INFLUENCE OF LEAF REMOVAL AND REFLECTIVE MULCH ON PHENOLIC COMPOSITION OF WHITE WINES

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Abstract

Aim: The aim of this research was to evaluate if leaf removal and red geotextile reflective mulch "Vitexsol" could affect phenolic composition of wines from three white cultivars, Riesling italico, Traminer, and Manzoni bianco.

Methods and results: A two-year study was conducted to evaluate the effects of leaf removal and reflective mulch on the phenolic composition of white wines from Zagreb vineyards in northwestern Croatia. Leaf removal (LR) and reflective mulch (RM) "Vitexsol", made from weave of aluminum platelets protected by a transparent film and sewn together with red polypropylene threads, were tested separately and combined (LR + RM) on vines of Traminer, Riesling italico and Manzoni bianco in 2008 and 2009. LR and RM had no consistent effect on must sugar content and titratable acidity. All treatments resulted in higher total phenol and flavan-3-ol content in wines of all cultivars, but in different years. LR + RM generally resulted in the highest phenolic acid and individual flavan-3-ol content in all wines except Traminer. RM treatment had the least effect on phenolic composition of wines.

Conclusion: LR + RM generally resulted in the highest content of most phenolic compounds, especially when compared to control wines.

Significance and impact of the study: This work provides some useful informations for adjusting vineyard practices and thus optimizing phenolic quality of white wines.

Key words: leaf removal, reflective mulch, phenols, flavan-3-ols, white wine

Résumé

Objectif: L'objectif de cette étude était d'évaluer si l'effeuillage et le tissu géotextile réfléchissant rouge "Vitexsol", pouvaient affecter la composition phénolique des vins de trois cultivars blancs, Riesling Italico, Traminer et Manzoni bianco.

Méthodes et résultats: Une étude de deux ans a été menée pour évaluer les effets d'effeuillage et de paillis réfléchissant sur la composition phénolique de vin blanc des vignobles de Zagreb au nord-ouest de la Croatie. L'effeuillage (LR) et le tissu réfléchissant (RM) "Vitexsol", fabriqué à partir de tissu de plaquettes d'aluminium protégé par un film transparent et cousu avec des fils en polypropylène rouge, ont été testés séparément et combinés (LR + RM) sur les vignes de Traminer, Riesling italico et Manzoni bianco en 2008 et 2009. LR et RM n'ont montré aucun effet systématique sur la teneur en sucre et sur l'acidité titrable du moût. LR + RM a généralement entrainé une teneur plus élevée en acides phénoliques et en composés flavan-3-ols dans tous les vins sauf Traminer. RM a eu le moins d'effet sur la composition phénolique des vins.

Conclusion: LR + RM a entrainé, de manière générale, une teneur élevée de la majorité des composés phénoliques, en particulier lorsqu'on les compare aux vins témoins.

Signification et impact de l'étude: Ce travail fournira quelques informations utiles pour ajuster les pratiques viticoles et ainsi optimiser la qualité phénolique des vins blancs.

Mots clés: effeuillage, tissu réfléchissant, phénols, flavan-3-ols, vin blanc

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INTRODUCTION

Phenols are present in grapes and wines in quite low concentrations, but with significant contribution to sensorial attributes of wines, such as color, bitterness, and astringency (Hufnagel and Hofmann 2008a, 2008b, Keller 2010). Phenols in wines are also important because of great health benefits as antioxidants in the human body (Kanner *et al*. 1994). In wines, these compounds are mostly originated from grape berries, and some are generated by yeast activity or extraction from oak wood barrels. As a result, grape variety, climatic conditions, vineyard practices and vinification techniques all affect the composition and content of phenolic compounds in wine (Jackson and Lombard 1993, Boulton *et al*. 1998, Downey *et al*. 2006, Keller 2010).

The phenolic compounds commonly found in white grapes and wines are non-flavonoid hydroxycinnamic acids, especially caffeic and caftaric acids (Chamkha *et al*. 2003). In white wines, small amounts of phenolics are considered desirable in providing body to the finished product, while their contribution to oxidative browning represents a less desirable role (Singleton and Noble 1976).

