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Acta Agriculturae Scandinavica, Section B — Soil & Plant Science

ISSN: 0906-4710 (Print) 1651-1913 (Online) Journal homepage: http://www.tandfonline.com/loi/sagb20

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To cite this article: Mato Drenjančević, Vladimir Jukić, Krunoslav Zmaić, Toni Kujundžić & Vesna Rastija (2017): Effects of early leaf removal on grape yield, chemical characteristics, and antioxidant activity of grape variety Cabernet Sauvignon and wine from eastern Croatia, Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, DOI: 10.1080/09064710.2017.1332238

To link to this article: http://dx.doi.org/10.1080/09064710.2017.1332238

Published online: 25 May 2017.

ACTA AGRICULTURAE SCANDINAVICA, SECTION B — SOIL & PLANT SCIENCE, 2017
https://doi.org/10.1080/09064710.2017.1332238

Effects of early leaf removal on grape yield, chemical characteristics, and antioxidant activity of grape variety Cabernet Sauvignon and wine from eastern Croatia

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ABSTRACT

The aim of this study was to determine the impact of two different treatments of early defoliation performed before blooming on: grape yield, chemical parameters, polyphenols content, and antioxidant activity of grape and red wine cv. Cabernet Sauvignon from the vineyard located in Ilok, the eastern continental region of Croatia. Two different treatments of leaf removal (LR) were performed: removal of 3 leafs (T1) and 6 leafs (T2) before blooming, together with control (no leaf removal) (T3) during two years (2013 and 2014). Crop yield and average cluster weights per vine were determined. Density, pH and titratable acidity were measured in must, while the total phenols, total anthocyanins and antioxidant activity were measured in the extract of grape skin and produced wine. The analysis of individual anthocyanins in wine was performed by HPLC method. T2 treatment significantly lowered the crop yield and the average cluster weights, and increased total phenols, total anthocyanins, antioxidant activity and most abundant individual anthocyanins in wine. Defoliation did not affect the other chemical parameters in must, grape skin extract and wine. Vintage year is statistically the most significant source of variability for density of must, antioxidant activity in grape skin extract, as well as pH and titratable acidity in wine. This study has showed that the early leaf removal treatment in eastern continental part of Croatia could be used for the production of smaller quantity of high quality Cabernet Sauvignon red wine abundant with anthocyanins.

ARTICLE HISTORY
Received 1 March 2017
Accepted 15 May 2017

KEYWORDS
Cabernet Sauvignon; early leaf removal; total polyphenols; antioxidant activity; anthocyanins

Introduction

Many ampelotechnical practices are performed in order to improve grape quality that directly affects wine composition and quality. Wine quality depends on must characteristics such as pH, total acidity, sugar and also wine alcohol content, acidity, and colour (González-Fernández et al. 2012). Wine aroma and flavour that derived from the large range of volatile compounds and their interactions are also important aspects of wine quality (Rapp & Mandery 1986).

Polyphenols play important role in sensory characteristics of wine, such as colour, astringency and bitterness and, therefore, play a major role in wine quality (Conde et al. 2007). Wines contain a wide range of polyphenols that include phenolic acids and flavonoids. Anthocyanins, in their flavilium cation form, are responsible for the colour of red berry varieties and red wines (Gutiérrez et al. 2005). Grapes exposed to the increased amount of sunlight are capable of enhanced biosynthesis of flavonoids (Bergqvist et al. 2001). Polyphenols are associated with wide spectrum of biological actions potentially beneficial for the human health, such as anticancerogenic, antidiabetic, neuroprotective, hormonal, antimicrobial, cardioprotective, antioxidant activity and other health effects of wine (Rastija 2011). Anthocyanins are water-soluble pigments present in red grape skins, which contribute to the development of red polymeric pigments during wine aging. The colour of red wine changes progressively during its life, due to process of polymerization and copigmentation (Mateus et al. 2003; Versari et al. 2008).

Leaf removal (LR) is a practice for improving a photosynthetic capacity and quality of crop plants and it could be applied from flowering to full vérasion. Potential benefits of LR include increased canopy air circulation, sunlight exposure of grapes and improved cluster microclimate, which leads to decreasing of cluster fungal diseases (English et al. 1990).

LR treatments may affect fruit set (flowers and berries per cluster), yield per vine (reduce berry weight and number of berries per cluster and cluster weight) and grape quality (fruit chemistry and skin/pulp ratio) (Koblet et al. 1994; Staff et al. 1997; Bennett et al. 2005; Nicolosi et al. 2012). The wanted outcomes of LR

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depend on timing and intensity of LR, cultivar, as well as the climate (Bledsoe et al. 1988). Early leaf removal (ELR) performed in an early stage of cluster development at bloom or before bloom is a more effective strategy for controlling berry size and berry cluster and, therefore, control vine crop and improve grape quality. Early defoliation may lead to better soluble solids accumulation, increase total anthocyanins and total polyphenols and colour intensity of wine (Smart et al. 1990; Tardaguila et al. 2010; Nicolosi et al. 2012; Vilanova et al. 2012). However, many studies have reported negative effects of LR, especially in warm climate. Excessive LR in warm climate can lead to sun-burned fruit, lowered concentration of anthocyanins and berry colour (Price et al. 1995).

