Bogicevic et al. Chemical and Biological Technologies in Agriculture (2015) 2:13
DOI 10.1186/s40538-015-0037-1

Chemical and Biological Technologies in Agriculture
a SpringerOpen Journal

RESEARCH Open Access

The effects of early leaf removal and cluster thinning treatments on berry growth and grape composition in cultivars Vranac and Cabernet Sauvignon

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Abstract

Background: The aim of the present work was to investigate the effect of early leaf removal and cluster thinning treatments in the Mediterranean climate on berry growth and how these two techniques affect phenolic profile (especially proathocyanidins) and color characteristics for later wine production. The study was conducted in 2011 in Podgorica, Montenegro. Two grapevine cultivars were selected to compare different ability in flavonoid accumulation: Vranac, with moderate accumulation and Cabernet Sauvignon, usually showing very good accumulation of polyphenols. Four treatments were compared: only leaf removal, only cluster thinning, leaf removal combined with cluster thinning, and no treatment that was used for control (control set).

Results: Early defoliation reduced the yield in both varieties. In Cabernet Sauvignon, defoliation initially delayed berry growth, but at the end, defoliation slightly affected almost all yield parameters (cluster weight, berry weight, and number of berries per cluster), while in cultivar Vranac, defoliation did not modify the berry growth and berry weight. In both varieties, cluster thinning did not affect the berry weight. In the treatments where both defoliation and cluster thinning was applied, a reduction of the cluster weight, berry weight, and berry numbers per cluster was observed. Cabernet Sauvignon showed a greater reactivity to the applied techniques, while Vranac was less reactive. At harvest, no damaged bunches (caused by sunburn) were found in defoliated treatment.

Conclusions: It can be concluded that for both varieties, early defoliation and cluster thinning lead to better soluble solids accumulation than in the control set. The treatments lead to raised concentration of anthocyanins and proanthocyanidins in both varieties. It is confirmed that the highest content of anthocyanins and proanthocyanidins was in the skin extracts of the grapes where both treatments were applied. This is followed by the treatment where only defoliation was applied. The enhanced contents of these compounds per berry in grape variety Vranac are the result of increased synthesis, while in Cabernet sauvignon variety, increased content was due to the less berry weight. The best wine characteristics (alcohol, color intensity, color hue, total anthocyanins, total polyphenols) were found in products, where defoliation was applied.

Keywords: Early defoliation; Grape yield; Berry composition; Proanthocyanidins; Anthocyanins; Polyphenols

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Background

In Montenegro, viticulture is mainly based on autochthonous grapevine varieties among which Vranac variety occupies about 70% of total autochthonous production. Besides autochthonous varieties, big attention is given to international grapevine varieties, in the first place to Cabernet Sauvignon, i.e., to its agrobiological technological and economics characteristics in agro-ecological conditions of sub-region Podgorica.

In modern viticulture, canopy management plays a key role, and it is widely recognized as an important factor in the composition of the resulting wines. Leaf removal in the fruiting zone, both manual and mechanical, is a common practice in high vigor, vertically trained vine canopies [1,2] and it could be applied from flowering to full veraison, thus improving fruit composition and reducing herbaceous wine character [3]. Defoliation is a consolidated practice for improving source-sink relations, photosynthetic capacity, and quality of crop plants; in Mediterranean environment, leaf removal is usually carried out in July during break out of color [4]. Early leaf removal performed in early stage of fruit development is an innovative viticultural practice for regulating yield components and improving grape quality [5-9]. Palliotti et al. [10] reported that vineyard efficiency was influenced by this practice, i.e., yield per vine and cluster weight were limited. Removing the leaves from cluster zone increases the evaporative potential within the fruit zone, lowering the humidity and making the cluster microclimate less conducive for the development of fungal diseases [11]. In the Sangiovese variety, characterized by highly compact clusters, early defoliation significantly reduced the fruit set, yield per shoot, cluster weight, number of berries per cluster, and cluster compactness [6,7,12]. Autochtonous variety, Vranac is also characterized by highly compacted clusters; we decided to apply these agro-techniques in order to reduce the yield parameters hoping that it would have the same beneficial effects as in the case of Sangiovese variety. Some studies reported that the influence of leaf removal on yield components depended on the variety. Leaf removal decreased the yield per vine and cluster weight in Merlot and Sangiovese, while the berry size was unaffected in both varieties. Only in grape variety Merlot, the number of berries per cluster and cluster compactness decreased, while in Cabernet Sauvignon variety, the effect of leaf removal was restrained by berry size [13].

