Effect of Cluster and Berry Thinning on Merlot and Cabernet Sauvignon Wines Composition

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Abstract

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The manipulation of grape yield is widely practised to improve grape and wine quality. Merlot and Cabernet Sauvignon vines in the Zagreb vineyard hills, north-western Croatia, were subjected to three crop removal treatments [cluster thinning (CT), berry thinning (BT), CT+BT, and untreated control] in a randomised block design experiment. CT and CT+BT treatments reduced the grape yield but increased the mean cluster weight compared to control vines. BT alone had a little effect on the yield components. Control grapes generally had the lowest soluble solids (°Oe) and highest titratable acidity (g/l). In most cases, control wines had the lowest total phenols, flavan-3-ols, and anthocyanin concentrations, while CT+BT treatment had the highest ones followed by CT treatment. It was concluded that CT+BT produced wines with generally increased total phenols, flavan-3-ols, and anthocyanins, as well as many individual phenolic compounds. Thus, grape yield per vine seems to be strongly connected with the grape and wine compositions. The final cost-effectiveness of this canopy interventions still remains questionable.

Keywords: crop removal; anthocyanins; flavan-3-ols; phenols

Red wine cultivars are considered to have fruit maturity problems in Red vine cool-climate regions like north-western part of Croatia. Merlot has large clusters, leading to a potential of excess crop and delayed ripening (King *et al*. 2012). On the other hand, Cabernet Sauvignon is a late ripening grape cultivar, which often remains below optimal ripeness in regions with short growing seasons.

There are positive effects of crop removal, including advanced fruit maturity, decreased acidity, and increased anthocyanins and phenolics in Cabernet Sauvignon (Petrie & Clingeleffer 2006). General increases in berry, must, and wine phenols and anthocyanins from Merlot and Cabernet Sauvignon cluster thinning treatments were confirmed by Di Profio *et al*. (2011a,b) and Mazza *et al*. (1999). Positive effects of cluster thinning on Pinot noir wines were reported by Reynolds *et al*. (1996), while Guidoni *et al*. (2002) found increased soluble solids, skin anthocyanins, and flavonoids in cluster-thinned Nebbiolo grapes.

However, the cluster removal produced a small or no effect on Cabernet Sauvignon wine composition, as reported by many authors (Chapman *et al*. 2004; Keller *et al*. 2005; Nuzzo & Matthews 2006). There is little information available on berry thinning practice in the vineyard. Thus, berry thinning is a unique feature of the present study. Gil *et al*. (2013) pointed out that this practice could be of interest, due to the assumption that, with minimal crop yield reduction, grape and wine quality still could be improved. Such a study could help provide a rationale for crop yield management in unfavourable years and climates.

The objective of the present study was to evaluate the effects of the cluster and/or berry thinning on Merlot and Cabernet Sauvignon yield components, fruit composition, and wine phenolic composition, and to test the hypothesis that the crop removal would improve fruit maturity and lead to improved wine phenolic composition.

MATERIAL AND METHODS

Experimental design, yield, basic must composition. The experiment was conducted over the 2010 and 2011 growing seasons in the experimental field Jazbina (Zagreb vineyard hills), which is a part of

the Department of Viticulture and Enology, Faculty of Agriculture in Zagreb. Merlot (clone R3) and Cabernet Sauvignon (clone R5) vines used in this trial were grafted to 5BB rootstock, double Guyot trained, with 2.00 × 1.20 m spacing.

The experiment was a randomised block design, with four treatments in three replications. Each plot consisted of three grapevines (experimental unit), so there were nine grapevines in each treatment. The cultivars were located in separate blocks. Each experimental unit was separated by a few untreated vines. Before the cluster and berry thinning treatments, the vines were shoot-thinned to equalise the vegetative potentials, taking into account that every retained shoot carried at least one cluster. So, each vine carried 18 shoots (two on the spurs, and seven on each cane). All cluster and berry thinning treatments were performed 30 days after bloom (pea size). The treatments were as follows: Cluster thinning (CT) – removing every distal cluster, with only one basal cluster remaining on each shoot; Berry thinning (BT) – removing the upper part of every cluster on the vine; Cluster thinning + berry thinning (CT+BT) – removing the upper part of each basal cluster on the vine, remaining after CT; Control (C) – untreated.