The effects of ELR treatments depend on grape variety. LR decreased the yield per vine and cluster weight in Merlot and Sangiovese, while the berry size was unaffected in both varieties. The same treatment affected Cabernet Sauvignon and reduced the berry size (Yorgos et al. 2012). In the study of Bogicevic et al. (2015) the early defoliation reduced all yield parameters in Cabernet Sauvignon, but in the case of Vranac variety the treatments did not modify the berry growth and berry weight, but only the cluster weight.

Although the effects of LR on grape yield and quality have been widely studied, no research has been done about the benefits of ELR performed before blooming on red grape cultivars grown in the continental part of Croatia. Bubola et al. (2012) analysed the effects of LR on the concentration of phenolic and organic acids of cv. Istrian Malvasia white wine. Treatment before blooming significantly lowered the concentration of phenolic acids which could affect the improvement of organoleptic characteristic of white wine.

The objective of this study was to determine the effects of early defoliation on Cabernet Sauvignon, grown in vineyard in eastern continental region of Croatia. To explain the possible effects observed, we have measured yield parameters, density, pH, titratable acidity of must, as well as total phenols, total anthocyanins, antioxidant activity and individual anthocyanins content measured in extract of grape skin and wine.

Materials and methods

Plant material and experimental design

This study was carried out with grape cultivars *V. vinifera*, variety Cabernet Sauvignon, during 2013 and 2014. Vineyard, planted in 1999 on a eutric brown soil, developed on loess, located in Ilok (lat. 45. 212 801, lon. 19. 385 056, elevation 160 m), the eastern continental region of Croatia, subregion Podunavlje, vineyards Srijem. The vines were planted with 2.0 m spacing between rows and 0.9 m within rows, for a total of 5555 vines/ha. Vine training system was Guyot, with 12 buds per plant. The experiment was designed by random block formation consisting of three replicates. Two different treatments of basal leaf removal were performed: removal of 3 leafs (T1) and 6 leafs (T2) before blooming, and control (no leaf removal) (T3). The treatments consisted of 10 plants per repetition, which was 90 vines in total. The experimental field provided no irrigation system. The soil management practices were all mechanically performed and standard cultural practices in the continental Croatia area were applied to all of the treatments. Rainfall and daily mean temperature for 2013 and 2014 during the April-September were obtained from Meteorological and Hydrological Service of Croatia and presented in Table 1. Cumulative rainfall during April-September vegetation period in 2014 was higher than in 2013, while the daily mean temperatures were equal.

Table 1. Weather conditions in Ilok during vegetation period in 2013 and 2014.

	Mean daily temperature, °C		Rainfall, mm	
Month	2013	2014	2013	2014
April	13.54	13.30	30.60	55.60
May	17.05	16.27	164.10	236.40
June	20.29	20.68	103.40	22.40
July	23.16	21.87	14.70	149.70
Aug	23.19	20.87	39.40	83.70
Sept	16.09	17.13	92.30	99.30
Mean temp./ °C	18.89	18.35		
Cumulative rainfall, mm			444.5	647.1

Crop yield

The date of harvest was determined according to content sugars and acidity and their relationship. For the yield assessment, the crop yield (CY/kg), number of clusters (NC) per vine, and average cluster weights (ACW/g) per vine were determined. Average cluster weights were calculated from yield and clusters per vine data. Two hundred berries were randomly chosen from each treatment and replicated for further analyses.

Grape juice analysis

Samples for grape juice analysis were taken after destemming and crushing of grapes and stored in freezer at −20°C, until analysis. From each replication in the vineyards one sample of grape juice was obtained, leading to three samples per treatment. The density of grape must (DM), an indication of grape ripeness and sugar content

used in winemaking, was determined by using digital refractometer (AR200, Topac, USA) and expressed in Oechsle scale (°Oe). pH was measured with an electronic pH metre (827 pH lab, Metrohm, Switzerland). Titratable acidity (TAM) was measured by using a 5.0 mL aliquot of juice and titrating against 0.1 M NaOH up to pH 8.2 and was expressed as the g L^{-1} of tartaric acid equivalents.

Grape skin extraction

Protocol for grape skin extraction was performed according to the method described by Novak et al. (2008). Extraction procedure was carried out three times. The three portions of grape skin extracts were mixed and the total volume of the extract was measured.

