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Effects of Severity of Post-flowering Leaf Removal on Berry Growth and Composition of Three Red *Vitis vinifera* L. Cultivars Grown under Semiarid Conditions

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ABSTRACT: The effects of the severity of post-flowering leaf removal on the growth and phenolic composition of berry skin and seeds were studied in three *Vitis vinifera* L. genotypes over two consecutive seasons, 2007 and 2008. The study was conducted in a commercial vertical shoot positioned (VSP)-trained nonirrigated vineyard of northern Greece, planted with cultivars Merlot, Cabernet Sauvignon, and Sangiovese. Three different severities of leaf removal in the fruit zone were applied manually at berry set: nondefoliated (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. Cluster compactness was reduced with the severity of defoliation only in Merlot, due to a decrease in berry number per cluster; berry fresh weight was unaffected in both cultivars. On the contrary, in Cabernet Sauvignon, yield was unaffected but berry size was restrained by leaf removal. Skin and seed mass followed variations in berry mass (except for seed mass in Sangiovese). 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 in the order FR > LR > ND but significantly reduced seed flavan-3-ols mainly as a result of the reduction in catechin and epicatechin amount. For these varieties, FR had lower seed flavan-3-ols than ND in both varieties, whereas LR had intermediate values. However, in Sangiovese, the highest seed phenolic content was recorded in LR. The results showed that post-flowering leaf removal improved the overall berry composition in Merlot and Cabernet Sauvignon but had limited effect in Sangiovese.

KEYWORDS: *leaf removal, lateral shoots, cluster microclimate, skin anthocyanins, seed flavan-3-ols*

INTRODUCTION

Flavonoid compounds of the grape skins and seeds are important determinants of wine color and flavor and include anthocyanins, flavonols, and proanthocyanidins. Anthocyanins are located in the skins of grape berries in red cultivars and accumulate after veraison,[1] although recent evidence has shown that expression of genes associated with the flavonoid pathway is triggered earlier.[2] Proanthocyanidins or condensed tannins are oligomers and polymers of flavan-3-ol monomers such as catechin and epicatechin and are biosynthesized in skins and seeds during the first phase of berry growth.[3]

Among the many seasonal practices that affect the phenolic profile of grapes,[4] cluster exposure by selective leaf removal is accepted as a powerful technique to manipulate flavonoid content of grapes and wines because increased light in the fruit zone is generally reported to increase skin anthocyanins.[5,6] Moreover, light environment of the grapes is reported to modify skin anthocyanin profile[7] and extractability.[8] However, fruit zone defoliation effects on grape composition are not always consistent depending on timing, severity of application,[9] and grapevine genotype.[10]

Recently, defoliation, either prebloom[6] or postbloom,[10] has been adopted as an effective means for both yield control and wine quality improvement. The positive effect of prebloom leaf removal on grape composition has been often attributed to lower cluster and berry size at harvest.[11] Moreover, cell division in the berry skin seems to be sensitive to temperature;[12,13] hence, exposed grapes have thicker berry skin and increased skin-to-pulp ratio.[11] At the whole vine level, prebloom defoliation was reported to increase leaf area-to-fruit ratio due to reduced fruit set and/or berry size[14,15] and to leaf area recovery after veraison.[16] In contrast, fruit zone defoliation at berry set was found to reduce whole vine photosynthesis at an early stage.[17] Moreover, postbloom defoliation was reported to be ineffective in lowering cluster weight and berry number per cluster in Graciano and Carignan, whereas final total leaf area per shoot was reduced, with no evident compensation for lateral leaf area.[10]

Of particular importance in defoliation trials is the interaction between light intensity and temperature because the concomitant increase in exposed berry temperature may be detrimental to flavonoid synthesis, especially under semiarid conditions.[18,19] Excessive leaf removal, resulting in extreme cluster exposure, has been reported to cause lower pigmentation in red grapes.[20] Yamane et al.[21] reported that

