

## Effect of timing of leaf removal on yield, grape primary composition and volatile composition of *Vitis vinifera* cv. Merlot grapevines

### Utjecaj termina djelomične defolijacije prinos, osnovni kemijski sastav te na sadržaj hlapljivih spojeva u grožđu sorte Merlot (*Vitis vinifera* L.)

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Received: November 22, 2023; accepted: June 10, 2024

#### ABSTRACT

Leaf removal in the grape zone is one of the most common viticultural practices to manipulate the microclimate of grapevines and reduce canopy density. The timing of defoliation plays an important role in the synthesis of volatile compounds in the grapes, as they are synthesized at different times during grape ripening. A two-year study (2018 and 2019) was conducted in a vineyard in the region of Croatian Uplands, sub-region Prigorje-Bilogora, to investigate the effects of different timing of leaf removal on the volatile profile of the grape variety Merlot (*Vitis vinifera* L.). The experiment was conducted with four treatments: no leaf and lateral shoot removal, basal leaf and lateral shoot removal before flowering, at bunch closure and at the beginning of veraison, with 4 basal leaves and all lateral shoots per shoot removed. Yield and leaf area to yield ratio were not affected by leaf removal, while early leaf removal (before flowering) reduced cluster and berry weight. Regardless of the time of defoliation, total acidity in the must decreased, while only defoliation at the beginning of the veraison led to an increase in sugar content in the must. Leaf removal reduced the content of C6 volatile compounds, which produce green and vegetal aromas. Changing the microclimate in the canopy by removing leaves can significantly improve the volatile composition of grapes and thus contribute to the improvement of grape quality.

**Keywords:** grapevine, volatile organic compounds, C6 alcohols and aldehydes, leaf removal

#### SAŽETAK

Djelomična defolijacija u zoni grožđa često je korištena ampelotehnička mjera u vinogradu u cilju manipuliranja mikroklimatom trsa te smanjenja bujnosti trsa. Termin provođenja zahvata djelomične defolijacije igra veliku ulogu u sintezi različitih hlapljivih spojeva u grožđu, s obzirom na to da se oni u bobici sintetiziraju u različito vrijeme tijekom dozrijevanja grožđa. Provedeno je dvogodišnje istraživanje (2018. i 2019.) na sorti Merlot (*Vitis vinifera* L.) uzgajanoj u regiji Središnja bregovita Hrvatska, podregija Prigorje-Bilogora, kako bi se procijenila opravdanost različitih termina provedbe zahvata djelomične defolijacije na hlapljive spojeve grožđa. Djelomična defolijacije izvedena je u tri termina-prije cvatnje, u vrijeme zatvaranja grozdova te na početku šare grožđa, uklanjanjem 4 bazalna lista te svih zaperaka, dok je četvrti tretman kontrola. Djelomična defolijacija nije utjecala na prinos te na omjer lisne površine i prinosa, dok je rana defolijacija (prije cvatnje) utjecala na smanjenje mase grozda i mase bobice. Bez obzira na termin provedbe, djelomična defolijacija utjecala je na smanjenje sadržaja ukupnih kiselina u grožđu, dok je jedino djelomična defolijacija na početku šare utjecala na povećanje sadržaja šećera u grožđu. Djelomična defolijacija utjecala je na smanjenje sadržaja hlapljivih C6 spojeva u grožđu, koji daju zelene i vegetalne arome. Modifikacijom mikroklimatskih uvjeta trsa pomoću zahvata djelomične defolijacije može se značajno poboljšati hlapljivi sastav grožđa, a samim time i pridonijeti poboljšanju kvalitete grožđa.

**Ključne riječi:** vinova loza, hlapljivi organski spojevi, C6 alkoholi i aldehidi, djelomična defolijacija

## INTRODUCTION

Leaf removal in the grape zone is one of the canopy management practices used to manipulate the vine microclimate by increasing solar radiation and temperature in the grape zone (Young et al., 2016; Torres et al., 2021), reducing vine vigor and increasing air circulation (Chorti et al., 2018), which affects the reduction of relative humidity in the grape zone and pests and diseases infections in the later phases of vegetation (Van der Weide et al., 2020; Wurz et al., 2020).