The positive aspects of leaf removal through increased fruit exposure to sunlight are well known. Leaf removal can increase soluble solids and reduce total acidity, as well as increase total phenol content in grapes (Smart *et al*. 1990). Besides other canopy management techniques, leaf removal is a common practice, especially in cooler regions, used to improve canopy microclimate and fruit ripening. Increased sunlight exposure causes the elevation of berry temperature, and both factors are closely related with fruit composition and quality.

Reflective mulches might also increase sunlight in the fruit zone, and consequently improve berry ripening and disease suppression (Hostetler *et al*. 2007a, 2007b). There are only few published works lately reporting about effects of reflective mulches or geotextiles on fruit composition and yield (Pearson 2004, Vanden Heuvel and Neto 2006, Hostetler *et al*. 2007a, 2007b, Sandler *et al*. 2009). Reflective mulch studies demonstrated various effects on yield, fruit composition and wine quality.

Riesling italico is the most widespread grape cultivar in Croatia, where it is also known as Graševina. Manzoni bianco, which is the progeny of Pinot blanc and White Riesling, is a cultivar recently introduced in Croatia that shows excellent potential to produce different types of wine, while Traminer is a very popular cultivar all around the world.

The aim of this research was to evaluate if leaf removal and red geotextile reflective mulch “Vitexsol” could affect phenolic composition of wines from three white cultivars, Riesling italico, Traminer, and Manzoni bianco and to provide some useful informations for adjusting vineyard practices and thus optimizing white wine phenolic quality. For example, flavan-3-ols and resveratrol are important because of great health benefits on human body. But when present at high concentrations, catechin and epicatechin can contribute to undesirable bitterness and astringency of wine, while high concentrations of hydroxybenzoic and hydroxycinnamic acids increase the risk of oxidation process as well as browning of wines.

MATERIALS AND METHODS

1. Study area and treatments

Research was performed in two consecutive years (2008 and 2009). Grape harvest as well as vinification process were conducted in the experimental field Jazbina, which is part of the Department of Viticulture and Enology, Faculty of Agriculture, University of Zagreb (Croatia). The white cultivars involved in this research were Riesling italico, Traminer, and Manzoni bianco. The vineyard was established in 1995, on the hills facing south-west, at about 250 m asl. All cultivars were grafted on *Vitis berlandieri* x *Vitis riparia* SO4 rootstock and double Guyot trained, with 2.00 x 1.20 m spacing.

The experiment was a random block design, with four treatments in three replications for each cultivar. Each plot consisted of three grapevines, with a few untreated vines as a guard between them, so there were 9 grapevines in each treatment. The treatments were as follows:

- Leaf removal (LR) – removal of 5 basal leaves from each shoot at veraison;
- Reflective mulch (RM) – made from a special weave of thin aluminum platelets protected by a transparent film and sewn together with red polypropylene threads as to provide multidirectional light reflection. “Vitexsol” was placed on the soil, directly under the vines, at veraison;
- Leaf removal and reflective mulch (LR + RM) – reflective mulch together with defoliation; and
- Control (C) – without any treatments.

Before LR and RM treatments, vines were shoot-thinned and cluster-thinned in aim to equalize vegetative and generative potential.

Grapes were harvested manually, each cultivar separately after reaching full ripeness. Each treatment and replicate was harvested and processed separately. After crushing and destemming, the grapes were macerated for 6 hours, then pressed and put into 15-L stainless steel tanks. Must samples were collected immediately after pressing, for soluble solid and titratable acidity analysis. Each lot was sulfited with 10 g/100 L. All fermentations were carried out with *Saccharomyces bayanus* Lallemand EC1118, with temperature control (16-18 °C), to completion. At the end of fermentation, the wine was racked, and samples were frozen for further analysis.

2. Sugar and titratable acidity analysis

Sugar content in musts was determined by refractometer (expressed in °Brix) and titratable acidity of the must (g/L) was estimated using the coloration pattern volumetric method according to the official methods of the European Union (EEC, 1990).

3. Spectrophotometric measurements

Total phenolic content was done with Folin-Ciocalteu method (Singleton and Rossi 1965). Results were expressed as mg gallic acid equivalents per liter of wine (mg GAE/L). Total flavan-3-ol content was determined by reaction of flavonoids with vanillin reagent in the acid medium (Ough and Amerine 1988). 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 (Analytik Jena, Germany).