Cluster thinning is described as the suppression of flowers or clusters before full maturation [14]. Therefore, cluster thinning has a direct effect on the source/sink ratio; having less sinks (fruits) photosynthetic assimilation might be improved, increasing grape quality [15]. It inducts physiological adjustments in the plant, improving the maturation's kinetics. Plus, this operation improves canopy sanitary conditions as thinning allows more enlightenment and fresh air penetration in the vegetation and clusters [16]. Cluster thinning's most evident effect is apparently crop load reduction, but its decrease is not equivalent to the thinning's intensity. Martins [17] found that, for the same intensity of thinning, in two consecutive years, the decline in production has been uneven. In fact, the vine compensates the stack lost, increasing the berry's volume and weight [18]. For this reason, Climaco et al. [19] suggests that cluster thinning must only be executed in the years when vineyards have such a fertility that may possibly undermine production quality. Cluster thinning advances grape maturity, improves grape quality, and also influences the chromatic characteristic of the wine [20].

The aim of the present work was to investigate the effect of early leaf removal and cluster thinning treatments on the berry growth and how these two techniques affect phenolic profile (especially proathocyanidins) and color characteristics on wine production in the Mediterranean climate. Two grapevine cultivars with different abilities in flavonoid accumulation were compared: Vranac with moderate accumulation of flavonoid and Cabernet Sauvignon with very good accumulation of flavonoid.

Methods

The trial was carried out during the 2011 growing season in the commercial vineyard of the Plantaze company in the Cemovsko field in Podgorica (Montenegro), planted with both local Vranac variety as well as Cabernet Sauvignon. The study was conducted in vineyards with uniform growing conditions. The treatments of variety Vranac were established in 10-year-old vineyard, grafted onto Kober 5BB rootstock, trained to a modified double Guyot training system, rows spaced 2.8 m apart and with 0.9 m between plants in the row. The grapevines of Cabernet Sauvignon were planted in 2005 (clone R5), grafted onto 1103P rootstock, and trained to a Guyot training system. The vine had a between-row and within-row spacing of 2.60 m × 0.70 m.

Winter pruning, for both varieties, was carried out leaving 14 buds per vine. In the first week of May, when the shoots reached 20 cm to each vine, shoot thinning was applied and ten shoots per plant were retained.

Yield components

At harvest, cluster number as well as the total yield was recorded on 15 tagged vines per treatment. In the laboratory, the following variables of 25 randomly selected clusters for each treatment were estimated: cluster weight, cluster and berry length and width, and berry number and weight.

Ratio skin/berry is expressed in percents where both weights were individually measured.

Grape juice analysis

The soluble solids of grape juice were determined by refractrometry and pH values achieved by pH meter and titratable acidity (TA) with 0.1 N NaOH and bromothymol blue as indicator (expressed as g/L of tartaric acid equivalents).

Grape skin analysis

For the two grape varieties studied, on days 161, 172, 185, 200, 213, and 222 and harvest time 231, 20 berries were sampled and weighed for each treatment in triplicate. Berry skins were removed manually from the pulp using a laboratory scalpel, weighed, and quickly placed in 50 ml hydro-alcoholic buffer at pH 3.2, containing 2 g/L $Na_2S_2O_5$ and 12% of ethanol. The samples were stored at −20°C until analysis for phenolic compounds was carried out. The total phenolic content of skin extracts of grapes was determined using the Folin-Ciocalteu method [21]. Determination of total anthocyanins was performed using the method described by Di Stefano et al. [22] and total proanthocyanidins described by Di Stefano and Cravero [23]. Total anthocyanins, phenolics, and proanthocyanidins were expressed in mg per kg grapes and mg per berry. All analyses were repeated three times.