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Table 1. Mean Temperature (T), Growing Degree Days (GDD, Base 10 °C), and Summation of Rainfall (P) Recorded from April to September during the Two Seasons of Study (2007 and 2008)

month	2007			2008			average 1931−2003		
	T (°C)	GDD (°C)	P (mm)	T (°C)	GDD (°C)	P (mm)	T (°C)	GDD (°C)	P (mm)
April	14.9	147	14.4	14.9	154	85.2	14.5	135	39.6
May	20.9	339	19.5	19.3	292	27.6	19.4	291	46.3
June	25.9	476	25.4	25.4	462	16.2	23.7	411	35.9
July	28.0	557	26.8	26.7	517	23.8	26.2	502	26.2
August	26.5	520	27.4	27.6	543	1.6	25.9	492	19.3
September	20.9	326	20.9	21.1	333	56.4	22.1	363	28.6
mean (T) or summation (GDD, P)	22.9	2365	134.4	22.5	2301	210.8	22.0	2195	195.9

anthocyanin accumulation in the skins was significantly higher at 20 °C than at 30 °C, whereas Mori et al.[22] observed a significant reduction of anthocyanin content of Cabernet Sauvignon grapes at 35 °C as compared to 25 °C.

Contrary to skin anthocyanins, limited data exist regarding the effect of cluster exposure to light on seed proanthocyanidins. Sunlight exposure increased the accumulation of skin proanthocyanidins in Shiraz[23] and Pinot noir[24] but had minimal influence on seed phenolics. Shading of Cabernet Sauvignon berries reduced the transcription of the specific proanthocyanidin biosynthesis genes in the skins during berry development, but no significant effect was observed in the seeds.[25] However, other works[26] reported that shaded fruit had increased seed tannins at ripeness but mainly as a result of increased seed weight.

Most research on fruit zone defoliation 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 typically exceed 30 °C. The aim of the present work was to investigate the effect of different treatments of post-flowering leaf removal on the growth and phenolic composition of grape skins and seeds in three nonirrigated field-grown *Vitis vinifera* L. varieties under the semiarid climate of northern Greece.

MATERIALS AND METHODS

Experimental Conditions and Vine Parameters. The trial was conducted during two growing seasons (2007−2008) in a 15-year-old commercial vineyard in Thessaloniki, northern Greece (40° 84′ N, 22° 79′ E), planted with *V. vinifera* L. cvs. Merlot, Cabernet Sauvignon, and Sangiovese, grafted onto 1103 Paulsen. Vine spacing was 1.2 m on the row, and row spacing was 2.0 m (4160 vines/ha). Vines were trained to a bilateral vertical shoot positioned (VSP) spur pruned cordon, at 12 nodes per vine. Rows were oriented northwest to southeast. The vineyard was located on a deep loamy soil and was managed according to standard agronomical practices of the region, without irrigation. The number and timing of seasonal practices (i.e., trimming) were similar for all varieties and treatments. Average climatic conditions of the area of the experiment and climatic conditions during the two experimental seasons are shown in Table 1.

Three different severities of leaf removal in the fruit zone were applied manually: natural shade in the fruit zone (nondefoliated, 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). Defoliation was applied at berry set of each year (E-L 29: berries 4 mm in diameter), on June 5, 2007, and June 8, 2008, for Merlot and on June 10 of both 2007 and 2008 for Sangiovese and Cabernet Sauvignon. Three adjacent rows in each variety were selected to build a randomized block design with each row as a block. Within each row (block), three panels (plots) of 10 consecutive vines (12 m of cordon length) were randomly assigned to the treatments ND, LR, and FR. For all parameters studied, only the mean per plot was used in data analysis.

Berry Sampling and Must Analysis. Grapes of each variety were harvested at commercial harvest (simultaneously with the rest of the vineyard), and total yield per plant was weighed. Merlot was picked on August 16, 2007, and August 23, 2008; Sangiovese on August 26, 2007, and August 29, 2008; and Cabernet Sauvignon on September 6, 2007, and September 10, 2008. Ten basal clusters (one cluster per vine) were randomly sampled in each plot and immediately weighed. All berries per cluster were counted and weighed to determine individual berry fresh weight. Cluster density (compactness) was estimated as the number of berries per centimeter of cluster length. A subsample of 200 berries per plot was pressed, and the must was analyzed for total soluble solids (TSS) by refractometry and for titratable acidity by titrimetry with 0.1 N NaOH using phenolphthalein as indicator.

