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THE INFLUENCE OF VITICULTURAL PRACTICES ON THE BERRY COMPOSITION OF MERLOT VARIETY GROWN IN THE WEST OF ROMANIA CLIMATE

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Abstract

Recent climatic conditions have favoured the expansion of red varieties in previously unsuitable areas for high-quality winemaking. The high degree of temperature variability during the growing season makes it challenging for berries to fully ripen in time for harvest. The objective of this research was to improve the berry quality and sensory characteristics of the Merlot variety in the Recaş vineyards area by implementing various viticultural practices. The timing and intensity of leaf removal and cluster thinning were examined in experiments carried out in 2020 and 2021. The cluster thinning had a minimal effect on basic berry chemistry at harvest in 2020 but increased pruning weight and cluster weight. Vine vigour and essential berry chemistry were not strongly influenced by clusters thinning in 2021. The level of titratable acidity was lower after leaf removal, but other essential quality indicators remained unchanged. Viticultural practises not only increased the berry quality but also the anthocyanin content of berries.

Key words*: berry quality, cluster thinning, leaf removal, sugars, titratable acidity.*

INTRODUCTION

Grape cultivation is one of the most important agricultural activities on the planet. According to FAO statistics (2021), the global area cultivated with grapevines in 2021 was 6.73 million hectares, with 73.5 tonnes of grapes for wine and table, resulting in 755 352 081 hl of wine. The world's largest grape production is concentrated in four countries: Italy, Spain, France, and China (FAOStat, 2021). Grapevine cultivation is restricted by climate regions, with annual isotherms ranging from 10°C to 20°C, with the possibility of expansion in favourable microclimates (Kosik et al., 2017). Different viticultural practises can compensate for less-than-ideal conditions for Merlot grapes, which do not reach full maturity every year (Anić et al., 2021). To achieve the best quality of the grapes and the wine, winemakers must understand how to fully ripen this variety in order to prevent unpleasant, grassy aromas (Trujillo et al., 2022). The process of berry growth and development occurs through cell division in the first stage; the second stage is the veraison stage, during which the accumulation of sugars in the pulp and skins, of anthocyanins, and potassium begins and towards the end, the colouring and softening of the berries; the third stage is the ripening stage, during which sugars accumulate primarily in the pulp and anthocyanins in the skins, especially in red varieties (CooMbe & McCarthy, 2000; Nan et al., 2019). A balance between vegetative and productive growth is required for optimal compound accumulation in berries (Tomasi et al., 2020). The crop load (the ratio of grape yield and the weight of the pruning wood) is accepted as an indicator of grapevine balance, with optimal values ranging from 5 to 10 (Silvestroni et al., 2019). Another useful tool for assessing vine balance is the ratio of yield to leaf area (expressed in cm^2/kg or m^2/kg), with leaf photosynthesis activating the production of carbohydrates that are transported to the berries (Del Zozzo et al., 2022). The management system, in addition to the vigour of the variety, influences photosynthesis efficiency and, indirectly, crop load (Nistor et al., 2021). A crop load that is too high (over 15 clusters) restrains the proper berry quality achieved at harvest, and a crop load that is too low (less than five bunches) is not economically efficient (Candar

et al., 2020). Sucrose is transported to the berries after photosynthesis in the leaves, where sugars measured as total soluble solids (TSS) accumulate between 18.5 and 27ºBrix, depending on variety, viticultural practises, and environmental conditions (Lu et al., 2022). According to some studies, thinning leaves or bunches reduced production and increased sugar content compared to vineyards with high grape production (Sivilotti et al., 2020), but other studies show that these practises had little to no effect on the amount of sugar that accumulated in berries up until harvest (Williams et al., 2023). After fermentation, grape seed compounds such as sugars, organic acids, polyphenols, and aromas significantly influence the wine's final quality (Mesić et al., 2020).
The study therefore concentrated on achieving the best Merlot grape quality (sugar, anthocyanins, pH, TA) by adjusting the crop load, leaf removal and cluster thinning at various growth stages during the 2020 and 2021 growing seasons.