Wine analysis

Eight microvinifications were carried out to study the influence of the agricultural treatments to wine composition and quality. All treatments were individually harvested manually at the moment of optimal phenological and technological maturation. For each treatment, approximately 100 kg of grapes were stored overnight in a cool chamber (4°C) and the following day warmed to room temperature before being slightly crushed. All musts were immediately inoculated with selected *Saccharomyces cerevisiae* yeast (Lalvin BDX, Lallemand Inc., Montreal, Canada) and Go-ferm protect (30 g hL^{-1}), yeast nutrient, Fermaid E (25 g hL^{-1}) was added during fermentation of both varieties. Fermentations were conducted in tanks at 25°C for 10 days. The total polyphenols [21], total anthocyanins [22], and total proanthocyanidins [23] of the wine were determined by UV-vis spectrophotometry. Spectrophotometric measurements of absorbance at 420, 520, and 620 nm were made using a 1 mm quartz cuvette. Color intensity was calculated by adding absorbance values at 420, 520, and 620 nm [24]. The tonality of the wine is defined as the ratio of absorbance at 420 and 520 nm [24].

Statistical analysis

Within each variety analysis of variance ANOVA was used to test the main effect using SPSS software (IBM SPSS version19). Comparison of means was performed using Duncan test at $p < 0.05$.

Results and discussion

Berry growth

In the first phase of berry growth, with the Cabernet Sauvignon variety, early defoliation affected the berry growth, causing delay in development compared to those of the control. However, before veraison, defoliated treatment showed a higher average berry weight with no significant differences (Table 1A). During the ripening stage, the NLR-CT did not modify the growth of the berry in confront to the control. The treatment previously defoliated (LR-NCT) showed a delay in growth after veraison; even if at harvest; it did not show a difference by control and thinned treatment. The treatment defoliated and then thinned (LR-CT) stopped in advance the growth and showed an early transition in over ripening. As a result at harvest, the treatment LR-CT showed the lowest an average weight of berry (Table 1B).

In cultivar Vranac during the first part of the berry growth, until veraison, in response to defoliation, there were no significant differences in the berry weight (Table 2A). However, the treatment defoliated presented before veraison a higher berry weight. In the course of ripening, the cluster thinning did not affect the berry growth. The defoliated treatment (LR-NCT), similar to Cabernet Sauvignon, had a developmental delay but reached similar values at harvest to the other treatments.

Overall, all types of treatments showed the maximum weight of the berries in the DOY 213 and decrease in the last 20 days (Table 2B).

Yield components

Table 3 shows the yield production per vine of four experimental conditions for two varieties. The defoliation reduced the yield in both varieties. The impact of this practice on the yield is higher in Cabernet Sauvignon: defoliation reduced the yield by 36% and defoliation with subsequent cluster thinning reduced it by 63%. Reduction was less pronounced in the Vranac variety:

Table 1 Berry weight (g) in Cabernet Sauvignon

DOY		161	172	185	200
A	NLR	6.01±0.84[a]	12.01±1.42[a]	15.98±1.17[a]	15.28±0.62[a]
	LR	6.62±0.63[a]	11.64±0.75[a]	12.02±1.64[b]	16.24±1.48[a]
DOY		213	222	231	
B	NLR-NCT	22.30±0.60[a]	21.70±0.60[a]	23.75±1.27[a]	
	LR-NCT	20.16±0.34[a]	18.49±0.89[b]	24.14±0.98[a]	
	NLR-CT	20.70±1.48[a]	22.01±0.74[a]	21.52±1.47[ab]	
	LR-CT	21.61±2.89[a]	18.47±0.91[b]	19.41±2.15[b]	

To before veraison (A) and after veraison to harvest (B). Treatments: leaf removal (LR), not leaf removal (NLR), leaf removal-not cluster thinning (LR-NCT), not leaf removal-cluster thinning (NLR-CT), leaf removal-cluster thinning (LR-CT), and control (NLR-NCT).