4. HPLC analysis

Levels of individual phenolic compounds were measured by a HPLC system (Agilent 1100 Series, Palo Alto, USA). These compounds were separated on Luna C18 column (Phenomenex, SAD) (250 × 4.6 mm i.d.) with gradient elution. The gradient consisted of two eluents: (A) water/phosphoric acid (99.5/0.5; v/v) and (B) acetonitrile/water/phosphoric acid (50/49.5/0.5; v/v/v). Flow rate was 1 mL/min. The gradient conditions were as follows: 100 % A for 2 min, from 2 min to 7 min 20 % B, from 7 min to 25 min 40 % B, followed by hold to 31 min, from 31 min to 40 min 100 % B. Equilibrium time to original conditions was 10 min Compounds were detected by DAD and FLD detectors. Peaks were identified by comparisons of UV-VIS spectra of each peak with the corresponding spectra of standard compounds and by comparisons of their retention times. Quantification was based on peak areas using external standards. Linear calibration curves for standards (peak area vs. concentration) were constructed. Wine samples were defrost, filtered by PTFE membrane filters (45 µm) and directly injected to HPLC system. Injected volume was 20 µL.

5. Statistical analysis

All variables were examined separately by year and cultivar using one-way analysis of variance (ANOVA). Means separation by Duncan's multiple range test was used to establish whether there were significant differences among the treatments ($p \leq 0.05$). A canonical discriminant analysis was performed to evaluate the utility of phenolic compounds in wine samples for discrimination between treatments within every cultivar. Squared Mahalanobis distance was calculated between centroids of treatments based on phenolic composition, and significance of these differences was determined. Data were analyzed using SAS statistical software, version 9.0 (SAS Institute, Cary, NC).

RESULTS

1. Soluble solids and titratable acidity

Must soluble solids were unaffected by treatments applied in year 2008 for all three cultivars (Table 1). In the second year of research (2009), significant differences can be observed within cultivars Riesling italico and Traminer, where the highest level of soluble solids was measured in control and RM musts.

Must titratable acidity was more sensitive to applied treatments. Riesling italico LR musts had the lowest titratable acidity level in both years. Regarding LR + RM treatment, somewhat contradictory results were obtained: LR + RM Riesling italico and Traminer musts from 2009 had the highest titratable acidity level, while Manzoni bianco LR + RM must from the same year had the lowest titratable acidity level.

2. Total phenols and flavan-3-ols

Treatments varied significantly in 2008 in wine total phenol content for cvs. Riesling italico and Traminer, and no significant differences were determined for Manzoni bianco (Table 2). In contrast, only cv. Manzoni bianco showed significant differences between treatments in year 2009. Generally, LR + RM increased total phenol content in wines of Riesling italico (2008) and Manzoni bianco (2009),

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Table 1. Reflective mulch (RM) and leaf removal (LR) effect on must soluble solid content and titratable acidity of three cultivars.

Year	Treatment	Must soluble solids (Brix)			Must titratable acidity (g/L)		
		Riesling italico	Traminer	Manzoni bianco	Riesling italico	Traminer	Manzoni bianco
2008	LR	25.8	24.4	25.6	5.13 c	4.03	5.13
	LR+RM	25.6	24.6	25.6	5.31 bc	3.70	4.90
	Control	26.0	24.4	25.6	5.53 a	3.84	5.06
	RM	25.4	24.2	25.6	5.37 ab	3.78	5.00
		ns	ns	ns	**	ns	ns
2009	LR	20.4 b	22.2 c	23.8	5.81 b	5.83 b	5.91 a
	LR+RM	19.6 c	22.4 bc	23.8	6.31 a	6.31 a	5.34 b
	Control	20.8 a	23.2 ab	24.4	5.93 b	5.93 b	5.87 a
	RM	20.6 ab	23.4 a	23.8	5.92 b	5.95 b	5.80 a
		**	*	ns	*	*	*

*, **, and ns indicate significant at $p = 0.05$, $p = 0.01$, and not significant, respectively.
Means with the same letter are not significantly different within cultivars and years (mean separation by Duncan's multiple range test at $p \leq 0.05$).