Berry Anthocyanins and Total Phenolics. Phenolic compounds were analyzed in whole berries by using the analytical protocol of Iland et al.[27] Fifty berries from each plot were transferred into a 125 mL plastic beaker and were homogenized using a Polytron PT 1200 with dispersing aggregate PT-DA 07/2 SYN-E082, at 25.000 rpm for 30 s. It was found that 30 s was sufficient to break up the berries so that the seeds were thoroughly broken and mixed into the mash of flesh and skins. After this procedure, no visible pieces of seeds or skins were observed in the homogenized sample. In this way a representative portion of the homogenate could be sampled. One gram of homogenate (in triplicate) was transferred into a pretared centrifuge tube (10−15 mL). Ten milliliters of 50% v/v aqueous ethanol, pH 2 (1 M HCl), was added and mixed for 1 h. After centrifugation at 3500 rpm for 10 min, the supernatant was used to measure the absorbance as follows: 0.5 mL of the supernatant was transferred into 10 mL of 1 M HC1 and mixed thoroughly. After 3 h, absorbance at 520 nm was recorded in a 10 mm Hellma (6030-OG) glass cell and also at 280 nm in a 10 mm OPTECH quartz cell. A Jasco V-530, double-beam UV−vis spectrophotometer was used for recording the absorbances. Anthocyanins (expressed as mg anthocyanins per g berry) were calculated from the absorbance measurement at 520 nm. Total phenolics [expressed as absorbance units (au) per g berry weight] were calculated from the measurement of absorbance at 280 nm.

Determination of Individual Anthocyanins by HPLC. One hundred berries from each treatment and replicate were weighed and manually skinned, and the skins were weighed and freeze-dried. The freeze-dried tissues were then extracted with 100 mL of 1% v/v HCl in methanol. Extraction was carried out by stirring for 48 h and repeated three times in triplicate. Extracts were pooled, and this mixture (in triplicate) was used for further analysis either immediately or after deep-freezing (−70 °C) for no longer than 4 days. Anthocyanin analysis was carried out according to the method of Arnous et al.[28] Identification was based on comparing retention times of the peaks detected with those of standard compounds and on UV−vis online spectral data. Seven different anthocyanins were determined: 3-*O*-monoglucosides of delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn), and malvidin (Mv); malvidin 3-*O*-coumaroylglucoside (MvC); and malvidin 3-*O*-acetylglucoside (MvA). Determinations were carried out with an external standard method (malvidin-3-*O*-

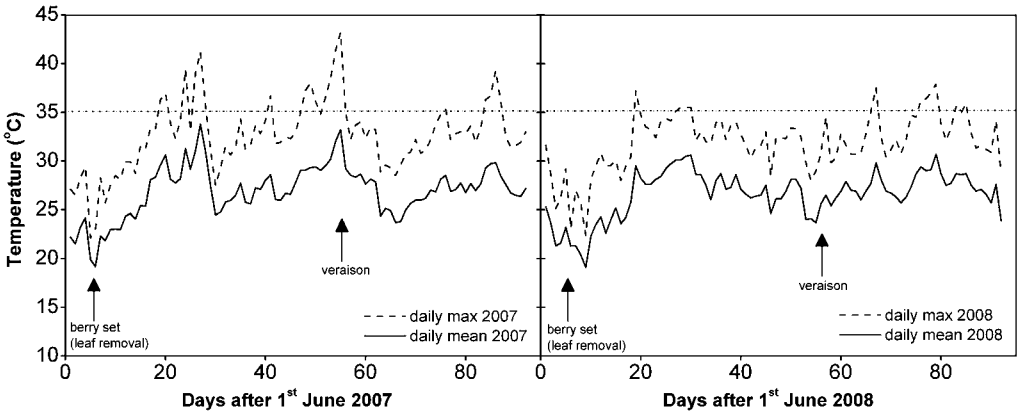

Figure 1. Evolution of daily mean and maximum temperature of the area of study (from June 1) during 2007 and 2008.

glucoside calibration curve). All peaks were quantified as malvidin-3-*O*-glucoside (Mv), and the results were expressed as milligrams of malvidin-3-*O*-glucoside equivalents per fresh berry weight and per berry. All analyses were performed in duplicate.