The grapes were harvested manually at commercial harvest times. The clusters from all treatments were counted to obtain the mean cluster number per vine, as well as average grape yield (kg) per vine and cluster weight (g). To determine the average weight of single berry, the randomised sample of 100 berries was weighed for every treatment. Immediately after crushing and destemming, must samples were collected for sugar and titratable acidity (TA) analyses. Sugar content in the musts was determined by refractometer (expressed in °Brix) and titratable acidity of the musts (g/l) was estimated using the coloration pattern volumetric method according to the official methods of the European Union (EEC 1990). The musts were put into 15-l stainless steel tanks. Each lot was sulfited with 100 ml/100 l of 5% sulphurous acid. The wines were fermented in the contact with skin for 8 days, with the cap plunged daily. All fermentations were carried out with *Saccharomyces cerevisiae* Lallemand 245, with room temperature control (20°C), to completion. At the end of the fermentation, the wines were pressed, and the obtained samples were frozen for further analysis.

Spectrophotometric measurements. Total phenol content was determined with the Folin-Ciocalteu method (Singleton & Rossi 1965). The results were expressed as mg gallic acid equivalents per liter of wine (mg GAE/l).

Total flavan-3-ols content was determined by the reaction of flavonoids with vanillin reagent in the acid medium (Amerine & Ough 1988). The results were expressed as mg (+)-catechin equivalents per liter of wine (mg CAT/l).

All spectrophotometric measurements were performed on Specord 40 UV-vis spectrophotometer (Analytic Jena, Jena, Germany).

Determination of phenolic compounds in wine. HPLC separation, identification and quantification of wine phenolic compounds were performed on Agilent 1100 Series system (Agilent, Waldbronn, Germany), equipped with DAD and FLD coupled to Agilent ChemStation (Version B.01.03) data-processing station. The wine samples were filtered through 0.45 µm PTFE membrane filters and then injected (20 µl) on a reversed-phase column Luna Phenyl-Hexyl (4.6 × 250 mm; 5 µm particle; Phenomenex, Torrance, USA), thermostatted at 50°C. The solvents were water/phosphoric acid (99.5 : 0.5, v/v – solvent A) and acetonitrile/water/phosphoric acid (50 : 49.5 : 0.5, v/v/v – solvent B), and the flow rate was 0.9 ml/minute. The linear gradient for solvent B was: 0 min, 0%; 7 min, 20%; 35 min, 40%; 40 min, 40%; 45 min, 80%; 50 min, 100%; 60 0%. Hydroxybenzoic acids were detected at 280 nm, *p*-hydroxycinnamic at 320 nm, flavonols at 360 nm, and anthocyanins (all 3-monoglucosides) at 518 nm. Flavan-3-ols were detected at λ_{ex} = 225 nm and λ_{em} = 320 nm. Phenolic compounds were identified by matching the retention time of each chromatographic peak with external standards and DAD spectrum. Quantification of the individual phenolic peaks was performed by the external standard method.

Statistical analysis. All variables were examined separately by year and cultivar using one-way analysis of variance (ANOVA). The data were analysed using SAS statistical Software, Version 9.0 (SAS Institute, Cary, USA).

RESULTS AND DISCUSSIONS

Yields. The yield parameters are presented in Table 1. Clusters per vine were reduced by cluster thinning treatments by ~40% for cv. Merlot, and by ~50% for cv. Cabernet Sauvignon. It is obvious that CT and CT+BT treatments reduced the grape yields in both years with both cultivars. BT treatment also reduced Cabernet Sauvignon yield in 2011. The yield on a percentage basis for cv. Merlot was reduced similar

Table 1. Effect of cluster and berry thinning on Merlot and Cabernet Sauvignon yield components at harvest 2010–2011, Jazbina, Croatia

