing dry conditions. Given the raising cost and limited availability of labour, cultural practices suitable for adaptation to climate change must be mechanisable and sustainable, and therefore they are limited to canopy management, such as leaf removal and shoot topping, or the use of antitranspirant sprays.

Antitranspirants limit gas exchange either by creating physical barriers on the leaf surface or by promoting stomata closure, resulting in reduced photosynthesis and delayed sugar accumulation. The p-menth-1-ene dimer (Cyclohexene, 1-methyl-4-(1-methylethyl)-, dimer, $C_{20}H_{34}$), also known as pinolene, has been demonstrated to form a thin film on the leaf surface, leading to decreased transpiration in both leaves and grape bunches, as well as reduced net CO_2 assimilation (Fahey and Rogers, 2018). Moreover, it has been shown to enhance water use efficiency in treated vines (Palliotti *et al.,* 2013a; Di Vaio *et al.,* 2019; Silvestroni *et al.,* 2020). This led to reduced berry dehydration (Fahey & Rogers, 2018) and a significant decrease in sugar accumulation (Brillante *et al.,* 2016; Di Vaio *et al.,* 2019; Silvestroni *et al.,* 2020), resulting in wines with lower alcohol content (Palliotti *et al.,* 2013a; Brillante *et al.,* 2016; Di Vaio *et al.,* 2020). While Di Vaio *et al.* (2019) reported an increase in total anthocyanin content in the musts, phenolic substances generally appear to have a negative response to pinolene application, leading to lower concentrations of these compounds (Palliotti *et al.,* 2013a; Brillante *et al.,* 2016; Di Vaio *et al.,* 2020; Silvestroni *et al.,* 2020).

Late-season source limitation practices (Previtali *et al.,* 2022), can also slow down sugar accumulation and acid degradation by modifying the leaf area to yield ratio (Bobeica *et al.,* 2015; Van Leewuen *et al.,* 2019b), similarly to late leaf removal and late shoot topping. Furthermore, since vine water consumption is directly linked to leaf area, reducing the leaf area can limit water use (Williams & Ayars, 2005), making canopy management a viable approach to modulating water status and adapting vines to annual climate variability (Pascual *et al.,* 2015). However, the effect of canopy size reduction on water consumption is not always easily predictable, as it is influenced by the interactions of vine growth with environmental conditions (Poni *et al.,* 2023) and the degree of the reduction obtained (Abad *et al.,* 2018).

Leaf removal is a viticultural practice commonly used to modulate grape composition and microclimate. The timing of this practice impacts its effectiveness (Bledsoe *et al*., 1988) due to changing canopy photosynthetic functionality over time (Poni *et al.,* 1994). Early intervention decreases fruit-set and bunch compactness, enhancing quality and rot tolerance (Intrieri *et al.,* 2008; Sivilotti *et al.,* 2016; Poni & Gatti, 2017; VanderWeide *et al*., 2021, Mataffo *et al.,* 2023). Late interventions near veraison slow ripening without affecting acidity or must pH (Lanari *et al.,* 2013; Palliotti *et al.,* 2013b; Caccavello *et al.,* 2017; Poni & Gatti, 2017; Lu *et al.,* 2022). Leaf position is another important factor. Removing basal leaves increases grape exposure, which boosts anthocyanin and polyphenol content while reducing humidity (Tarricone *et al.,* 2020; Cataldo *et al.,* 2021; Iorio *et al.,* 2022). In contrast, removing apical leaves helps prevent overexposure, thereby moderating sugar accumulation and delaying berry ripening (Zhang *et al.,* 2017; Vercesi *et al.,* 2024).

Similarly, late shoot topping proved to be an effective tool to delay berry ripening (De Toda *et al.,* 2013; Filippetti *et al.,* 2014; Valentini *et al.,* 2018; Lu *et al.,* 2023), and the reduction of the canopy may also reduce vine water use and subsequent stress conditions (Poni *et al.,* 2023).

The impacts on phenolic composition exhibit varying degrees of consistency. Certain studies indicate heightened anthocyanin concentrations with the implementation of leaf removal (Verdenal *et al*., 2019; Alatzas *et al.,* 2023) and shoot topping (De Toda *et al.,* 2013; Tessarin *et al.,* 2017). Conversely, other investigations report no consequential effect in anthocyanins (Filippetti *et al.,* 2014; Valentini *et al.,* 2018; Lu *et al.,* 2022; Tessarin *et al.*, 2022). Lanari *et al.* (2013) found different effects on the anthocyanins content of Montepulciano and Sangiovese, highlighting cultivar-specific responses. The efficacy of these practices is further influenced by the severity of implementation and the degree of bunch exposure. Both low and high grape exposure to sunlight hinder anthocyanin accumulation (Chorti *et al.,* 2010). However, grape overexposure, especially late in the season as temperatures rise, can reduce their accumulation. In fact, Cabernet-Sauvignon grapes showed significant anthocyanin reduction at 35 °C respect to 25 °C (Mori *et al.,* 2007). Similar outcomes were noted in other studies as well (Bobeica *et al.,* 2015; Caccavello *et al.,* 2017; Cataldo *et al.,* 2021).

The application of these practices needs to be reconsidered in a more cautious way (Poni *et al.,* 2023), to avoid sunburns and overheating, particularly in light of the effects brought about by climate change.

In this study, we compared three different degrees of source-limitation practices with various impacts on photosynthesis and on the fruit zone microclimate, particularly shade onto clusters. We compared above-cluster leaf removal, to the use of an antitranspirant spray (pinolene), reported to reduce A_N more than g_s (Brillante *et al.,* 2016) and used as a source-limitation agent in previous works (Palliotti *et al.,* 2010 among others), to shoot-topping, all performed during grape ripening. Our hypothesis:

- Above cluster leaf removal will have the mildest effect on source limitation and a moderate impact on cluster exposure, as the leaves that will be removed will not be at their maximum photosynthetic activity and should not expose the clusters nor offer sun penetration during the central part of the day.
- Pinolene will have a moderate impact on source limitation and no impact on cluster exposure; as leaves will not be removed but the photosynthetic efficiency of the whole canopy will be reduced (although mildly at the leaf level);
- Topping will have the strongest impact on source limitation, as it will remove the youngest leaves with higher photosynthetic activity, and the strongest impact on cluster exposure, as it will remove the portion of the canopy hanging and shading the sides, while also increasing sun penetration from the top during midday.

All the treatments were compared to an untreated control, with the aim to provide a comprehensive evaluation of their effects on plant physiology, grape, and wine quality in the hot and arid climate conditions of the San Joaquin Valley of California, the largest vineyard area of the USA.

MATERIALS AND METHODS

1. Experimental site and plat material

The trial was carried out at California State University Fresno (Fresno, CA), in a twenty-five-year-old vineyard planted with *Vitis vinifera*, L. cv. Cabernet-Sauvignon on Freedom during the 2021 and 2022 growing seasons. Plants were spur-pruned (13.3 two-bud spurs/m) in a bilateral cordon and trained in a California Sprawling system. Cordon wire was positioned at 140 cm from the ground and a foliage support wire was placed at 30 cm above the cordon wire. Rows were oriented east-west and vine spacing was 1.8 m between the plants and 3.6 m between the rows. Plants were drip-irrigated during the whole growing season according to the grower's practices, corresponding roughly to 3,000 m^3 water/ha total (300 mm). The actual crop coefficient of these plants was not measured, but it is estimated to fall between 0.7 and 1 at full canopy size, according to visual assessment of the percent shaded area (Williams & Ayar, 2005). Meteorological data were taken from California Irrigation Management Information System (CIMIS) of the California Department of Water Resources (DWR) network station installed on site.