Determination of Individual Seed Polyphenols by HPLC. Berries collected for anthocyanin assessment were manually deseeded, and the seeds were weighed, frozen in liquid nitrogen, and stored in the freezer (−20 °C) until analyzed according to method described by Guendez et al.[29] A lot comprising 2 g of seeds was ground with a pestle and mortar; subsequently, the powder was placed in a vial, and 8 mL of ethyl acetate was added and vortexed for 3 min. The extract was twice centrifuged at 6000 rpm for 5 min, at 4 °C. The clear extracts were then pooled and taken to dryness in a rotary vacuum evaporator at 35 °C, and the resulting residues were dissolved in 8 mL of methanol, containing 5% (v/v) perchloric acid/water. The solution was filtered through Gelman GHP Acrodisc 13 syringe filters (0.45 μm) prior to analyses. Chromatographic analyses were carried out as described previously.[30] Peaks were identified by comparison of retention times and ultraviolet (UV) spectra with commercial standards. Eight representative polyphenols were determined: gallic acid (GA), (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin-3-*O*-gallate (ECG), (−)-epigallocatechin-3-*O*-gallate (EGCG), (−)-epigallocatechin (EGC), procyanidin B_1, and procyanidin B_2. Procyanidins are expressed as mg/L (+)-catechin equivalents, whereas the rest of the compounds are expressed against their own calibration curves. All analyses were performed in duplicate.

Statistics. 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 SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). Comparison of means was performed using Duncan's multiple-range test at $p < 0.05$.

RESULTS AND DISCUSSION

Climatic Conditions. Total rainfall, calculated from April to September, was 134.4 mm in 2007 and 210.8 mm in 2008 (Table 1). Rainfall for the three summer months in 2007 (79.6 mm) was close to long-term average (75.8 mm) but was lower in 2008 (mainly in August), although in 2008 vines benefited from higher spring rainfall. Average temperature during the summer months was higher than the long-term average in both years of the trial (26.8 °C in 2007 and 26.5 °C in 2008, compared to the average of 25.2 °C). According to the seasonal pattern of temperature (Figure 1), daily maximum temperature exceeded 30 °C for most of the summer period. A higher number of days with extreme temperatures (>35 °C) was observed in 2007 than in 2008 (20 and 13, respectively), mainly during the first period of berry growth, that is, prior to veraison (15 and 5, respectively). With regard to the study period, accumulated heat expressed as growing degree days (GDD, calculated from daily mean temperatures, base 10 °C) was similar between years for June (476 and 462, respectively, for 2007 and 2008) but was higher in 2007 for July (557 compared to 517 in 2008) and in 2008 for August (543 compared to 520 in 2007). Accumulated heat over the growth season was higher than the long-term average for both seasons (Table 1). Despite the relatively hot conditions of both years, no sunburnt fruit was detected in any of the three cultivars during this trial.

Yield Components. All yield components (except for number of clusters per vine) were higher in 2008 than in 2007 for Merlot, whereas the opposite was observed for yield in Sangiovese (Table 2). No year effect was observed for yield

Table 2. Effect of Year (n = 9) and Severity of Leaf Removal (n = 6) on Yield Components[a]

	yield (kg/vine)	cluster no./vine	cluster fresh wt (g)	total berries/cluster	cluster density (g/cm)	berry fw (g/100 berries)
			Merlot			
2007	2.46 b	19.4	158 b	139 b	7.0 b	118 a
2008	4.32 a	20.6	252 a	255 a	13.3 a	93 b
FR	2.03 b	17.3 b	148 b	157 b	8.4 b	100
LR	3.65 a	20.9 a	232 a	215 a	10.6 a	107
ND	4.50 a	21.9 a	234 a	220 a	11.4 a	109
y × tr[b]	ns	ns	ns	ns	ns	ns
			Cabernet Sauvignon			
2007	3.26	19.0	198	191	10.9	99
2008	3.59	20.8	188	181	10.7	96
FR	3.45	21.1	178	182	10.7	89 b
LR	3.24	18.5	196	189	11.1	98 ab
ND	3.58	20.0	206	187	10.5	105 a
y × tr	ns	ns	ns	ns	ns	ns
			Sangiovese			
2007	6.19 a	16.0	388 a	225	9.4	174 a
2008	4.44 b	17.1	318 b	227	11.9	123 b
FR	4.78 b	15.9	320 b	202	9.8	144
LR	5.05 b	15.9	379 a	238	11.1	148
ND	6.12 a	17.9	360 a	237	10.9	153
y × tr	ns	ns	ns	ns	ns	**

[a]Values represent measurements taken at ripeness stage. In the same column, statistically significant differences between years and treatments within varieties are indicated by different letters ($p < 0.05$). FR, full leaf removal in the cluster zone; LR, lateral shoot removal in the cluster zone; ND, nondefoliated; fw, fresh weight. [b]*, **, and *** represent significance of the year × treatment (y × tr) interaction at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns, not significant.