Table 2 Berry weight (g) in Vranac

DOY		161	172	185	200
A	NLR	6.03±1.30[a]	21.13±1.51[a]	24.16±1.24[a]	27.79±3.85[a]
	LR	7.09±1.16[a]	19.98±2.07[a]	24.56±1.93[a]	29.90±3.37[a]
DOY		213	222	231	
B	NLR-NCT	45.30±1.78[a]	40.73±3.97[a]	39.48±1.97[a]	
	LR-NCT	40.81±2.66[a]	38.05±2.25[a]	40.43±2.18[a]	
	NLR-CT	42.54±4.94[a]	45.53±5.76[a]	38.36±1.34[a]	
	LR-CT	44.43±1.71[a]	42.24±5.61[a]	39.19±1.74[a]	

To before veraison (A) and after veraison to harvest (B). Treatments: leaf removal (LR), not leaf removal (NLR), leaf removal-not cluster thinning (LR-NCT), not leaf removal-cluster thinning (NLR-CT), leaf removal-cluster thinning (LR-CT), and control (NLR-NCT).

defoliation reduced the yield by 23%, while defoliation followed by cluster thinning reduced it by 46% compared to the controlling set.

In Cabernet Sauvignon, the defoliation reduced the average weight of the bunch and total berry numbers per cluster. On the other hand, the cluster thinning had no influence on the berry weight. The treatment 'defoliated-cluster thinning' had lower bunch weight and berry weight. The defoliation treatment resulted in the lowest number of berries per cluster, while the 'cluster thinning' resulted in the highest number of berries per bunch. As a consequence, the density of the cluster is higher in the treatment NLR-CT. There are no significant differences in the ratio skin/berry (Table 3).

In cultivar Vranac, early leaf removal slightly reduced the cluster weight. This treatment also resulted in a slightly increased ratio of skin vs. berry weight. There were no recorded differences in the berry weight and in the number of berries per cluster. However, early leaf removal followed by cluster thinning resulted in a lower berry weight and number of berries which determine the lowest average cluster weight.

Grape juice analysis

In both varieties and after all tested treatments, the result was a good accumulation of sugars. Consequently, the control set showed the lowest value in the content of sugar. The best accumulation of soluble solids in Vranac was achieved by the early defoliation followed by the cluster thinning (LR-CT), while only the cluster thinning treatment (NLR-CT) resulted in a greater accumulation of sugars in the variety Cabernet Sauvignon (Table 4). Regarding the total acids, it is noticed that early defoliation induced higher content of total acids in Cabernet Sauvignon variety, when compared to Vranac variety where these treatments had no influence on this parameter.

Total polyphenols in berry skins

Early defoliation in Cabernet Sauvignon in the early stages of berry growth lead to increase in total berry polyphenols (Table 5A). Before veraison, there were no significant differences in the total polyphenols content between the treatments. Increased content in mg/kg grapes was due to the less berry weight and not due to the increase of synthesis (Table 5B).

The total phenols in the variety Vranac, up to veraison, increased due to the effect of early leaf removal (Table 6A). Polyphenols content in mg per berry was also increased in defoliated treatment, due to increased synthesis in the berries (Table 6B).

Total anthocyanins in berry skins

Table 7A shows the total anthocyanin content (mg/kg grapes) in Cabernet Sauvignon from veraison to harvest. No significant differences between the treatments were found, except at harvest time. Results for the control set (NLR-NCT) and early defoliation treatment only (LR-NCT) resulted in lower total anthocyanins when compared to results after cluster thinning only (NLR-CT) and early defoliation

Table 3 Yield components and cluster and berry characteristics at harvest recorded in Cabernet Sauvignon and Vranac

		Clusters/Vine	Yield/vine (kg)	Cluster wt (g)	Berry wt (g)	No of berries/cluster	Compactness index	Skins/berry %
Cabernet Sauvignon	NLR-NCT	18	1.48±0.48[c]	134±3.8[b]	1.13±0.02[b]	113±3.3[b]	0.18±0.01[a]	17.17±2.1[a]
	LR-NCT	17	0.96±0.33[b]	117±3.9[b]	1.13±0.02[b]	97±3.2[ab]	0.18±0.01[a]	20.48±2.3[a]
	NLR-CT	10	0.91±0.32[b]	163±4.2[c]	1.08±0.02[b]	152±3.9[c]	0.28±0.02[b]	18.59±2.4[a]
	LR-CT	9	0.56±0.17[a]	86±4.3[a]	0.94±0.02[a]	89±4.4[a]	0.22±0.02[a]	17.18±3.5[a]
Vranac	NLR-NCT	13	2.35±0.14[b]	176±8.0[a]	1.96±0.03[a]	89±4.2[a]	0.45±0.01[a]	16.10±2.8[a]
	LR-NCT	11	1.82±0.43[a]	161±8.1[a]	1.96±0.03[a]	80±3.6[a]	0.42±0.01[a]	19.86±3.0[a]
	NLR-CT	9	1.64±0.28[a]	170±7.4[a]	1.94±0.03[a]	89±4.1[a]	0.47±0.03[a]	14.73±3.4[a]
	LR-CT	9	1.27±0.15[a]	147±8.2[a]	1.87±0.03[a]	75±4.5[a]	0.32±0.01[b]	16.73±3.2[a]