By improving the microclimatic conditions of the vine and the balance between the ratio of older, less photosynthetically active leaves and younger, more photosynthetically active leaves (Intrieri et al., 2008), leaf removal can improve the composition of the grapes by increasing the content of polyphenolic and volatile compounds (Alatzas et al., 2023; Hickey et al., 2018; Pascual et al., 2017). In the research by Šuklje et al. (2014), wines obtained from grapes from the leaf removal treatment were associated with tropical fruit aromas, while wines obtained from control grapes were associated with aromas of green pepper, asparagus and grass.

An increase in temperature in the grape zone caused by leaf removal leads to an increase in respiration intensity and a decrease in the total acidity of the grapes (Alatzas et al., 2023; Hickey et al., 2018). Partial defoliation in the grape zone increases the sun exposure of the basal buds (Wang et al., 2020a) and promotes bud differentiation, which can lead to an increase in yield in the next season (Sánchez & Dokoozlian, 2005).

By improving the vine microclimate, the removal of basal leaves promotes the synthesis of volatile compounds such as monoterpenes and C13 norisoprenoids (Alessandrini et al., 2018; Kwasniewski et al., 2010; Young et al., 2016), and there is a decrease in the content of some volatile compounds such as methoxypyrazines (Ferrari et al., 2017; Gregan and Jordan, 2016) and C6 compounds (Komm and Moyer, 2015; Sun et al., 2023). Removing leaves in the grape zone increases the light intensity around the grapes and stimulates the expression of metabolic genes responsible for the biosynthesis of

volatile compounds or their precursors in grapes (Alatzas et al., 2023; Yue et al., 2020).

The removal of leaves in the grape zone can be performed with different intensities and at different times (Cataldo et al., 2021; Yue et al., 2020; Verdenal et al., 2019), and depending on this, it can affect the composition of the grapes differently. The timing of leaf removal plays an important role in the content of volatile compounds in grapes, as different volatile compounds in grapes are synthesized at different times during grape ripening (Wang et al., 2018).

C6 alcohols and aldehydes are a group of compounds that originate from grapes, but can also be formed during alcoholic fermentation by the action of yeasts. In grape berries, aldehydes are formed by lipoxygenase, whereby fatty acids are oxidized by lipoxygenase (LOX) to fatty acid hydroperoxides, which are degraded to aldehydes by hydroperoxide lyase (Zhu et al., 2012). The C6 volatile compounds transform from acetate esters to aldehydes and finally to alcohols during grape ripening (Kalua and Boss, 2009), but can be converted into corresponding alcohols (Schwab et al., 2008) and esters (Dennis et al., 2012) in wine by alcohol dehydrogenase.

Many factors influence the content of C6 compounds in grapes, such as variety, growing region, canopy management practices and environmental conditions (Kalua and Boss, 2009; Schüttler et al., 2015). Their content in grapes increases significantly immediately after ripening, followed by a decrease in content until harvest (Kalua and Boss, 2009). In wines, they give an unpleasant grassy aroma of unripe fruit (Koundouras, 2018).

The effects of different timing of leaf removal on the grape primary composition and volatile content is important to many grape growers and wine producers. The aim of this study is to investigate the influence of different timings of leaf removal- before flowering, at bunch closure and at the beginning of veraison, on the yield, grape primary composition and the content of C6 aldehydes and alcohols of the Merlot variety grown under the environmental conditions of the region of Croatian Uplands, sub-region Prigorje-Bilogora.