MATERIALS AND METHODS

Plant materials

The Merlot (grafted on Kober 5 BB rootstocks) plots were planted in the Recaș vineyards in 2009 and were used for research in 2020 and 2021 growing seasons. The Recaș vineyards are located 24 kilometres from Timișoara, at latitude 45°48'4.18"N and longitude 21°30'42.89"E. The Recaș vineyards cover an area of more than 1000 hectares spread out over hills with calcareous or clay soils.
The experimental plots had 10 rows and 5 vines per row with a simple Guyot training, a planting distance of 2.2 m between rows and 1 m between vines per row, and an average density of 4,545 vines/hectare. During dry pruning, 45 buds per vine were retained. The vigorous shoots were hedged during the growing season when their tips reached a height of 30 cm above the highest trellis. During the growing season, pest and disease control measures were implemented based on past performance, alerts, and weather conditions. The Timișoara meteorological station and satellite data for the Recaș location (https://freemeteo.ro/vremea/ recas/) were used to determine the monthly precipitation and daily temperature during the growing season.

Field experiment

The vines in the experiment were divided into eight blocks of five vines each, for a total of 40 vines. Blocks were randomly assigned to the fruit set, pre-veraison, veraison, and before ripening stages. The fruiting load was set at 15, 30, and 45 bunches per vine; the first four basal leaves on the shoot were removed to expose the clusters, as Merlot is a medium-vigorous variety; apical clusters that remained less developed were removed. Leaf and bunch thinning were done simultaneously.

Measurement of shoot growth

At flowering, the total number of shoots was counted, of which six were marked for length monitoring (cm/day) every seven days, beginning from the second week of June until veraison in the last week of July, using a measuring tape. The shoot growth measurements were useful for estimating daily growth, correlated with phenological stages and the effects of crop load and leaf removal on their length.

Cluster and berry sampling

From fruit set to ripening, 50 berries were collected from each selected vine at 14-day intervals. The samples were placed in labelled plastic bags and transported to the laboratory in a freezing bag, where they were kept at -20ºC for future analysis. The total weight of bunches on a vine, the average weight of a cluster, and the average weight of berries in a cluster were all recorded.
Clusters on marked shoots were collected separately, and berries were measured for sugars, titratable acidity, and pH. Before analysis, the berries were defrosted and crushed, and the naturally drained juice was decanted into 50 ml cylinders. The sugar content of the must was determined using a Brix HI96813 digital refractometer (Hanna Instruments, Inc). Total acidity (TA H_2SO_4) and pH were measured with HI 84532 titrator (Hanna Instruments, Inc).

Statistical Analysis

Analyses of variance (ANOVA) for yield and berry components were performed using GraphPadPrism Software, Version 8.4.2 (San Diego, California, USA, 2020) and XLStat (by Addinsoft, 2018, Statistical and Data Analysis Solution Version 2018.7.5). Comparisons were made by the LSD test with significance levels of 0.05.

RESULTS AND DISCUSSIONS

Climate conditions

Following a less rainy spring in 2020 (Figure 1), there was abundant precipitation (over 100 mm) during the shoot growing stage (June, July) until veraison, with the phenological stages developing at a constant rate. There was much less rain and much higher temperatures during the same time period in 2021. August was the warmest and driest month, following veraison in both growing seasons. It was relatively wet in 2020 during grape ripening, which began in the second decade of September, but very dry in 2021.

The maximum air temperature reached more than 35°C during the summer of 2021, but no severe sunburn damage to grape berries was observed.

Shoot length growth was unaffected by leaf and bunch thinning. Regardless of the fruiting load, the lateral bunches were the most exposed to the sun after the leaves were removed.

Vegetative growth in 2020-2021

Leaves or clusters thinning had no effect on the length of the shoots, but the earlier these treatments were done, the greater the impact on the growth rate of the shoots (Figures 2 and 3).