Vines subjected to early defoliation (LR-NCT), cluster thinning (NLR-CT), early defoliation and cluster thinning (LR-CT), or control (NLR-NCT).

Table 4 Must composition at harvest recorded in Cabernet Sauvignon and Vranac

		TSS (Brix)	Titratable acidity (g tartaric acid/L)	pH
Cabernet Sauvignon	NLR-NCT	22.40	7.40	3.59
	LR-NCT	24.40	8.90	3.53
	NLR-CT	25.60	7.57	3.59
	LR-CT	24.80	7.95	3.58
Vranac	NLR-NCT	22.00	6.77	3.57
	LR-NCT	22.80	6.60	3.62
	NLR-CT	22.80	6.75	3.61
	LR-CT	24.60	6.53	3.59

Vines subjected to early defoliation (LR-NCT), cluster thinning (NLR-CT), early defoliation, and cluster thinning (LR-CT) or control (NLR-NCT). The reported values are from must just crushed for microvinification.

followed by the cluster thinning treatments (LR-CT) which resulted in higher total athocyanins concentration. This effect seems to be related to berry growth and not to the increase of the synthesis, as can be seen in Table 7B.

No significant differences between the treatments during maturation were found in the total anthocyanin content for the cultivar Vranac, except that at DOY 222, lowest content in the thinned treatment (Table 8A) is observed. However, the highest concentration was in the treatment defoliated-thinned. The concentration of anthocyanins per berry is not associated to berry growth albeit to the increase in their synthesis (Table 8B). Besides, agricultural practices defoliation and cluster thinning have had an impact to the content of anthocyanins, increasing it compared to the control.

Total proanthocyanidins in berry skins

The content of proanthocyanidins in Cabernet Sauvignon, from berry set to before veraison, reacts in the same way as polyphenols: defoliation causing retardation of the berry growth and increased content of proanthocyanidins (Table 9A). Even in this case, the effect was not due to the increased synthesis per berry (Table 9B).

After veraison, for all treatments, a further increase in the content of proanthocyanidins was observed, which could be due to the persistence in the synthesis or different extraction of proanthocyanidins from the skins. All viticultural practices led to higher content of proanthocyanidins at harvest, without significant differences (Table 10A,B).

In Vranac, before veraison, greater accumulation of total proanthocyanidins and proanthocyanidins per berry in the treatment defoliated was observed (Table 11A,B). At harvest, the highest contents per berry and in mg/kg grapes was observed in the treatment defoliated-cluster thinned. However, treatments defoliation and cluster thinning enhanced proanthocyanidins concentration compared to the controlling set (Table 12A,B).

Table 5 Total polyphenols and polyphenols per berry in Cabernet Sauvignon from berry set to before veraison

A - total polyphenols (mg/kg)			
DOY	172	185	200
NLR	5377±827[a]	4372±327[a]	3384±303[a]
LR	5772±479[a]	4782±529[a]	3375±450[a]
B - polyphenols per berry (mg)			
DOY	172	185	200
NLR	3.19±0.36[a]	3.49±0.38[a]	2.58±0.26[a]
LR	3.35±0.31[a]	2.85±0.21[b]	2.73±0.19[a]

Treatments: leaf removal (LR) and not leaf removal (NLR).

Table 6 Total polyphenols and polyphenols per berry in Vranac from berry set to before veraison

A - total polyphenols (mg/kg)			
DOY	172	185	200
NLR	3006±401[a]	2358±148[a]	1894±326[a]
LR	3505±147[a]	2922±295[b]	2248±357[a]
B - polyphenols per berry (mg)			
DOY	172	185	200
NLR	3.18±0.51[a]	2.84±0.15[a]	2.62±0.47[a]
LR	3.49±0.26[a]	3.59±0.51[b]	3.32±0.26[b]

Treatments: leaf removal (LR) and not leaf removal (NLR).