Table 2. Reflective mulch (RM) and leaf removal (LR) effect on total phenol and flavan-3-ol content in wine of three cultivars

Year	Treatment	Total phenols (mg/L)			Flavan-3-ols (mg/L)		
		Riesling italico	Traminer	Manzoni bianco	Riesling italico	Traminer	Manzoni bianco
2008	LR	249.4 c	266 a	280.9	3.57 b	4.45 a	4.13
	LR+RM	307.1 a	201 b	274.5	6.23 a	2.87 b	4.18
	Control	261.6 bc	228 ab	265.4	3.25 b	3.69 ab	4.10
	RM	281.7 ab	218 b	249.9	4.49 b	3.20 b	3.98
		**	*	ns	*	*	ns
2009	LR	233.9	162.3	217.8 b	5.32	1.85	3.18
	LR+RM	252.5	181.4	232.4 a	6.07	2.49	3.51
	Control	236.7	169.7	221.8 b	4.51	2.45	3.46
	RM	245.4	160.4	224.4 b	4.61	2.21	3.60
		ns	ns	**	ns	ns	ns

*, **, and ns indicate significant at $p = 0.05$, $p = 0.01$, and not significant. respectively.
Means with the same letter are not significantly different within cultivars and years (mean separation by Duncan's multiple range test at $p \leq 0.05$).

but also decreased total phenol content in Traminer wine (2008).

Results of flavan-3-ol content in wines varied significantly between treatments. LR treatment resulted in the highest flavan-3-ol content in Traminer wine (2008), LR + RM in an increase of flavan-3-ols in Riesling italico wine (2008), while RM treatment resulted in the highest flavan-3-ol content in Manzoni bianco wine (2009).

3. Wine phenolic composition

Riesling italico. Control wines of Riesling italico had the lowest content of all measured individual phenolic compounds, in at least one experimental year. Content of epicatechin was significantly lower in control wines in both years. On the other hand, LR + RM wines had the highest content of all compounds in at least one experimental year, with a significantly higher content of vanillic acid in both years (Table 3).

Canonical discriminant analysis between wines of Riesling italico from different treatments from two years of research based on eight phenolic compounds shows highly significant squared Mahalanobis distances between control and LR wines, and between control and LR + RM wines (Table 4).

Table 3. Reflective mulch (RM) and leaf removal (LR) effect on wine phenolic composition (mg/L) of three cultivars

Year	Treatment	Gallic acid	Vanillic acid	Catechin	Epicatechin	Resveratrol	Caftaric acid	Caffeic acid	Coumaric acid
Riesling italico									
2008	LR	5.10	0.19 a	2.29	2.44	1.06	3.17	2.31	5.42
	LR+RM	5.34	0.20 a	2.36	2.35	1.08	3.45	2.42	5.33
	Control	5.20	0.13 c	2.30	1.87	0.97	3.40	2.22	5.18
	RM	5.45	0.16 b	2.53	2.31	0.94	3.46	2.38	5.32
		ns	**	ns	ns	ns	ns	ns	ns
2009	LR	5.18	0.35	2.20	1.56 a	1.35 a	3.89	1.55	6.91 a
	LR+RM	5.48	0.39	2.38	1.65 a	1.45 a	4.46	1.59	7.03 a
	Control	4.53	0.35	2.07	1.22 b	1.11 b	3.84	1.46	6.32 c
	RM	5.13	0.31	2.15	1.48 a	1.36 a	4.03	1.37	6.63 b
		ns	ns	ns	*	*	ns	ns	**
Manzoni bianco									
2008	LR	4.66	0.21	1.95 b	1.49	0.26	3.64	1.73	3.61
	LR+RM	4.76	0.21	2.11 a	1.40	0.26	3.61	1.66	3.79
	Control	4.44	0.20	1.90 c	1.49	0.28	3.61	1.67	3.86
	RM	4.74	0.24	1.98 b	1.45	0.26	3.62	1.62	3.43
		ns	ns	**	ns	ns	ns	ns	ns
2009	LR	4.77 a	0.11	1.78 b	1.23 b	0.28	3.32 b	2.04	3.29
	LR+RM	4.87 a	0.12	1.98 a	1.38 a	0.31	3.49 a	1.93	3.45
	Control	4.22 b	0.10	1.65 b	1.17 b	0.25	3.19 c	1.86	3.34
	RM	4.88 a	0.12	1.80 b	1.27 b	0.23	3.30 bc	1.93	3.40
		**	ns	**	**	ns	*	ns	ns
Traminer									
2008	LR	3.44	0.10	2.89	1.78	0.15 bc	3.83	0.75	2.15
	LR+RM	3.42	0.12	2.76	1.83	0.17 ab	3.67	0.50	2.26
	Control	3.79	0.03	2.38	1.65	0.19 a	3.48	0.31	2.18
	RM	3.42	0.06	2.85	1.70	0.12 c	3.79	0.77	2.17
		ns	ns	ns	ns	**	ns	ns	ns
2009	LR	4.20	0.06	2.58	1.46	0.14	3.30	0.43	1.56
	LR+RM	3.82	0.07	2.52	1.52	0.20	3.39	0.40	1.94
	Control	3.49	0.06	2.20	1.67	0.12	3.62	0.55	1.75
	RM	3.90	0.10	2.81	1.59	0.17	3.48	0.44	1.83
		ns	ns	ns	ns	ns	ns	ns	ns