Microvinification

Fermentations were carried out per each treatment in triplicate for both years of experiment. Grapes were destemmed, crushed, treated with 15 mg L^{-1} SO_2 and inoculated with *Saccharomyces cerevisiae* (Uvaferm BDX). Fermentations were conducted in 5-litre glass fermentors at a temperature of 25°C. Pomace was mixed twice a day. After seven days of fermentation and maceration, pomace was pressed using a small mechanical press. Wines were sulfited with 20 mg L^{-1} SO_2. Three months after the end of fermentation wines were taken for the analyses.

Determination of total polyphenols

Total polyphenols in grape berry extract/wine were measured spectrophotometrically at 765 nm (UV8500, Lab Alliance, State College, PA) after the reaction with Folin–Ciocalteu phenol reagent, according to the Folin-Ciocalteu micro method (Waterhouse 2013). Total polyphenols in grape berry extract were expressed as g of gallic acid equivalents (GAE) per kg of fruit's fresh weight (TPS/g kg^{-1}), and g GAE per L of wine (TPW/g L^{-1}). All measurements were performed in triplicate and reported as mean ± standard deviation (SD).

Determination of total anthocyanins

Total anthocyanins were determined by pH-differential method (Giusti & Wrolstad 2001). The total anthocyanin content was expressed in mg of cyanidin-glucoside equivalents (CGE) per kg of fruit's fresh weight (TAS/g kg^{-1}) or mg per 1 L of wine (TAW/mg L^{-1}), using a molar extinction coefficient (ε) of cyanidin-glucoside of 26 900 L mol^{-1} cm^{-1} and molar weight (MW) (449.2 g mol^{-1}). Data presented are mean ± standard deviation (SD) of three measurements.

Determination of antioxidant activity

The method consisted of spectrophotometric measurement of the colour change intensity in solution depending on the amount of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al. 1995). Antioxidant activity of samples is expressed in terms of g gallic acid equivalents per 1 kg of grapes (g GAE kg^{-1}), and g GAE per litre of wine (g GAE L^{-1}).

High-performance liquid chromatography (HPLC): separation and detection of anthocyanins

The samples were stored at −20°C previous to HPLC analysis. For sample preparation wine samples were centrifuged for 3 min. The supernatant was transferred into HPLC-vials. The anthocyanins of red wine samples were analysed according to OIV-MA-AS315-11 with only few modifications (OIV 2007).

Anthocyanins were analysed by direct injection of the samples, previously filtered through a 0.45 µm pore size membrane filter, in an Agilent 1100/1200 series HPLC system equipped with an Agilent photodiode array detector (DAD). The separation was performed at 20°C on a reversed phase column: LiChrospher 100 RP 18 (5 µm) in LiChroCart 250–4 (MERCK) with guard column LiChroCart 4 mm RP 18 (MERCK). The solvents were: (A) water/formic acid/acetonitrile (87:10:3, *v:v:v*) and (B) water/formic acid/acetonitrile (40:10:50, *v:v:v*). The flow rate was 0.4 mL min^{-1}. The following gradient of eluents was used: 6%–30% (B) 0–15 min, 30%–50% (B) 15–30 min, 50%–60% (B) 30–35 min, 60%–6% (b) 35–41 min. The detection wavelength was 520 nm. The seven different anthocyanin compounds were identified by comparing their retention times of available standard substances (cyanidin-3,5- diglucoside, cyanidin-3-glucoside, delphinidin-3-glucoside, malvidin-3,5-diglucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, all obtained from Sigma-Aldrich (St. Louis, Missouri, USA) or spectral characteristics with data given in the literature (Burns et al. 2002; Ryan & Revilla 2003; Radovanović & Radovanović 2010; Li et al. 2013). Quantification was made by means of a calibration curve ($R^2 = 1$) obtained by injecting standard solutions of malvidin-3-monoglucoside chloride with seven different concentrations. Results were expressed as malvidine-3-glucoside equivalents (mg L^{-1}). All analyses were done in duplicate and results expressed as mean value.

Statistical analysis

One-way analysis of variance (ANOVA) and cluster analysis were performed using STATISTICA 8.0 (StatSoft, Inc., Tulsa, USA). One-way analysis of variance (ANOVA) was performed at the significance level 0.05 using Fisher's least significant different (LSD) test in order to determine the significant difference between treatments and years of vintage.