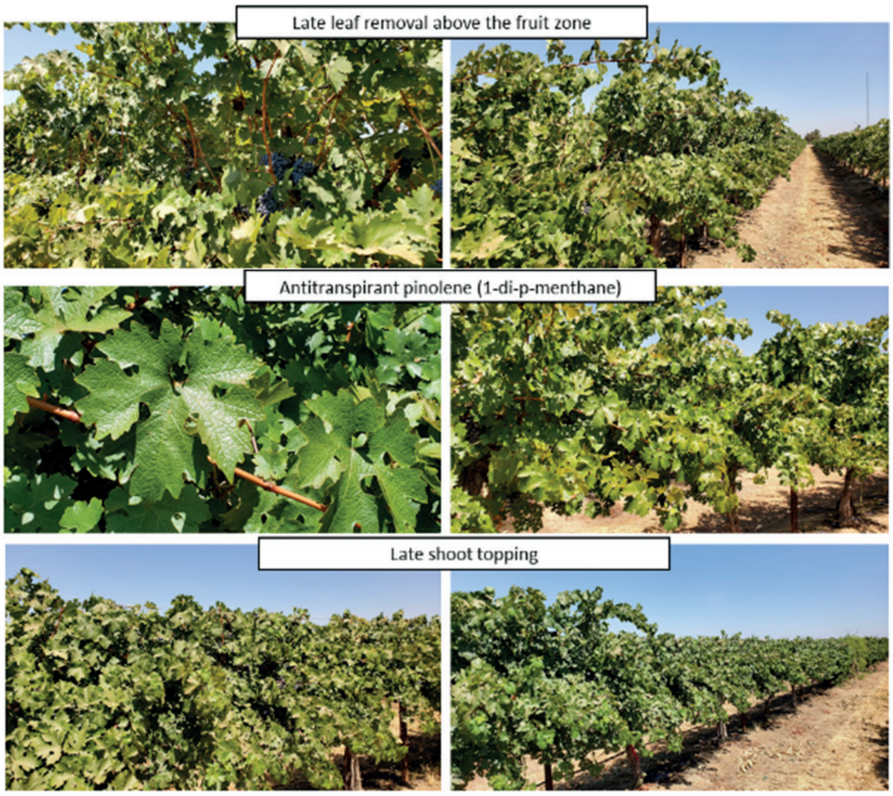

FIGURE 1. Treatments applied in this study.

2. Experimental design and treatment application

The trial was conducted using a completely randomised block design, with four treatments and four replicates of 8 vines (32 vines per treatment). Treatments consisted of: i) untreated control (C), ii) late leaf removal above the fruit zone (LR), iii) antitranspirant pinolene spraying (P), and iv) late shoot-topping (T), as shown in Figure 1.

All treatments were applied manually at stage 36 of the modified EL scale (Coombe *et al.,* 1995), on 20 August 2021 and 12 August 2022. The intent of the treatments was to reduce photosynthetic activity while maintaining shade. Leaf removal was performed by removing about 30 cm of leaves in the middle portion of each shoot. Pinolene [di-1-p-menthene ($C_{20}H_{34}$), Vapor Gard, Miller Chemical, USA] was mixed in a 5% solution with water and sprayed until the point of runoff with a backpack sprayer on the whole canopy. Topping was performed by removing the apical portion of the shoots at a height of 60 cm above the cordon wire. With topping the aim was to remove the most photosynthetically active apical leaves at the expense of greater sun penetration. With above-cluster leaf removal (mid-section of the shoots), the aim was to reduce sun penetration in the fruit zone compared to apical topping at the expense of removing less photosynthetically active leaves. The occasional higher cluster on very tall spurs was exposed, but that was a negligible percentage of fruit. With pinolene, the aim was to limit photosynthesis through a reduction in gas exchange while maintaining shading from all the leaves on the canopy.

3. Canopy measurements

The total leaf area per shoot was determined using a leaf area meter (LI-3100, LiCOR, NE, USA), on four shoots per replicate sampled from four different vines (for a total of 16 shoots per treatment) on 30 September 2021 and 23 September 2022. Light canopy interception was measured at noon on 10 September 2021 and 9 September 2022 using a line quantum sensor (LI-191R, LiCOR, NE, USA) inserted in the fruit zone and rightly above and parallel to the cordon, on four plants per replicate. Sunlight interception was calculated as % of ambient photosynthetic active radiation (PAR). Prior to data acquisition in each experimental unit, ambient light was acquired in an open road close to the field.

4. Leaf gas exchange and solar noon stem water potentials

Leaf gas exchange was measured weekly during the central hours of the day (11:30–13:30 solar time) from early September until harvest. In each replicate, four mature and sun-exposed leaves from four grapevines were sampled from the middle section of the main shoot. Measurements were taken using a LI-6800 portable photosynthesis system (LiCOR, NE, USA). Carbon assimilation rate (A_N, µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (g_s, mmol H_2O m^{-2} s^{-1}) were acquired by measuring inlet and outlet CO_2 (set at 410 ppm) and H_2O relative concentration (following ambient relative humidity at the time of the measurement). The intrinsic water use efficiency (WUEi) was derived as the ratio between net assimilation (A_N) and stomatal conductance (g_s).

Stem water potential (Ψ_{stem}) was measured on one fully expanded leaf per vine on four vines per replicate weekly from the application of the treatments to harvest. One hour before the measurement, each leaf was bagged with a mylar bag, and between 12:30 and 14:30, leaves were sampled and measured for water potential using a portable pressure chamber (PMS Instruments, OR, USA).

5. Grape composition

For each year of the study, grape ripening was monitored weekly, starting from 27 August 2021, and 12 August 2022, until harvest. In 2021, as the vineyard was commercially hand-harvested, the plants that were not used for yield assessment were held out from harvest and sampled for grape composition two weeks after the harvest date. Two samples of 24 and 48 berries per replicate were taken. The 24-berry set was used for the analysis of anthocyanin, while the 48-berry set was used for the analysis of total soluble solids, pH, and titratable acidity.

The 48-berry samples were crushed. The juice was used to determine total soluble solids (TSS), through digital refractometry (Fisherbrand, Thermo Fisher Scientific Inc.), pH was measured using a pHmeter (Accumet, AB315, Thermo Fisher Scientific Inc.), and the titratable acidity (TA) was measured through automatic titration with NaOH, 0.1N until a pH of 8.2 (Easy Plus, Mettler-Toledo, LLC, OH, USA).

The 24-berry sets were weighted and frozen to be later used to measure anthocyanins as described in Martínez-Lüscher *et al.* (2019). The skins were separated from the flesh with a scalpel, freeze-dried and then ground. An aliquot of 50 mg of the powder was extracted with methanol: water: 7 M hydrochloric acid (70:29:1, V:V:V). Extracts were filtered (0.45 µm, Thermo Fisher Scientific, San Jose, CA, United States) and analysed using reversed-phase high-performance liquid chromatography (HPLC) coupled to a diode array detector (DAD). The HPLC system was an Agilent 1260 series (Agilent, Santa Clara, CA, United States) with a reversed-phase C18 column LiChrospher® 100, 250 mm × 4 mm with a 5 µm particle size and a 4 mm guard column of the same material. Flow was set to 0.5 ml per minute and column temperature was set to 40 °C. Two mobile phases with a concentration of 5.5 % aqueous formic acid and formic acid in acetonitrile were used to ensure the following proportions (V/V) of acetonitrile were maintained: 0–8 min 8 %, at 25 min 12.2 %, at 35 min 16.9 %, at 70 min 35.7 %, 70–75 min 65 %, and 80–90 min 8 %. Peaks from chromatograms were then identified, observing the absorbance at 520 nm, and the area of each peak was compared to malvidin standards obtained routinely during data acquisition and used to determine the equivalent concentration of the various anthocyanins.

6. Assessment of vine yield and sun damage

At the time of the commercial harvest (30 September 2021, and 23 September 2022), yield components were assessed as total yield per vine, the number of clusters per vine, and average cluster weight; average berry weight was instead obtained on berry samples as described earlier. In 2021, 100 clusters per treatment were used to quantify visible damages due to excessive solar radiation using a scale of no damage, mild damage, moderate damage, and severe damage as reported in Martínez-Lüscher *et al.,* 2019 (a picture scale can be found in Figure 2 of this reference and it was used for the current study). In 2022, the same measurement was carried out taking samples of 25 clusters per replicate.

7. Wine making

Grapes from each treatment were manually harvested into picking bins of a max. 8 kg. The harvest date corresponded to the commercial picking decision for the field, when grapes reached 24 °Bx. Bins were stored in a cold room at 10 °C for 3 days. Each treatment was crushed separately, and must lots of 38 L were created (3 replicates per treatment, total of 12 independent fermentations). Potassium metabisulfite was added at 30 ppm to every wine lot at crush. All wines were inoculated with the same commercial yeast (*Saccharomyces cerevisiae*, Enartis WS) according to standard dosage recommendation (25 g/hL). Yeast assimilable nitrogen (YAN) was adjusted in each treatment by adding diammonium phosphate (DAP) to reach a concentration of YAN (in mg/L) equal to ten times the content in soluble solids (in °Brix). Cap management was done by manual punch-downs once a day in the morning, and must samples were collected for monitoring the progress of fermentation by measuring sugar concentration and temperature. At the end of alcoholic fermentation, wines were pressed using a vertical press, racked into 19 L stainless steel kegs, and allowed to settle in a cold room at 50 °F for four weeks. After settling, wines were racked and filtered using a benchtop filter (Buon Vino Super Jet Filter) with cellulose pads of 5–7 microns pore size. Free sulfur dioxide was adjusted in all finished wines to 30 ppm. Wines were bottled in 750 mL bottles with agglomerate corks and kept in storage at 10 °C until analysis.

