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Influence of basal leaf removal on the chemical composition of Sauvignon Blanc and Riesling wines

Bernard Kozina [1]*, Marko Karoglan [1], Stanka Herjavec [1], Ana Jeromel [1] and Sandi Orlic [2]

*[1]Department of Viticulture and Enology, Faculty of Agriculture, University of Zagreb, Croatia. [2]Department of Microbiology, Faculty of Agriculture, University of Zagreb, Croatia. *e-mail: bkozina60@yahoo.com*

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Abstract

The influence of partial leaf removal of grapevines on sugar, acids and pH must content was evaluated and changes in wine volatile composition and overall quality were defined. The experiment was carried out in 2002 with Sauvignon Blanc and Riesling varieties. Treatments were a) control, or no leaf removal, b) removal of 4 leaves per shoot and c) removal of 8 leaves per shoot. At the end of fermentation analysis of standard chemical compounds, organic acids and aroma compounds were determined by means of HPLC and GC-MS. Basal leaf removal increased soluble solids and reduced titratable acidity and pH. In some cases increase in free volatile and potential volatile terpene levels was noticed. Results of study indicate that basal leaf removal had different influence on the wine chemical composition and quality that depends upon the variety tested. In Sauvignon Blanc wines basal leaves removal significantly reduced tartaric and malic acid content and increased the content of free and bound monoterpenes. In Riesling wines basal leaves removal had significant influence only on tartaric acid content while there was no marked difference in the sum of free and bound monoterpenes. Sensory evaluation showed that the quality of Sauvignon Blanc wines could be much improved while the quality of Riesling wines remained more or less the same.

Key words: Basal leaf removal, organic acids, monoterpenes, Sauvignon, Riesling.

Introduction

It is well known that canopy manipulation can lead to significant improvements in yield and fruit composition. Wolf *et al.* [1] increased sugar content and reduced titratable acidity in Chardonnay by hedging, lateral shoot removal and basal leaf removal, while Bledsoe *et al.* [2] demonstrated improvements in fruit composition of Sauvignon Blanc by leaf removal. The benefits of basal leaf removal are the best when trimming occurs between blooming and verasion. Earlier removal tends to increase inflorescence necrosis, decrease berry quality and disrupts subsequent bud break [3]. Removal around verasion may be detrimental by reducing sugar accumulation by the fruit [4]. The impact of basal leaf removal, usually involving the leaves positioned immediately above and below and opposite the fruit cluster, is clearly influenced by the number of leaves removed [3]. Changes generally associated with basal leaf removal include a reduction in titratable acidity, associated with a reduction in the uptake of potassium and enhanced degradation of malic acid. Tartaric and citric acid levels are not affected [5]. According to Kliewer *et al.* [6] basal leaf removal also increase soluble solids and reduce titratable acidity and pH. Reynolds and Wardle [7] found that basal leaf removal increased free volatile and potential volatile terpene levels in Gewürztraminer and Riesling berries, as well as Smith *et al.* [8] reported that leaf removal increased several free and bound aroma compounds in Sauvignon Blanc. According to Jackson [5], levels of grassy, herbaceous or vegetable odours of Sauvignon Blanc wines decline, whereas fruity aromas may rise or remain unaffected, while Zoecklein *et al.* [9] detected positive influence of fruit zone leaf thinning on the concentration of glycosyl-glucose, an indicator of the potential flavour content, in Riesling grapes. Monoterpenes play an important role in grape and wine flavour, specifically in Gewürztraminer, Riesling and the Muscat group [10]. The dominating monoterpene alcohols found in several Muscat-type varieties and others like Chardonnay, Riesling or Gewürztraminer are linalool (floral and citrus), geraniol (freshly cut grass), citronellol and α-terpineol [11]. The major portions of these compounds exist in grapes in glycosidically bound forms [12]. The ratio of conjugated to free monoterpenols can be as high as 15:1 [13]. These bound forms are generally non-volatile and non-odorous but represent an important source of fragrant compounds [14]. They can be released by acid hydrolysis and/or glucosidase enzymes activity during vinification process [15]. Other flavour precursors liberated from glucosidases include aliphatic or cyclic alcohols, such as hexanol, 2-phenylethanol, benzyl alcohol, C13 norisoprenoids, phenol acids and volatile phenols [5]. According to Zoecklein *et al.*[9] the sum of bound aromatic alcohols (benzyl alcohol and 2-phenylethanol) was higher in fruit of leaf-removed canopies than controls. Amongst all the terpene compounds, linalool is the one in highest concentration in the Muscat group, and is generally always above its threshold value [16]. Also, there are the polyhydroxylated forms of monoterpenes. These higher oxidation state monoterpenes (linalool oxides and terpene diols and triols) can be present in significant concentrations, but have high threshold values, making their contribution to grape aroma minor[16], but even though they are odourless compounds, they can break down to give pleasant and potent volatiles, i.e., diendiol, hotrienol and nerol oxides[17].