*, **, and ns indicate significant at $p = 0.05$, $p = 0.01$, and not significant, respectively.
Means with the same letter are not significantly different within cultivars and years (mean separation by Duncan's multiple range test at $p \leq 0.05$).

Other distances are not significant. This means that LR and LR + RM treatments resulted in significant changes in wine phenolic composition of cv. Riesling italico. Based on position of wines from different treatments on scatter plot obtained from first and second canonical variables, and correlation of phenolic compounds with first two canonical variables (Figure 1), we can see that distance between control and all of the treatments is based on lower content of all phenolic compounds in control wines, except caftaric acid.

Manzoni bianco. LR + RM treatment resulted in a significantly higher content of catechin in Manzoni bianco wines in both years. Besides that, all phenolic compounds except vanillic and caffeic acids were significantly higher in LR + RM wines in at least one experimental year. Control wines had the lowest content of gallic acid in both years.

Squared Mahalanobis distance between control and all of the treatments based on the phenolic composition of wines from two years of study was significant. The greatest distance was determined between control and LR + RM wines. Distance between other treatments was not significant (Table 4).

Distance between control wines of Manzoni bianco and LR + RM wines can be explained by the lower epicatechin, catechin and caftaric acid contents in control wines, and distance between control, LR and RM wines by the lower vanillic acid content in control wines. Distance between LR + RM, LR and RM wines, which can be seen on the same scatter plot, is explained by the higher resveratrol and coumaric acid contents in LR + RM wines (Figure 2).

Traminer. Experimental treatments did not affect phenolic composition of Traminer wines in 2009. In 2008, control wines had significantly lower content of vanillic and caffeic acids. RM treatment induced the lowest content of resveratrol in wine. LR and RM treatments separately induced a higher content of caffeic acid, while LR + RM treatment induced an increase in vanillic acid content in wine.

Using the results of phenolic composition of wines from two years of research in canonical discriminant analysis, no significant distances were determined between treatments, but even so, the greatest distance was determined between control wines and LR wines, and between control wines and RM wines (Table 4). Those distances can be explained by the lower content of catechin and vanillic, caffeic, gallic and caftaric acids in control wines (Figure 3).

Table 4. Squared Mahalanobis distances between cultivars and different treatments of every cultivar based on phenolic profile of wines from two years of study.

Treatments within cultivars				
Riesling italico				
	LR	LR+RM	Control	RM
LR	0	5.3 ns	17.5**	5.4 ns
LR+RM		0	21.5**	6.9 ns
Control			0	7.0 ns
RM				0
Manzoni bianco				
LR	0	8.9 ns	17.7**	4.3 ns
LR+RM		0	35.8**	8.9 ns
Control			0	34.6**
RM				0
Traminer				
LR	0	3.5 ns	8.4 ns	0.3 ns
LR+RM		0	3.9 ns	3.3 ns
Control			0	8.5 ns
RM				0

*, **, and ns indicate significant at $p = 0.05$, $p = 0.01$, and not significant, respectively.

Figure 1. Correlation analysis for cv. Riesling italico wines. (A) Position of cv. Riesling italico wines from different treatments in the space defined by the first two canonical variables from canonical discriminant analysis using their phenolic profile from two years of study (RM – reflective mulch, LR – leaf removal, C – control). (B) Vector diagram of correlation of wine phenolic compounds and first two canonical variables based on the different treatments shown in a.

