The impact of cluster thinning and leaf removal timing on the grape quality and concentration of monomeric anthocyanins in Cabernet-Sauvignon and Probus (Vitis vinifera L.) wines

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ABSTRACT

Aim: Leaf removal around clusters and cluster thinning are techniques usually applied in cool-climate vineyards in order to achieve optimal grape maturity. However, the impact of the timing of these two operations differs across varieties. Thus, the aim of the present work was to investigate the effects of cluster thinning and leaf removal timing (performed at three specific time points) on grape quality and monomeric anthocyanins in the wines of Cabernet-Sauvignon and Probus (Kadarka × Cabernet-Sauvignon, Vitis vinifera L.).

Methods and results: The experiment was conducted in Sremski Karlovci (Northern Serbia) in 2014, 2015, and 2016. Leaf removal was applied on six basal nodes of each shoot at three time points, 7 days after flowering, 30 days after flowering, and at veraison, i.e., at the onset of berry ripening. After cluster thinning, which was performed 7 days after flowering, one cluster per shoot was retained. On the treated vines, leaf removal treatment and cluster thinning were applied only once. Leaf removal was more effective than cluster thinning in respect to grape quality. Leaf removal, applied 7 and 30 days after flowering, decreased titratable acidity in Cabernet-Sauvignon, while in Probus, an interaction of leaf removal and year was observed. Moreover, early leaf removal decreased the incidence of Botrytis sp. in Probus.

The varieties reacted differently to cluster thinning in respect to grape quality: cluster thinning increased total soluble solids in Probus and lowered titratable acidity in Cabernet-Sauvignon. In 2015, both cluster thinning and leaf removal yielded changes in the anthocyanin ratios in the wines. Cluster thinning increased total and acylated anthocyanins in the wine of Cabernet-Sauvignon compared to wine derived from unthinned vines. The peonidin content was 40% higher in the Cabernet-Sauvignon wine if the vines were subjected to leaf removal treatments.

Conclusions: Cluster thinning and leaf removal affected both Cabernet-Sauvignon and Probus (Vitis vinifera L.) grape quality and wine composition. Early leaf removal was the most effective treatment in both varieties. Therefore, combined application of cluster thinning and early leaf removal is highly recommended in the production of high-quality red wines in Serbia.

Significance and impact of the study: Timing of leaf removal application was usually investigated around flowering and veraison. Our results suggested that leaf removal between these two phenological stages also improves grape quality and changes the ratio of the monomeric anthocyanins in the wine.

KEYWORDS

leaf removal, cluster thinning, Cabernet-Sauvignon, Probus, quality

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/2505
INTRODUCTION

In most of the wine regions worldwide, the production of high-quality wine is a challenge. Among many environmental factors, climate has the greatest impact on vine development and grape quality. Wine-producing regions are characterised by mean climatic conditions, which are major drivers of wine quality in relation to its origin (van Leeuwen and Dariet, 2016). However, even in a given wine region these conditions vary from year to year.

In addition to climate, grape quality depends on the grape variety and viticulture practices. The viticulture practices of cluster thinning (CT) and leaf removal (LR) are commonly performed to improve grape quality.

CT can increase total soluble solid concentration (TSS) (Reynolds et al., 1994; Valdes et al., 2009) and pH (Valdes et al., 2009) of the grape juice. It can also speed up ripening (Barros et al., 2018), which could be useful especially in regions with unfavourable conditions during grape ripening. This technique also increases ethylene production in some fruits, indicating advance maturity (Lopez et al., 2011). However, in other trials limited or no effects of CT on the grape quality were shown (Ough and Nagaoka, 1984; Keller et al., 2005).

LR in the fruit zone is one of the most important and commonly applied canopy management operations in viticulture. This technique is performed on grapevines to improve light penetration and air circulation around the clusters. It is also applied to increase penetration of fungicide sprays and decrease disease incidence. LR can lead to increased levels of TSS (Bledsoe, 1988; Intrieri et al., 2008; Kemp, 2010), total anthocyanins (Tardaguila et al., 2010; Drenjančević et al., 2017), and decreased titratable acidity (TA) (Petrie et al., 2003). However, different results can be observed depending on the climate, variety and time of application.

The right moment for LR varies depending on the region, variety, and type of wine produced. In the past, it was usually performed around veraison (onset of ripening). Aćimović et al. (2016) found that removal of fewer than six leaves did not significantly affect the final yield per vine and some grape quality parameters.

Recently, positive a effect of early LR (around flowering) on the grape quality was observed (Moreno et al., 2017). Early LR significantly decreases fruit set, which in turn increases cluster looseness and tolerance to rot (Poni et al., 2006; Diago et al., 2010). Also, early LR can significantly decrease yield (Tardaguila et al., 2010) and cluster weight (Petrie et al., 2003; Intrieri et al., 2008). One of the most positive effects of LR is reducing the incidence of Botrytis cinerea (Sivilotti et al., 2016).