Table 3. Effect of Year (n = 9) and Severity of Leaf Removal (n = 6) on the Growth of Berry Skin and Seed Mass and on Must Composition at Ripeness Stage[a]

	skin fw (g/100 berries)	seeds fw (g/100 berries)	skin to berry fw ratio (%)	seeds to berry fw ratio (%)	TSS (°Brix)	titratable acidity (g tartaric acid/L)
Merlot						
2007	19.7 a	6.0	16.7	5.1 b	25.4	4.8 b
2008	14.1 b	5.7	15.2	6.2 a	25.1	6.3 a
FR	17.1	5.5	17.1	5.7	26.0	5.9 a
LR	16.1	6.0	15.4	5.7	24.7	5.4 ab
ND	17.5	5.9	16.1	5.5	25.1	5.3 b
y × tr[b]	ns	ns	ns	ns	ns	ns
Cabernet Sauvignon						
2007	16.7	5.9	16.9	6.0	24.4 a	5.6 b
2008	15.4	5.9	16.1	6.1	22.0 b	7.8 a
FR	14.5 b	5.3 b	16.2	6.0	22.9	6.8
LR	16.5 ab	5.9 ab	16.8	6.1	23.3	6.6
ND	17.2 a	6.4 a	16.5	6.1	23.3	6.6
y × tr	ns	ns	ns	ns	ns	ns
Sangiovese						
2007	25.1 a	8.7	14.5	5.0 b	20.6	6.0 b
2008	19.0 b	9.2	15.6	7.7 a	21.2	8.0 a
FR	21.7	9.1	15.0	6.4	19.8	7.2
LR	21.7	9.2	14.9	6.5	21.5	7.0
ND	22.8	8.6	15.3	6.2	21.5	6.8
y × tr	ns	ns	**	ns	ns	ns

[a]In the same column, statistically significant differences between years and treatments within varieties are indicated by different letters (p < 0.05). FR, full leaf removal in the cluster zone; LR, lateral shoot removal in the cluster zone; ND, nondefoliated; fw, fresh weight. [b]*, **, and *** represent significance of the year × treatment (y × tr) interaction at p < 0.05, p < 0.01, and p < 0.001, respectively; ns, not significant.

parameters in Cabernet Sauvignon. Moreover, there was no year × treatment interaction for yield parameters in any of the cultivars (Table 2).

Number of clusters per vine was similar among treatments in Cabernet Sauvignon and Sangiovese but was lower in FR in Merlot. Defoliation had a significant effect on yield and cluster weight in Merlot and Sangiovese but not in Cabernet Sauvignon (Table 2). Yield per vine was reduced by defoliation in Merlot and Sangiovese, although the intermediate defoliation (LR) was effective only in Sangiovese (Table 2). The impact of defoliation on yield was particularly severe in Merlot, with FR reducing it by >50% on a two-year basis. Reduction was less severe in Sangiovese (22% in FR as compared to ND). Similar results were observed for mean cluster weight with lower values in FR than either LR or ND. In Merlot, cluster weight was decreased by 37% and in Sangiovese by 12%. No effect on yield per vine and cluster weight was recorded in Cabernet Sauvignon (Table 2). In pooled data over years, variation in cluster weight accounted for the major part of the variability in yield in Merlot and Sangiovese (r^2 = 0.89 and 0.65, respectively; p < 0.001) but not in Cabernet Sauvignon (r^2 = 0.22). However, the lower yield of the FR vines in Merlot was also partially due to the reduced number of clusters in this treatment (Table 2).

Number of berries per cluster was significantly decreased by defoliation in Merlot, with FR inducing a two-year-averaged 30% reduction in berry number per cluster as compared to ND. In this variety, the reduction in berry number was in turn reflected in a decrease in cluster compactness (Table 2). Increased intensity of defoliation tended to decrease berry size, but a significant difference between FR and ND vines was observed only in Cabernet Sauvignon (Table 2). A significant year × treatment effect was observed for Sangiovese for which berry size was higher in ND in 2007 (193.1 g/100 berries compared to 156.3 and 172.4 g/100 berries in FR and LR, respectively) and in FR in 2008 (131.2 g/100 berries compared to 123.5 and 113.3 g/100 berries in LR and ND, respectively). In pooled data over years, variation in the number of berries per cluster accounted for the major part of the variability in cluster weight in all varieties (r^2 = 0.75 for Merlot, r^2 = 0.80 for Cabernet Sauvignon, and r^2 = 0.69 in Sangiovese; p < 0.001), whereas no correlation was observed between cluster and berry weight (r^2 = 0.05, 0.07, and 0.10, respectively).