Results and discussion

Results of measuring: crop yield (CY), number of clusters per vine (NC) and average cluster weights per vine (ACW/g) are shown in Table 2. All of data reports are the mean values of three measurements. According to analysis of variance, removal of 6 leafs before blooming significantly reduced the CY and NC 2013. In 2014, the highest NC was recorded for vine where 6 leafs were removed, but CY, and consequently ACW, were reduced. The differences for all three parameters between 2013 and 2014 were not observed. Effects of early defoliation on the decrease of yield and average cluster weights are in accordance with studies of several authors (Koblet et al. 1994; Diago et al. 2010; Petrie et al. 2000; Nicolosi et al. 2012). A decrease in berry weight is the result of lower cell numbers within each berry and a reduction in the final size of these cells. Effects of ELR and vintage on the density of grape must (DM/°Oe), pH, and titratable acidity (TAM/g L^{-1}) observed in must are shown in Table 2. Statistically significant differences have not been observed between those three parameters within the treatments, consistent with pervious work of Bubola et al. (2012), Osrečak et al. (2015) and Sandler et al. (2009) performed on white varieties. ELR has already shown to be effective in reducing the yield by 30–70% due to a decrease in berries set and reduced cluster compactness (Poni et al. 2006; Intrieri et al. 2008). According to the results of this study, only the vintage year is the largest and a statistically significant sources of variability for these parameters of must. Lower cumulative rainfall during vegetation period in 2013 resulted in higher DM and TAM. This is in agreement with Castellarin et al. (2007) and Ferrer et al. (2014), who reported that the increase in sugar content is respond to the water deficit.

Total phenols (TPS/g kg^{-1}), total anthocyanins (TAS/g kg^{-1}), and antioxidant activity measured in the extract of grape skin (AOAS/gGAE kg^{-1}) during the period 2013–2014 are presented in Table 2. ELR treatments revealed no significant differences of TPS, TAS, and AOAS. The results are in accordance with study of Poni et al. (2008) where berry pigmentation and total phenols in grape was not improved by early defoliation performed on 'Sangiovese' variety. On the contrary, many studies have shown that with ELR berry weight is decreased and harvest sugar content increased, as well as skin anthocyanins (Scheiner et al. 2010; Tardaguila et al. 2010). However, LR could be a large stress for a plant, as an over-exposure to the sun, that lead to reduction of phenolic compounds concentration and increase of antioxidant activity as stress respond. Study of Bubola et al. (2012) reports that before blooming, treatments significantly lowered the concentration of phenolic acids in grape juice. The biosynthesis and accumulation of anthocyanins in Cabernet Sauvignon is

Table 2. Effects of early leaf removal and vintage on: crop yield (CY/kg); number of clusters per vine NC); average cluster weights per vine (ACW/g), density of grape must (DM/°Oe), pH, and titratable acidity (TAM/g L^{-1}) observed in must; total phenols (TPS/g kg^{-1}), total anthocyanins (TAS/g kg^{-1}), and antioxidant activity measured in extract of grape skin (AOAS/gGAE kg^{-1}); total phenols (TPW/g L^{-1}), total anthocyanins (TAW/mg L^{-1}), and antioxidant activity (AOAW/gGAE L^{-1}), pH, total acidity(g L^{-1}) and ethanol (% *v:v*) measured in wine, during the 2013–2014.

Sample	Parameters	T1 2013	T2 2013	T3 2013	T1 2014	T2 2014	T3 2014
Grape	CY/kg	1.44 ± 0.27A	1.05 ± 0.11B	1.40 ± 0.13A	1.20 ± 0.04B	1.07 ± 0.04B	1.65 ± 0.34A
	NC	22.83 ± 2.20Aa	16.23 ± 4.50Bb	20.74 ± 0.86A	17.53 ± 1.10Bb	25.22 ± 5.00Aa	20.84 ± 0.97A
	ACW/g	64.20 ± 18.70	57.10 ± 9.80	67.40 ± 5.60	68.50 ± 6.19A	43.54 ± 7.72B	79.02 ± 13.84A
Must	DM/°Oe	91.33 ± 2.51a	94.33 ± 0.58a	91.00 ± 5.29a	78.33 ± 0.58b	78.33 ± 3.21b	78.00 ± 2b
	pH	3.55 ± 0.17	3.58 ± 0.08	3.56 ± 0.04	3.48 ± 0.07	3.50 ± 0.06	3.53 ± 0.05
	TAM/g L^{-1}	8.56 ± 1.10	7.65 ± 0.23	7.78 ± 1.73	7.07 ± 0.83	7.20 ± 0.55	6.62 ± 0.57
Grape skin	TPS/mg kg^{-1}	5.20 ± 1.04	5.40 ± 1.21	4.80 ± 0.38b	6.6 ± 0.68	5.71 ± 0.12	6.93 ± 0.77a
extract	TAS/mg kg^{-1}	2.39 ± 0.36	2.09 ± 0.47	2.05 ± 0.15	2.17 ± 0.37	1.77 ± 0.15	2.32 ± 0.37
	AOAS/gGAE kg^{-1}	305.30 ± 3.12b	326.67 ± 28.12b	329.30 ± 6.35b	383.56 ± 17.57a	393.04 ± 14.70a	369.34 ± 18.45a
Wine	TPW/mg L^{-1}	2.11 ± 0.33Ba	2.96 ± 0.37Aa	1.64 ± 0.56B	1.48 ± 0.14b	1.44 ± 0.06b	1.55 ± 0.02
	TAW/mg L^{-1}	183.10 ± 27B	222.35 ± 11A	163.23 ± 26.00B	197.00 ± 20.42	206.00 ± 15.13	185.67 ± 10.6
	AOAW/gGAE L^{-1}	94.0 ± 5.00a	96 ± 5.00	101.56 ± 3.72a	76.67 ± 10.97Bb	91.67 ± 4.16A	87.00 ± 10.15ABb
	pH	3.54 ± 0.06	3.54 ± 0.03a	3.51 ± 0.05a	3.46 ± 0.07	3.44 ± 0.05b	3.40 ± 0.03b
	Total acidity / g L^{-1}	5.87 ± 0.64	5.80 ± 0.26	5.90 ± 0.46b	6.83 ± 0.76	7.03 ± 1.19	7.60 ± 0.72a
	Alcohol	10.13 ± 0.16Bb	10.61 ± 0.15A	10.07 ± 0.37Bb	10.81 ± 0.35a	10.99 ± 0.26	11.06 ± 0.18a