8. Wine Analysis

Must and finished wine analysis include titratable acidity (TA), pH, and °Brix. Fermentation °Brix and temperature were monitored with a hydrometer and digital thermometer (Easy Dens, Anton Paar). Colour intensity (absorbance at 420, 520, and 620 nm) was measured with a Perkin Elmer Lambda 25 Spectrophotometer using quartz cuvettes of 2 mm path length. Wine anthocyanins were measured using HPLC-DAD with the same method described in section 5 of the Materials and methods for grapes.

9. Statistical analysis

Statistical analysis was performed using R version 4.3.1– package “agricolae” (de Mendiburu, 2019). Data were subjected to one-way analysis of variance (ANOVA). When the results of ANOVA were significant at $p \leq 0.05$, data were then subjected to Tukey’s HSD test. Graphical representations were also obtained with R.

RESULTS AND DISCUSSION

1. Meteorological conditions

Meteorological data are shown in Figure 2 and Table S1. Considering the period from 1 April to 31 October, minimum temperature averages were slightly lower in 2021 (-0.3 °C), while both mean temperatures and maximum temperatures were higher in 2022 (+0.6 °C and +0.4 °C respectively). In 2022 a higher number of days with temperatures exceeding 38 °C was also registered. Even though both seasons were particularly dry, especially during summer, in 2022 the rainfall from April to October was half that of 2021. Additionally, in 2022, there was less rainfall during the first months of the year compared to 2021, which probably made it more challenging to establish sufficient water reserves in the soil for the growing season. In 2022, the air temperatures recorded during the period between the application of treatments and the harvest were higher compared to 2021. Mean and maximum temperatures surpassed 30 °C and 40 °C respectively, particularly towards the latter part of August. Total ET_0 was of 1,551 mm in 2021, with 1,280 mm from 1 April to 31 October, and 1,632 mm in 2022, with 1,338 mm from 1 April to 31 October.

2. Leaf area and light interception

Figure 3 shows the total leaf area/shoot for both growing seasons, including leaf area on laterals. In both years, C and P showed the highest values that were not different, as they

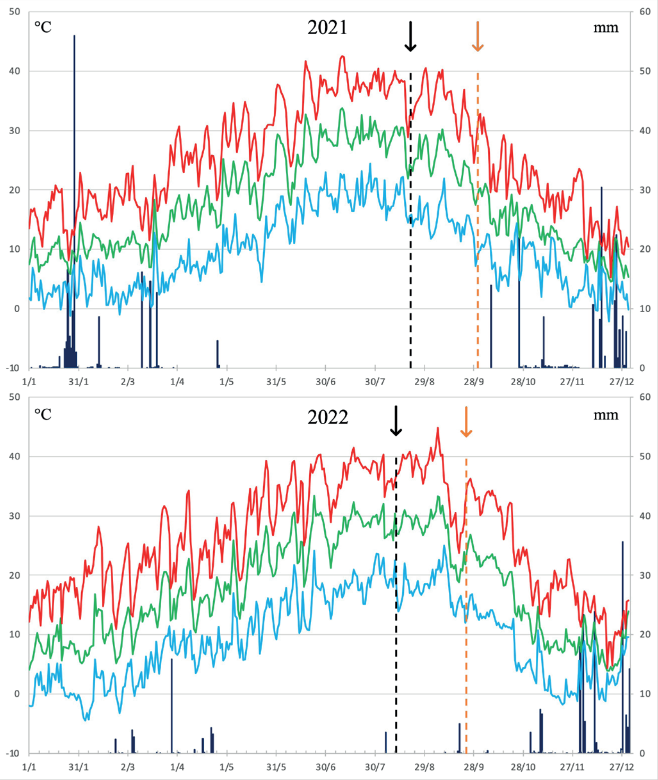

FIGURE 2. Climatological trends in 2021 and 2022 in the study area. Red, green, and cyan lines indicate maximum, mean, and minimum air temperatures (°C) respectively. Blue bars indicate daily rainfall (mm). Black arrows and dashed lines indicate the treatment application date. Orange arrows and dashed lines indicate the harvest date.

were not subjected to canopy manipulation. Specifically, the highest value was registered for control vines (2,715 cm^2) in 2021, and for pinolene (3,054 cm^2) in 2022.

In both years, compared to the control, canopy manipulation led to a significant decrease of total leaf area. Amongst all treatments, T registered the lowest values in both years, with 1,049 cm^2 and 845 cm^2 in 2021 and 2022 respectively. Similarly, LR caused a reduction of total leaf area, leading to 1,582 and 1,882 cm^2 for the two years of analysis. Although the differences between the two canopy manipulation practices were not statistically significant, T was a more aggressive practice that reduced shoot leaf area by 61 % and 62 %, while LR reduced it by 42 % and 23 % in 2021 and 2022 respectively. Reductions in total leaf area/shoot with canopy-manipulation practices were similar to those reported in other studies (Palliotti *et al.,* 2013a; Filippetti *et al.,* 2014; Valentini *et al.,* 2018; Lu *et al.,* 2022; Tessarin *et al.,* 2022).

The percentage of light in the fruiting zone with respect to the ambient light was affected by the treatments (Figure 3). As T was the treatment that reduced shoot leaf area, it led to the highest exposure of clusters at noon compared to the other treatments. Specifically, in 2021 light percentage in T vines was significantly increased by 8 % and by 11 % in 2022 with respect to C. Although not significant, LR also exhibited a trend towards higher exposure of the fruiting zone (6 % in 2021 and 11 % in 2022). It's important to note that the measurements were taken at noon, when the sun was perpendicular to the ground, while leaves were removed on the sides (Figure 1), therefore increasing light penetration when the sun was oblique to the ground. It can be assumed that if measurements had been taken in the morning or afternoon, the percentage of light in the fruit zone would have been higher and possibly significantly different from C. C vines exhibited the lowest light percentage in the fruit zone in both years, at 4 % and 6 % respectively. P and C vines were not significantly different, although slightly higher values were recorded for P, but this variation is not directly attributed to the treatment itself, although P increased the glossiness of leaves (Figure 1), which could have an impact on the amount of reflected light.

3. Midday stem water potential

Trends for the stem water potential (Ψ_{stem}) of the four treatments are shown in Figure 4. Values in 2022 were generally lower, indicating an elevated stress condition in the vines because of the higher temperatures and reduced rainfall during that vintage (Figure 2). In 2021, P leaves exhibited the lowest value (-1.77 MPa), which coincidentally is also the minimum value recorded across both years. In 2022, both P and LR treatments recorded the lowest value (-1.65 MPa). In both vintages, T vines recorded higher values compared to the other treatments, especially at the end of the season, and this effect was significant in most of the measurement dates. This effect is related to the strong reduction of the canopy that was induced with the treatment, removing more than 60 % of total leaf area in both years. Poni *et al.* (2023) already reported that summer pruning practices can reduce

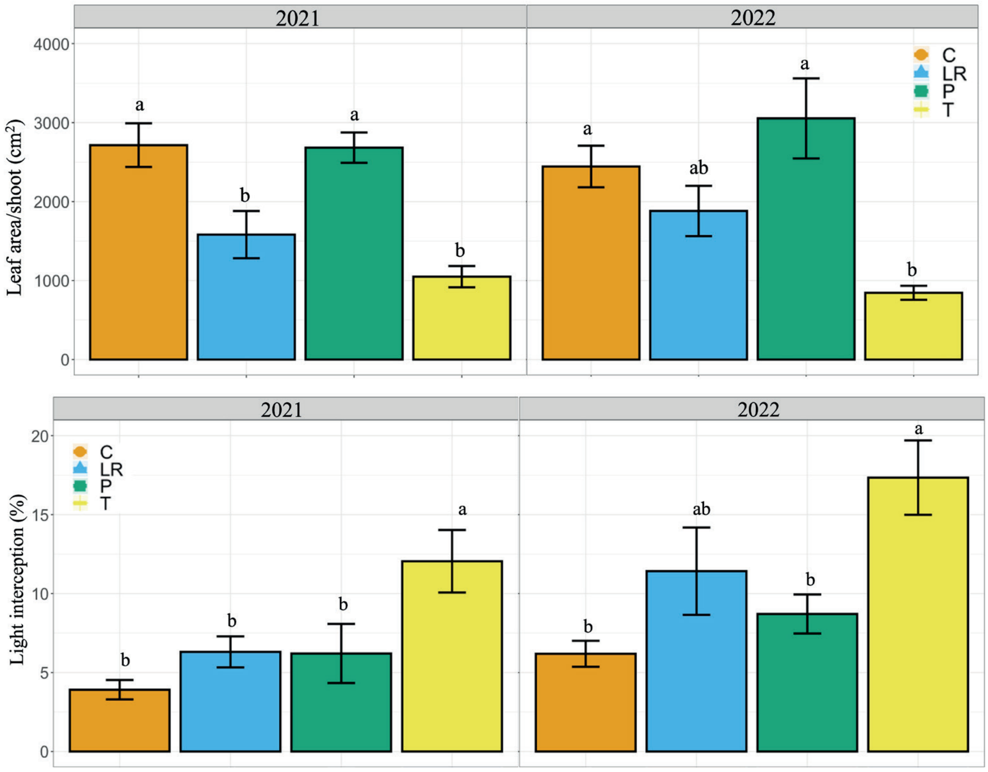

FIGURE 3. Total leaf area/shoot and percentage of light in the fruiting zone in 2021 and 2022 for the four treatments: control (C), late above-cluster leaf removal (LR), pinolene (P) and late topping (T). When ANOVA was significant, different letters indicate differences between treatments with p-value ≤ 0.05 (Tukey-HSD test); error bars represent standard error of the mean (n = 16).