Leaf removal typically reduces yield when applied before flowering[10] because fruit set is mainly determined by carbohydrate supply between flowering and berry set.[31] Removing basal leaves at bloom reduces total assimilate production because, at this stage, the lower portion of the shoot contributes more than the upper part to whole-vine photosynthesis.[17] Previous work on Sauvignon blanc,[32] Sangiovese,[6,15] and Barbera[33] also reported decreased yield, cluster size, and fruit set in prebloom defoliated vines. Yield components were not affected by prebloom defoliation in Grenache vines,[34] which, according to the authors, was due to the low severity of intervention.

Contrary to prebloom leaf removal, postbloom defoliation was reported to be ineffective in significantly lowering cluster weight and berry number per cluster in Graciano and Carignan,[10] but in this work, lateral shoots were not removed. However, in a three-year trial with field-grown Trebbiano,[14] yield components were markedly reduced by defoliation at fruit set, mainly due to a reduction in the number of berries per cluster. Other studies similarly show that berry abortion can occur in response to postbloom defoliation.[17,35,36] Our results (Table 2) suggest that the effect of postbloom leaf removal on yield components was cultivar-dependent because the number

Table 4. Individual Skin Anthocyanin[a] Concentrations and Proportion of Total Anthocyanins (TSA) across Varieties[b]

	mg/100 g berry fresh weight			% TSA		
	Merlot	Cabernet Sauvignon	Sangiovese	Merlot	Cabernet Sauvignon	Sangiovese
Dp	15.8 ± 1.9	9.8 ± 1.0	11.5 ± 0.8	9.5 ± 0.8	6.6 ± 0.4	11.5 ± 0.6
Cy	6.1 ± 1.0	2.1 ± 0.4	7.3 ± 0.6	3.7 ± 0.4	1.1 ± 0.2	8.5 ± 0.4
Pt	16.2 ± 1.5	11.5 ± 0.7	13.4 ± 0.8	9.8 ± 0.5	6.7 ± 0.3	15.6 ± 0.5
Pn	20.2 ± 2.8	11.1 ± 0.6	11.0 ± 0.7	12.4 ± 1.4	6.5 ± 0.3	12.8 ± 0.4
Mv	84.1 ± 7.3	118.3 ± 7.3	42.5 ± 2.4	51.0 ± 2.2	68.2 ± 0.6	49.9 ± 1.2
MvC	18.3 ± 1.8	15.2 ± 1.2	1.5 ± 0.3	11.2 ± 0.8	8.7 ± 0.3	1.6 ± 0.3
MvA	4.0 ± 0.6	3.7 ± 0.7		2.3 ± 0.3	2.1 ± 0.3	
3′-OH	26.3 ± 3.8	13.1 ± 0.9	18.3 ± 1.1	16.1 ± 1.8	7.6 ± 0.4	21.4 ± 0.5
3′,5′-OH	138.3 ± 10.7	160.2 ± 9.8	67.2 ± 3.5	83.9 ± 1.8	92.4 ± 0.4	78.6 ± 0.5
3′/3′,5′-OH	0.202 ± 0.026	0.083 ± 0.004	0.272 ± 0.008			
TSA	164.7 ± 12.0	176.3 ± 10.4	85.5 ± 4.5			

[a]Dp, delphinidin-3-*O*-glucoside; Cy, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; MvC, malvidin 3-*O*-coumaroylglucoside; MvA, malvidin 3-*O*-acetylglucoside. [b]3′-OH, 3′-hydroxylated anthocyanins: all Cy and Pn derivatives. 3′,5′-OH, 3′,5′-hydroxylated anthocyanins: all Dp, Pt, and Mv derivatives. Values are the mean ± standard error over years and treatments (n = 18).

of berries per cluster and cluster size were responsive to leaf removal severity only in Merlot.