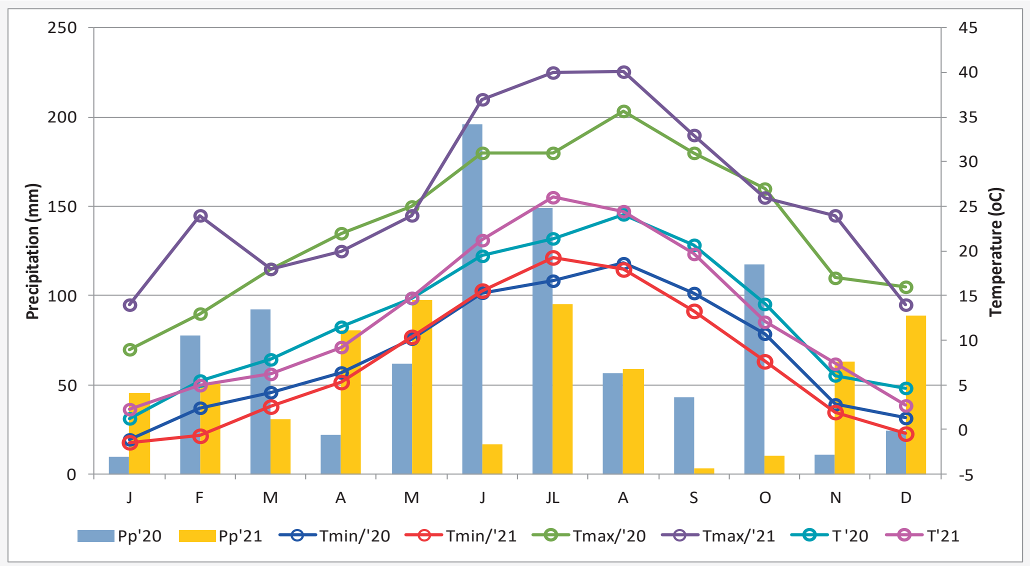

Figure 1. Temperature and precipitation during 2020-2021
(Pp - precipitation; Tmin - minimum temperature; Tmax - maximum temperature; T - average temperature)

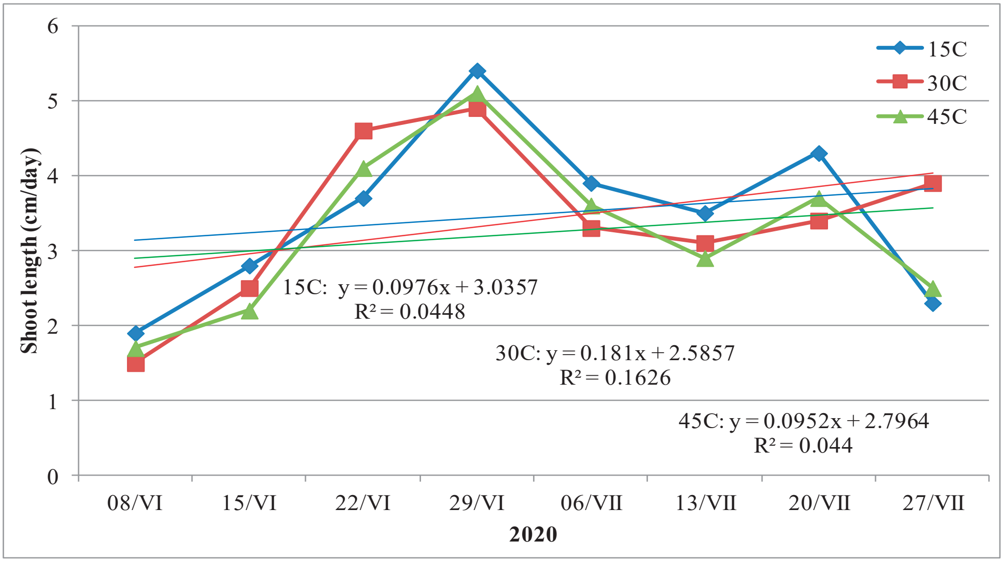

Figure 2. Shoot growth (cm/day) from the beginning of June to the end of July in 2020 (15°C, 30°C and 45°C are abbreviations for crop load on vine; data were collected from vines selected for leaf removal and cluster thinning)