The objective of this study was to evaluate the influence of partial leaf removal of the Sauvignon Blanc and Riesling grapevines on sugar, acids and pH must content and to define changes in wine volatile composition and overall quality.

Material and Methods

Field trials: The experiment was carried out in 2002 on Sauvignon Blanc and Riesling grapes that were grown in the same vineyard, located in Department of Viticulture and Enology, Faculty of Agriculture Research Centre in continental wine region of Croatia, subregion Prigorje. Productive situation considered for both varieties was the same as follows: Guyot pruning, grafted on Kober 5BB, planted at 2,00 m × 1,20 m, planted in north-south rows with fruit clusters formed 1,0 m above the ground.

Treatments were arranged in a completely randomized design that consisted of 10 replicates of 6 vine plots. Treatments were a) control, or no leaf removal, b) removal of 4 leaves per shoot and c) removal of 8 leaves per shoot for both varieties. Leaves were removed by hands around vérasion time.

Wine production: All 10 replicates for each treatment were pooled together to provide adequate material for 100 litre inox tanks fermentation. The free-run juice was treated with 50 mg/l SO_2 and allowed to settle overnight. Must alcoholic fermentation was carried out with selected *Saccharomyces paradoxus* RO54 strain obtained from the Department of Microbiology, Faculty of Agriculture, University of Zagreb. Yeast strain culture was preincubated in sterilized grape must for 48 h at 25°C and finally inoculated at $8x10^6$ CFU/ml. Fermentation was allowed to proceed at 18°C to dryness. Standard post fermentation procedures as racking, filtration and bottling were subsequently employed.

Chemical analyses: The common analyses of wine components were carried out on the must and wines using standard methods[18]. Organic acid (citric, tartaric, malic and lactic) analyses were performed on the HPLC Modules Hewlett Packard 1050 Series comprising a quaternary pump, an online degaser, manual injector, a VW detector linked to a HP (Hewlett Packard) 3395 Integrator. The chromatographic separations were done on Bio Rad Aminex HPX 87H (300 m x 7.8 mm i.d.) organic acid cation exchange column heated to 65°C. The mobile phase was 0.065% (v/v) H_3PO_4 in double glass distilled water with a flow rate of 0.6 ml/min. Detection of organic acids was measured by absorption at 210 nm. Acids were quantified by integration of peak height and calibrated with an external standard. Higher alcohols, ethyl acetate and acetaldehyde analyses were performed on FISION gas chromatograph from distillate of wine and 3-methyl-2 butanol as internal standard, by method of Gabri and Salvagiotto [19]. Volatile wine components (esters, fatty acids, monoterpenes) were determined on CARLO ERBA gas chromatograph, on two capillary columns (CarboWax and DBWax) by solid-phase extraction (SPE) using XAD-2 cartridges and elution with pentane: dichloromethane (2:1) solvent. Temperature programming was: 5 min isothermal at 55°C then linear temperature rise of 2.5°C/min to 175°C (10 min) then linear temperature rise of 2.5°C/min to 188°C (10 min), and linear temperature rise of 10°C/min to 192°C (55 min). Injector and detector temperature was 250°C, split mode 1:30 and carrier gas used was hydrogen (2 ml/min). The analyses were performed in the Istituto Agrario di San Michele all´Adige, Italy.

Statistical analysis: One-way analysis of variance an Least Significant Difference (LSD) comparison tests were used to statistically interpret differences in mean values, if any, at 95 and 99% accuracy level.

Sensory analysis: The wines from the 2002 harvest season were subjected to sensory evaluation by a panel comprising 15 members of the Croatian Enology Society, all of them highly experienced in wine sensory testing, especially Sauvignon Blanc and Riesling. Wine of each treatment was compared by a paired sample test and ranking method to determine aroma and overall quality differences between tested wines. Evaluation took place in the Department of Viticulture and Enology in Zagreb, 6 months after the first decantation. Determination of statistical significance for both test results was done according to literature [20].