The varieties react differently to CT and time of LR. Moreover, there is a lack of knowledge on how LR applied at other times during berry development in combination with previously applied CT will affect the grape and wine quality.

The aim of this study was to investigate the effects of CT (performed 7 days after flowering) and timing of LR (7 days after flowering, 30 days after flowering, at veraison) on grape and wine quality parameters of Cabernet-Sauvignon and Probus (Kadarka × Cabernet-Sauvignon, Vitis vinifera L.).

MATERIALS AND METHODS

The experiment was conducted over a 3-year period (2014–2016) at the experimental field of the University of Novi Sad, Faculty of Agriculture, situated in Sremski Karlovcı – Fruska Gora (45º10’ N, 20º10’ E). Fruska Gora is one of the most important Serbian wine-growing districts and is located in the Srem region. Cabernet-Sauvignon and Probus (VIVC variety number 9719) vines, grafted on SO4 rootstock, were planted in 2000, in a northeast-southwest orientation with 2.8 m spacing between rows and 1.6 m separation between pair of vines in a row. Vines were vertical shoot positioned (VSP) Guyot pruned with one cane and one spur (14 buds per vine). LR was applied on six basal nodes of each shoot. After CT, which was performed 7 days after flowering, one cluster per shoot remained.

Eight treatments were compared in the present study (Figure 1), four of which did not involve CT: (1) ED - LR performed 7 days after flowering; (2) MD - LR performed 30 days after flowering; (3) LD - LR performed at veraison, and (4) UN - no LR was performed. The remaining four treatments involved the same LR strategies as above, but also included cluster thinning: (5) ED+CT - both LR and cluster thinning were performed 7 days after flowering; (6) MD+CT - LR performed 30 days after flowering, with cluster thinning applied 7 days after flowering; (7) LD+CT - LR performed at veraison, while cluster thinning was applied 7 days after flowering; and (8) UN+CT - cluster...
thinning was applied 7 days after flowering without LR. On the treated vines, LR treatment and CT were applied only once. A fully randomised block design was applied in the experiment. Each treatment included three replicates, with eight vines per replicate.

1. Analyses

Yield (kg/m²) was determined at harvest by weighing all the grapes of each replicate. Average cluster weight (g) was obtained by weighing ten clusters per replicate. Botrytis incidence was determined as a percentage by visual assessment of the cluster health status (% of bunches infected) at harvest time. Berry weight (g) was determined in random samples of 30 berries per replicate. Then, these berries were collected in a plastic bag and stored in the freezer at -20 °C until required for the analysis of the total anthocyanins, skin weight, weight of seeds and number of seeds. Moreover, total soluble solids (TSS) content in the juice (%) was detected using an Oechsle hydrometer after crushing all the grapes at harvest. Titratable acidity (g/L) of the juice was analysed by adding 10 % NaOH drop-by-drop until the acids were neutralised.

One month after the harvest, the frozen berries were taken for further analyses. The seeds were then separated, weighed and counted. The berry skins were separated, weighed and extracted in ethanol/water/hydrochloric acid (in the 70:29:1 v:v:v ratio) solution overnight for total anthocyanins analysis. Then, the absorbance value at 540 nm was read using a spectrophotometer and was converted to the malvidin-3-O-glucoside concentration values by multiplying the absorbance by 16.17 and by the dilution factor.

2. Microvinifications

From each replicate, grapes were destemmed, crushed, and 15 mg L⁻¹ SO₂ was added before being inoculated with Saccharomyces cerevisiae (Uvaferm BDX). Fermentations were conducted in 5 L glass fermenters at a temperature of 25 °C. The pomace was mixed twice a day. After eight days of fermentation and maceration, the liquid phase (wine) was separated. Wines were racked twice, at 14 and 60 d after the end of fermentation. Then, the wines were bottled and stored at 12 °C. After 6 months, the samples were collected and stored at -20 °C until required for analysis.

3. High-performance liquid chromatography (HPLC) of anthocyanins

HPLC analyses included the wines produced in 2015, 3 months after the samples were frozen. Prior to HPLC analyses, wines were centrifuged for 3 min, and the supernatant was transferred into HPLC vials. Sample preparation was performed according to the OIV-MA-AS315-11 protocol (OIV, 2007). Anthocyanins were injected into an Agilent 1100/1200 series HPLC system equipped with an Agilent photodiode array detector (DAD). Separation was performed on a
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reversed-phase column LiChrospher 100 RP 18 (5 µm) in LiChroCart 250-4 (MERCK) with a guard column LiChroCart 4 mm RP 18 (MERCK), at a temperature of 20 °C. The following HPLC-grade solvents were used: water/formic acid/acetonitrile (87:10:3, v:v:v) as solvent A, and water/formic acid/acetonitrile (40:10:50, v:v:v) as solvent B. Elution was performed at a flow rate of 0.4 ml/min, using a gradient elution, starting with 6 % (B), increasing to 30 % (B) after 15 min, 50 % (B) at 30 min, and 60 % (B) at 35 min, before decreasing to 6 % (B) at 41 min. The detection wavelength of 520 nm was utilised for all measurements. Anthocyanin compounds were identified by comparing the retention time with available standards, or the spectral characteristics with data published in the pertinent literature (Burns et al., 2002; Ryan and Revilla, 2003; Radovanović and Radovanović, 2010). Anthocyanins were quantified using a seven-point external calibration curve (R² = 0.9997) obtained by injecting standard solutions of