Treatment	Clusters/vine		Grape yield (kg/vine)		Clusters weight (g)		Berry weight (g)	
	2010	2011	2010	2011	2010	2011	2010	2011
Merlot								
C	30.6	29.7	4.30^{a}	4.07^{a}	140.2^{c}	137.2^{c}	1.72^{c}	1.70^{c}
CT	18.0	18.0	2.86^{b}	2.81^{b}	159.2^{a}	156.2^{a}	1.88^{b}	1.84^{b}
BT	32.3	30.0	4.29^{a}	3.95^{a}	132.7^{d}	131.7^{d}	1.84^{b}	1.83^{b}
CT+BT	18.0	18.0	2.72^{b}	2.68^{b}	151.3^{b}	148.6^{b}	2.01^{a}	1.99^{a}
ANOVA Year		–		**		**		**
ANOVA Treatment		–		***		***		***
ANOVA Y × T		–		ns		ns		ns
Cabernet Sauvignon								
C	38.3	35.3	3.96^{a}	3.62^{a}	103.5^{c}	102.6c	1.38^{d}	1.36^{d}
CT	18.0	18.0	2.18^{b}	2.14^{c}	121.1^{a}	118.7^{a}	1.59^{b}	1.57^{b}
BT	39.3	36.7	3.75^{a}	3.41^{b}	95.5^{d}	93.0^{d}	1.50^{c}	1.49^{c}
CT+BT	18.0	18.0	2.13^{b}	2.07^{c}	118.3^{b}	115.2^{b}	1.72^{a}	1.69^{a}
ANOVA Year		–		***		**		**
ANOVA Treatment		–		***		***		***
ANOVA Y × T		–		ns		ns		ns

CT – cluster thinning; BT – berry thinning; C – control; values followed by the same letter do not differ significantly at $P = 0.05$; significant at $^{***}P \leq 0.001$, $^{**}P = 0.01$, $^{*}P = 0.05$, ns – non-significant

to clusters per vine, while the yield percentage-wise in Cabernet Sauvignon was not reduced as much as clusters per vine. Cluster weights, as expected, increased from the BT treatment (lowest), and CT treatment (highest) in both years for both cultivars. BT treatment reduced Merlot cluster weight by ~5%, while Cabernet Sauvignon cluster weight was reduced by ≥ 10%. On the other hand, CT treatment increased Merlot cluster weight by ~14%, while Cabernet Sauvignon cluster weight was increased by more than 25%. All thinning treatments increased the berry weight, especially CT+BT treatment providing the highest berry weight in both years and both cultivars. Contrary to expectations, Cabernet Sauvignon berry weight was reduced by BT alone, when compared with CT and CT+BT treatments. Di Profio *et al*. (2011a) reported that the mean cluster weight tended to increase when clusters per vine were reduced by more than yield per vine. The increase in cluster weight is probably the consequence of the increased berry size due to yield compensation (Reynolds *et al*. 1994). The noteworthy yield reductions in both cultivars must be taken into account when applying similar treatments, because of significant economic implications for vineyard operators (King *et a*l. 2012). The reduced yield per vine achieved by cluster thinning has been previously reported (Reynolds *et al*. 1995; Miller & Howell 1998; Keller *et al*. 2005; Dami *et al*. 2006; Di Profio *et al*. 2011a; King *et al*. 2012; Sun *et al*. 2012), as well as concomitant increases in cluster weight (Reynolds *et al*. 1995; Dami *et al*. 2006; King *et al.* 2012; Sun *et al*. 2012).

The yield was slightly reduced in 2011 as a result of the excessive vigour of the vines in 2010, resulting in poor floral bud initiation, similar to the report by Sun *et al*. (2012). The excessive vigour was the consequence of excessive rainfall during the vegetation period.

An important influence of the experimental years was also detected. Significantly lower yield components are a possible consequence of drought conditions in 2011, which enhanced the transpiration process, and thus lowered the cluster and berry weight values.

Fruit composition. Merlot fruit maturity was delayed in the control vines in both years in terms of Brix. The treatments also led to lower TA in grapes in 2011, but not in 2010. CT+ BT enhanced Cabernet Sauvignon maturity in 2010, in terms of increased Brix and decreased TA. In the same year, control grapes had the highest TA. In 2011, there were no differences between the treatments.