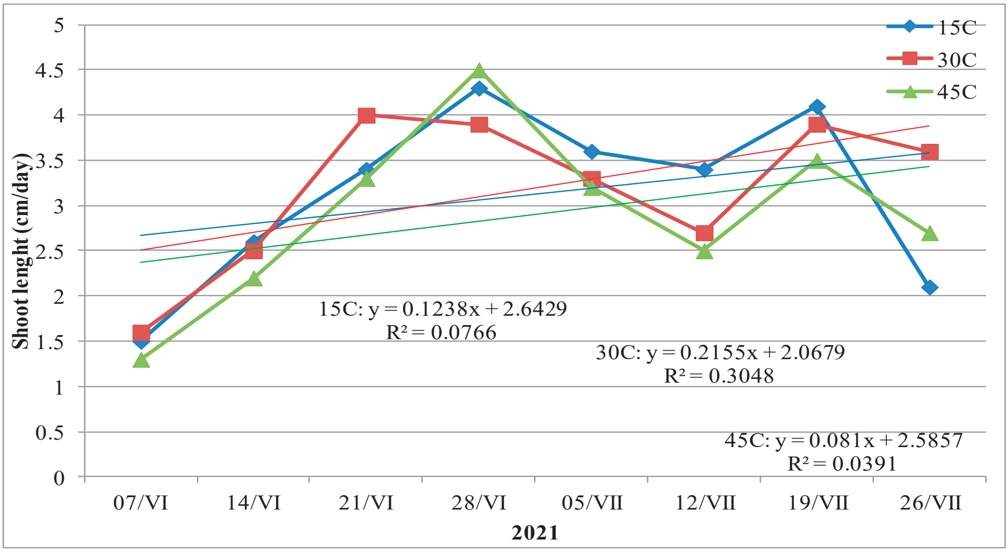

Figure 3. Shoot growth (cm/day) from the beginning of June to the end of July in 2021 (15°C, 30°C and 45°C are abbreviations for crop load on vine; data were collected from vines selected for leaf removal and cluster thinning)

With the exception of the crop load of 30 clusters in 2021, which positively influenced shoot length growth, cluster exposure had no effect on shoot growth rate.

Grape production components

Cluster thinning during berries setting, with a crop load of 15 clusters, significantly decreased grape production per vine (Tables 1 and 2).

Table 1. The influence of basal leaf removal and cluster thinning on crop yield (2020)

Crop load	Yield (kg/vine)			
	Berry set	Pre-veraison	Veraison	Before ripening
15 clusters	1.19[b]	1.29[b]	1.59[b]	1.59[b]
30 clusters	3.40[a]	2.65[a]	3.30[a]	3.21[a]
45 clusters	2.43[a]	3.20[a]	4.65[a]	4.98[a]

[a, b] Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

However, there was no significant difference in the yield after leaf thinning at 30 and 45 clusters of crop load. During the 2021 growing season, only the 45 cluster crop load per vine showed significant yield differences when cluster thinning was done before, during or after veraison.

Table 2. The influence of basal leaf removal and cluster thinning on crop yield (2021)

Crop load	Yield (kg/vine)			
	Berry set	Pre-veraison	Veraison	Before ripening
15 clusters	1.27[b]	1.56[b]	1.65[c]	1.53[b]
30 clusters	3.52[a]	2.71[ab]	3.42[b]	3.15[b]
45 clusters	2.94[a]	3.38[a]	4.80[a]	4.45[a]

[a, b, c] Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

Leaf and severe cluster thinning (crop load of 15 clusters) at berries setting significantly decreased grape yield in the 2021 growing season (Table 2) compared to a crop load of 45 clusters per vine. In the berry setting stage, grape yield did not differ significantly between 30 and 45 clusters of crop load. When leaf and bunch thinning were performed at veraison, grape production differed for all three crop loads, with the higher number of clusters per vine yielding the most. Regarding cluster weight, the interaction between the three factors - leaf removal, cluster thinning, and cluster weight respectively was significant.