![Graph showing average monthly precipitation and temperatures during the 2014-2016 period.](image)

**FIGURE 2.** Average monthly precipitation and temperatures during the 2014-2016 period.

**TABLE 1.** Yield, cluster weight and incidence of Botrytis sp. for Cabernet-Sauvignon and Probus (2014-2016).

| Treatment | Cluster thinning | Leaf removal | Average | Statistical significance |
|-----------|------------------|--------------|---------|--------------------------|
|           | CT               | No CT        | ED      | LD                       | UN                       |
|           | 0.49             | 0.89         | 0.55*   | 0.66*                    | 0.80*                    |
|           | 0.92             | 1.48         | 1.16    | 1.21                     | 1.20                     |
|           | 145              | 136          | 123     | 150                      | 146                      |
|           | 239              | 206          | 209     | 234                      | 217                      |
|           | 3.6              | 4.3          | 3.4     | 4.3                      | 4.1                      |
|           | 12.6             | 11.6         | 9.5     | 11.9*                    | 14.3*                    |
|           |                  |              |         |                          |                          |
|          Cabernet-Sauvignon | Probus      | Cabernet-Sauvignon | Probus | Cabernet-Sauvignon | Probus |
|          Cluster weight (g)         | Botrytis sp. (%)       |
|          141               | 222              | 170     | 10.2                         |
|          2014               | 2015             | 2016     | 10.3                         |
|          2014-2016           |                  |          |                               |
|          CT **              | LR **            | CT × LR  | CT × Y                       |
|          ns               | ** ns            | ns       | ns                           |
|          **               | ns               | *        | ns                           |
|          ns               | ns               | ns       | ns                           |
|          **               | ns               | ns       | ns                           |
|          **               | ns               | ns       | ns                           |
|          **               | ns               | ns       | ns                           |
|          ns               | ns               | ns       | ns                           |
|          ns               | ns               | ns       | ns                           |
|          ns               | ns               | ns       | ns                           |

Factorial ANOVA with three factors (CT, LR and Y). ** indicate a significant difference among leaf removal factor levels. *p < 0.05, **p < 0.01, ns, nonsignificant.
Intraday repeatability and reproducibility were determined using an acidic ethanol-water extract (EtOH/H₂O/HCl, 70:29:1, v:v:v) of grape skins from the ‘Pinot noir’ cultivar (VIVC variety number 9279). Repeatability and reproducibility were expressed as relative standard deviations (RSD) of five anthocyanin monoglucosides (delphinidin, cyanidin, petunidin, peonidin, and malvidin). For intraday repeatability, the extract was injected into the HPLC system eight times within 24 h. Intraday variation was evaluated on five consecutive days.

4. Statistical analyses

Statistical analyses were performed using R software. The mean values were expressed as relative standard deviations (RSD) of five anthocyanin monoglucosides (delphinidin, cyanidin, petunidin, peonidin, and malvidin). For intraday repeatability, the extract was injected into the HPLC system eight times during 24 h. Intraday variation was evaluated on five consecutive days. All analyses were performed in triplicate and results were expressed as mean values.

**RESULTS**

Weather conditions for the experimental site during (2014-2016) are shown in Figure 2. 2014 was extremely rainy, especially in May and July, but 2015 and 2016 were drier and hotter. In 2014, rainy weather caused berry cracking in Probus, which reduced berry yield and quality. Flowering occurred in the last 10 days of May in Probus, which reduced yield and quality. In 2015 and 2016, flowers were subjected to CT. Depending on the year and variety, the yield was reduced by 45% on average. In 2014, weather affected yield more significantly than in other years. The data was processed by multifactorial ANOVA. Duncan’s test was used to test the significance of differences (p < 0.05) among the mean values of measured parameters. The normality of distribution was tested by using an Anderson-Darling test. When the data was not normally distributed, the nonparametric Kruskal-Wallis test was applied. Graphs were generated using the ggplot2 package.