Generally, berry weight is reported to decrease with postflowering defoliation[14] due to the limiting leaf area during stage I of berry growth.[37] According to previous studies, leaf removal after fruit set strongly reduced berry growth of Cabernet Sauvignon,[37] and despite leaf area and berry growth rate restoration after veraison, final berry size was lower in defoliated shoots. This was also observed in our trial with Cabernet Sauvignon. The absence of difference in berry size among treatments in Merlot could be the result of compensatory growth due to increased assimilate partitioning in the remaining berries of the exposed clusters after berry abortion.

Skin growth showed no year × treatment effect, except for the relative skin weight in Sangiovese (Table 3), which showed no differences in 2007 among treatments but was higher in ND in 2008 (16.6% compared to 15.9 and 14.4% in LR and FR, respectively). Comparing the two years of study showed that 2007 values were higher than 2008 values except for Cabernet Sauvignon. With regard to defoliation, skin fresh weight at harvest was similar among treatments in Merlot and Sangiovese, but it was higher in ND as compared to FR in Cabernet Sauvignon, which is likely to be the result of the higher berry weight in ND for this cultivar (Table 3). Overall, skin mass followed variations in berry mass in all varieties, as shown by the positive linear correlation between these parameters (r^2 = 0.62 for Merlot, r^2 = 0.62 for Cabernet Sauvignon, and r^2 = 0.77 in Sangiovese; $p < 0.001$), confirming previous reports of a close correlation between skin and total berry growth in Cabernet Sauvignon[38] and Syrah.[39] As a result, the skin to berry ratio was unaffected by defoliation (Table 3) in all cultivars.

In previous works with Barbera[33] and Sangiovese,[11] skin development was enhanced by leaf removal as compared to the flesh, but in these studies defoliation was applied prior to bloom. Skin growth is reported to be promoted by a long-lasting exposure of berries to high light and/or temperature due to enhanced cell division in the pericarp, 3−4 weeks after flowering.[13] However, cell division in the pericarp seems to be particularly sensitive to extreme temperatures as the skin thickness of berries kept at 40 °C during the postflowering period was lower compared to berries kept at 25 °C.[12] It is therefore possible that the higher skin-to-berry weight ratios found in defoliated vines of previous studies[40] were due to either the longer period of exposure to light (prebloom compared to postbloom in our study) or the milder climatic conditions as compared to the area of the present study, allowing cluster temperature to rise to levels more conducive for skin growth.[40]

Seed growth was similar among years with no year × treatment interaction (Table 3). Similarly to skin weight, seed weight was affected by defoliation only in Cabernet Sauvignon, ND berries having significantly heavier seeds than FR. Similar results were provided by Ristic et al.[26] for Shiraz. The relative contribution of seeds to the total berry weight (and thus to its total phenolic content) is important in the assessment of cluster exposure effects on red winemaking.[3] According to our results, relative seed weights were similar among treatments, in all three varieties. Similarly to skin growth, seed growth correlated linearly and positively with total berry weight in Cabernet Sauvignon and Merlot (r^2 = 0.33 for Merlot, $p < 0.05$; and r^2 = 0.73 for Cabernet Sauvignon, $p < 0.001$) but not in Sangiovese (r^2 = 0.12; not significant). In previous studies working with prebloom leaf removal, seed mass and seed-to-berry weight ratio were increased in defoliated shoots in Barbera but not in Lambrusco grapes grown under similar conditions,[40] providing evidence that seed growth response to defoliation is probably cultivar dependent.

Must Composition. Averaged over years, soluble solids at harvest were similar among years (except for Cabernet Sauvignon) and treatments, without interaction with season (Table 3). Previous studies under a cooler climate[6,14] reported a positive effect of defoliation on must soluble solids. In these studies defoliation was applied prior to flowering, which is reported to increase leaf area-to-fruit ratio during berry ripening.[11] However, under conditions more similar to the Greek climate,[10] grape soluble solids and wine alcohol were not affected by defoliation in Carignan, irrespective of the timing of intervention. In the latter study, defoliation did not alter shoot vegetative pattern nor induce any compensatory lateral growth. It is possible that, due to the limiting environment of our study (high daily temperatures) as well as the timing of defoliation (postbloom), the source compensation observed in other works was not high enough to warrant an improvement in the sugaring process.