## MATERIAL AND METHODS

The research was conducted in 2018 and 2019 on the experimental field Jazbina of the Department of Viticulture and Enology of the Faculty of Agriculture, University of Zagreb (45°51'23,6"N, 16°00'24,9"E), on the grape variety Merlot, planted in 2005 and grafted on *Vitis berlandieri* x *Vitis riparia* SO4. The vineyard is located on a slope with a slightly south-west exposure at an altitude of 252 meters. The rows are arranged in a northwest-southeast direction and the average slope of the terrain is about 10%. The distance between the rows is 2.1 m and the distance between the vines is 1.2 m, resulting in a total of 4000 vines/ha. The height of the vine trunk is 80 cm and the training system is double Guyot, with two spurs and two canes left by winter pruning, giving an average load of 20-24 buds. Shoot trimming was done during the berry setting to avoid excessive shading of the grape zone. The vines were subjected to the usual cultivation methods for the Croatian Uplands. Data for daily air temperatures and precipitation for both seasons were obtained from a weather station in the vineyard.

The experiment was conducted with four treatments: Control- no leaf and lateral shoot removal; LR 1- basal leaf and lateral shoot removal before flowering (E-L stage 18); LR 2- basal leaf and lateral shoot removal at bunch closure (E-L stage 32); LR 3- basal leaf and lateral shoot removal at the beginning of veraison (E-L stage 35), according to the modified Eichhorn-Lorenz (E-L) scale (Coombe, 1995), with four basal leaves and all lateral shoots removed per shoot. The leaf removal treatments were repeated on the same vines in both years. The experiment was designed in a randomized block design with three replicates. Within the replicates, each treatment included three neighboring vines, which means that each treatment is represented by 9 vines.

The onset of the main phenological stages was determined visually. To determine the onset of the phenological stage, it had to be recorded on at least 50% of the vines observed. Budbreak was recorded when the tips of the young leaves were visible (E-L stage 4), flowering when 50% of the flower caps fell off (E-L stage

23), berry set when the berries were about 2-3 mm in diameter (E-L stage 27), bunch closure when the berries began to touch (E-L stage 32) and veraison when 50% of the berries changed color (E-L stage 35). The leaf area was calculated according to the method of Lopes and Pinto (2005) for each vine in the experiment.

The grape harvest was determined by weekly monitoring the content of soluble solids, titratable acidity and pH. Merlot was harvested manually on September 19, 2018, and September 20, 2019, in the morning hours. The yield was measured on a vine basis and the number of clusters per vine was counted. The clusters from each repetition of each treatment were pressed by hand to obtain the must. The soluble solids were determined with a refractometer, the total acidity with the titration method according to O.I.V. (2001) and the pH with a pH meter (Hanna instruments Edge). A sample of 100 berries was taken from each replicate, from which the skin was removed for the determination of volatile compounds.

The grape skins were stored in a freezer at -20 °C until extraction and analysis of the volatile compounds. Before analysis, skin samples were frozen overnight at -80 °C and dried by lyophilization. The procedure for analyzing volatile compounds is based on the method described by Šikuten et al. (2021). The analysis of volatile compounds was performed using a Gas Chromatographer coupled with an ISQ 7000 TriPlus quadrupole mass spectrometer, with analytes previously isolated by SPME-Arrow extraction. Samples of dry grape skins were ground to a fine powder using a MiniG grinder and stored in a freezer at -20 °C until analysis. The 100 mg samples were weighed into a glass vial with a volume of 20 mL and sealed with a stopper with a PTFE/silicone septum cap. The incubation and adsorption temperature were set to 60 °C and the incubation and adsorption time to 10 and 46 minutes, respectively. The desorption temperature was 250 °C and the duration was 7 minutes. For the chromatographic analysis, a Wax column with the dimensions 60 m x 0.25 mm x 0.25 µm was used with a linear temperature program in the temperature range from 40 to 210 °C with a temperature increase of 2 °C per minute. The mass

spectra were recorded by monitoring the current of all ions in the range from 20 to 500 m/z, with the electron energy set to 70 eV. The compounds were identified by comparing the retention times, retention indices and mass spectra with those of the NIST 17 and Wiley 12 databases. The results are expressed in absolute peak areas, which is common in SPME analysis of solid samples when a commercially available sample matrix is not available (Šikuten et al., 2021).