T1 = Removal of 3 leafs; T2 = 6 leafs; T3 = control (no leaf removal). Different upper case letters in each row indicate statistically significant differences ($p < 0.05$) between treatments in the same year by the Fisher's least significant different (LSD) test. Different lower case letters in each row indicate statistically significant differences (*$p < 0.05$) between years by the Fisher test.

Table 3. Effects of early leaf removal and vintage on individual anthocyanins in wine (mg L^{-1}).

Compound	T1 2013	T2 2013	T3 2013	T1 2014	T2 2014	T3 2014
Delphinidin- 3-glucoside	0.41 ± 0.21b	0.76 ± 0.1b	0.6 ± 0.11b	3.07 ± 1.16a	3.21 ± 0.78a	2.3 ± 2.04a
Cyanidin-3-glucoside	0.04 ± 0.08b	0.16 ± 0.08b	0.09 ± 0.1b	0.56 ± 0.31a	0.61 ± 0.13a	0.43 ± 0.4a
Malvidin-3,5-diglucoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Petunidin-3-glucoside	1.83 ± 0.26b	2.43 ± 0.13b	2.15 ± 0.25b	7.62 ± 0.81ABa	8.43 ± 0.43Aa	6.31 ± 1.61Ba
Peonidin-3-glucoside	0.44 ± 0.07b	0.82 ± 0.1b	0.66 ± 0.9b	3.91 ± 0.43ABa	4.15 ± 0.22Aa	2.93 ± 0.8Ba
Malvidin-3-glucoside	15.03 ± 4.44b	18.74 ± 2.03b	18.3 ± 0.89b	50.25 ± 9.05Aa	59.19 ± 7.48Aa	39.47 ± 21.26Ba
Delphinidin-3-(acetyl) glucoside	0.21 ± 0.18b	0.39 ± 0.1b	0.34 ± 0.18b	2.72 ± 0.8a	2.75 ± 0.60a	2 ± 1.49a
Cyanidin -3-(acetyl) glucoside	0.09 ± 0.16	n.d.	n.d.b	0.62 ± 0.32B	0.29 ± 0.09B	2.19 ± 1.46Aa
Petunidin -3-(acetyl) glucoside	0.12 ± 0.13b	0.25 ± 0.9b	0.15 ± 0.1b	1.87 ± 0.57a	1.99 ± 0.47a	1.47 ± 1.01a
Delphinidin-3-O-(6-*p*-coumaroyl)glucoside	n.d.	n.d.	n.d.b	0.03 ± 0.02	n.d.	0.07 ± 0.01a
Peonidin-3-(acetyl) glucoside	0.01 ± 0.0b	0.05 ± 0.04b	0.01 ± 0.0b	1.75 ± 0.65Aa	2 ± 0.32Aa	1.01 ± 0.97Ba
Malvidin-3-(acetyl) glucoside	3.74 ± 1.73b	4.61 ± 0.62b	4.72 ± 0.28b	15.89 ± 3.14Aa	17.4 ± 3.09Aa	11.15 ± 6.67Ba
Cyanidin 3-*O*-(6′′-*p*-coumaroyl)glucoside	n.d.	n.d.	n.d.b	n.d.B	n.d.B	0.1 ± 0.09Aa
Petunidin-3-*O*-(6-*p*-coumaroyl)glucoside	n.d.	n.d.	n.d.b	0.04 ± 0.03	0.06 ± 0.05	0.09 ± 0.07a
Malvidin-3-*O*-(6-*p*-coumaroyl)glucoside (*cis* isomer)	n.d.b	n.d.b	n.d.	0.15 ± 0.11ABa	0.25 ± 0.21Aa	0.1 ± 0.15B
Peonidin-3-*O*-(6-*p*-coumaroyl)glucoside	n.d.b	n.d.b	n.d.b	O.78 ± 0.43a	0.65 ± 0.13a	0.69 ± 0.64a
Malvidin-3-*O*-(6-*p*-coumaroyl)glucoside (*trans* isomer)	0.41 ± 0.21b	0.76 ± 0.1b	0.6 ± 0.11b	3.07 ± 1.16a	3.21 ± 0.78a	2.3 ± 2.03a
Cyanidin-3,5-*O*-diglucoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not detected; *T1 = Removal of 3 leafs; T2 = 6 leafs; T3 = control (no leaf removal). Different upper case letters in in each row indicate statistically significant differences (*$p < 0.05$) between treatments by the Fisher's least significant different (LSD) test. Different lower case letters in each row indicate statistically significant differences (*$p < 0.05$) between years by the Fisher test.

quite sensitive to extent cluster exposure to the sun and over-heating (Bergqvist et al. 2001).

