Effects of post-flowering leaf removal on the phenolic composition of three red *Vitis vinifera* L. cultivars under semiarid conditions

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Abstract

Among seasonal practices that affect grape quality, selective leaf removal at the cluster zone is accepted as a powerful technique to manipulate flavonoid content of grapes and wines. However, most of research on fruit-zone defoliation has been conducted under temperate climate and it remains uncertain if cluster exposure would be a recommendable practice in semiarid viticultural areas. In this study, we investigated, over two consecutive seasons, the effect of the severity of post-flowering leaf removal on the growth and phenolic composition of berry skin and seeds in three *Vitis vinifera* L. genotypes (Merlot, Cabernet Sauvignon and Sangiovese) under the semiarid conditions of Northern Greece. Three different severities of leaf removal in the fruit-zone were applied manually at berry set: non defoliated (ND), removal of the lateral shoots of the first six basal nodes (LR) and full removal of the total leaf area (main leaves and lateral shoots) of the first six basal nodes (FR). Grape samples were obtained at commercial harvest. Leaf removal decreased yield per vine and cluster weight in Merlot and Sangiovese but berry fresh weight was unaffected in both cultivars. On the contrary, in Cabernet Sauvignon yield was unaffected but berry size was reduced by leaf removal. Fruit zone leaf removal did not affect must soluble solids and increased titratable acidity only in Merlot. Defoliation increased skin anthocyanins in Merlot and Cabernet Sauvignon but significantly reduced seed flavan-3-ols mainly as a result of the reduction in catechin and epicatechin amount. However, leaf removal had limited effect in Sangiovese phenolic profile.

Key words: leaf removal, lateral shoots, cluster microclimate, skin anthocyanins, seed flavan-3-ols.

Introduction

It is generally reported that increased light in the fruit zone enhances skin anthocyanin content (Dokoozlian and Kliewer, 1996). Therefore, selective leaf removal is widely used as effective technique to manipulate the colour properties of grapes and wines. Contrary to anthocyanins, few data exist on the effect of cluster exposure on skin and seed proanthocyanidins. Sunlight exposure was reported to increase the accumulation of skin proanthocyanidins in Shiraz but had minimal influence on seed phenolics (Downey et al., 2004). However, the effect of leaf removal on grape composition is not always consistent depending on timing and severity of application, and grapevine genotype (Tardaguila et al., 2010).

Most of research on leaf removal has been conducted under temperate climate and it remains uncertain if early cluster exposure would be a recommendable practice in semiarid viticultural areas where daily summer temperatures exceed 30^{o}C. The interaction between light intensity and temperature is particularly important, since the increased temperature in exposed berries is reported to negatively affect flavonoid synthesis (Spayd et al., 2002).

The aim of the present work was to investigate the effect of different treatments of leaf removal at berry set on the growth and phenolic composition of grape components, in three non irrigated field-grown *Vitis vinifera* L. varieties, under the semi-arid climate of Northern Greece.

Materials and Methods

The experiment was conducted during 2007 and 2008 in a commercial vineyard in Thessaloniki, Northern Greece, planted with *Vitis vinifera* L. cvs Merlot, Cabernet Sauvignon and Sangiovese, grafted onto 1103 Paulsen. Vines were trained to a bilateral vertical shoot positioned spur pruned cordon, with NW to SE orientation. The vineyard was located on a deep loamy soil and was managed without irrigation. Three different intensities of leaf removal in the fruit-zone were applied at beery set, and replicated three times in randomized blocs: non defoliated (ND), removal of the lateral shoots of the first six basal nodes (LR) and full removal of the total leaf area (main leaves and lateral shoots) of the first six basal nodes (FR). At harvest, 10 basal clusters were randomly sampled in each plot to determine yield and weight of beery components. Total soluble solids (TSS) by refractometry and titratable acidity by titrimetry with 0.1 N NaOH were estimated in a sample of 200 berries per plot. Individual skin anthocyanins and seed flavan-3-ols were analyzed with HPLC as previously described (Koundouras et al., 2009).