All data were analyzed by two-way analysis of variance (treatment, year) using XLSTAT software v.2020.3.1. (Addinsoft, New York, NY, USA).

## RESULTS AND DISCUSSION

Mesoclimatic conditions in 2018 and 2019 in terms of precipitation and air temperature are shown in Table 1 and were discussed in detail in Anić et al. (2021). In brief, the lower temperatures in April and May 2019 led to a delay in flowering by 16 days compared to 2018 (Table 2). However, temperatures from flowering to harvest were sufficient for the grape ripening process and the harvest

took place only one day later in 2019 than in 2018. In terms of precipitation, the 2019 season experienced 33% more precipitation from budbreak to berry set than in 2018, while 2018 experienced more precipitation from berry set to veraison (142%). In the final phase of grape ripening, there were no differences in the amount of precipitation recorded in both seasons.

The main objective of the study was to show the influence of the timing of leaf removal on the yield, grape primary composition, and the content of C6 compounds in grapes of the Merlot variety under the continental conditions of Croatia.

Leaf removal in the grape zone is an effective measure to increase bud fertility (Wang et al., 2020), which can lead to better bud differentiation and an increase in the number of clusters per bud in the next growing season. Leaf removal had no effect on the number of clusters per vine compared to the control (Table 3), which is consistent with the studies of Bubola et al. (2020) and Feng et al. (2015).

**Table 1.** Temperature conditions and precipitation (Jazbina, 2018 and 2019)

Month	Daily mean air temperature in °C		Precipitation (mm)	
	2018	2019	2018	2019
April	16.3	13.0	71	80.6
May	19.3	13.1	149.3	164.3
June	21.2	23.5	139.1	59.0
July	22.6	22.8	86.8	71.1
August	24.5	23.6	49.5	43.6
September	19.0	18.0	62.5	160.9
MvT <sup>1</sup>	19.7	18.4		
MaT <sup>2</sup>	13.3	13.4		
ΣvO <sup>3</sup>			585.1	623.2
ΣgO <sup>4</sup>			940	1037

<sup>1</sup> mean air temperature during vegetation (1.4.-31.10.)

<sup>2</sup> mean annual air temperature

<sup>3</sup> precipitation during vegetation (1.4.-31.10.)

<sup>4</sup> total annual precipitation

**Table 2.** Day of the year for the beginning of different phenological stages of the Merlot variety (Jazbina, 2018 and 2019)

Month	Day of the year		Date	
	2018	2019	2018	2019
Budbreak	90	92	March 31	April 2
Flowering	151	167	May 31	June 16
Berry set	162	178	June 11	June 27
Bunch closure	185	198	July 4	July 17
Veraison	207	221	July 26	August 8
Harvest	261	262	September 19	September 20

Kliwer and Dokoozlian (2005) showed that the appropriate ratio of leaf area to yield to support the development and ripening of grapes is 0.8 - 1.2 m<sup>2</sup>/kg, which was also shown in our study (Table 3). Leaf removal had no effect on yield and leaf area to yield ratio. In the experiment of Van der Weide et al. (2020), early defoliation had no effect on yield. On the other hand, Lopes et al. (2020) and Sabbatini and Howell (2010) showed that

the removal of 6 basal leaves before or during flowering reduced the yield. Since basal leaves are the main source of assimilates between flowering and berry set (Pallioti et al., 2011), basal leaf removal can affect the balance between yield and leaf area, which can negatively impact carbohydrate reserves in the vine (Frioni et al., 2018). This can reduce the differentiation of flower primordia and reduce the fertility of the buds in the next growing