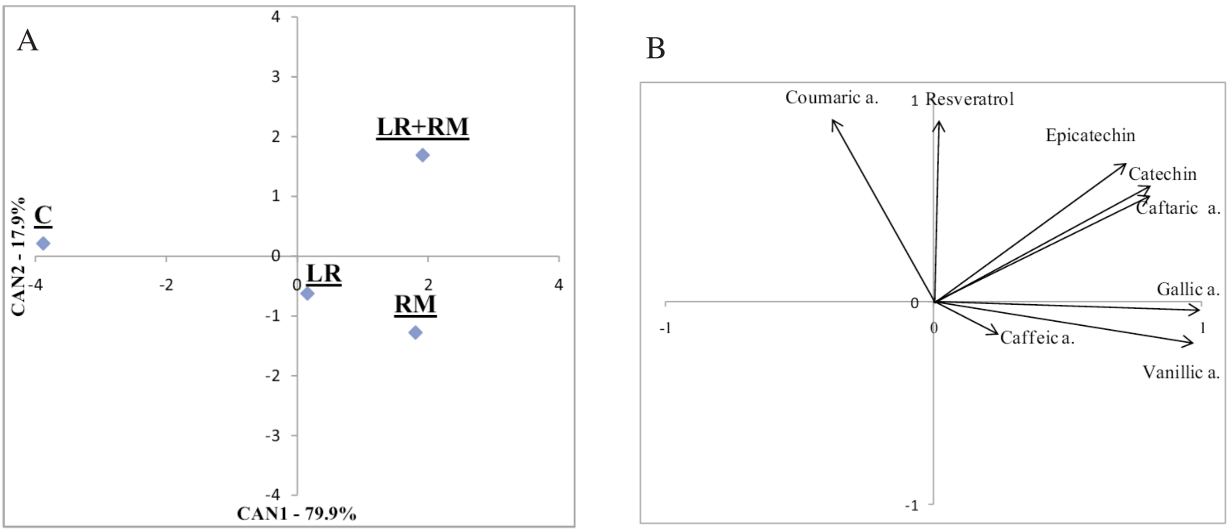

Figure 2. Correlation analysis for cv. Manzoni bianco wines. (A) Position of cv. Manzoni bianco wines from different treatments in the space defined by the first two canonical variables from canonical discriminant analysis using their phenolic profile from two years of study (RM – reflective mulch, LR – leaf removal, C – control). (B) Vector diagram of correlation of wine phenolic compounds and first two canonical variables based on the different treatments shown in a.

DISCUSSION

1. Soluble solids and titratable acidity

According to the results of Riesling italico and Traminer musts analysis, it could be pointed out that LR treatment played the key role in reducing soluble solids in some experimental musts. It is not surprising knowing that LR was earlier reported to reduce berry soluble solids by leaving inadequate leaf area to support the ripening of the remaining (Zoecklein *et al.* 1992). Soluble solid content in Manzoni bianco musts from both years was unaffected by any of treatments applied, consistent with previous work of Vanden Heuvel *et al.* (2007) and Sandler *et al.* (2009). On the other hand, silver aluminized reflective mulch increased soluble solids of Cabernet franc berries at harvest (Coventry *et al.* 2005), but black and white reflective geotextiles decreased soluble solids of Cabernet franc, probably because of increased vine density due to elimination of competing weeds (Hostetler *et al.* 2007b). Generally,