| Treatment | Berry weight (g) Cabernet-Sauvignon | Probus | Skin weight (g) Cabernet-Sauvignon | Probus | Number of seeds/berry Cabernet-Sauvignon | Probus | Weight of seeds/berry (g) Cabernet-Sauvignon | Probus |
|-----------|-------------------------------------|--------|------------------------------------|--------|-----------------------------------------|--------|---------------------------------------------|--------|
| CT        | 1.38                                | 1.76   | 0.61                               | 0.68   | 1.9                                     | 1.9    | 0.08                                        | 0.09   |
| No CT     | 1.45                                | 1.83   | 0.39                               | 0.64   | 1.9                                     | 1.9    | 0.08                                        | 0.09   |
| Leaf removal | ED                        | 1.40    | 1.81                               | 0.40   | 0.66                                    | 2.0    | 0.08                                        | 0.09   |
|           | MD                                  | 1.46    | 1.81                               | 0.42   | 0.64                                    | 1.9    | 0.08                                        | 0.09   |
|           | LD                                  | 1.44    | 1.86                               | 0.40   | 0.75                                    | 2.0    | 0.08                                        | 0.09   |
|           | UN                                  | 1.35    | 1.67                               | 0.37   | 0.62                                    | 1.8    | 0.07                                        | 0.08   |
| Average   | 1.48                                | 2.13   | 0.58                               | 0.84   | 1.9                                     | 1.8    | 0.08                                        | 0.08   |
|           | 1.33                                | 1.98   | 0.32                               | 0.58   | 1.9                                     | 2.0    | 0.08                                        | 0.09   |
|           | 1.20                                | 1.80   | 0.27                               | 0.52   | 1.9                                     | 2.0    | 0.09                                        | 0.09   |
|           | 2014-2016                           | 1.41    | 1.79                               | 0.40   | 0.66                                    | 1.9    | 0.08                                        | 0.09   |

**TABLE 2.** Berry weight, skin weight number and weight of seeds per berry for Cabernet-Sauvignon and Probus (2014-2016).

| Statistical significance | 2014 | 2015 | 2016 | 2014–2016 |
|--------------------------|------|------|------|-----------|
| CT                       | ns   | ns   | ns   | ns        |
| LR                       | ns   | ns   | ns   | ns        |
| Y                        | **   | **   | **   | **        |
| CT × LR                  | ns   | ns   | ns   | ns        |
| LR × Y                   | ns   | ns   | ns   | ns        |
| CT × LR × Y              | ns   | ns   | ns   | ns        |

Factorial ANOVA with three factors (CT, LR and Y). *p < 0.05. **p < 0.01. ns, nonsignificant.
### TABLE 3. TSS, TA and total anthocyanins of Cabernet-Sauvignon and Probus (2014-2016).

|                  | TSS (%) | TA (g/L) | Total Anthocyanins (mg/L) |
|------------------|---------|----------|---------------------------|
|                  | Cabernet-Sauvignon | Probus | Cabernet-Sauvignon | Probus | Cabernet-Sauvignon | Probus |
| **Cluster thinning** |         |          |                          |        |                      |        |
| CT               | 21.3    | 21.0<sup>A</sup> | 7.1<sup>B</sup> | 7.1 | 701 | 1449 |
| No CT            | 21.5    | 19.5<sup>a</sup> | 7.4<sup>A</sup> | 6.6 | 674 | 1515 |
| **Leaf removal**  |         |          |                          |        |                      |        |
| ED               | 21.5    | 20.8     | 7.0<sup>b</sup> | 6.1 | 765<sup>a</sup> | 1568 |
| MD               | 21.4    | 20.1     | 7.0<sup>b</sup> | 6.7 | 643<sup>b</sup> | 1423 |
| LD               | 21.5    | 20.2     | 7.3<sup>ab</sup> | 6.6 | 684<sup>ab</sup> | 1393 |
| UN               | 21.3    | 19.9     | 7.6<sup>a</sup> | 8.0 | 657<sup>b</sup> | 1537 |
| **Average**      |         |          |                          |        |                      |        |
| 2014             | 22.9    | 21.0     | 7.8                      | 8.8   | 766 | 1422 |
| 2015             | 19.9    | 18.9     | 5.9                      | 5.2   | 631 | 1420 |
| 2016             | 21.5    | 20.9     | 8.0                      | 6.6   | 664 | 1602 |
| 2014–2016        | 21.4    | 20.3     | 7.2                      | 6.9   | 687 | 1481 |
| **Statistical significance** |         |          |                          |        |                      |        |
| CT               | ns      | **       | **                       | *     | ns               | ns     |
| LR               | ns      | ns       | **                       | **    | *                | ns     |
| Y                | **      | **       | **                       | **    | **               | ns     |
| CT × LR          | ns      | ns       | ns                       | ns    | ns               | ns     |
| CT × Y           | ns      | ns       | **                       | ns    | ns               | ns     |
| LR × Y           | **      | ns       | **                       | ns    | ns               | ns     |
| CT × LR × Y      | ns      | ns       | ns                       | ns    | ns               | ns     |

Factorial ANOVA with three factors (CT, LR and Y).

<sup>A,B</sup> indicate a significant difference between CT and NoCT at p < 0.05.