single-vine water use, which aligns with our findings. In the case of LR, this trend was observed in 2021 but not in 2022. In this case, the different response is most probably related to the different intensity of the treatment, which was lower in the second year of study, as already discussed. Other authors have shown that in warm climate, reducing leaf area only limits water use in the driest conditions and with high yields (Poni *et al.,* 2006; Abad *et al.,* 2018). Although the second year was warmer, yields were lower. This observation also aligns with Abad *et al*. (2018), who found that canopy reduction effects on water demand are non-linear, with more substantial reductions yielding the best results. Consequently, our results suggest that it is possible to decrease water stress by reducing the total leaf area by at least 40 %, while reductions of less than one-third do not determine mitigations to water stress conditions. Nevertheless, since stem water potential measurements were taken at midday, this may have affected the accuracy of our results. Accuracy can diminish under extreme temperatures and at elevated levels of water deficit (Deloire *et al.,* 2020); therefore, conducting measurements at alternative times could potentially improve precision and sensitivity (Williams & Araujo, 2002; Heyns, 2017).

Although unexpected, pinolene did not alleviate water stress in the treated vines. However, examining the gas exchange values, which will be discussed further, P only showed a tendency to reduce transpiration and stomatal conductance, with minimal to no significant difference compared to C, in contrast to findings from other studies (Palliotti *et al.,* 2013a; Di Vaio *et al.,* 2019). Therefore, it is likely that in this trial, the modulation of gas exchange was not sufficient to significantly reduce water loss and prevent the onset of stress conditions.

4. Leaf gas exchange and water use efficiency

Figure 5 shows leaf gas exchange data in the four treatments in 2021. Considering net assimilation (A_N), T showed the highest values compared to the other treatments, with the maximum value registered at the beginning of the measurements (10.54 µmol CO_2 m^2s^{-1}). On the other hand, P reduced the photosynthetic activity of the treated vines compared to C, even though the observed difference was not statistically significant. In the case of stomatal conductance, g_s, the highest value was 0.21 mol H_2O m^2 s^{-1}, registered by T on the first sampling point date, while the lowest value was 0.08 mol H_2O m^{-2} s^{-1}, registered by both P and LR on the last date. No treatment showed significant differences when compared to C vines. However, T maintained the greatest g_s in all the observation dates, while P the lowest, and this difference was statistically significant on one of the dates. Both g_s and A_N decreased across all treatments as the season progressed; this was a result of increased water stress, as reported in Figure 4, and because of leaf aging, which reduced photosynthetic functionality, consistent with findings by Lupo *et al.* (2023). Further analysis via principal components analysis (PCA), shown in Figure S1, supports these results. As expected, physiological parameters like A_N and g_s were directly correlated with stem water potential. Consequently, treatments that mitigate stress conditions, such as T, demonstrated improved vine performance. In this context, T was the most effective treatment, showing distinct clustering from the control in PCA results.

Pinolene, known for its antitranspirant properties due to its film-forming capacity (Fahey and Rogers, 2018), exhibited similar effects after we applied it to Cabernet-Sauvignon. However, contrary to findings in other studies (Palliotti *et al.,* 2013a; Brillante *et al.,* 2016; Di Vaio *et al.,* 2019; Silvestroni *et al.,* 2020), water use efficiency in treated plants was not enhanced. This may be due in part to the heightened climatic stress during our study period compared to those prior investigations. It is worth noting that the observed reduction in g_s was not statistically significant compared to C, probably due to the more stressful conditions of our trial that diminished C functionality, which might explain the absence of differences

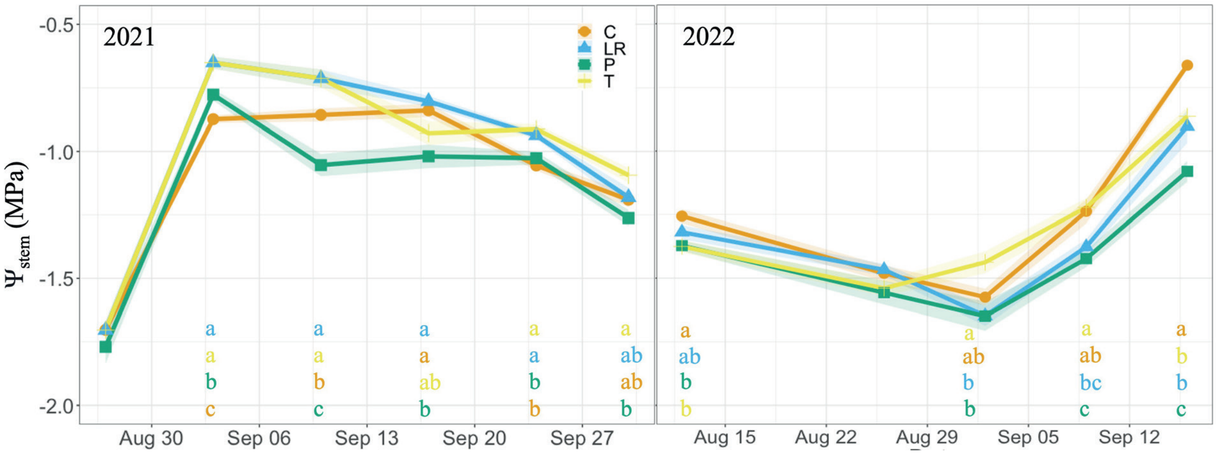

FIGURE 4. Trends for stem water potential (Ψ_{stem}) in 2021 and 2022. When ANOVA was significant, different letters indicates differences between treatments with p-value ≤ 0.05 (Tukey-HSD test). Shaded regions indicate the standard error of the mean. C indicates control, LR indicates leaf removal, P indicates pinolene and T indicates late topping. Treatments were applied on 20 August 2021 and 12 August 2022. Harvest took place on 30 September 2021 and 23 September 2022.

in intrinsic water use efficiency. Although the concentration rate was already higher than that reported in previous studies (Brillante *et al.,* 2016; Di Vaio *et al.,* 2019), it is possible that using a higher concentration of pinolene could yield different results.

Several studies have reported evidence of increased photosynthetic activity as a form of compensation following a reduction in the source-sink ratio (Petrie *et al.,* 2003; Kliewer & Dokoozlian, 2005; Bobeica *et al.,* 2015). In this study, a similar trend was observed in T vines throughout the season, but not in LR vines. We believe that the absence of this result in LR vines may be linked to the smaller reduction in the source (leaves) that these vines underwent, as discussed earlier. Hence, this reduced reduction in the source may not have been sufficient to trigger the overcompensation mechanisms at the leaf level in the plants. This interpretation aligns with the PCA results, where LR showed a weaker correlation with physiological parameters, such as A_N and g_s compared to T.

5. Berry ripening

Figure 6 displays the evolution of total soluble solids and must pH for 2021 and 2022. In 2022, ripening occurred more rapidly than in the previous year, leading to harvest one week earlier. The highest sugar concentration between both years was 27.7 °Brix, recorded for P in 2022.