Within each variety, a two-factor (year and leaf removal treatment) analysis of variance (ANOVA) was used to test the corresponding main effects and interactions using the SPSS software (version 17.0, SPSS Inc., IL, USA). Comparison of means were performed using Duncan's multiple range test at $p<0.05$.

Results and Discussion

Yield per vine was reduced by FR and LR treatments in Merlot and Sangiovese (Table 1). Defoliation decreased yield by approximately 50% in Melrot and by 22% in Sangiovese (FR as compared to ND). Similar results were observed for cluster weight with lower values in the FR vines. However, in Cabernet Sauvignon, no effects on yield per vine and cluster weight were recorded. Leaf removal at bloom is reported to reduce yield, cluster size and fruit set in different varieties (Intrieri et al., 2008; Poni and Bernizzoni, 2010), mainly due to lower assimilate production caused by the reduced leaf area (Petri et al., 2003). Post-bloom defoliation can also be effective in reducing yield components (Poni et al., 2004) but its effect is less significant and depends on grape cultivar, as our results suggest.

Berry weight was reduced with leaf removal only in Cabernet Sauvignon, as previously reported (Ollat and Gaudillère, 1998), possibly due to the limiting leaf area during green berry stage. However, no effect of leaf removal on berry size was detected in Merlot and Sangiovese. Skin weight at harvest was similar among treatments in Merlot and Sangiovese but it was higher in ND in Cabernet Sauvignon as a result of the higher berry size in this treatment and variety. Similarly to skin mass, seed growth was only affected by defoliation in Cabernet Sauvignon (ND>FR) as also reported in other studies (Ristic et al., 2007). Must soluble solids were not different among defoliation treatments (Table 1) which is in agreement with experiments under similar conditions (Tardaguila et al., 2010). Titratable acidity was increased by leaf removal only in Merlot, possibly related to a positive effect of defoliation on tartaric acid synthesis reported in previous works (Tardaguila et al., 2010).

Table 1. Effect of leaf removal on yield components; FR, full leaf removal in the cluster zone; LR, lateral shoot removal in the cluster zone; ND, non defoliated. Values represent two year averages.

	Yield (kg/vine)	Bunch weight (g)	Berry fw (g/100 berries)	Skin fw (g/100 berries)	Seeds fw (g/100 berries)	TSS (Brix)	Titratable acidity (g/L)
Merlot							
FR	2.03 b	148 b	100	17.1	5.5	26.0	5.9 a
LR	3.65 a	232 a	107	16.1	6.0	24.7	5.4 ab
ND	4.50 a	234 a	109	17.5	5.9	25.1	5.3 b
Cabernet Sauvignon							
FR	3.45	178	89 b	14.5 b	5.3 b	22.9	6.8
LR	3.24	196	98 ab	16.5 ab	5.9 ab	23.3	6.6
ND	3.58	206	105 a	17.2 a	6.4 a	23.3	6.6
Sangiovese							
FR	4.78 b	320 b	144	21.7	9.1	19.8	7.2
LR	5.05 b	379 a	148	21.7	9.2	21.5	7.0
ND	6.12 a	360 a	153	22.8	8.6	21.5	6.8

In each column, statistically significant differences between irrigation treatments within a variety are indicated by different letters ($p<0.05$).

Leaf removal had a significant effect on the concentration of all individual anthocyanins in Merlot and Cabernet Sauvignon (Table 2), with FR vines having the higher amounts as compared to ND. LR generally presented intermediate values or similar to ND. The total amount of anthocyanins in the berries, expressed as sum of individual anthocyanins, was higher in FR as compared to ND in Merlot and Cabernet Sauvignon. The results coincide with other works reporting that increased exposure of grapes to sunlight is associated with higher levels of skin anthocyanins (Jeong et al., 2004). However, no differences were observed among treatments in Sangiovese, (Table 2) suggesting that the response of skin anthocyanins to light exposure is probably cultivar-dependent.