**Table 3.** Leaf removal effect on Merlot yield components (Jazbina, 2018 and 2019)

	Yield per vine (kg)	Cluster weight (g)	Berry weight (g)	Clusters /vine	Leaf area/yield (m <sup>2</sup> /kg)
Treatment					
Control	4.05	171.8 <sup>a5</sup>	1.79 <sup>a</sup>	25.7	0.98
LR 1 <sup>1</sup>	3.21	135.5 <sup>b</sup>	1.57 <sup>c</sup>	22.9	0.82
LR 2 <sup>2</sup>	3.38	144.4 <sup>ab</sup>	1.63 <sup>bc</sup>	26.8	0.83
LR 3 <sup>3</sup>	3.93	152.2 <sup>ab</sup>	1.75 <sup>ab</sup>	25.8	0.79
Sign. <sup>4</sup>	ns	*	*	ns	ns
Year					
2018	3.81	139.1	1.85	26.9	0.86
2019	3.87	160.8	1.52	23.7	0.85
Sign.	ns	*	**	*	ns
Treatment x year	*	ns	**	ns	ns

<sup>1</sup> leaf removal performed before flowering<sup>2</sup> leaf removal performed at bunch closure<sup>3</sup> leaf removal performed at veraison<sup>4</sup> Data were analyzed using two-way mixed model ANOVA; for significant difference among values, means were separated using Tukey's test. ns, non-significant; \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.0001$ <sup>5</sup> Different letters identify significantly different means

season. If defoliation takes place around or after bunch closure, leaf removal generally has no effect on yield components, as shown in Table 3, but also in research by Feng et al. (2015), Kwasniewski et al. (2010), Masetti et al. (2016) and Young et al. (2016).

The effects of defoliation on vine growth and yield components depend on the timing and intensity of the treatment. Leaf removal before flowering led to a reduction in bunch and berry weight, while leaf removal at bunch closure only led to a reduction in berry weight compared to the control treatment (Table 3). In the studies of Intrieri et al. (2008) and Lopes et al. (2020), the removal of basal leaves before flowering led to a reduction in yield, as the photosynthetically active leaves were removed at a time when the flowers have a high demand for assimilates, which affects the carbon balance of the vine.

Climatic conditions during the growing season play an important role in grape ripening (Masetti et al., 2016). Table 1 shows that low air temperatures in the spring months

delayed the growth of the vines in 2019 (Table 2), but the air temperatures during grape ripening were appropriate and the grapes reached the appropriate technological maturity, although with slightly less favorable values of the grape primary composition compared to the previous year (Table 4).

The timing of leaf removal played an important role in the accumulation of sugars, total acids and pH values of Merlot grapes in 2018 and 2019 (Table 4). Leaf removal at the beginning of veraison led to an increase in sugar content, while earlier timings had no effect on the sugar content in the grape juice. Studies show that sugar accumulation is higher in grapes exposed to sunlight (Song et al., 2015), as the increased solar radiation from defoliation can increase the activity of enzymes involved in the regulation of sugar metabolism in grapes (Rienth et al., 2016).

Intensive leaf removal (Cataldo et al., 2021) or leaf removal before flowering can be expected to disrupt grape ripening, reduce berry set and consequently

**Table 4.** Leaf removal effect on Merlot grape juice primary composition (2018 and 2019)

	Soluble solids (Brix)	Titrateable acidity (g/L)	pH
Treatment			
Control	23.2 <sup>b5</sup>	6.4 <sup>a</sup>	3.41 <sup>b</sup>
LR 1 <sup>1</sup>	23.4 <sup>b</sup>	6.1 <sup>b</sup>	3.41 <sup>b</sup>
LR 2 <sup>2</sup>	23.2 <sup>b</sup>	6.0 <sup>b</sup>	3.39 <sup>c</sup>
LR 3 <sup>3</sup>	23.9 <sup>a</sup>	5.9 <sup>c</sup>	3.42 <sup>a</sup>
Sign. <sup>4</sup>	***	***	***
Year			
2018	24.2	5.7	3.52
2019	22.5	6.5	3.31
Sign.	***	***	***
Treatment x year	***	***	***

<sup>1</sup> leaf removal performed before flowering

<sup>2</sup> leaf removal performed at bunch closure

<sup>3</sup> leaf removal performed at veraison

<sup>4</sup> Data were analyzed using two-way mixed model ANOVA; for significant difference among values, means were separated using Tukey's test. ns, non-significant; \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.0001$

<sup>5</sup> Different letters identify significantly different means

reduce grape and berry weight, as was also shown in our research, but also in the research of Intrieria et al. (2008).