In 2021, no significant difference in total soluble solids was observed from ripening through harvest for all treatments. T was the only treatment capable of significantly decreasing sugar accumulation, although only at the end of the season (post-harvest date) when more pronounced effects of the applied treatments were observed. Specifically, TSS content was lower in P and T compared to C vines (reductions of -1.4 and -2.1 °Brix respectively), with a significant difference in the case of T. In 2022, sugar concentration in T was the lowest compared to the other treatments, similar to the previous year. However, in contrast to the prior year, P exhibited the highest sugar concentration throughout the entire second half of the season. It was interesting to note that in 2021 sugar accumulation followed a linear pattern with a slow rate, in contrast with 2022, where a fast accumulation rate and a maximum sugar concentration was reached one week prior to harvest, followed by a decrease of approximately 2 to 3 °Bx in LR and P respectively, but not in C and T. Despite at harvest the difference was no longer significant, T reduced the pace of sugar accumulation in the berries. This trend is in accordance with findings from other studies (Filippetti *et al.,* 2014; Valentini *et al.,* 2018; Lu *et al.,* 2023;

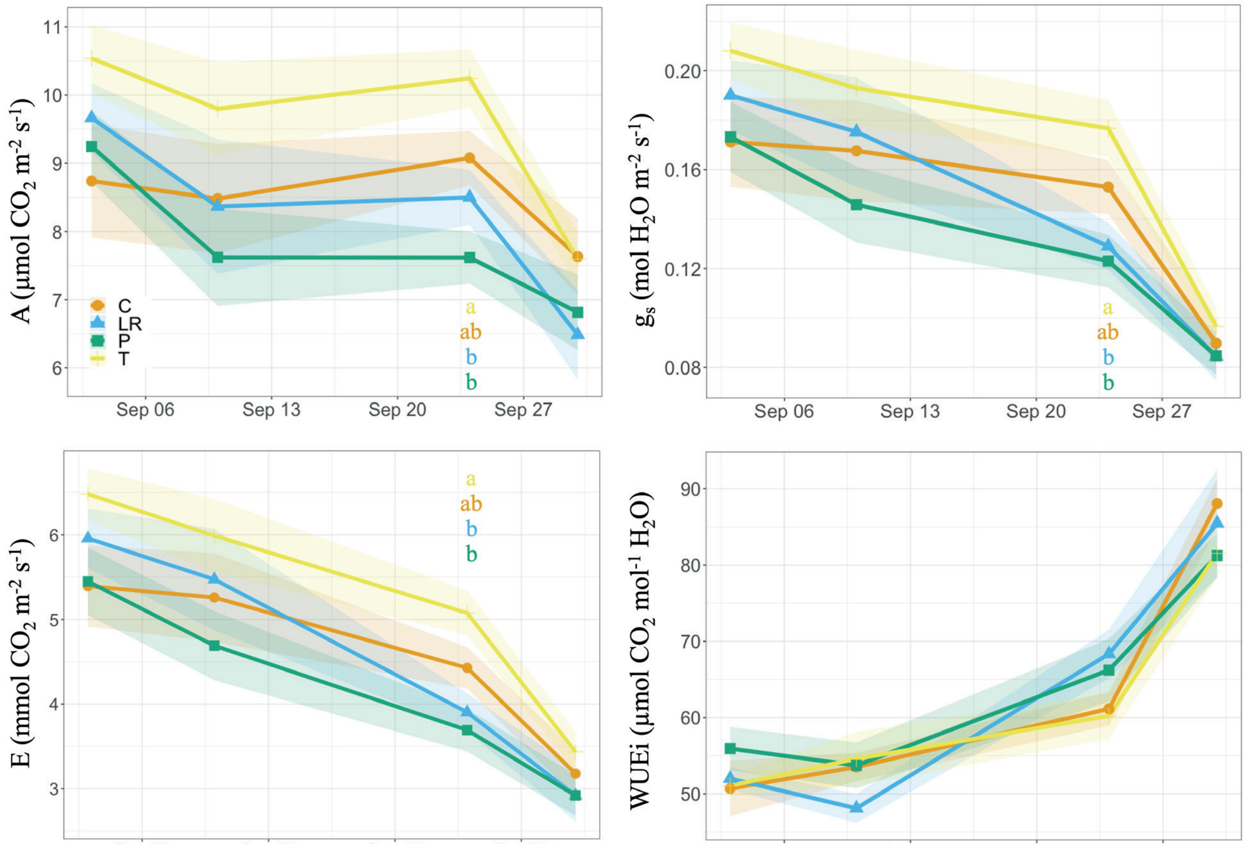

FIGURE 5. Trends for net photosynthesis (A), stomatal conductance (g_s), transpiration (E) and intrinsic water use efficiency (WUEi) in 2021. When ANOVA was significant, different letters indicates differences between treatments with p-value ≤ 0.05 (Tukey-HSD test). Shaded ribbons indicate the standard error of the mean. C indicates control, LR indicates late above-cluster leaf removal, P indicates pinolene and T indicates late topping. Treatments were applied on August 20, 2021. Harvest took place on September 30, 2021.

Poni *et al.,* 2023), which already reported the effectiveness of late trimming in delaying berry ripening.

Several studies have already proven that late leaf removal can delay ripening by slowing down sugar accumulation (Lanari *et al.,* 2013; Caccavello *et al.,* 2017; Poni & Gatti, 2017). However, our results did not show any effect on berry ripening, which is similar to the findings of Tessarin *et al.* (2022). Palliotti *et al*. (2013b) observed that to determine a significant reduction in sugar accumulation, vine leaf area should be reduced by 30–35 %. In our case, this reduction was achieved only in 2021 but not in 2022, as previously discussed. Additionally, to fully understand the mechanisms governing the effects of defoliation, the age and the functionality of the leaves must be considered. As indicated by Poni *et al.* (2023), throughout the season leaf functionality shifts towards the top of the shoots, as leaves at the bottom tend to be older. Therefore, we suggest that the lack of observed effects on grape ripening may be due to the removal of the leaves from the middle section of the shoots, and not from the upper section, thus limiting the impact on vine photosynthesis.

In 2021, differences in must pH between treatments were significant only in the first half of the season. At harvest, none of the treatments produced a significant modification of pH compared to control. The lowest pH value was 4.16, recorded for P, while the highest was 4.74 for LR, the highest value observed in both years. Starting from mid-September, LR consistently had the highest pH among all treatments, while although not statistically significant anymore, both P and T lowered the pH compared to C. In 2022, a similar pattern emerged for P, primarily resulting in the lowest pH compared to the other treatments, with a minimum value of 3.75, also the lowest over both years. However, topping, which had restrained pH in the previous year, showed higher values, while LR, which exhibited the highest values in 2021, led to a lower must pH. Overall, none of the treatments were able to mitigate the drop in pH which is typically associated with the respiration metabolism of organic acids (notably malic acid) in grapes. The high pH values shown across all treatments at harvest would typically require a significant addition of tartaric acid for winemaking purposes in order to decrease pH (ideally below 4.0) and increase titratable acidity.

6. Yield components, grape composition, and sun damage at harvest

Yield components and grape composition at harvest are shown in Table 1. Crop level was higher in 2021 than in 2022, with an average yield per vine of 12 kg and 8.3 kg respectively. Similarly, average berry weight in 2022 was lower by 0.14 g, possibly due to the hotter and drier climate of this year, resulting in increased water stress. Martínez-

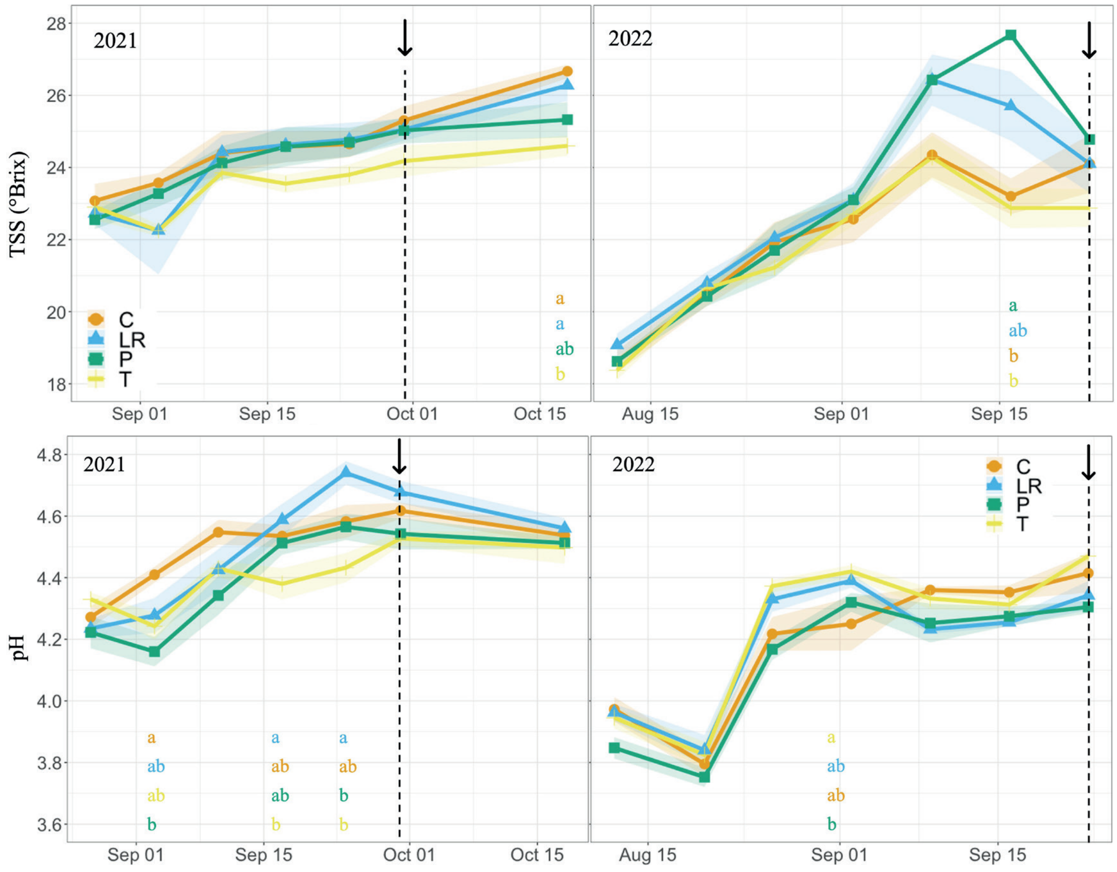

FIGURE 6. Trends for total soluble solids (TSS) and pH in 2021 and 2022. When ANOVA was significant, different letters indicates differences between treatments with p-value ≤ 0.05 (Tukey post hoc test). Shaded regions indicate the standard error of the mean. C indicates control, LR indicates leaf removal, P indicates pinolene and T indicates late topping. Black arrow indicates the harvest date.