Table 3. The influence of basal leaf removal and cluster thinning on cluster weight (2020)

Crop load	Cluster weight (g)			
	Berry set	Pre-veraison	Veraison	Before ripening
15 clusters	89[a]	101[a]	122[a]	105[a]
30 clusters	76[c]	81[b]	93[b]	80[b]
45 clusters	59[c]	68[c]	76[c]	86[b]

[a, b, c] Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

The lower number of clusters per vine obviously resulted in larger and heavier clusters when compared to the other two crop loads of 30 and 45 clusters per vine. However, cluster weight increased only for 15 crop loads when cluster thinning was done before and after veraison (Table 3). Regardless of crop load, the difference in cluster weight at berry set was insignificant. In fact, when analyzing bunch weight for the four phenological phases, no significant differences were found as a result of crop load influence.

Heavier clusters were also observed in the 2021 growing season at 15 and 45 clusters per vine (Table 4). The smallest weight differences between bunches, regardless of crop load, were when leaf removal and bunch thinning were done at veraison; the differences were insignificant at other growing stages.

Table 4. The influence of basal leaf removal and cluster thinning on cluster weight (2021)

Crop load	Cluster weight (g)			
	Berry set	Pre-veraison	Veraison	Before ripening
15 clusters	96[a]	106[a]	115[a, b]	108[a]
30 clusters	68[b]	74[b]	124[a]	96[ab]
45 clusters	86[a]	84[b]	106[ab]	78[b]

[a, b] Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

In the 2020 growing season, no significant differences in sugar accumulation (°Brix) were found for the levels of the cluster thinning (Table 5). The effect of leaf removal on sugar accumulation was more obvious when the treatments were done at veraison, and the berries were exposed to sunlight before ripening began.

Table 5. The influence of leaf removal and cluster thinning on TSS (°Brix) (2020)

Treatment	TSS (°Brix)			
	Berry set	Pre-veraison	Veraison	Before ripening
Leaf removal	15.3[a]	18.4[a]	20.8[a]	21.9[b]
Cluster thinning	16.9[a]	19.1[a]	21.5[a]	22.6[a]

[a, b] Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

Table 6. The influence of leaf removal and cluster thinning on TSS (°Brix) (2021)

Treatment	TSS (°Brix)			
	Berry set	Pre-veraison	Veraison	Before ripening
Leaf removal	16.5[b]	19.6[a]	21.4[a]	22.9[b]
Cluster thinning	17.1[a]	20.4[a]	22.3[a]	23.6[a]

[a,b] Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

When grape berries were exposed to sunlight through leaf removal and cluster thinning during the same phenological phase in the 2021 growing season, the differences in sugar accumulation were insignificant (Table 6). However, when the treatments were applied at different phenological stages, there were significant differences in sugar accumulation, taking into account that sugar accumulation increases as the berries reach maturity.

Table 7. Berry components depending of growing season timing of leaf removal and cluster thinning

Berry components	Berry set	Pre-veraison	Veraison	Before ripening
	Treatment during 2020 growing season			
pH	3.4[a]	3.6[a]	3.8[a]	3.8[a]
TA (g/L H_2S0_4)	5.5[a]	4.9[a]	4.7[a]	4.3[a]
Anthocyanins (mg/g)	0.71[a]	0.69[a]	0.63[a]	0.72[a]
	Treatment during 2021 growing season			
pH	3.2[a]	3.5[a]	3.3[a]	3.5[a]
TA (g/L H_2S0_4)	5.7[a]	5.0[a]	4.9[a]	4.5[a]
Anthocyanins (mg/g)	0.73[a]	0.68[a]	0.65[a]	0.76[a]

[a, b] Means within columns that are not followed by the same letter differ significantly at $p \leq 0.05$

In both growing seasons, except for sugars, no significant relationships were found between treatment timing and the other components of berry juice (TA, pH, and anthocyanins) as a result of grape berry exposure to sunlight after leaf removal and cluster thinning (Table 7).