<sup>ab</sup> indicate a significant difference among leaf removal factor levels at p < 0.05.

<sup>*</sup>p < 0.05, <sup>**</sup>p < 0.01, ns, nonsignificant.
The year significantly affected the incidence of *Botrytis* sp. in both varieties. Moreover, ED significantly reduced *Botrytis* sp. incidence in Probus compared to undefoliated vines.

The treatments had no effect on berry weight, skin weight, number and weight of seeds per berry (Table 2). However, differences in berry and skin weight across the years were observed in both varieties. Mean ± standard error for all parameters related to the grape quality across the years are shown in Supplementary Tables 2, 3, 4 and 5.

CT increased TSS content in Probus, but no effect was shown on Cabernet-Sauvignon (Table 3). Interaction LR × Y affected TSS content in Cabernet-Sauvignon, while Probus was unaffected by LR. CT and LR treatments decreased titratable acidity in Cabernet-Sauvignon. Interactions CT × Y and LR × Y affected TA in Probus (Supplementary Table 1). The highest TA was recorded in 2014 in undefoliated vines (11.2), and the lowest was recorded in 2015 in ED treatment (4.8 g/L) (Supplementary Table 1). ED increased total anthocyanins in the grape skin compared to undefoliated vines. CT showed no effect on total anthocyanins in the grape skin of both varieties. Interaction LR × Y affected total anthocyanins in Probus (Supplementary Figure 1).

CT, ED and LD increased total anthocyanins in the wine of Cabernet-Sauvignon (Figure 3). For Probus, interaction CT × LR affected total anthocyanins. The highest content of the total anthocyanins (169.8 (mg/L)) was observed in Probus wine that received the treatment ED + CT (Supplementary Figure 1).

LD treatments decreased the tri-substituted anthocyanins content in Cabernet-Sauvignon wine compared to UN (Figure 4). In Probus, no effect was observed. In all treatments, di-substituted anthocyanins were present (up to 9 %) in the wines of both varieties. In the wines of Cabernet-Sauvignon, the lowest percentage of methoxylated anthocyanins was observed in ED treatment (90.9 %).

CT and LD increased acylated anthocyanins in Cabernet-Sauvignon, while in Probus no effect was shown (Figure 5). In the wines of Cabernet-Sauvignon and Probus, acylated anthocyanins were present at up to 27 and 24 %, respectively. In Cabernet-Sauvignon, MD and LD showed a lower percentage of coumaroylated anthocyanins, compared to undefoliated vines. In both varieties, monoglucoside percentage was unaffected by the treatments.

CT decreased the percentage of malvidin in Probus, while Cabernet-Sauvignon was unaffected (Figure 6). The varieties reacted differently to LR treatments: in Cabernet-Sauvignon, LR treatments ED and LD decreased the percentage of malvidin, whereas in Probus MD treatment increased it.
in wine. However, the varieties reacted differently to these treatments, as evident from the variation in grape quality. Moreover, year - either alone or in interaction with other factors - affected all tested parameters except seed number and weight.

CT was always conducted 7 days after flowering, as its timing had a limited effect on the grape and wine quality (King et al., 2015). In the climate conditions of Serbia, it is advisable to perform this operation after flowering because of unpredictable weather conditions, which can adversely affect berry-set. Thus, an additional crop removal before berry-set would be undesirable.

ED affected grape yield differently depending on the variety. Tardaguila et al. (2010) reported similar results for Cabernet-Sauvignon to those we observed: they found that the yield was reduced by 30–70% by early LR. Bešlic et al. (2013) investigated the effect of early LR on the yield parameters of Cabernet-Sauvignon and Prokupac, and observed that early LR decreased berry size and number of berries per cluster, which lowered the yield.

Moreover, berry cracking of Probus, in rainy 2014, increased the incidence of Botrytis sp., particularly in UN. Lower incidence of Botrytis in treatments involving LR could be related to better aeration of clusters and lower bunch compactness in ED. Lower incidence of Botrytis as a result of early LR treatment was also observed by Palliotti et al. (2012). However, as there was a high variation in Botrytis incidence among the plots subjected to the same treatment, in future research the incidence should be recorded for each vine separately.

Although the difference was not statistically significant, berry weight was higher in samples subjected to CT treatments. Gli Munoz et al. (2009) also observed that CT tends to increase berry weight of Tempranillo and Syrah.

TSS increased in Probus following CT treatments, while no effect on Cabernet-Sauvignon was observed. Probus has around 30% heavier clusters compared to Cabernet-Sauvignon, which could be the reason for a different response to CT. In addition, Gil et al. (2009) reported a varietal behaviour responding to CT; while Tempranillo significantly increased TSS following CT, no effects were shown for Shiraz. High temperatures have been shown to contribute to lower TSS (Greer and Weston, 2010) and TA (Buttrose et al., 1971; Brandt et al., 2019). Karoglan et al. (2014) and Zhuang et al. (2014) observed that TSS content

**DISCUSSION**

Our findings suggest that CT and LR improved grape quality and modified the anthocyanin ratios
in the grape juice of red varieties was unaffected by CT.