TABLE 1. Yield components and grape composition at harvest.

Year	Treat.	Clusters/vine (n°)	Yield/vine (kg)	Berry weight (g)	TSS (°Brix)	Sugars/berry (g)	pH	TA (g/l)	Total anthocyanins (mg/kg)
2021	C	163	12.4	1.06	24.7	0.27	4.54	3.42	232.7
	LR	138	12.0	1.05	24.8	0.26	4.56	3.65	214.2
	P	139	11.2	1.10	24.7	0.28	4.51	3.06	238.8
	T	151	12.4	1.00	23.8	0.24	4.50	2.98	206.6
sign.		ns	ns	ns	ns	ns	ns	ns	ns
2022	C	88 b	9.3	0.99 a	24.1	0.24 a	4.42	4.39	156.4
	LR	144 a	8.8	0.95 a	24.1	0.23 a	4.34	3.86	113.8
	P	86 b	8.0	0.73 b	24.8	0.18 b	4.31	3.95	82.7
	T	127 a	9.2	0.95 a	22.9	0.22 ab	4.47	3.82	150.9
sign.		***	ns	*	ns	*	ns	ns	ns

*Grape composition at harvest for all treatments in 2021 and 2022. TSS indicates total soluble solids, TA indicates titratable acidity, abw indicates average berry weight. In case of significant ANOVA, different letters across a column indicate significant difference between treatments with a p-value < = 0.05 (T-test). C indicates control, LR indicates leaf removal, P indicates pinolene and T indicates late topping. Total anthocyanins in 2021 were measured on a sampling date after harvest, on October 18, 2021.

Lüscher *et al.* (2017b) already reported similar impact on yield in the San Joaquin Valley of California when precipitation before budbreak is reduced, because of reduced cluster number. In 2022, the average cluster number across all treatments was reduced by 33 % with respect to 2021.

Cluster number per vine and average berry weight were not significantly different in 2021, nor was the total yield per vine. In 2022 we observed a higher number of clusters per vine for plants subjected to LR and T and a significantly lower berry weight for P vines, reduced by 0.26 g when compared to the control. A possible explanation regarding the increase in cluster number in LR and T, which lacks data to be confirmed, could be an increase in shoots from latent buds as a result of the increased light in the cordon zone following the first year of treatment. Because these vines are not shoot-thinned, more secondary shoots could have increased the cluster number. This hypothesis is corroborated by the fact that a substantial increase in cluster number did not correspond to a significant increase in yield, which remained similar to C in LR and T, as secondary shoots produce small and light clusters.

Although the total yield per vine in 2022 was not significantly different between treatments, the reduced average berry weight observed for pinolene resulted in the lowest crop level registered in both years, just 8 kg per vine. This was over 1 kg more compared to C in the same year, as well as when compared to the other treatments. In contrast to Fahey and Rogers (2018), who reported that pinolene can reduce berry dehydration in treated plants, our observations showed only a slight increase in berry weight in 2021 and a notable reduction in 2022. As previously discussed, the 2022 vintage was exceptionally hot and dry, certainly impacting the water status of the plants and leading to increased stress conditions compared to the previous year. Therefore, we hypothesise that the reduction in berry weight observed in 2022 is due to pinolene's removal of waxes on the berry surface, thus exposing the grapes to higher dehydration in vintages where plants are particularly susceptible to water stress conditions (example pictures in Figure S2).

Grape must composition at harvest was not affected by the applied treatments. Considering all treatment together, the average total soluble solids content was of 24.5 °Brix in 2021 and 24.0 °Brix in 2022. In both years, the highest total soluble solids content was 24.8 °Brix, measured in LR in 2021 and in P in 2022, while T always showed the lowest values: 23.8 and 22.9 °Brix for 2021 and 2022 respectively. The average must pH was 4.53 in 2021 and 4.39 in 2022, with the greatest value observed in LR in 2021 (4.56) and the lowest in P in 2022 (4.31). Considering the two vintages altogether, titratable acidity was slightly higher in 2022 (4.00 g/L) than in 2021 (average of 3.27 g/L). This result was unexpected, as higher temperatures are known to increase respiration rates and the metabolism of malic acid (Keller, 2020). The lowest value was 2.98 g/L for T in 2021, while the highest was 4.39 g/L for C in 2022. From a winemaking perspective, the possibility of preserving organic acids in grapes would be a desirable attribute; nevertheless, none of the treatments influenced pH or TA.

Pinolene already proved to effectively delay ripening in other studies (Brillante *et al.,* 2016; Di Vaio *et al.,* 2019; Silvestroni *et al.,* 2020). In this trial, a slight decrease in TSS accumulation was observed in 2021 but not in 2022. In our opinion, the difference found in the cluster number per vine between the first and the second year could play a key role in the explanation of the different outcomes observed. As a matter of fact, in 2022 we found a significantly lower number of clusters in pinolene-treated vines. The sugar content per berry values supports this theory, as it was significantly lower in P vines compared to the control (0.18 g compared to 0.24 g), indicating that if not for the difference in the number of clusters, we might have seen a lower TSS concentration in P vines.

Grape production was also assessed, considering the extent of sun damage on clusters (Figure 7). In 2021 we observed a higher frequency of clusters with no visible damages compared to 2022. At the same time, we also found the highest percentage of severe damage in 2021, with almost 60 % of clusters from the LR treatment seriously compromised by sun damages. In both years, the assessment of berry damage from C and P grapes did not show differences, and both treatments had the best quality among all the treatments under analysis. While in 2021 the extent of the damages on T was similar to C and P, in 2022 we registered a higher frequency of mild and moderate damage (classified as B and C), while fewer clusters had severe damages. In the case of LR, we found the highest percentage of damaged clusters in both years, especially with severe intensity.

As expected, the canopy manipulation operated with both leaf removal and shoot topping resulted in an overexposure of the clusters to high solar radiation, which is detrimental for grape quality and leads to a higher risk of sunburn and damage (Poni *et al.,* 2023). Damages in T were overall milder than LR, and this is a consequence of the different exposures determined by the two treatments. By removing leaves, which were operated on both sides of the canopy, clusters were exposed to direct sunlight for an extended duration during the morning and the afternoon. On the contrary, shoot topping concentrated the exposure of the fruiting zone mainly during the central hours of the day.

Berry anthocyanins evolution is shown in Figure 8. The highest concentration recorded was 357.4 mg/kg of berry in 2021 and 343.5 mg/kg of berry in 2022, both for P. Except for one date in 2021, no significant difference was observed between the treatments in both years. On this occasion, T exhibited the lowest concentration, with just 127.8 mg/kg of berry. After an initial accumulation, anthocyanin concentration tended to decrease in the latter part of the season, independently from the treatment applied. P showed the highest concentration in 2021 and throughout 2022, except for the harvest date, when it was the lowest, although the difference was not significant. At harvest, total anthocyanins (Table 1), as well as berry anthocyanin profile (Table S2), were unaffected by the treatments.

Di Vaio *et al.* (2019) already reported an increase in anthocyanins concentration thanks to the application of pinolene, while other studies have highlighted a negative effect on the phenolic substances (Palliotti *et al.,* 2013a; Brillante *et al.,* 2016; Di Vaio *et al.,* 2020; Silvestroni *et al.,* 2020). Our results do not align with any of the cited theories, rather suggestting there is no effect in this regard.

LR and T exhibited the lowest concentration in 2021. In 2022 this trend was confirmed for LR but not for T, which ended up with the highest content at harvest after C vines. The role of light and temperature on anthocyanin content has been largely described. While increased exposure can promote their accumulation (De Toda *et al.,* 2015; Tessarin *et al.,* 2018), a reduction in their content may derive from both under and overexposure (Mori *et al.,* 2007; Chorti *et al.,* 2010). On the other hand, other studies did not highlight a direct effect of both leaf removal (Filippetti *et al.,* 2014; Valentini *et al.,* 2018) and shoot topping (Poni & Gatti 2017; Tessarin *et al.,* 2022) on berry anthocyanins concentration. It appears that the level of exposure we obtained was not high enough to determine an increase or a decrease of these compounds at the berry level, rendering our results more in line with these latter findings.