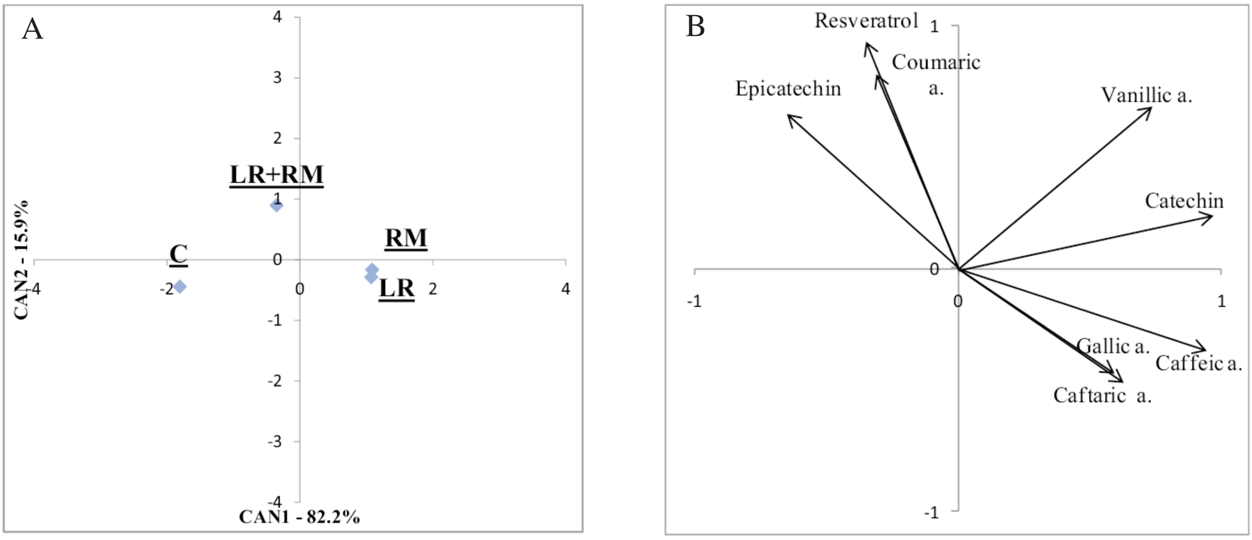

Figure 3. Correlation analysis for cv. Traminer wines. (A) Position of cv. Traminer wines from different treatments in the space defined by the first two canonical variables from canonical discriminant analysis using their phenolic profile from two years of study (RM – reflective mulch, LR – leaf removal, C – control). (B) Vector diagram of correlation of wine phenolic compounds and first two canonical variables based on the different treatments shown in a.

white geotextile expressed more positive effects than the black one, but with no significant differences when compared to control treatments. The same authors found no significant differences in Pinot noir fruit soluble solids, pH and titratable acidity (Hostetler *et al*. 2007a).

Impact of treatments applied on titratable acidity content in must was not consistent across cultivars or years. LR + RM treatment reduced titratable acidity only in Manzoni bianco must in 2009. It is very surprising assuming increased sunlight and higher temperatures in the cluster zone in RM treatment. RM led to less perceived acidity in Riesling wines, according to Reynolds *et al*. (2008). In 2008, we observed somehow expected results, where Riesling italico control must had the highest, and LR must the lowest titratable acidity level. Reductions in titratable acidity due to LR treatment were linked to lower malic acid concentrations in fruit exposed to direct sunlight or higher temperatures (Zoecklein *et al*. 1992). But, there is many studies showing no consistent effects of RM on pH or total acidity in grapes (Hostetler *et al*. 2007a, 2007b, Vanden Heuvel *et al*. 2007, Sandler *et al*. 2009). When RM was applied for a shorter period in late summer, there were no effects on soluble solids, pH or total phenolic concentrations (Pearson 2004, Vanden Heuvel and Neto 2006).

Generally, grape composition can be affected not only by the total amount of photosynthetically active radiation (PAR) intercepted by clusters, but also by the ratio of red and far-red radiation (R: FR), which regulates the levels of phytochrome involved in many aspects of vine metabolism and growth (Smart *et al*. 1988). Red and black mulches reflect similar amounts of PAR, but light reflected by red plastic is higher in R: FR, while light from white and green mulches has low R: FR due to strong absorption of red light by the mulches.

2. Total phenols

Generally, LR + RM showed the greatest effect on increasing of total phenol content in wines. Silver aluminized reflective mulch increased total phenolics and flavonols of Cabernet franc berries at harvest (Coventry *et al*. 2005). On the other hand, silver aluminized reflective mulch, as well as crushed quahog shells, did not affect total phenols in Merlot wines (Sandler *et al*. 2009). This is similar to the work of Vanden Heuvel *et al*. (2007) and Hostetler *et al*. (2007a, 2007b). LR treatment resulted in the highest total phenol content in 2008 Traminer wines, but also expressed opposite effect causing the lowest content in Riesling italico wines from the same year. Low total phenol content in LR wines is in accordance with Prajitna *et al*. (2007), who stated that more leaves means more assimilates for phenolics synthesis.