The titratable acidity of cluster thinned Probus vines tended to be higher than in control vines, while in Cabernet-Sauvignon the opposite was true. di Profio et al. (2011) and Reščić et al. (2015) reported that CT decreased titratable acidity, while Valdes et al. (2009) failed to observe any effect. LR usually reduces titratable acidity (Bledsoe et al., 1988; Petrie and Clingeleffer, 2006) or has no effect (Kemp, 2010; Sivilotti et al., 2016). In the present study, a decrease in titratable acidity was noted in Cabernet-Sauvignon, whereby Probus was affected by LR × year interaction. The effects of LR applied around veraison (onset of ripening) on grape quality were less consistent, possibly due to the competition in the accumulation of photoassimilates between fruits and roots, which starts around veraison (Morinaga et al., 2003).

Poni et al. (2006) have shown that the increase in seasonal carbon supply per crop unit (up to 38 %) is the main factor behind the enhanced grape quality in defoliated vines compared to controls. These authors further noted that quality improvement can be attributed to a combination of lower yield, lower canopy age and photosynthesis compensation. Verdenal et al. (2017) observed that enhanced wine quality could be related to greater skin thickness following LR. Surprisingly, the total anthocyanin content in the skin of Probus variety was also unaffected by year. These findings could be related to the higher difference in the berry composition within the Probus cluster compared to Cabernet-Sauvignon. In the present study, a higher standard error was noted for all tested Probus grape quality parameters.

The temperature during the growing season is directly related to grape maturity. Therefore, higher total anthocyanins in the skin and wine observed in our research could be the consequence of a temperature increase caused by LR. This is crucial to fulfilling thermal requirements needed for fruit maturation in cool summers (Frioni et al., 2017).
Ristic et al. (2007) found that exclusion of sunlight from the cluster decreased total anthocyanins.

The results yielded by the present study indicate that LR increased peonidin and its derivatives in the Cabernet-Sauvignon wine and ED increased the hydroxylated anthocyanin content. It also affected hue and colour stability, which are influenced by the hydroxylation and methylation pattern of the B ring of the anthocyanidins (He et al., 2010). An improvement in Cabernet-Sauvignon and Uni Blanc grape and wine quality as a result of the combined effects of CT and LR was also observed by Song et al. (2018). In the future, it would be interesting to explore the effects of LR and CT on berry skin thickness.

**CONCLUSIONS**

CT and LR affected grape and wine quality in both varieties. Among the LR treatments, ED was the most effective in both varieties. The anthocyanins content in Cabernet-Sauvignon wine was increased by CT and LR, but a lower yield in ED and CT did not compromise wine quality improvement. However, Probus was more influenced by weather conditions during the season. No negative effect of ED on grape and wine quality was observed. Moreover, ED decreased the incidence of Botrytis sp. in Probus, so ED treatment should be performed each year in the Probus vineyards to prevent the incidence of Botrytis sp.

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**REFERENCES**

Ačimović D., Tozzini L., Green A., Sivilotti P. and Sabbatini P., 2016. Identification of a defoliation severity threshold for changing fruitset, bunch morphology and fruit composition in Pinot noir. *A. J. Grape Wine Res.* 22, 399-408. doi:10.1111/ajgw.12235

Barros M.I.L.F., Fröhlich D.B., Mello L.L., Manica-Berto R., Malgarim M.B., Costa V.B. and Mello-Farias P., 2018. Impact of Cluster Thinning on Quality of “Malbec” Grapes in Encruzilhada do Sul-RS. *Am. J. Plant Sci.* 9, 495-506. doi:10.4236/ajps.2018.93037

Bešlic Z., Todić S. and Matijašević S., 2013. Effect of timing of basal leaf removal on yield components and grape quality of grapevine cvs Cabernet-Sauvignon and Prokupac (*Vitis vinifera* L.). *Bulg. J. Agric. Sci.* 19, 96-102.

Bledsoe A.M., Kliwer W.M. and Marois J.J., 1988. Effects of timing and severity of leaf removal on yield and fruit composition of Sauvignon blanc grapevines. *Am. J. Enol. Vitic.* 39, 49-54.