Total phenols in wine (TPW/g L^{-1}), total anthocyanins (TAW/g L^{-1}), and antioxidant activity measured in the produced wine (AOAW/gGAE L^{-1}) during the period 2013–2014 are shown in Table 2. Removal of 6 leafs resulted in statistically significant increased TPW and TAW in 2013, while in 2014 this effect was not observed. Antioxidant activity was enhanced by the same treatment only in wine from 2014. These results are in agreement with results obtained by other authors (Guidoni et al. 2008; Osrečak et al. 2016).

In the present study, the main factor of variability for TPS (only for untreated grapes) and AOAS was the year. Weather conditions in 2014 were more favourable for biosynthesis of polyphenols, and thereby an enhanced antioxidant activity of grape. Increased rainfall during vegetation period in 2014 probably resulted in dilution effect in the grape pulp, which lowered concentration of phenols and accordingly, antioxidant activity. However, opposite results for the same parameters in wine from 2013 were observed, where increased levels of total phenols and antioxidant activity have been measured. Guidoni et al. (2008) state that LR may improve grape health and quality in microclimatic conditions unfavourable for ripening or in less sun-exposed vineyards. Vintage did not significantly affect the total anthocyanins in grape skin and wine.

Effects of ELR and vintage on concentration of individual anthocyanins in wine are presented in Table 3. Among the 17 identified anthocyanins, six of them increased concentration by leaf removal in 2014: petunidin-3-glucoside, malvidin-3-glucoside, malvidin-3-glucoside, peonidin-3-(acetyl) glucoside, malvidin-3-(acetyl) glucoside, malvidin-3-O-(6-p-coumaroyl)glucoside (*cis* isomer). In the same year ELR treatments decreased the concentration of cyanidin-3-(acetyl) glucoside, while the rest of the anthocyanins did not have a significant effect. Effect of ELR on anthocyanins concentration was not observed in wine from 2013. Late LR treatment (performed at verasion) also positively influenced malvidin-3-glucoside biosynthesis in Teran wines (Osrečak et al. 2016). Similar treatment of cv. Uva Longanesi vine from Brasil resulted without significant difference in concentration of malvidin-3-glucoside but a significant increase of other anthocyanins was found in untreated wines (Tessarin et al. 2014). The relationship between anthocyanin composition and defoliation seems to be cultivar dependent. Generally, although solar radiation exposure could increase anthocyanin concentration, excessive berry temperature could be a detrimental for their synthesis and accumulation (Matus et al. 2009).

In accordance to study of Ferrer et al. (2014) and Barbagallo et al. (2011), concentration of individual anthocyanins has shown the greatest variation between the years. Most of the compounds have increased concentrations in wine from 2014 (Table 3). Opposite of results of Ferrer et al. (2014) and Roby et al. (2004) studies, excess rainfall during vegetation period in 2014 enhanced the concentration of anthocyanins. This was probably due to effects of the other environmental conditions, such as mean daily temperature that remained at the same level for both years.

In summary, among the investigated treatments, early removal of six leafs had the strongest effect on the grape yield, chemical characteristics, and antioxidant activity of grape variety Cabernet Sauvignon. This treatment decreased a crop yield, cluster number, and in rainier

year, average cluster weights per vine. However, increase in total phenols, total anthocyanins and antioxidant activity in wine was observed with this technique, but not in both season.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Vukovar-Srijem County, Croatia (project, Influence of early leaf removal on Cabernet Sauvignon variety [grant number 369]).

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References

Barbagallo M, Guidoni S, Hunter JJ. 2011. Berry size and qualitative characteristics of *Vitis vinifera* L. cv. Syrah. S Afr J Enol Vitic. 32:129–136.

Bennett J, Jarvis P, Creasy GL, Trought MCT. 2005. Influence of defoliation on overwintering carbohydrate reserves, return bloom, and yield of mature Chardonnay grapevines. Am J Enol Viticult. 56:386–393.

Bergqvist J, Dokoozlian N, Ebisuda N. 2001. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the Central San Joaquin Valley of California. Am J Enol Viticult. 52:1–7.