Table 2. Effect of leaf removal on skin anthocyanins (mg/100 g berry fresh weight) (Dp, delphinidin-3-*O*-glucoside; Cy, cyanidine-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; MvC, malvidin 3-*O*-coumarateglucoside); FR, full leaf removal in the bunch zone; LR, lateral shoot removal in the bunch zone; ND, non defoliated. Values represent two year averages.

	Dp	Cy	Pt	Pn	Mv	MvC	Total
Merlot							
FR	23.7 a	9.5 a	23.2 a	29.2 a	109.9 a	22.6 a	218.1 a
LR	13.8 b	4.9 b	14.7 b	18.1 b	78.4 b	17.5 b	147.4 b
ND	9.8 c	3.9 b	10.8 c	13.4 c	63.9 b	14.7 c	116.5 b
Cabernet Sauvignon							
FR	15.4 a	3.4 a	14.6 a	13.5 a	144.1 a	18.7 a	209.7 a
LR	10.4 b	1.4 b	11.0 b	10.9 ab	116.6 b	15.0 b	165.3 b
ND	8.6 b	1.4 b	9.0 b	8.7 b	94.4 c	11.9 c	134.0 c
Sangiovese							
FR	11.3	7.9	14.9	10.3	43.0	1.4	88.8
LR	10.0	7.5	13.4	11.2	42.5	1.2	85.8
ND	8.2	6.6	11.8	11.5	42.1	1.9	82.1

In each column, statistically significant differences between irrigation treatments within a variety are indicated by different letters ($p<0.05$).

In the conditions of this study, defoliation in the fruit zone decreased the levels of free flavan-3-ol monomers in grape seeds in Merlot and Cabernet Sauvignon. Among seed polyphenols, catechin (C) and epicatechin (EC) were higher in Merlot and Cabernet Sauvignon in ND vines without significant differences for the minor compounds (Table 3). The total free flavan-3-ol amount (calculated as the sum of individual polyphenols detected), was also higher in ND vines as compared to FR ones in these cultivars, with intermediate values for LR. According to previous works, shaded grapes (Cortel and Kennedy, 2006) and dense canopies (Koundouras et al., 2009) had increased seed tannins at ripeness. However, in Sangiovese, the highest C and EC levels, as well as total seed polyphenols, were recorded in LR.

Conclusions

Leaf removal at berry set was shown to improve the overall berry composition in Merlot and Cabernet Sauvignon (higher levels of skin anthocyanins and a lower contribution of the seeds to the total pool of berry tannins) but had limited effect in

Sangiovese. Additional knowledge is required in order to elucidate the effect of light exposure on each category of grape phenolic compounds of the grape across different varieties and climatic conditions.

Table 3. Effect of leaf removal on seed flavan-3-ols (mg/100 g berry fresh weight) (GA, gallic acid; C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-*O*-gallate; EGCG, (-)-epigallocatechin-3-*O*-gallate; EGC, (-)-epigallocatechin); FR, full leaf removal in the bunch zone; LR, lateral shoot removal in the bunch zone; ND, non defoliated. Values represent two year averages.

	GA	C	EC	ECG	EGCG	EGC	Total
Merlot							
FR	0.8	21.9 b	24.5 b	8.0 b	1.4	1.6	58.2 b
LR	0.9	23.2 b	28.5 a	9.6 a	1.1	1.9	65.2 ab
ND	0.9	27.7 a	29.3 a	9.5 a	1.5	1.9	70.8 a
Cabernet Sauvignon							
FR	0.5 b	19.1 b	15.0 b	1.0	1.2	1.1	37.9 b
LR	0.6 a	21.1 ab	15.5 ab	1.0	1.1	1.2	40.5 ab
ND	0.7 a	23.9 a	19.1 a	1.5	1.3	1.5	48.0 a
Sangiovese							
FR	0.8	11.0 ab	15.7 b	5.4 a	1.1	1.7	35.7 b
LR	0.8	13.6 a	20.2 a	6.3 a	1.3	1.9	44.1 a
ND	0.7	9.5 b	16.9 ab	3.4 b	0.8	1.7	33.0 b

In each column, statistically significant differences between irrigation treatments within a variety are indicated by different letters ($p<0.05$).

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