It was difficult to find a correlation between treatments (leaf removal, cluster thinning, and treatment timing). Furthermore, there was a lot of rain, and it was very hot in June and July 2020, which stimulated the growth of the shoots and made measurements harder to manage. Grape yield increased, however, when the crop load per vine was 15 or 30 clusters. When the treatments were applied at berry set, the production increased for the higher crop load of 45 clusters. When the treatments were applied early in the growing season, at berry set, pre-veraison, or veraison but not before ripening, bunch weight increased at a low crop load (15 clusters). The weight of the berries in the cluster increased as a result of leaf removal and cluster thinning, which improved grape yield. Early leaf removal (at berry set) affected berries by sunlight and, consequently, sugar accumulation; however, during both growing seasons, key berry juice constituents (pH, TA, and anthocyanins) were not significantly influenced by the timing of treatments and crop load.

Leaf removal when the temperature was very high at the end of July and especially in August was beneficial for reducing the malic acid content of berry juice (Ghiglieno et al., 2020); sunlight and temperature were also important for anthocyanin accumulation (Tarricone et al., 2020). The lowering of crop load, or vine vigour, had no noticeable impact on berry ripening or on the main components of the berries, such as sugars which could explain the absence of significant differences between the chemical components of berry juice. The berry content in various qualitative components influenced not only the amount of sugar but also the grape yield. The anthocyanin content was influenced by the interaction of treatments (leaf removal and cluster thinning) as well as the increase in temperature at the cluster level as a result of leaf removal (Alba et al., 2022). Previous research found that when the crop load is balanced with the canopy, the accumulation of sugars or other qualitative components from berries is unaffected in hot climates (Anić et al., 2021; King et al., 2012; Pieri, 2010).

Sugar accumulation was relatively stable despite differences in humidity between the two growing seasons, indicating that viticultural practises had a relatively low influence on this qualitative component compared to anthocyanin content (O'Brien et al., 2021; de Rosas et al., 2022).

However, contradictory findings were found regarding sugar accumulation, which was influenced by viticultural practises and environmental conditions (Navrátilová et al., 2020; Aru et al., 2022); TSS content was found to be higher after the cluster thinning or leaf removal in some studies (Chorti et al., 2010; Wang et al., 2022). Usually, the presence of more sugar has always been associated with ripened berries, serving as an indicator of berry maturity (Gris et al., 2010; Previtali et al., 2021).

Coarfă & Popa (2020) found an evolution of 38 g of sugar content over a period of 13 days, from 187.5 to 225.8 g/L, during the gradual harvesting of Merlot berries from the final decade of August to the second decade of September 2017 (both months with rainfall deficiency). In a similar climate (temperature and rainfall) from July to September (2020), Onache et al., (2021) found that Merlot grapes ripened approximately 10 days earlier than the previous year 2019 due to the highest temperatures recorded during the growing season, with 210 g/L sugar content, 3.06 total acidity (H_2SO_4), and a lower anthocyanin content of 464.87 mg/L.

CONCLUSIONS

The research in 2020 and 2021 were quite similar, with an emphasis on the effect of crop load, leaf removal, and grape berry exposure to sunlight on Merlot grapes. Similar outcomes were observed in both seasons for grape yield and berry quality components, with minor differences influenced by precipitation during the pre-veraison and veraison time frames. Small grape yield differences were also observed between the treatments, especially between the grape yield after leaf removal and after the cluster thinning.

In comparison to 2020, however, the grapevine has been less affected by leaf removal and cluster thinning treatments in 2021. The impact of these treatments on shoots growth has been negligible. The research results from both growing seasons show that cluster thinning had little effect on grape yield and berry components in the 2021 growing season. When the number of clusters was reduced in the berry set or pre-veraison stages, the vines had higher vigour and larger clusters as a result of the climatic conditions of the 2020 growing season. Finally, grapevine balance is determined by the canopy vigour of the grape variety and the timing of leaf removal, both of which can influence grape berry quality improvement.

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