Merlot expressed greater sensitivity to the treatments, especially in terms of Brix values, which were the lowest in the control grapes and also had the highest yields in both years. These findings are in accordance with many previous works (Guidoni *et al*. 2002, 2008; Dami *et al.* 2006; Di Profio 2011a; King *et al*. 2012). It

Table 2. Effect of cluster and berry thinning on Merlot and Cabernet Sauvignon fruit composition at harvest 2010–2011, Jazbina, Croatia

Treatment	Merlot				Cabernet sauvignon			
	soluble solids (°Brix)		titratable acidity (g/l)		soluble solids (°Brix)		titratable acidity (g/l)	
	2010	2011	2010	2011	2010	2011	2010	2011
C	23.2^{c}	22.2^{ba}	5.7^{a}	5.6^{a}	21.2^{b}	23.4^{a}	9.2^{a}	6.8^{a}
CT	24.2^{ab}	23.6^{a}	5.3^{a}	5.0^{b}	21.8^{ab}	23.6^{a}	8.3^{ab}	7.1^{a}
BT	24.4^{a}	23.2^{a}	5.5^{a}	5.2^{b}	21.4^{b}	23.4^{a}	7.9^{b}	7.0^{a}
CT+BT	23.8^{b}	23.2^{a}	6.0^{a}	5.2^{b}	22.4^{a}	24.2^{a}	7.9^{b}	7.1^{a}
ANOVA Year	***^{b}		*		***		***	
ANOVA Treatment	***		ns		*		ns	
ANOVA Y × T	ns		ns		ns		ns	

CT – cluster thinning; BT – berry thinning; C – control; values followed by the same letter do not differ significantly at $P = 0.05$; significant at ***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$, ns – non-significant

is also noteworthy that the TA was higher in 2010, which could be attributed to a cooler than normal season. However, cluster and/or berry thinning had little or no influence on TA, similar to the observation by Keller *et al*. (2005), although the literature is not consistent on the effect of cluster thinning on TA. Generally, the fruit composition responses were as expected because of the increasing yields, which resulted in the delayed fruit ripening (Dami *et al*. 2006).

Significant influence of experimental years on the fruit composition was also observed, but in different ways. Soluble solids content was lower in Merlot grapes in 2011, and at the same time, higher in Cabernet Sauvignon grapes. Titratable acidity was lower in 2011. This is somewhat expected since in warmer climate conditions TA content decreases as a consequence of enhanced respiration process.

Wine phenolic composition. *Merlot.* CT+BT treatment resulted in the highest total phenols in both years. The same trend was noticed for coumaric acid, epicatechin, and rutin, although the majority of phenolic compounds were the highest in CT+BT wines, at least in one year. On the other hand, gallic and caffeic acids, *trans*-resveratrol, delphinidin, and petunidin concentrations were the lowest in control wines in both years. However, as most phenolic compounds concentrations were the lowest in control wines, it can be assumed that phenols concentrations in Merlot wines were strongly correlated with the yield per vine. Thinning treatments did not affect epicatechin gallate and isorhamnetine concentrations in wine in either year.

Cabernet Sauvignon. The same trend was noticed in Cabernet Sauvignon wines as with Merlot, with the highest total phenols in CT+BT wines, and the lowest in control wines. CT+BT treatment resulted in the highest levels of total phenols, flavan-3-ols, and anthocyanins levels in wine in both years. Besides that, gallic, caffeic, and coumaric acids, as well as catechin, *trans*-resveratrol, rutin, and isorhamnetin concentrations, were the highest in CT+BT wines in both years. Among anthocyanins, the CT+BT treatment provided the highest levels of peonidin and malvidin in wine in both years. Control wines, again, revealed the lowest concentrations of the majority of phenols. Total phenols and flavan-3-ols concentrations were the lowest in control wines in both years, as well as gallic acid, isorhamnetin and all individual anthocyanin compounds except petunidin. The correlation between phenols concentration and the yield was apparent in Cabernet Sauvignon wines, too.

Phenols concentrations, in general were higher in the warmer 2011 year. The increased temperature, either through direct heating by incident radiation or increased air temperature, would increase the rate of metabolic processes in the plant with an associated increase in metabolite accumulation (Dokoozlian & Kliewer 1996).

Cluster thinning in combination with berry thinning was the most effective treatment, followed by cluster thinning. Berry thinning had little or no effect on phenols concentrations in wine. Accordingly, berry thinning treatment itself seems insufficient and inadequate to improve the potential grape and wine quality. The control and BT treatments led to very similar yield parameters results, only with BT having higher berry weight. Consequential changes in skin:juice ratio might be the reason why BT treatment did not improve wine phenol quality.