Table 7 Total anthocyanins and anthocyanins per berry in Cabernet Sauvignon in 2011 from veraison to harvest

A - total anthocyanins (mg/kg)				
DOY	200	213	222	231
NLR-NCT	153±75[a]	1229±50[a]	1846±114[a]	2165±107[ab]
LR-NCT	89±13[a]	1360±127[a]	1845±559[a]	2104±195[a]
NLR-CT	86±53[a]	1356±75[a]	1954±167[a]	2439±129[bc]
LR-CT	96±26[a]	1499±173[a]	2043±65[a]	2522±230[c]
B - anthocyanins per berry (mg)				
DOY	200	213	222	231
NLR-NCT	0.11±0.05[a]	1.62±0.10[a]	2.00±0.10[ab]	2.57±0.15[a]
LR-NCT	0.07±0.01[a]	1.37±0.11[a]	1.69±0.46[a]	2.53±0.13[a]
NLR-CT	0.07±0.04[a]	1.40±0.08[a]	2.15±0.11[b]	2.62±0.19[a]
LR-CT	0.08±0.03[a]	1.64±0.40[a]	1.89±0.13[ab]	2.43±0.06[a]

Treatments: leaf removal-not cluster thinning (LR-NCT), not leaf removal-cluster thinning (NLR-CT), leaf removal-cluster thinning (LR-CT), and control (NLR-NCT).

Table 8 Total anthocyanins and anthocyanins per berry in Vranac in 2011 from veraison to harvest

A - total anthocyanins (mg/kg)				
DOY	200	213	222	231
NLR-NCT	225±88[a]	950±133[a]	1922±190[ab]	2059±238[a]
LR-NCT	205±16[a]	1061±176[a]	2173±133[a]	2195±69[a]
NLR-CT	217±134[a]	1124±133[a]	1755±175[b]	2228±144[a]
LR-CT	192±84[a]	1169±90[a]	2168±156[a]	2351±173[a]
B - anthocyanins per berry (mg)				
DOY	200	213	222	231
NLR-NCT	0.34±0.10[a]	2.14±0.21[a]	3.86±0.65[a]	4.06±0.51[a]
LR-NCT	0.29±0.03[a]	2.16±0.29[a]	4.11±0.42[ab]	4.44±0.38[a]
NLR-CT	0.27±0.16[a]	2.37±0.03[a]	4.26±0.72[ab]	4.25±0.70[a]
LR-CT	0.29±0.11[a]	2.59±0.11[a]	4.55±0.30[b]	4.60±0.17[a]

Treatments: leaf removal-not cluster thinning (LR-NCT), not leaf removal-cluster thinning (NLR-CT), leaf removal-cluster thinning (LR-CT), and control (NLR-NCT).

Table 10 Total proanthocyanidins and proanthocyanidins per berry in Cabernet Sauvignon from veraison to harvest

A - total proanthocyanidins (mg/kg)			
DOY	213	222	231
NLR-NCT	3819±511[a]	4520±87[a]	5119±1209[a]
LR-NCT	4928±624[a]	6072±586[ab]	5414±1162[a]
NLR-CT	4257±219[a]	5245±1006[a]	6074±802[a]
LR-CT	4613±150[a]	7283±1428[b]	7157±999[a]
B - proanthocyanidins per berry (mg)			
DOY	213	222	231
NLR-NCT	5.01±0.57[a]	4.90±0.20[a]	6.04±1.19[a]
LR-NCT	4.96±0.56[a]	5.61±0.58[ab]	6.55±1.53[a]
NLR-CT	4.40±0.24[a]	5.78±1.21[ab]	6.55±1.12[a]
LR-CT	4.97±0.52[a]	6.71±1.24[b]	6.95±1.25[a]

Treatments: leaf removal-not cluster thinning (LR-NCT), not leaf removal-cluster thinning (NLR-CT), leaf removal-cluster thinning (LR-CT), and control (NLR-NCT).