Bledsoe AM, Kliewer WM, Marois JJ. 1988. Effects of timing and severity of leaf removal on yield and fruit composition of Sauvignon blanc grapevines. Am J Enol Viticult. 39:49–54.

Bogicevic M, Maras M, Mugoša M, Kodžulović V, Raičević J, Šućur S, Failla O. 2015. The effects of early leaf removal and cluster thinning treatments on berry growth and grape composition in cultivars Vranac and Cabernet Sauvignon. Chem Biol Techn Agric. 2:230.

Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 28:25–30.

Bubola M, Peršurić Đ, Kovačević Ganić K, Karoglan M, Kozina B. 2012. Effects of fruits zone leaf removal on the concentrations of phenolic and organic acids in Istrian Malvasia grape juice and wine. Food Technol Biotech. 50:159–166.

Burns J, Mullen W, Landrault N, Teissedre P-L, Lean MEJ, Crozier A. 2002. Variations in the profile and content of anthocyanins in wines made from Cabernet Sauvignon and hybrid grapes. J Agr Food Chem. 50:4096–4102.

Castellarin S, Matthews M, Di Gaspero G, Gambetta G. 2007. Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. Planta. 227:101–112.

Conde C, Silva P, Fontes N, Dias ACP, Tavares RM, Sousa MJ, Agasse A, Delrot S, Gerós H. 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food. 1:1–22.

Diago MP, Vilanova M, Tardaguila J. 2010. Effects of timing of manual and mechanical early defoliation on the aroma of *Vitis vinifera* L. Tempranillo wine. Am J Enol Viticult. 61:382–391.

English JT, Bledsoe AM, Marois JJ, Kliewer WM. 1990. Influence of grapevine canopy management on evaporative potential in the fruit zone. Am J Enol Viticult. 41:137–141.

Ferrer M, Echeverría G, Carbonneau A. 2014. Effect of berry weight and its components on the contents of sugars and anthocyanins of three varieties of *Vitis vinifera L.* under different water supply conditions. S Afr J Enol Vitic. 35:103–113.

Giusti MM, Wrolstad RE. 2001. Anthocyanins. Characterization and measurement with UV visible spectroscopy. In: R. E. Wrolstad, editor. Current protocols in food analytical chemistry. New York: Wiley; p. F1.2.1–F1.2.13.

González-Fernández AB, Marcelo V, Valenciano JB, Rodríguez-Pérez JR. 2012. Relationship between physical and chemical parameters for four commercial grape varieties from the Bierzo region (Spain). Sci Hortic-Amsterdam. 147:111–117.

Guidoni S, Oggero G, Cravero S, Rabino M, Cravero MO, Balsari P. 2008. Manual and mechanical leaf removal in the bunch zone (*Vitis vinifera* L., cv Barbera): effects on berry composition, health, yield and wine quality, in a warm temperate area. J Int Sci Vigne Vin. 42:49–58.

Gutiérrez IH, Lorenzo ES-P, Espinosa AV. 2005. Phenolic composition and magnitude of copigmentation in young and shortly aged red wines made from the cultivars, Cabernet Sauvignon, Cencibel, and Syrah. Food Chem. 92:269–283.

Intrieri C, Filippetti I, Allegro G, Centinari M, Poni S. 2008. Early defoliation (hand vs mechanical) for improved crop control

and grape composition in Sangiovese (*Vitis vinifera* L.). Aust J Grape Wine R. 14:25–32.

Koblet W, Candolfi-Vasconcelos MC, Zweifel W, Howell GS. 1994. Influence of leaf removal, rootstock, training system on yield and fruit composition of Pinot noir grapevines. Am J Enol Viticult. 45:181–187.

Li Y, Ma R, Xu Z, Wang J, Chen T, Chen F, Wang Z. 2013. Identification and quantification of anthocyanins in Kyoho grape juice-making pomace, Cabernet Sauvignon grape winemaking pomace and their fresh skin. J Sci Food Agr. 93:1404–1411.

Mateus N, Silva AMS, Rivas-Gonzalo JC, Santos-Buelga C, de Freitas V. 2003. A new class of blue anthocyanin-derived pigments isolated from red wines. J Agr Food Chem. 51:1919–1923.

Matus MJ, Loyola R, Vega A, Peña-Neira A, Bordeu E, Arce-Jonson P, Alcalde JA. 2009. Post-veraison sunlight exposure induces MYB-mediated transcriptional regulation of anthocyanin and flavonol synthesis in berry skins of *Vitis vinifera*. J Exp Bot. 60:853–867.

Nicolosi E, Continella A, Gentiloe A, Cicala A, Ferlito F. 2012. Influence of early leaf removal on autochthonous and international grapevines in Sicily. Sci Hortic-Amsterdam. 146:1–6.

Novak I, Janeiro P, Seruga M, Oliveira-Brett A. 2008. Ultrasound extracted flavonoids from four varieties of Portuguese red grape skins determined by reverse-phase high-performance liquid chromatography with electrochemical detection. Anal Chim Acta. 630:107–115.