Many similar studies found that cluster thinning tended to result in higher content of wine phenols (Prajitna *et al*. 2007; Di Profio *et al*. 2011b). The suggestion that grapes from low-crop vines accumu-

Table 3. Effect of cluster and berry thinning on phenols concentration (mg/l) in Merlot wines (2010–2011, Jazbina, Croatia)

Compound	2010				2011				ANOVA		
	C	CT	BT	CT+BT	C	CT	BT	CT+BT	year	treatment	Y × T
Total phenols	1796.64^{ca}	1989.68^{b}	1848.62^{c}	2143.40^{a}	2338.73^{c}	2401.40^{b}	2382.17^{b}	2607.63^{a}	***^{b}	***	*
Total flavan-3-ols	27.27^{b}	28.29^{b}	28.00^{b}	34.92^{a}	135.86^{b}	151.91^{a}	118.21^{c}	159.05^{a}	***	***	***
Gallic acid	50.18^{c}	60.57^{b}	59.58^{b}	64.07^{a}	22.41^{b}	25.56^{a}	25.09^{a}	26.54^{a}	***	***	***
Caftaric acid	24.05^{b}	30.82^{a}	29.93^{a}	33.51^{a}	98.75^{a}	98.78^{a}	94.09^{a}	99.16^{a}	***	ns	ns
Caffeic acid	1.81^{c}	3.35^{a}	2.67^{b}	3.60^{a}	18.84^{d}	22.45^{b}	20.62^{c}	25.88^{a}	***	***	***
Coummaric acid	3.12^{c}	3.80^{b}	3.52^{bc}	4.48^{a}	10.01^{b}	11.16^{b}	10.20^{b}	13.30^{a}	***	***	ns
Catechin	26.18^{c}	28.05^{ab}	27.30^{b}	28.77^{a}	29.63^{a}	30.37^{a}	29.67^{a}	30.65^{a}	***	***	ns
Epicatechin	15.88^{c}	17.68^{b}	17.77^{b}	20.11^{a}	16.56^{b}	21.88^{ab}	18.08^{b}	29.94^{a}	***	**	*
Epicatechin-galate	0.01^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.03^{a}	0.03^{a}	0.03^{a}	0.04^{a}	ns	ns	ns
trans-Resveratrol	0.34^{d}	0.43^{c}	0.71^{b}	0.89^{a}	0.36^{c}	1.29^{a}	1.21^{b}	1.30^{a}	***	***	***
Rutin	0.09^{b}	0.13^{a}	0.08^{b}	0.13^{a}	0.12^{c}	0.78^{b}	0.11^{c}	1.37^{a}	*	***	**
Quercetin	2.11^{b}	3.40^{a}	3.21^{ab}	3.64^{a}	35.33^{b}	35.58^{b}	35.44^{b}	46.14^{a}	***	***	***
Myricetin	0.05^{a}	0.07^{a}	0.08^{a}	0.08^{a}	2.75^{b}	2.99^{ab}	2.87^{ab}	3.73^{a}	***	ns	ns
Kaempferol	0.04^{a}	0.06^{a}	0.04^{a}	0.07^{a}	0.71^{b}	1.11^{a}	0.90^{ab}	1.12^{a}	***	*	ns
Isorhamnetin	0.07^{a}	0.09^{a}	0.08^{a}	0.11^{a}	0.14^{a}	0.15a	0.15^{a}	0.15^{a}	***	ns	ns
Total anthocyanins	367.80^{a}	429.41^{a}	405.27^{a}	430.05^{a}	536.54^{c}	576.07^{ab}	559.81^{bc}	599.19^{a}	***	*	ns
Dp-3-gl	9.89^{b}	16.23^{a}	15.89^{a}	16.25^{a}	12.49^{b}	16.85^{ab}	14.21^{ab}	21.28^{a}	ns	**	ns
Cy-3-gl	3.73^{b}	5.53^{a}	4.34^{b}	5.11^{a}	4.21^{b}	5.55^{a}	5.19^{ab}	5.68^{a}	*	***	ns
Pt-3-gl	18.06^{c}	23.42^{ab}	21.74^{b}	24.65^{a}	22.94^{b}	30.15^{a}	26.55^{ab}	31.59^{a}	***	**	ns
Pn-3-gl	8.01^{b}	9.34^{a}	8.68^{ab}	8.95^{a}	8.51^{b}	10.61^{ab}	9.22^{ab}	11.37^{a}	**	**	ns
Mv-3-gl	278.57^{b}	289.91^{b}	288.02^{b}	343.24^{a}	395.74^{a}	406.96^{a}	394.69^{a}	444.81^{a}	***	**	ns