Wine analysis

Table 13 shows descriptors of wines made from grapes of four experiments for two varieties. In the wines of cultivar Cabernet Sauvignon, higher alcohol content was found in the cluster thinning treatment (as a result of the best accumulation of sugar) and lowest in the control set. All the wines exhibit similar color hue. The value of total anthocyanins, polyphenols, proanthocyanidins, and color intensity was highest in the treatment defoliated-cluster thinned followed by the treatment defoliated. The lowest values of all parameters were found in the control set (no treatments). The ethanol content in Vranac wines, in accordance with sugar accumulation, was the same for the treatment 'defoliated' and treatment 'cluster thinning', and the highest in the treatment 'defoliated-cluster thinned' and the lowest in the control set. The values of color intensity and color hue were very similar for all the treatments, even though some slightly higher value was found in the treatment 'defoliated-cluster thinned'. The content of total anthocyanins, polyphenols, and proanthocyanidins is highest in the treatment 'defoliated-cluster thinned' followed by the treatment 'defoliated'. The best wine characteristics were found in products from the plots where defoliation was applied. These results could be due to better extraction of polyphenolic compounds in wine. Internal tasting of all experimental wines made of both varieties of grapes showed that wines made of grapes where leaf removal and cluster thinning were applied were characterized by fuller body, higher fruitiness aromas, and more intense color. All of this resulted in enhanced complexity of aromas.

Experimental

In the experimental design, four treatments were compared: a) not defoliated - not thinned (NLR-NCT), leaf removal - not cluster thinning (LR-NCT), not leaf removal - cluster thinning (NLR-CT), and leaf removal - cluster thinning (LR-CT).

Table 9 Total proanthocyanidins and proanthocyanidins per berry in Cabernet Sauvignon from berry set to before veraison

A - total proanthocyanidins (mg/kg)			
DOY	172	185	200
NLR	9037±1205[a]	7448±1000[a]	5682±436[a]
LR	10438±633[b]	9087±1174[b]	5992±1370[a]
B - proanthocyanidins per berry (mg)			
DOY	172	185	200
NLR	5.36±0.27[a]	5.91±0.58[a]	4.34±0.34[a]
LR	6.08±0.58[b]	5.41±0.63[a]	4.80±0.48[a]

Treatments: leaf removal(LR) and not leaf removal (NLR).

Table 11 Total proanthocyanidins and proanthocyanidins per berry in Vranac from berry set to before veraison

A - total proanthocyanidins (mg/kg)			
DOY	172	185	200
NLR	5470±624[a]	4837±582[a]	3518±784[a]
LR	6215±596[a]	5771±730[a]	3788±535[a]
B - proanthocyanidins per berry (mg)			
DOY	172	185	200
NLR	5.83±1.85[a]	5.83±0.63[a]	4.82±0.85[a]
LR	6.20±0.78[a]	7.14±1.41[b]	5.61±0.55[a]

Treatments: leaf removal (LR) and not leaf removal (NLR).

Table 12 Total proanthocyanidins and proanthocyanidins per berry in Vranac from veraison to harves

A - total proanthocyanidins (mg/kg)			
DOY	**213**	**222**	**231**
NLR-NCT	3155±235[a]	3455±845[a]	2494±455[a]
LR-NCT	3250±445[a]	3674±640[a]	2780±268[ab]
NLR-CT	2933±489[a]	2985±397[a]	3010±434[ab]
LR-CT	3439±606[a]	2777±736[a]	3731±756[b]
B - proanthocyanidins per berry (mg)			
DOY	**213**	**222**	**231**
NLR-NCT	7.16±0.78[a]	6.99±1.58[a]	4.94±1.08[a]
LR-NCT	6.67±1.31[a]	6.98±1.24[a]	5.64±2.45[ab]
NLR-CT	5.84±0.69[a]	7.32±0.11[a]	5.76±0.73[ab]
LR-CT	7.61±1.12[a]	6.00±2.22[a]	7.28±1.26[b]

Treatments: leaf removal-not cluster thinning (LR-NCT), not leaf removal-cluster thinning (NLR-CT), leaf removal-cluster thinning (LR-CT), and control (NLR-NCT).