Results and Discussion

Chemical composition: The results presented in Tables 1 and 2 show standard chemical composition of Sauvignon Blanc and Riesling musts and wines. Reduction in total acidity, pH, and organic acid content associated with leaf removal in this study are consistent with many previous studies [21, 22] and are partly a consequence of enhanced malic acid degradation in Sauvignon Blanc musts, while in the Riesling treatments stronger difference was noted in the tartaric acid content. According to Iacono *et al.* [4], leaf removal around verasion may influence reducing sugar accumulation but our results showed no marked difference in the must sugar content of both varieties.

Table 1. Standard chemical composition of Sauvignon Blanc must and wine.

Compound	Must after settling				Wine after fermentation		
	Control	4-leaves	8-leaves	LSD	Control	4-leaves	8-leaves
Reducing sugars g/l	231	226	228	n.s	1	1	1
Alcohol vol %	-	-	-		13.6	13.3	13.4
Total acidity g/l*	8.8**	8.0	7.9	5% = 0.2; 1% = 0.3	7.7	6.8	6.7
Volatile acidity g/l**	-	-	-		0.41	0.45	0.43
pH	3.00	3.06	3.07	n.s.	3.08	3.10	3.11
Tartaric acid g/l	6.4**	6.0	6.1	5% = 0.2; 1% = 0.3	5.2	5.0	5.1
Malic acid g/l	3.8**	3.1	3.2	5% = 0.1; 1% = 0.2	3.5	3.0	3.1
Lactic acid g/l	-	-	-		0.1	0.2	0.1
Citric acid g/l	0.3	0.3	0.3	n.s.	0.3	0.3	0.3

* as tartaric acid; ** as acetic acid

Table 2. Standard chemical composition of Riesling must and wine.

Compound	Must after settling				Wine after fermentation		
	Control	4-leaves	8-leaves	LSD	Control	4-leaves	8-leaves
Reducing sugars g/l	201	207	202	n.s.	1	1	1
Alcohol vol %	-	-	-		12.0	12.2	12.0
Total acidity g/l*	10.7**	9.8	9.7	5% = 0.2; 1% = 0.3	9.0	8.4	8.5
Volatile acidity g/l**	-	-	-		0.35	0.40	0.39
pH	2.90	2.93	2.93	n.s.	2.95	2.98	2.96
Tartaric acid g/l	9.2**	8.6	8.7	5% = 0.1;1% = 0.2	8.0	7.6	7.5
Malic acid g/l	2.2	2.2	2.3	n.s.	2.0	2.1	2.1
Lactic acid g/l	-	-	-		0.2	0.2	0.1
Citric acid g/l	0.2	0.2	0.2	n.s.	0.2	0.2	0.2

* as tartaric acid; ** as acetic acid

The higher alcohols are formed during fermentation from decarboxylation and reduction of the corresponding α-keto-acids or by carbohydrate degradation[23]. According to Zambonelli [24] higher alcohol concentrations can vary from 100 up to 500 mg/l, while the most abundant higher alcohol that captures more than 50% of total higher alcohol content is 3-methyl-1-butanol. According to Rapp and Mandery [25], when their concentrations are below 400 mg/l they usually contribute to the desirable complexity of wine.

Results of higher alcohol concentrations presented in Table 3 show no difference between control and basal leaf removal treatments in both varieties. Isoamyl alcohol was the most abundant one, and the total sum ranging between 375.1 and 390.9 mg/l in Sauvignon Blanc wines and 318.5 and 393.4 mg/l in Riesling wines confirmed positive characteristics of used *S. paradoxus* strain RO54 [26]. Benzyl alcohol was the only higher alcohol whose concentrations strongly vary between control and leaves removed treatments in both varieties, probably because it is one of few higher alcohols derivate from grapes and not produced during alcohol fermentation [5]. Control wines had the highest amount of this alcohol while between the basal leaf removal treatments the difference was not so pronounced.

During the alcoholic fermentation many esters can be formed, but the most significant ones are acetate esters of higher alcohols and ethyl esters of fatty acids. Ethyl acetate is the main ester occurring in wine with concentrations from 50 to 200 mg/l [18]. According to Cavazza and Grando [27] acetate esters contribute to the formation of fruity wine aroma. Yeasts synthesize much the same fatty acids irrespective of the nature of raw material used. However, the amount and portion of medium-chain fatty acids released into the fermentation medium is strictly dependent on yeast strain, the composition of the medium and fermentation conditions such as pH, temperature, presence/absence of major and minor nutrients and degree of aeration [28].