3. Non-flavonoids

It can be concluded that hydroxybenzoic acid level was mostly the lowest in control wines, and the highest in LR + RM wines. The effect of LR + RM was most evident in Riesling italico wines, where vanillic acid level was highest in both years. The difference in the sum of hydroxybenzoic acids was the greatest between control and LR + RM Riesling italico wines from 2009, amounted to almost 1 mg/L.

The same trend was observed with hydroxycinnamate level in wines. So, LR + RM wines generally had the highest and control wines the lowest levels of these compounds. Again, Riesling italico wines were more sensitive to the LR + RM treatment, while Traminer wines were mostly unaffected by any of the treatments. Despite the fact that the biosynthesis of hydroxycinnamates in grapes reaches its peak before veraison (Adams, 2006), and that hydroxycinnamate level responds positively only to early performed leaf removal (Lemut *et al*. 2011), it seems that LR and RM treatment at veraison still modified accumulation of these compounds, to a certain extent.

Lowest content of resveratrol in control Riesling italico wines, as well as highest resveratrol content in LR + RM Riesling italico and Manzoni bianco wines is in accordance with Goldberg *et al*. (1999), who found highest resveratrol concentrations in warm conditions. Higher resveratrol content could be expected within LR treatments, because of UV light exposure. Higher resveratrol content is also often associated with cooler climates, because of higher incidence of fungal infections.

4. Flavan-3-ols

Experimental treatments did not show consistent effect on flavan-3-ol content in wines, but it is obvious that none of the control wine samples had the highest content of flavan-3-ols. According to Dokoozlian and Kliewer (1996), increasing light increased the flavonoid content of grapes. Control wines of all cultivars mostly had the lowest level of catechin and epicatechin. This is very similar to the work of Goldberg *et al*. (1998, 1999), who stated that dry sunny conditions increase the concentrations of catechins in white (but not red) wines. Experimental years 2008 and 2009 were very dry and sunny with only 600 and 366 mm of rainfall, respectively, and with more than 1500 hours of sunlight during the vegetation period. Together with applied treatments of LR and RM, we can state that conditions in vineyard with extremely high temperatures and with more direct sunlight in the cluster zone affected increasing of catechin and epicatechin levels in wine.

On the contrary, white reflective woven material as well as silver aluminized reflective mulch and crushed quahog shells did not affect total flavonols of Merlot (Sandler *et al*. 2009).

Furthermore, total flavonols were unaffected by white woven synthetic mulch in Pinot noir and Merlot grapes and wines (Vanden Heuvel *et al*. 2007). That is similar to the work of Hostetler *et al*. (2007a, 2007b).

Similar as with the non-flavonoids, the biosynthesis of flavan-3-ols begins long before veraison when the treatments in the present study were performed. Furthermore, this study does not deal with the source of flavan-3-ols, so it is possible that some of the detected compounds originated from grape seeds, whose biosynthesis could not be affected by light interception. Besides that, mainly polymerized flavan-3-ols are found in wines from exposed grapes (Price *et al*. 1995), so polymerization could also be the reason for low concentrations of monomeric flavan-3-ols in some of the experimental wines.

In summary, techniques including leaf removal and reflective mulch (LR + RM) showed the greatest effects on must and wine composition, particularly on Riesling italico and Manzoni bianco cultivars.

CONCLUSION

Hydroxycinnamic acid was the most abundant phenolic compound in wines, followed by hydroxybenzoic acid. Leaf removal together with reflective mulch treatment (LR + RM) generally resulted in the highest content of most phenolic compounds, especially when compared to control wines, which is very important regarding health benefits. But considering the fact that polyphenols, especially hydroxycinnamic acids, play an important role as precursors of oxidation process, and that flavan-3-ols contribute to undesirable bitterness and astringency, it is doubtful if we can claim that mentioned treatments influenced the improvement of wine quality. This should be confirmed with organoleptic evaluation of wines, which we did not include in this research. But it is also undoubtedly confirmed that leaf removal and reflective mulch treatments can increase the level of phenolic compounds in wine, which is particularly applicable in cooler climates and in climatic unfavorable years. Research on leaf removal and reflective mulch influence on red wine phenolic composition is ongoing.

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