Brandt M., Scheidegger M., Rauhut D., Patz C.D., Will F., Zorn H. and Stoll M., 2019. The influence of temperature and solar radiation on phenols in berry skin and maturity parameters of *Vitis vinifera* L. cv. Riesling. *Oeno One* 53, 2. doi:10.20870/oeno-one.2019.53.2.2424

Burns J., Mullen W., Landrault N., Teissedre P.L., Lean M.E.J. and Crozier A., 2002. Variations in the profile and content of anthocyanins in wines made from Cabernet-Sauvignon and hybrid grapes. *J. Agric. Food Chem.* 50:4096-4102. doi:10.1021/jf011233s

Buttrose M.S., Hale C.R. and Kliwer W.M., 1971. Effect of temperature on the composition of ‘Cabernet-Sauvignon’ berries. *Amer. J. Enol. Vitic.* 22, 71-75.

di Proffio F., Reynolds A.G. and Kasimos A., 2011. Canopy management and enzyme impacts on Merlot, Cabernet franc, and Cabernet-Sauvignon. I. Yield and berry composition. *Am. J. Enol. Vitic.*, 62, 139-151. doi:10.5344/ajev.2010.10024

Diago M.P., Vilanova M. and Tardaguila J., 2010. Effects of timing of early defoliation (manual and mechanical) on the aroma attributes of Tempranillo (*Vitis vinifera* L.) wines. *Am. J. Enol. Vitic.* 61, 382-391.

Drenjančević M., Jukić V., Zmaić K., Kujundžić T., and Rastija V., 2017. Effects of early leaf removal on grape yield, chemical characteristics, and antioxidant activity of grape variety Cabernet-Sauvignon and wine from eastern Croatia. *Acta Agric. Scand. B Soil Plant Sci.* 67, 705-711. doi:10.1080/09064710.2017.1332238

Frioni T., Zhuang S., Palliotti A., Sivilotti P., Falchi R. and P. Sabbatini, 2017. Leaf removal and cluster thinning efficiencies are highly modulated by environmental conditions in cool climate viticulture. *Am. J. Enol. Vitic.* 68, 325-335. doi:10.5344/ajev.2017.16098

Greer D.H. and Weston C., 2010. Heat stress affects flowering, berry growth, sugar accumulation and photosynthesis of *Vitis vinifera* cv. Semillon grapevines grown in a controlled environment. *Funct. Plant Biol.* 37, 206-214. doi:10.1071/FP09209

He F., Mu L., Yan G.L., Liang N.N., Pan Q.H., Wang Y., Reeves M.J., and Duan C.Q., 2010, 37, 206-214. doi:10.1071/FP09209

He F., Mu L., Yan G.L., Liang N.N., Pan Q.H., Wang Y., Reeves M.J., and Duan C.Q., 2010. Biosynthesis of anthocyanins and their regulation in grapes. *Molecules*, 15, 9057-9091. doi:10.3390/molecules15129057

Intrieri C., Filippetti I., Allegro G., Centinari M. and Poni S., 2008. Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* 14, 25-32. doi:10.1111/j.1755-0238.2008.00004.x

Karoglan M., Osrečak M., Maslov L. and Kozina B., 2014. Effect of cluster and berry...
thinning on Merlot and Cabernet-Sauvignon wines composition. *Czech J. Food Sci.*, 32, 470-476. doi:10.17221/598/2013-CJFS

Keller M., Millis L.J., Wample R.L., Spayd S., 2005. Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. *Am. J. Enol. Vitic.* 56, 91-103.

Kemp B.S., 2010. The effect of the timing of leaf removal on berry ripening, flavour and aroma compounds in pinot noir wines. Ph. D. thesis, Lincoln University, Canterbury, New Zealand.

King P.D., Smart R.E. and McClellan D.J., 2015. Timing of crop removal has limited effect on Merlot grape and wine composition. *Agric. Sci.* 6, 456-465. doi:10.4236/as.2015.64045

Lopez G., Larrigaudiere C., Girona J., Behboudian M.H. and Marsal J., 2011. Fruit thinning in 'Conference' pear grown under deficit irrigation: Implications for fruit quality at harvest and after cold storage. *Sci. Hortic.* 129, 64-70. doi:10.1016/j.scienta.2011.03.007

Moreno D., Valdés E., Uriarte D., Gamero E., Talaverano I. and Vilanova M., 2017. Early leaf removal applied in warm climatic conditions: Impact on Tempranillo wine volatiles. *Food Res. Int.*, 98, 50-58. doi: 10.1016/j.foodres.2016.09.017.

Morinaga K., Imai S., Yakushiji H. and Koshita Y., 2003. Effects of fruit load on partitioning of 15N and 13C, respiration, and growth of grapevine roots at different fruit stages. *Sci. Hortic.* 239-253. doi:10.1016/S0304-4238(02)00199-1

OIV, 2007. OIV-MA-AS315-11 protocol-Determination of 9 major anthocyanins in red and rosé wines using HPLC (Oeno 22/2003, Oeno 12/2007).