The changes in sugar content during grape ripening depend on sugar accumulation, sugar metabolism and the degree of dilution of the berry. Sugar accumulation in grapes is highly influenced by the balance between leaf surface area and yield (leaf-to-fruit ratio). If this ratio is too low ( $< 0.7 \text{ m}^2/\text{kg}$ ), sugar accumulation is reduced (Kliwer and Dokoozlian, 2005). The leaf removal before flowering and at bunch closure had no effect on the sugar content of the grapes in both study years, which is consistent with the studies by Chorti et al. (2018), Feng et al. (2015) and Riesterer-Loper et al. (2019) and can be explained by the recovery mechanism and the increased photosynthetic activity of the remaining leaves (Mataffo et al., 2023).

Leaf removal led to a reduction in titratable acidity at harvest, regardless of timing, with the lowest level observed when leaf removal was performed at the beginning of veraison. A reduction in total acidity in grapes in defoliation treatments could be due to an increase in sunlight and temperature within the grape zone, which could lead to malic acid degradation due to increased cellular respiration (Rienth et al., 2016). The timing of defoliation affected the pH of the grapes, although no significant difference was observed from a technological point of view. Some studies have shown that leaf removal affects the total acidity of grape juice (Alatzas et al., 2023; Lopes et al., 2020; Mosetti et al., 2016; Riesterer-Loper et al., 2019; Yue et al., 2020; Verdental et al., 2019), while others show no influence of leaf removal on the primary composition of grapes (Feng et al., 2015; Young et al., 2016).

Most volatile organic compounds are produced during alcoholic fermentation, but some of them can also come from grapes. There are numerous studies on the influence of genotype, environmental factors and canopy management practices on the volatile composition of grapes (Alessandrini et al., 2018; Feng et al., 2015; Joubert et al., 2016; Rienth et al., 2021). Understanding how environmental factors influence the formation of

volatile compounds in grapes and their degradation during grape ripening is essential for the development of strategies to adapt canopy management practices to achieve the desired aroma profile of wine.

Although the effect of defoliation in the grape zone on the phenolic composition of grapes has been frequently studied in red grape varieties (Chorti et al., 2018; Hickey et al., 2018; Osrečak et al., 2016), there are few studies on the influence of leaf removal on the volatile composition of grapes in red grape varieties (Feng et al., 2015).

The two main groups of volatile compounds associated with green aromas are methoxypyrazines and volatile C6 compounds. High levels of green and vegetal aromas in red wines are thought to have a negative impact on the aroma of the wine itself (Preston et al., 2008). The content of C6 compounds is associated with unripe grapes (Gao et al., 2019). Both C6 alcohols and aldehydes in grapes are formed as a product of fatty acid oxidation (Ferreira and Lopez, 2019), with the conversion of acetate esters to aldehydes and finally to alcohols occurring during grape ripening (Kalua and Boss, 2009). The C6 aldehydes hexanal and 2-hexenal are associated with unripe grapes, as their content decreases during grape ripening (Gao et al., 2019).

The C6 volatile compounds detected in the grape skin were the aldehydes (E)-2-hexenal and hexanal as well as the alcohols 1-hexanol, (E)-3-hexen-1-ol and (E)-2-hexenol. Leaf removal decreased the content of C6 volatile compounds in the grape skin (Table 5), with the greatest decrease occurring when the leaves were removed before flowering.