CT – cluster thinning; BT – Berry thinning; C – control; Y × T – year × treatment; values followed by the same letter do not differ significantly at $P = 0.05$; significant at $^{***}P \leq 0.001$, $^{**}P \leq 0.01$, $^{*}P \leq 0.05$, ns – non-significant

late more phenolic compounds than those produced by high-crop vines is a general statement derived from this work, which is in agreement with Prajitna *et al.* (2007). The same authors stated that cluster thinning might have increased polyphenols accumulation indirectly by advancing fruit maturity or more directly by altering the source to sink balance and as such might have increased the substrate levels necessary for polyphenols synthesis.

Despite the increase in the berry size due to the yield compensation, higher contents of phenols were still measured in CT+BT and CT wines than those in the control wines. This is in accordance with Reynolds *et al.* (1994) and Di Profio *et al.* (2011a), the latter having suggested that it is not likely that the increases in anthocyanins were due primarily to a higher skin:juice ratio. The decreases in Brix and phenolic compounds in control fruit and wines observed in this work might indicate a relationship between sugar-based and phenolic maturity. Similar explanation has been suggested by Guidoni *et al.* (2008), Di Profio *et al.* (2011b), and others. The individual and/or total anthocyanins contents responded positively to CT+BT treatment, where Cabernet Sauvignon was somewhat more responsive than Merlot, similar to Di Profio *et al.* (2011b).

Both cluster thinning treatments significantly increased *trans*-resveratrol in wines in both years. Similar findings were reported by Prajitna *et al.* (2007), who suggested that the total level of phenolic compounds present in wine is a better indicator than resveratrol in determining the health benefits of wine. In our study, almost every phenolic compound was increased by CT+BT treatment, when compared to control.

Epicatechin-galate content in either cultivar was not affected by the experimental years. The same pattern was detected for delphinidin. Total anthocyanins content in Cabernet sauvignon wines did not differ due to the experimental years.

Finally, cluster and berry thinning are practised to reduce the grapevine yield and advance ripening, in terms of Brix, TA, and other grape and wine compounds. Its practicality has often been questioned

Table 4. Effect of cluster and berry thinning on phenols concentration (mg/l) in Cabernet Sauvignon wines, 2010–2011, Jazbina, Croatia