Ough C.S. and Nagaoka R., 1984. Effect of thinning and vineyard yields on grape and wine composition and wine quality of Cabernet-Sauvignon. *Am. J. Enol. Vitic.* 55, 30-34

Palliotti A., Gardi T., Berrios J.G., Civardi S., and Poni S., 2012. Early source limitation as a tool for yield control and wine quality improvement in a high-yielding red *Vitis vinifera* L. cultivar. *Sci. Hortic.* 145, 10-16. doi:10.1016/j.scienta.2012.07.019

Petrie P.R and Clingeleffer P.R., 2006. Crop thinning (hand versus mechanical), grape maturity and anthocyanin concentration: outcomes from irrigated Cabernet-Sauvignon (*Vitis vinifera* L.) in a warm climate. *Aust. J. Grape Wine Res.* 12, 21-29. doi:10.1111/j.17550228.2006.tb00040.x

Petrie P.R., Trought M.C.T., Howell G.S., and Buchan G., 2003. The effect of leaf removal and canopy height on whole-vine gas exchange and fruit development of *Vitis vinifera* L. Sauvignon blanc. *Funct. Plant Biol.* 30, 711-717. doi:10.1071/FP02188

Poni S., Casalini L., Bernizzoni F., Civardi S. and Intrieri C., 2006. Effects of early defoliation on shoot photosynthesis, yield components, and grape quality. *Am. J. Enol. Vitic.* 57, 397-407.

Radovanović B. and Radovanović A., 2010. Free radical scavenging activity and anthocyanin profile of Cabernet-Sauvignon wines from the Balkan region. *Molecules* 15, 4213-4226. doi:10.3390/molecules15064213

Reščić J., Mikulic-Petkovsek M., Stampar F., Zupan A. and Rusjan D., 2015. The impact of cluster thinning on fertility and berry and wine composition of “Blauer Portugieser” (*Vitis vinifera* L.) grapevine variety. *J. Int. Sci. Vigne Vin*, 49(4), 275-291. doi:10.20870/oeno-one.2015.49.4.16

Reynolds A., Price S., Wardle D. and Watson B., 1994. Fruit environment and crop level effects on Pinot noir. I. Vine performance and fruit composition in the British Columbia. *Am. J. Enol. Vitic.*, 45, 452-459.

Ristic R., Downey P.G., Iland K., Bindon I.L., Francics M., Herderich, S. and Robinson S.P., 2007. Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. *Aust. J. Grape Wine Res.* 13, 53-65. doi:10.1111/j.1755-0238.2007.tb00235.x

Ryan J.M. and Revilla E., 2003. Anthocyanin composition of Cabernet-Sauvignon and Tempranillo Grapes at different stages of ripening. *J. Agric. Food Chem.* 51, 3372-3378. doi:10.1021/jf020849u

Sivilliotti P., Herrera J.C, Lisjak K., Česnik B.H., Sabbitini P., Peterlunger E., and Castellarin S.D., 2016. Impact of leaf removal, applied before and after flowering, on anthocyanin, tannin, and methoxypyraraze concentrations in ‘Merlot’ (*Vitis vinifera* L.) Grapes and Wines. *J. Agric. Food Chem.* 64, 4487-4496. doi:10.1021/acs.jafc.6b01013.

Song C.Z., Wang C., Xie S., and Zhang Y.W., 2018. Effects of leaf removal and cluster thinning on berry quality of *Vitis vinifera* cultivars in the region of Weibei Drvland in China. *J. Integr. Agric.* 17, 1620-1630. doi:10.1016/S2095-3119(18)61990-2

Tardaguila J., Martínez de Toda F., Poni, S., and Diago M.P., 2010. Impact of Early Leaf Removal on Yield and Fruit and Wine Composition of *Vitis vinifera* L. Graciano and Carignan. *Am. J. Enol. Vitic.* 61, 372-38.

Valdes E.M., Moreno Gamero E.D., Uriarte D., del Henar Prieto M., Manzano R., Picon J. and Intrigliolo S.D., 2009. Effects of cluster thinning and irrigation amount on water relations, growth, yield and fruit and wine composition of Tempranillo grapes in Extremadura (Spain). *J. Int. Sci. Vigne Vin.* 43, 67-76. doi:10.20870/oeno-one.2009.43.2.799

van Leeuwen C. and Dariet P., 2016. The impact of climate change on viticulture and wine quality. *J.W.E.* 11, 150-167. doi:10.1017/jwe.2015.21

Verdenal T., Zufferey V., Dienes N.A., Gindro K., Belcher S., Lorenzini F., Rösti J., Koestel C.,
Spring J.L. and Viret O., 2017. Pre-flowering defoliation affects berry structure and enhances wine sensory parameters. _Oeno One_ 51, 263-275. doi:10.20870/eno-one.2017.51.2.1808

Zhuang, S., Sabbatini, P., Tozzini, L., Green, A., Acimovic, D., Howell, G.S., Castellarin, S., 2014. Impact of cluster thinning and basal leaf removal on fruit quality of Cabernet Franc (_Vitis vinifera_ L.) grapevines grown in cool climate conditions. _Hort. Science_ 49(6), 750-756. doi:10.21273/HORTSCI.49.6.750