7. Wine composition

Wine produced from the different treatments was analysed and results are reported in Table 2. Overall, alcohol content was higher in 2022 than in 2021, In 2022, the lowest alcohol content was 15.2 % for the C treatment. In both years, LR exhibited the highest alcohol concentration, with a maximum of 18.8 % in 2022. This was likely due to a higher incidence of sun damage and raisining as shown by the results from

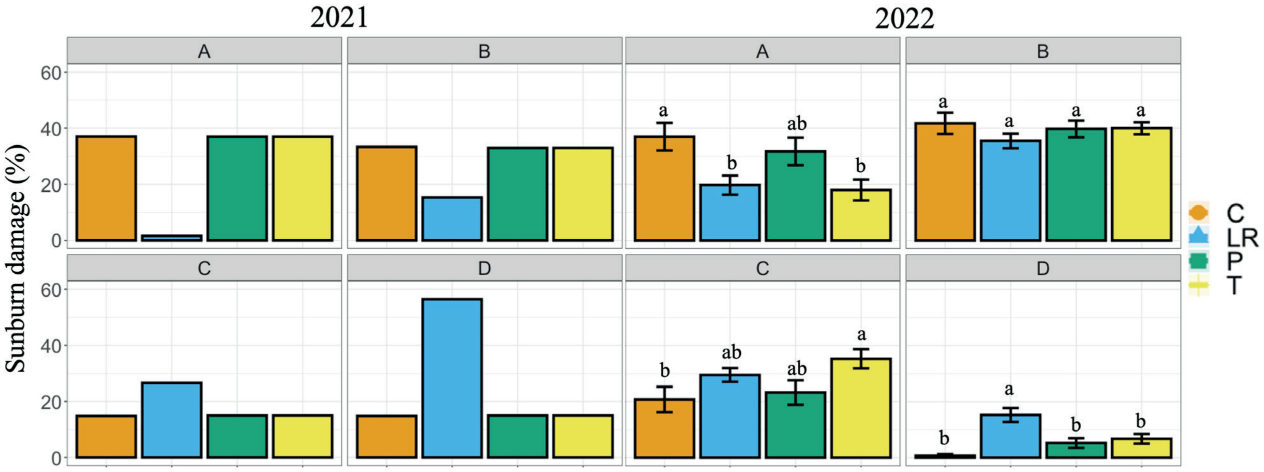

FIGURE 7. Percentage of no damages (A), mild damages (B), moderate damages (C) and severe damages (D) on the clusters at harvest. When ANOVA was significant, different letters indicates differences between treatments with p-value ≤ 0.05 (Tukey-HSD test). C indicates control, LR indicates leaf removal, P indicates pinolene and T indicates late topping.

Figure 7. Berry shrivelling and sun damages are well known to increase the concentration of soluble solids in must. These berries were likely excluded from the grape composition samples shown earlier.

In 2022, T and LR treatments reduced wine acidity (4.81 and 5.01 g/L respectively), even though each treatment had higher content when compared to the previous year. LR also exhibited a higher pH in the same year. For this study, acidity in wines was not modified in any treatments. However, conventional winemaking practices would typically necessitate the addition of tartaric acid to counterbalance the high pH in the musts. Standard targets for titratable acidity exceeding 5 g/L, and pH values below 3.8 are generally preferred to optimise wine colour and enhance the efficacy of sulfur dioxide against spoilage microorganisms and oxidation (Ribéreau-Gayon *et al.*, 2006).

Optical density was measured at 420, 520, and 620 nm to assess wine colour. In 2021, significant differences were found only at 620 nm, and in this case, LR resulted in the highest value of 0.11, which corresponds to nearly 3 % of the total colour intensity (sum of the three optical densities). In 2022 a significant drop of all colour parameters was observed in comparison with 2021 (~70 % less colour), which is related to the decrease of anthocyanins on berries. Regardless of this difference, we observed significant differences in all wavelengths, where LR had the highest colour intensity followed by T. Wine from the control always showed the lowest colour values. In 2022, LR confirmed the trend observed in 2021, maintaining the highest values at each wavelength with significant differences compared to most treatments. An improvement in wine colour was observed by Tessarin *et al*. (2022). Our results over two consecutive years confirm that leaf removal can effectively improve wine colour. However, we also believe that in the second year, the higher alcohol content in the wine played a key role in facilitating colour extraction, thus intensifying the effects of the defoliation. In addition, as presented in Figure 7, LR had a higher number of dehydrated berries that increased the amount skin/juice ratio in the musts, while they were excluded from analysis of anthocyanins reported on berry analysis (Table S2).

Total anthocyanins content was significantly higher in 2021 compared to 2022 in all treatments. In 2021, C had the highest total anthocyanin concentration, whereas in 2022, T had the highest concentration. Although the berry content at harvest did not differ between treatments, LR resulted in the lowest concentration of total anthocyanins in the wine in both years. As expected, the major contributor to total anthocyanins were glucosides in both years (average 57 % across all treatments in 2021 and 50 % in 2022). Acylated anthocyanins (acetylated, and coumarylated) in C were significantly higher than all treatments, whereas in 2022, T was the treatment with the highest concentration.

Interestingly, in 2022, the percentage of acetylated anthocyanins was higher (approximately 46 %) compared to 2021 (37 %). These ratios are relevant for winemaking, because acylated anthocyanins are known to contribute colour stability in wines by preventing bleaching by sulfur dioxide and preventing pigment degradation by means of copigmentation (Brouillard & Dangles, 1994; Giusti & Wrolstad, 2003; Waterhouse *et al*., 2016). In the case of our results, while the concentration of acylated anthocyanins were lower for all treatments in 2022, the colour is expected to be more stable during wine ageing. Similarly, T showed a higher rate of 3’4’5’ hydroxylated compounds than the other treatments. In the hottest 2022, the amount of these compounds was 50 % higher in T than in C, although this difference was not statistically significant. The 3’4’5’ hydroxylated anthocyanins are more stable in wine.

An interesting aspect of the data is the lack of strong correlation between anthocyanins in the berries and in the wine. Principal Component Analysis (PCA) data from both 2021 and 2022 indicate a clear separation between berry

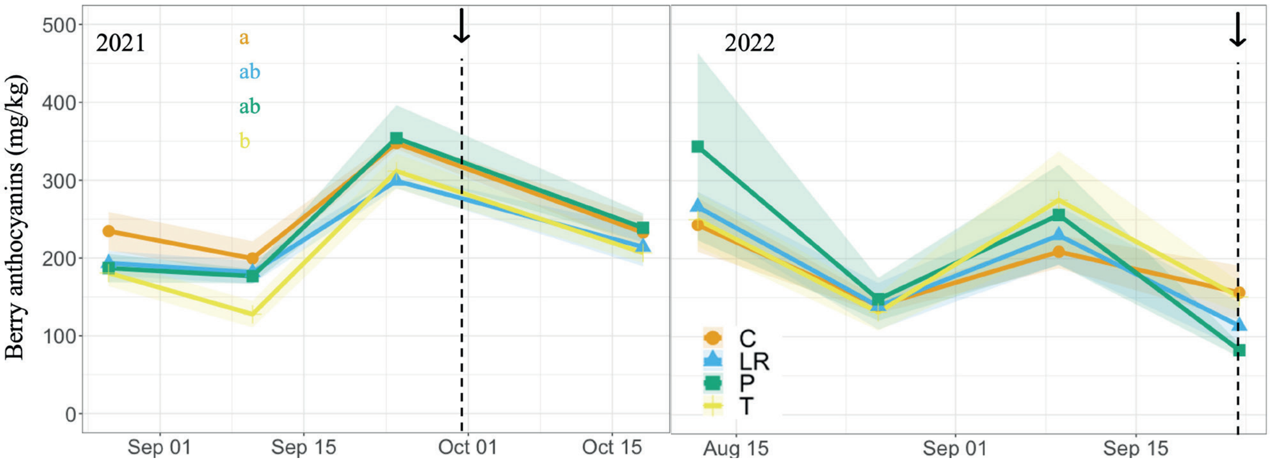

FIGURE 8. Berry anthocyanins evolution in 2021 and 2022. When ANOVA was significant, different letters indicate differences between treatments with p-value ≤ 0.05 (T-test). Shaded regions indicate the standard error of the mean. C indicates control, LR indicates leaf removal, P indicates pinolene and T indicates late topping. The black arrow indicates the harvest date. In 2021, the experimental vines that were not used for yield assessment were sampled two weeks after the harvest date to observe the effect of treatments with a longer hang time.