Compound	2010				2011				ANOVA		
	C	CT	BT	CT+BT	C	CT	BT	CT+BT	year	treatment	Y × T
Total phenols	2181.56^{ca}	2326.35^{b}	2327.53^{b}	2513.38^{a}	2317.67^{c}	2447.60^{b}	2424.20^{b}	2516.30^{a}	***^{b}	***	***
Total flavan-3-ols	40.29^{c}	40.88^{bc}	46.75^{b}	54.25^{a}	130.33^{c}	145.23^{b}	137.37^{bc}	159.10^{a}	***	***	ns
Gallic acid	15.70^{d}	21.31^{b}	19.20^{c}	38.23^{a}	40.00^{d}	49.48^{b}	45.85^{c}	55.53^{a}	***	***	***
Caftaric acid	88.01^{a}	94.81^{a}	89.94^{a}	96.07^{a}	98.87^{a}	105.93^{a}	100.64^{a}	107.45^{a}	ns	*	ns
Caffeic acid	3.78^{b}	4.14^{b}	4.06^{b}	5.36^{a}	10.71^{c}	18.69^{b}	17.57^{b}	22.93^{a}	***	***	***
Coummaric acid	3.12^{c}	4.31^{b}	3.42^{c}	5.71^{a}	7.03^{c}	12.12^{b}	8.22^{c}	15.17^{a}	***	***	***
Catechin	34.48^{b}	36.58^{b}	35.44^{b}	47.58^{a}	36.88^{c}	46.43^{b}	37.13^{c}	54.39^{a}	***	***	**
Epicatechin	13.37^{b}	14.17^{b}	13.47^{b}	17.61^{a}	13.03^{b}	18.83^{a}	15.74^{ab}	19.70^{a}	*	**	ns
Epicatechin-galate	0.05^{a}	0.06^{a}	0.06^{a}	0.06^{a}	0.07^{a}	0.09^{a}	0.08^{a}	0.09^{a}	ns	ns	ns
trans-Resveratrol	1.06^{c}	1.21^{ab}	1.15^{bc}	1.31^{a}	1.14^{c}	1.93^{b}	1.27^{c}	2.24^{a}	***	***	***
Rutin	0.12^{c}	1.61^{b}	0.93^{c}	2.36^{a}	0.15^{d}	1.74^{b}	1.02^{c}	3.37^{a}	***	***	***
Quercetin	3.54^{b}	4.31^{a}	3.72^{b}	4.48^{a}	4.63^{c}	8.72^{ab}	8.13^{b}	9.29^{a}	***	***	***
Myricetin	0.09^{a}	0.14^{a}	0.13^{a}	0.15^{a}	2.11^{c}	3.60^{a}	2.43^{b}	3.71^{a}	***	***	***
Kaempferol	0.13^{a}	0.15^{a}	0.14^{a}	0.16^{a}	1.32^{c}	1.86^{a}	1.56^{b}	1.87^{a}	***	***	***
Isorhamnetin	0.14^{b}	0.16^{ab}	0.15^{ab}	0.18^{a}	0.87^{b}	1.05^{ab}	0.91^{b}	1.25^{a}	***	*	*
Total anthocyanins	450.16^{c}	494.05^{b}	487.07^{b}	592.80^{a}	477.47^{b}	513.30^{ab}	501.20^{b}	550.40^{a}	ns	***	*
Dp-3-gl	14.46^{c}	16.73^{bc}	20.68^{ab}	22.29^{a}	13.40^{b}	20.73^{a}	20.50^{a}	21.41^{a}	ns	***	***
Cy-3-gl	0.76^{d}	7.76^{b}	3.61^{c}	8.62^{a}	1.98^{c}	8.31^{a}	3.84^{b}	8.43^{a}	***	***	***
Pt-3-gl	23.09^{b}	28.33^{a}	24.02^{b}	31.30^{a}	21.13^{b}	23.43^{ab}	22.88^{ab}	26.08^{a}	***	***	*
Pn-3-gl	5.30^{d}	12.09^{b}	5.89^{c}	15.30^{a}	5.62^{d}	13.23^{b}	7.96^{c}	13.84^{a}	***	***	***
Mv-3-gl	283.33^{d}	347.68^{b}	328.16^{c}	446.07^{a}	335.98^{d}	383.14^{b}	354.16^{c}	408.83^{a}	***	***	***

CT – cluster thinning; BT – Berry thinning; C – control; Y × T – year × treatment; values followed by the same letter do not differ significantly at $P = 0.05$; significant at $^{***}P \leq 0.001$, $^{**}P \leq 0.01$, $^{*}P \leq 0.05$, ns – non-significant

because of the increased production costs and lost yields. Sun *et al*. (2012) on the ground of economic analysis claimed that the bottle prices would have to increase by $0.02 to $0.41 to compensate for the additional labour costs and lost yield. New analytical methods have been introduced that allow the growers to calculate their optimal yields and grape prices, and make specific, quantitatively justified cluster thinning decisions in the field (Preszler *et al*. 2010).

CONCLUSIONS

The maturity of red grape cultivars is often below optimum in the north-western region of Croatia, especially for late ripening grape cultivars, such as Cabernet Sauvignon, and in relatively cooler seasons. Crop removal and other canopy interventions are therefore widely used in those vineyards. Accordingly, the results indicate that it is possible to improve the wine phenolic composition by using cluster thinning, especially in combination with berry thinning. Cluster thinning in combination with berry thinning had the greatest effect on total phenols concentration in wines from both cultivars in both years, including higher concentrations of many individual phenolic compounds. This suggests the importance in source-sink ratios in the accumulation of secondary metabolites in grapes and subsequent wines. Consequently, it is doubtless that the crop removal is an efficient "tool" that should be practised in cool-climate regions, especially for red grape cultivars. However, considerably higher prices of grapes will be required the compensate for the yield loss and additional production costs resulting from the time required to perform the thinning operations, and this is questionable under the existing grapevine market conditions. Cluster and/or berry thinning should therefore be an additional practice in high yield and cooler growing season conditions. Further research should be focused on the economic feasibility of such canopy operations.

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