Defoliated treatment was applied in full bloom on day of year (DOY) 152 corresponding to the phenophase 23 according to the grapevine growth stage classification proposed by Coombe [25], which consisted of manual removal of the first eight basal leaves of each shoot. All lateral shoots were retained. Cluster thinning was conducted on DOY 200, at mid veraison, at stage 35 [25], where the distal cluster was removed leaving one cluster per shoot. The elementary experimental plot was composed of 20 consecutive vines; each treatment was replicated in three elementary plots, randomly positioned in the vineyard.

Conclusions

Objectives of the research program were to study the effect of some environmental and physiological aspects on the intensity of flavonoid synthesis. The study was conducted in 2011 in Podgorica, Montenegro. Two grapevine cultivars were selected to compare their ability in flavonoid accumulation: autochtonous variety Vranac, with moderate accumulation of flavonoids and Cabernet Sauvignon with good accumulation of polyphenols. The following experimental treatments were compared: early leaf removal (flowering time), cluster thinning (veraison time) and combination of both treatments. The early defoliation reduced the yield per vine in Cabernet Sauvignon and Vranac. In Cabernet Sauvignon, defoliation initially delayed berry growth, but at harvest, only the treatment 'defoliation-cluster thinning' had significantly lower berry weight. In cultivar Vranac, defoliation did not modify the berry growth and berry weight. In both varieties, cluster thinning had no effect on the berry weight. In the treatment 'defoliated-thinned', reduction of the cluster weight, berry weight, and berry number per cluster is observed. This is probably the consequence of a lower fruit set, where the defoliation had a greater impact on the first cluster. Cabernet Sauvignon showed a greater reactivity to the applied techniques, compared to Vranac. At harvest, no damaged bunches (caused by sunburn) were found in defoliated treatment. Early defoliation and cluster thinning in both varieties raised the concentration of anthocyanins and proanthocyanidins. The enhanced contents of these compounds per berry in grape variety Vranac are the result of increased synthesis, while in Cabernet sauvignon variety, increased content was due to the less berry weight. Defoliation and cluster thinning led to better soluble solid accumulation than in the control sets (no treatments applied). The skin extracts contained the highest content of anthocyanins and proanthocyanidins in the treatment defoliated-thinned followed by the treatment thinned, while these contents were higher in wines from the vineyards where defoliation was applied. It could be due to better extraction of these compounds during winemaking. Additional work is in progress to verify that early leaf removal and cluster thinning do indeed result in better quality of Vranac wine.

Table 13 Chemical composition in Cabernet Sauvignon and Vranac wines

		Alcohol content (% vol)	Colour intensity	Colour hue	Total anthocyanins (mg/L)	Total polyphenols (mg/L)	Total proanthocyanidins (mg/L)
Cabernet Sauvignon	**NLR-NCT**	13.43	1.42	0.63	295	1897	872
	LR-NCT	14.59	1.76	0.62	333	2311	1112
	NLR-CT	15.36	1.69	0.64	318	2028	910
	LR-CT	14.94	1.89	0.64	353	2749	1224
Vranac	**NLR-NCT**	13.31	1.86	0.57	389	1532	308
	LR-NCT	13.67	1.86	0.59	392	1711	314
	NLR-CT	13.68	1.85	0.59	344	1548	292
	LR-CT	14.68	2.06	0.61	467	1842	555

Treatments: leaf removal-not cluster thinning (LR-NCT), not leaf removal-cluster thinning (NLR-CT), leaf removal-cluster thinning (LR-CT), and control (NLR-NCT).

Competing interests
The authors declare that they have no competing interests.

Authors' contribution
VM was the project coordinator and together with OF and MB contributed to project conception and overall experimental design. MB participated in experimental designing, performed spectrophotometric analysis and statistical data analysis. MM, VK, JR, SŠ participated in experimental designing, performed experimental treatments, vinification and analysis of quality control of grapes and wines. VM and OF supervised final data analysis and made the concept of results and conclusions. All co-authors participated in critical reading of the manuscript and approved the final manuscript.

Acknowledgment
This research was supported by the following project, Ecophysiological and molecular aspects of the synthesis and polymerization of flavanols in the grapes of Vranac and Cabernet sauvignon cultivars and their impact on the quality of wine", co-funded by the Ministry of Science of Montenegro.

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Received: 1 October 2014 Accepted: 15 March 2015
Published online: 08 May 2015

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