OIV-MA-AS315-11. 2007. HPLC-Determination of nine major anthocyanins in red and rosé wine. Method: OIV-MA-AS315-11, 2007. Compendium of International Methods of Analysis of Wines and Musts. Available from: http://www.oiv.int/public/medias/2540/oiv-ma-as315-11.pdf.

Osrečak M, Karlogan M, Kozina B. 2016. Influence of leaf removal and reflective mulch on phenolic composition and antioxidant activity of Merlot, Teran and Plavac maliwines (*Vitis vinifera* L.). Sci Hortic-Amsterdam. 209:261–269.

Osrečak M, Karlogan M, Kozina B, Preiner D. 2015. Influence of leaf removal and reflective mulch on phenolic composition of white wines. J Int Sci Vigne Vin. 49:183–193.

Petrie PR, Trought MCT, Howell GS. 2000. Fruit composition and ripening of Pinot noir (*Vitis vinifera L.*) in relation to leaf area. Aust J Grape Wine R. 6:46–51.

Poni S, Bernizzoni F, Civardi S. 2008. The effect of early leaf removal on whole-canopy gas exchange and vine performance of *Vitis vinifera* L. 'Sangiovese'. Vitis. 47:1–6.

Poni S, Casalini L, Bernizzoni F, Civardi S, Intrieri C. 2006. Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. Am J Enol Viticult. 57:397–407.

Price SF, Breen PJ, Valladao M, Watson BT. 1995. Cluster sun exposure and quercetin in Pinot noir grapes and wine. Am J Enol Viticult. 46:187–194.

Radovanović B, Radovanović A. 2010. Free radical scavenging activity and anthocyanin profile of Cabernet Sauvignon wines from the Balkan region. Molecules. 15:4213–4226.

Rapp A, Mandery H. 1986. Wine aroma. Experientia. 42:873–884.

Rastija V. 2011. An overview of innovations in analysis and beneficial health effects of wine polyphenols. Mini-Rev Med Chem. 11:1–17.

Roby G, Harbertson JF, Douglas AA, Matthews MA. 2004. Berry size and vine water deficits as factors in wine grape composition: Anthocyanins and tannins. Aust J Grape Wine R. 10:100–107.

Ryan, J.-M., & Revilla E. (2003). Anthocyanin composition of Cabernet Sauvignon and Tempranillo grapes at different stages of ripening. J Agr Food Chem, 51, 3372–3378.

Sandler HA, Brock II PE, Vanden Heuvel JE. 2009. Effects of three reflective mulches on yield and fruit composition of coastal New England winegrapes. Am J Enol Viticult. 60:332–338.

Scheiner J, Sacks G, Pan B, Ennahli S, Tralton L, Wise A, Lerch S, Vanden Heuvel J. 2010. Impact of severity and timing of basal leaf removal on 3-isobutyl-2-methoxypyrazine concentrations in red winegrapes. Am J Enol Viticult. 61:358–364.

Smart RE, Dick JK, Gravett IM, Fisher BM. 1990. Canopy management to improve grape yield and wine quality principles and practices. S Afr J Enol Vitic. 1:3–17.

Staff SL, Percival DC, Sullivan JA, Fisher KH. 1997. Fruit zone leaf removal influences vegetative, yield, disease, fruit composition, and wine sensory attributes of *Vitis vinifera* L. 'Optima' and 'Cabernet franc'. Can J Plant Sci. 77:149–153.

Tardaguila J, Martinez de Toda F, Poni S, Diago MP. 2010. Impact of early leaf removal on yield and fruit and wine composition of *Vitis vinifera* L. Graciano and Carignan. Am J Enol Viticult. 61:372–381.

Tessarin P, Boliani AC, Bothelo RV, Rusin C, Versari A, Parpinello GP, Rombolá AD. 2014. Effects of late defoliations on chemical and sensory characteristics of cv. Uva Longanesi wines. J Soil Sci Plant Nut. 14:1021–1038.

Versari A, Boulton RB, Parpinello GP. 2008. A comparison of analytical methods for measuring the color components of red wines. Food Chem. 106:397–402.

Vilanova M, Diago MP, Genisheva Z, Oliveira JM, Tardaguila J. 2012. Early leaf removal impact on volatile composition of Tempranillo wines. J Sci Food Agr. 92:935–942.

Waterhouse A. 2013. Folin-Ciocalteau micro method for total phenol in wine. [cited 2013 Jun 3]. Available from: http://waterhouse.ucdavis.edu/phenol/folinmicro.htm.

Yorgos K, Georgiadou A, Tikos P, Kallithraka S, Koundouras S. 2012. Effects of severity of post-flowering leaf removal on berry growth and composition of three red *Vitis vinifera* L. Cultivars grown under semiarid conditions. J Agr Food Chem. 60:6000–6010.