The stronger difference between tested treatments was noted in fatty acids and volatile esters concentrations (Tables 4 and 5) in both varieties. The main reason for that was probably the difference in the composition of medium and presence/absence of minor and major nutrients associated with changes in their uptake between treatments during grape maturation period. It is interested to notice that in the Riesling wines the number of removed leaves had no marked influence on the concentration of these compounds. At the contrary, in the Sauvignon Blanc 8 removed leaves treatment wines had the lowest amount of fatty acids and volatile esters while the difference between control and 4 removed leaves treatment was not so pronounced.

The basal leaf removal treatment, especially that with 8 leaves, positively influenced the concentrations of free and bound volatile terpenes in Sauvignon Blanc wines what is in agreement with previous studies [8, 29] (Table 6). In Riesling wines basal leaf removal treatment had opposite effect meaning that the sum of free and bound volatile terpens was higher in the control wines compared with 4 and 8 removed leaves treatments (Table 7). Between analysed monoterpene alcohols in both varieties the most abundant were linalool and α-terpineol. Sum of bound aromatic alcohols (benzyl alcohol and 2-phenylethanol) was highest in the 4 removed leaves treatment and lowest in the 8 removed leaves treatment in both varieties tested what is partly in accordance with Zoecklein *et al.* [9] who found higher concentrations in leaf-removed treatments than in control.

Sensory properties of wines: Sauvignon Blanc and Riesling wines were sensory evaluated by paired sample test and ranking method and the results are presented in Tables 8 and 9. The main aim was to define the differences, if any, in aroma profile and overall quality between control and basal leaf removed treatment wines. According to the results presented it seams that basal leaf removal had positively influenced the quality of Sauvignon Blanc wines while in the Riesling wines the difference between treatments was not so marked. In comparison with the control wine, the basal leaf removal Sauvignon Blanc wines, especially that with 8 removed leaves, were of significantly better quality, what was probably the result of their lower acidity, especially malic acid content, and higher monoterpene content. According to the Riesling wines, sensory evaluation results presented removal of 8 leaves negatively influenced the Riesling wine quality while the difference between the control and 4 leaves removed treatment wines was not so pronounced.

Table 3. Sauvignon Blanc and Riesling wines higher alcohol and acetaldehyde (mg l^{-1}) concentrations.

Wine	Compound	Control	4-leaves	8-leaves
Sauvignon Blanc	1- Propanol	17.5	14.5	14.5
	Hexanol	0.5	0.6	0.5
	Isobutanol	44.5	41.5	45
	Isoamyl alcohol	223.5	239.5	258.5
	2 -Phenyl ethanol	37.9	44.1	37.6
	Benzyl alcohol	67	43	19
	∑ Higher alcohols	417	403	394
	Acetaldehyde	61.5	80	77.5
Riesling	1- Propanol	11.5	12.0	10.5
	Hexanol	0.7	0.4	0.4
	Isobutanol	42.5	44.0	39.5
	Isoamyl alcohol	210.0	215.5	225.0
	2 -Phenyl ethanol	45.7	33.6	38.9
	Benzyl alcohol	83.0	13.0	21.0
	∑ Higher alcohols	393.4	318.5	335.3
	Acetaldehyde	40.5	40.5	44.0

Table 4. Sauvignon Blanc and Riesling wines fatty acids (µg l^{-1}) concentrations.

Wine	Compound	Control	4-leaves	8-leaves
Sauvignon Blanc	Isovaleric acid	708	675	1165
	Caproic acid	2480	2226	1042
	Caprylic acid	4475	3957	1249
	Capric acid	989	841	257
	∑ Fatty acids	8653	7698	3712
Riesling	Isovaleric acid	1109	876	888
	Caproic acid	2084	999	1299
	Caprylic acid	3399	1300	1767
	Capric acid	670	278	297
	∑ Fatty acids	7262	3452	4250

Table 5. Sauvignon Blanc and Riesling wines volatile esters (µg l^{-1}) concentrations.

Wine	Compound	Control	4-leaves	8-leaves
Sauvignon Blanc	Ethyl acetate	37	38,5	34
	Isobutyl acetate	109	70	22
	Isoamyl acetate	2886	2039	297
	Hexyl acetate	45	45	7
	Phenyl ethyl acetate	354	284	63
	Ethyl butyrate	246	203	109
	Ethyl caproate	614	504	198
	Ethyl caprylate	839	667	127
	Ethyl caprate	172	141	22
	Diethyl succinate	230	222	441
	∑ Volatile esters	5532	4218	1320
Riesling	Ethyl acetate	32,5	31	25,5
	Isobutyl acetate	75	23	22
	Isoamyl acetate	202	320	482
	Hexyl acetate	47	4	7
	Phenyl ethyl acetate	261	55	82
	Ethyl butyrate	204	109	139
	Ethyl caproate	478	206	291
	Ethyl caprylate	548	140	214
	Ethyl caprate	113	26	37
	Diethyl succinate	205	443	339
	∑ Volatile esters	2170	1357	1643

Table 6. Sauvignon Blanc wines free and bound monoterpenes (µg l^{-1}) concentrations.