TABLE 2. Finished wine composition in 2021 and 2022.

	year	C	LR	P	T	sign.
Alcohol (%)	2021	15.3 ab	15.5 a	15.1 b	15.2 b	**
	2022	15.2 c	18.8 a	16.7 b	17.1 b	***
pH	2021	4.50	4.50	4.51	4.45	ns
	2022	4.40 b	5.03 a	4.65 b	4.68 b	**
TA (g/l)	2021	2.95	2.80	3.34	3.17	ns
	2022	5.60 a	5.01 b	5.68 a	4.81 b	**
OD 420 nm	2021	1.57	2.04	1.68	1.54	ns
	2022	0.32 b	0.43 a	0.35 ab	0.42 a	*
OD 520 nm	2021	1.26	1.67	1.35	1.24	ns
	2022	0.33 c	0.45 a	0.38 bc	0.43 ab	*
OD 620 nm	2021	0.07 b	0.11 a	0.08 ab	0.07 b	*
	2022	0.13 b	0.18 a	0.14 b	0.16 ab	**
Colour Intensity	2021	2.91	3.81	3.11	2.86	ns
	2022	0.77 c	1.06 a	0.87 bc	1.00 ab	**
Anthocyanin (mg/L)						
Delphinidin	2021	3.6 b	4.2 a	3.7 b	3.5 b	*
	2022	1.8	0.9	1.2	2.1	ns
Cyanidin	2021	3.9 b	4.7 a	4.1 b	3.8 b	***
	2022	1.7	1.2	1.4	2.4	ns
Petunidin	2021	0.7	0.6	0.6	0.6	ns
	2022	0.7 ab	0.5 b	0.6 ab	0.8 a	*
Peonidin	2021	3.8	2.7	4.5	1.9	ns
	2022	1.3	0.6	1.2	2.1	ns
Malvidin	2021	185.3 a	152.7 b	168.7 ab	156.8 b	***
	2022	66.7 ab	23.3 c	41.7 bc	93.9 a	***
Total Glucosides	2021	112.9 a	93.2 b	101.5 b	93.7 b	***
	2022	35.7 ab	13.1 c	23.1 bc	50.0 a	***
Total Acetylated	2021	68.1 a	60.5 b	66.2 ab	61.7 ab	*
	2022	32.9 ab	12.3 c	20.9 bc	47.0 a	**
Total Coumarilated	2021	16.3 a	11.2 c	13.8 b	11.3 bc	***
	2022	3.5 b	1.1 c	2.1 c	5.7 a	***
Total 3′4′ Hydroxylated	2021	7.8	7.4	8.5	5.7	ns
	2022	2.9 b	1.8 b	2.6 bc	5.5 a	**
Total 3′4′5′ Hydroxylated	2021	189.5 a	157.5 b	173.0 ab	161.0 b	***
	2022	69.3 ab	24.7 c	43.5 bc	97.3 a	***
Ratio of 3′4′5′/3′4 hydroxylated	2021	25.1	21.4	21.5	28.4	ns
	2022	23.6 a	13.4 b	16.7 b	16.3 b	**
Total Anthocyanins	2021	197.3 a	164.9 b	181.6 ab	166.7 b	**
	2022	72.2 ab	26.5 c	46.1 bc	102.8 a	***

*Wine composition in 2021 and 2022. In case of significant ANOVA, different letters across a column indicate significant differences between treatments with a p-value < = 0.05 (Tukey post hoc test). TA indicates titratable acidity, OD indicates the optical density of the wines measured at 420 nm, 520 nm, and 620 nm. Delphinidin, Cyanidin, Petunidin, Peonidin, and Malvidin are the sum of all the corresponding compounds, including glucosides, acetylated and coumarilated (*e.g.*, Delphidin is the sum of Delphinidin-3-glucoside, Delphinidin-3-(acetyl)-glucoside, and Delphinidin-3-(cou)-glucoside). Total Anthocyanins are reported as the sum of Delphinidin, Cyanidin, Petunidin, Peonidin, and Malvidin in all forms. C indicates control, LR indicates leaf removal, P indicates pinolene, and T indicates late topping.

and wine anthocyanins (Figure S1), showing no strong correlation between their distributions. This suggests that the anthocyanin content in the wine is influenced by factors beyond berry anthocyanin concentration, such as alcohol content, which may enhance their extraction (He *et al.,* 2012). Additionally, the abundance of raisins and dehydrated berries in the winemaking process may have further skewed the final anthocyanin profile in wine (Tarara *et al.,* 2008; Torres *et al.,* 2020). Our results suggest a strong correlation between water stress and the final anthocyanin content in the wine. Treatments that induced more significant water stress, like P and C in 2021, resulted in a notably higher concentration of total anthocyanins in the wine. This could be due to the increased concentration of anthocyanins that develop as a physiological response to water stress, a phenomenon well-documented in various studies (Mirás-Avalos & Intrigliolo, 2017).

Depending on the level of exposure and bunch temperature, studies on the behaviour of anthocyanins in response to increasing light exposure can be quite contradictory (Mori *et al.,* 2007; Chorti *et al.,* 2010; Alatzas *et al.,* 2023). Our results suggest that increased exposure to sunlight in

LR might lead to a lower concentration of total anthocyanins in the wine due to their degradation. This is consistent with results from many other trials in California (Martínez-Lüscher *et al.,* 2017a; Martínez-Lüscher *et al.,* 2020; Torres *et al.,* 2020; Torres *et al.,* 2021). In the case of T, the low concentration observed in 2021 may also be related to a reduced sugar accumulation, therefore to a delay in biosynthesis and not to degradation.

While the other treatments showed more inconsistency over the two years, LR consistently affected the concentration of all anthocyanin groups. LR resulted in an increased content of total delphinidins and cyanidins in 2021. When we consider the relative abundance of these components, it was increased significantly by LR in both years. LR also decreased the concentration in 2021 and their relative abundance in both years. Unexpectedly, LR had the highest colour intensity (with T) but the lowest concentration of anthocyanins each year.

These results suggest that anthocyanin concentration in the wine is subject to more complex dynamics, influenced by environmental factors like light exposure and water stress. However, the lack of a strict correlation between berry and wine anthocyanins implies that the winemaking process plays a crucial role in determining wine color quality and stability.

CONCLUSIONS

Late canopy management operated with above-cluster leaf removal and topping reduced leaf area per vine and increased light exposure in the cluster zone. Plant water status, indicated by the midday stem water potential, was generally improved by topping due to the reduction in canopy size. Leaf removal also had a positive effect on water status, but only in 2021, when the canopy reduction was more severe. Conversely, pinolene application ended up exacerbating water stress. Pinolene film forming capacity confirmed its efficacy in reducing gas exchanges, while topping led to an increase because of overcompensation phenomena.

Topping had positive effects in slowing down sugar accumulation, resulting in a lower concentration of total soluble solids at harvest. The wine colour was improved by topping in 2022, while the other characteristics were not altered.

At harvest, no difference between treatments was found regarding total yield, must pH and titratable acidity. However, the incidence of sun damages and shriveling was increased by light-exposing treatments (T and LR), which showed a higher frequency of damaged clusters. Pinolene led to a lower sugar content per berry at harvest and to a higher acidity in the wines.

Leaf removal reduced the total anthocyanins in the wine and altered their relative abundance. However, wine colour was significantly improved by leaf removal, aided by the higher alcohol content, which facilitated their extraction.

Late season canopy management can effectively reduce water stress, delay grape ripening and improve wine colour. However, overexposure should be avoided to prevent sunburn and damage that could compromise production, especially in areas with high solar radiation, like the San Joaquin Valley of California. On the other hand, antitranspirants effectively reduces gas exchanges and does not alter wine colour. Furthermore, the removal of waxes determined by pinolene can reduce berry size because of water loss, with the risk of lowering the productive level, at least in the hot and dry climates. The combination of canopy management and antitranspirant could be evaluated in future studies to assess the potential for synergic effects.

The results presented here provide valuable insights into canopy management for growers operating in hot climates. The choice between one technique or another should consider the meteorological conditions in order to reduce heat stress and improve grape production. In the conditions of Central California, late topping is the best solution evaluated in this study, as it maintains yield, improves wine colour, and delays sugar accumulation so that harvest can take place in a milder climate.

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AUTHOR CONTRIBUTION

LP led data analysis and wrote the first draft of the manuscript; GP carried out data collection, chemical analysis, and data management; ELP field data collection and student support; VL writing, training, and financial support to LP; MP wine making, writing; LB conceptualisation and scientific design, fundraising and resource allocation, group leadership, contribution to field data collection, chemical analysis, data analysis, and writing. All authors revised the manuscript and approved the current version.

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