The synthesis of C6 compounds is strongly influenced by changes in vine microclimate, with an increase in sunlight causing a decrease in the content of C6 compounds (Wang et al., 2020b). UV-B radiation regulates the metabolism of the C6 compounds n-hexanal and trans-2-hexenal in grape berries (Joubert et al., 2016). In the study by Bureau et al. (2000), it was found that shading of grapes affects the increase in the content of hexanal and (E)-2-hexenal, which could explain the decrease in the content of C6 compounds in our samples due to the increase in

solar radiation caused by the removal of leaves. In the study given by Komm and Moyer (2015), early defoliation affected the reduction of hexanal content in grapes of the Riesling variety. In a study by Feng et al. (2015), the removal of the basal leaves when berries were pea-size, did not affect the content of C6 compounds in grapes of the Pinot blanc variety, which was related to the lack of differences in the degree of ripeness of the grapes.

In the study by He et al. (2020), leaf removal affected the increase in the content of C6 alcohols, especially (Z)-3-hexenol, which is due to the increased expression of genes encoding the activity of the enzyme lipoxygenase and alcohol dehydrogenase (VviADH1), which is involved

in the lipoxygenase-hydroperoxide metabolic pathway, and also to the reduction of the C6 aldehydes hexanal and (E)-2-hexenal, which is due to the increased solar radiation caused by leaf removal. Early defoliation can influence the reduction (Vilanova et al., 2012) but also the increase (Gambacorta et al., 2022; Iorio et al., 2022) of the content of C6 compounds in the wine of the Tempranillo and Aglianico varieties.

Air temperature affects the content of C6 compounds in grapes, and it has been shown that the content of C6 compounds correlates negatively with the increase in air temperature from flowering to harvest (Lu et al., 2022; Wang et al., 2020b).

**Table 5.** Leaf removal effect on Merlot C6 alcohols and aldehydes content (2018 and 2019)

	(E)-2- hexenal	Hexanal	1- hexanol	(E)-3- hexen-1-ol	(E)-2-hexenol
Treatment					
Control	685 <sup>6</sup> a <sup>5</sup>	110 <sup>a</sup>	1080 <sup>a</sup>	55 <sup>a</sup>	1150 <sup>a</sup>
LR 1 <sup>1</sup>	405 <sup>d</sup>	nd	720 <sup>d</sup>	32 <sup>d</sup>	420 <sup>d</sup>
LR 2 <sup>2</sup>	600 <sup>b</sup>	7 <sup>c</sup>	865 <sup>b</sup>	44 <sup>b</sup>	930 <sup>c</sup>
LR 3 <sup>3</sup>	470 <sup>c</sup>	80 <sup>b</sup>	825 <sup>c</sup>	41 <sup>c</sup>	110 <sup>b</sup>
Sign. <sup>4</sup>	***	***	***	***	***
Year					
2018	390	55	780	40.5	857.5
2019	690	43.5	965	45.7	942.5
Sign.	***	***	***	***	***
Treatment x year	***	***	***	***	***

<sup>1</sup> leaf removal performed before flowering

<sup>2</sup> leaf removal performed at bunch closure

<sup>3</sup> leaf removal performed at veraison

<sup>4</sup> Data were analyzed using two-way mixed model ANOVA; for significant difference among values, means were separated using Tukey's test. ns, non-significant; \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.0001$

<sup>5</sup> Different letters identify significantly different means.

<sup>6</sup> values are expressed in absolute peak area  $\times 10^5$



## CONCLUSION

The composition and content of volatile compounds in grapes depend largely on the genotype of the vine, the environmental conditions during the ripening of the grapes and the canopy management practices in the vineyard. Early leaf removal reduced cluster and berry weight and the total acidity in the must, while leaf removal at the beginning of the veraison led to an increase in sugar content and pH and a decrease in total acidity in the must, which could lead to unbalanced wine. Leaf removal reduced the content of C6 volatile compounds in the grape skin. Since C6 alcohols and aldehydes have a negative effect on wine aroma, the removal of leaves in the grape zone can lead to a reduction in their content, which can have a positive effect on wine aroma.

The results represent a particular challenge for winegrowers to adapt the vineyard management strategy and thus the canopy management practices to the climatic conditions of the season and that, in the context of climate change and rising air temperatures, it is necessary to adapt the leaf removal treatment depending on the desired quality of grapes.

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