	Free monoterpens (FVT)			Bound monoterpens (PVT)		
	control	4-leaf	8-leaf	control	4-leaf	8-leaves
Oxide linalool fur.trans	-	-	-	13	34	49
Oxide linalool fur.cis	0.6	0.6	1.8	2.2	1.8	2.8
Oxide linalool pyr.trans	15.5	6.5	14.4	2.4	4.4	6.7
Oxide linalool pyr.cis	3.1	4.2	3.8	0.6	0.5	0.4
Σ Oxide linalool	**19.8**	**11.3**	**20.0**	**18.2**	**40.7**	**58.9**
Linalool	12.4	3.3	49.4	4.7	17	30
α-terpineol	3.5	2.0	32	23	24	28
Citronellol	-	-	-	1.4	2,0	2,0
Nerol	-	-	-	4.1	9,1	5
Geraniol	6.3	6.4	5.9	42	64	55
HO-diol (I)	49	83	162	34	94	94
HO-diol (II)	3.2	3.3	3.7	1.5	1.7	0,3
Σ monoterpens	**74.4**	**98.0**	**253.0**	**110.7**	**211.8**	**214.3**
Benzyl alcohol				699	622	663
2 -Phenyl ethanol				436	809	273
Σ				**1135**	**1431**	**936**

Table 7. Riesling wines free and bound monoterpenes (µg l^{-1}) concentrations.

	Free monoterpens (FVT)			Bound monoterpens (PVT)		
	control	4-leaves	8-leaves	control	4-leaves	8-leaves
Oxide linalool fur.trans	-	-	-	52	50	31
Oxide linalool fur.cis	0,7	2.4	0.4	61	55	43
Oxide linalool pyr.trans	6.4	11.7	2.6	21	19	15
Oxide linalool pyr.cis	1.9	3.4	3	4.6	3.2	3.3
Σ Oxide linalool	**9.0**	**17.5**	**6.0**	**138.6**	**127.2**	**92.3**
Linalool	13	70	59	22	33	24
α-terpineol	71	40	25	57	61	53
Citronellol	-	-	-	1.3	1.4	1.2
Nerol	-	-	-	6.8	8.2	5.8
Geraniol	4.7	11	5.4	40	34	31
HO-diol (I)	169	136	129	89	74	39
HO-diol (II)	3.2	0.6	0.7	2.9	3,9	1.5
Σ monoterpens	**260.9**	**257.6**	**219.1**	**219.0**	**215.5**	**155.5**
Benzyl alcohol				236	194	202
2 -Phenyl ethanol				318	487	202
Σ				**654**	**681**	**404**

Table 8. Results of Sauvignon Blanc and Riesling wine tasting by Paired sample test.

	Sauvignon Blanc						Riesling					
	Control	4 leaves	Control	8 leaves	4 leaves	8 leaves	Control	4 leaves	Control	8 leaves	4 leaves	8 leaves
Total	2	13*	1	14**	6	9	9	6	10	5	10	5

Note: * significant level p< 0.05 **significant level p< 0.01

Table 9. Results of Sauvignon Blanc and Riesling wine tasting by Ranking method.

	Sauvignon Blanc			Riesling		
	Control	4 leaves	8 leaves	Control	4 leaves	8 leaves
Rank total	44	24	22*	26	27	37

Note: * any rank total outside the range 23-37 is significant at P < 0

Conclusions

Results of this study indicate that basal leaf removal had different influence on the wine chemical composition and quality that depends upon the variety tested. In Sauvignon Blanc wines, basal leaves removal significantly reduced tartaric and malic acid content and increased the content of free and bound monoterpenes. No marked difference was noticed in the higher alcohol content with exception in benzyl alcohol with the highest concentrations in the control wines of both varieties tested. In Riesling wines basal leaves removal had significant influence only on tartaric acid content while there was no marked difference in the sum of free and bound monoterpenes. Sensory evaluation showed that the quality of Sauvignon Blanc wines could be much improved by application of basal leaf removal while the quality of Riesling wines remained more or less the same. Finally, it must be stated that this work constitutes a preliminary approach to the more complete study of different basal leaf removal treatments influence on the chemical composition and overall wine quality.

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