Titratable acidity (TA) was higher in all cultivars in 2008 compared to 2007. TA was increased by defoliation only in

Table 5. Year (n = 9) and Leaf Removal Severity (n = 3) Effects on Skin Anthocyanins[a] (Milligrams per 100 g Berry Fresh Weight) in Merlot[b]

		Dp	Cy	Pt	Pn	Mv	MvC	MvA	3′-OH	3′,5′-OH	3′/3′,5′-OH ratio
2007		19.8 a	8.7 a	18.6 a	28.4 a	64.8 b	12.2 b	2.8	37.2 a	118.4 a	0.30 a
2008		11.7 b	3.5 b	13.7 b	12.0 b	103.3 a	24.3 a	5.1	15.6 b	158.2 b	0.10 b
2007											
	FR	30.0 a	14.2 a	26.5 a	41.8 a	82.9 a	14.7 a	4.2	56.0 a	158.3 a	0.35 a
	LR	17.8 b	7.0 b	17.4 b	25.5 b	62.0 ab	11.4 ab	1.1	32.5 b	109.8 b	0.30 b
	ND	11.7 c	5.0 b	12.0 c	17.9 c	50.0 b	10.6 b	3.2	22.9 b	87.1 b	0.26 b
2008											
	FR	14.4 a	4.8	20.0 a	16.6 a	136.8 a	30.6 a	5.6	21.4 a	210.4 a	0.10
	LR	9.9 b	2.8	11.9 b	10.6 b	94.8 b	23.6 b	5.8	13.5 b	145.9 b	0.09
	ND	7.9 b	2.9	9.6 b	8.9 b	78.2 b	18.8 c	3.8	11.8 b	118.4 b	0.10

[a]Dp, delphinidin-3-*O*-glucoside; Cy, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; MvC, malvidin 3-*O*-coumaroylglucoside; MvA, malvidin 3-*O*-acetylglucoside. [b]3′-OH, 3′-hydroxylated anthocyanins: all Cy and Pn derivatives. 3′,5′-OH, 3′,5′-hydroxylated anthocyanins: all Dp, Pt, and Mv derivatives. FR, full leaf removal in the cluster zone; LR, lateral shoot removal in the cluster zone; ND, nondefoliated. In the same column, statistically significant differences between years (n = 9) and leaf removal treatments within a year (n = 3) are indicated by different letters ($p < 0.05$).

Table 6. Year (n = 9) and Leaf Removal Severity (n = 3) Effects on Skin Anthocyanins[a] (Milligrams per 100 g Berry Fresh Weight) in Cabernet Sauvignon[b]

		Dp	Cy	Pt	Pn	Mv	MvC	MvA	3′-OH	3′,5′-OH	3′/3′,5′-OH ratio
2007		12.6	2.0	12.3	11.7	103.7 b	11.9 b	1.9 b	13.7	142.4 b	0.09 a
2008		10.3	2.2	10.7	10.4	133.0 a	18.5 a	5.4 a	12.6	178.0 a	0.07 b
2007											
	FR	18.8 a	4.1	16.7 a	15.3 a	129.7 a	14.4 a	0.1	19.4 a	179.6 a	0.11
	LR	9.4 b	0.1	10.3 b	10.4 b	90.9 b	11.1 ab	2.2	10.4 b	123.9 b	0.08
	ND	9.7 b	1.9	10.0 b	9.3 b	90.4 b	10.1 b	3.5	11.3 b	123.8 b	0.09
2008											
	FR	12.0	2.8	12.4 a	11.8	158.5 a	22.9 a	7.6 a	14.8 a	213.4 a	0.07
	LR	11.5	2.8	11.7 ab	11.3	142.3 a	18.8 b	4.9 b	14.2 a	189.3 ab	0.07
	ND	7.5	0.9	8.0 b	8.1	98.3 b	13.6 c	3.8 c	9.0 b	131.3 b	0.07

[a]Dp, delphinidin-3-*O*-glucoside; Cy, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; MvC, malvidin 3-*O*-coumaroylglucoside; MvA, malvidin 3-*O*-acetylglucoside. [b]3′-OH, 3′-hydroxylated anthocyanins: all Cy and Pn derivatives. 3′,5′-OH, 3′,5′-hydroxylated anthocyanins: all Dp, Pt, and Mv derivatives. FR, full leaf removal in the cluster zone; LR, lateral shoot removal in the cluster zone; ND, nondefoliated. In the same column, statistically significant differences between years (n = 9) and leaf removal treatments within a year (n = 3) are indicated by different letters ($p < 0.05$).

Table 7. Year (n = 9) and Leaf Removal Severity (n = 3) Effects on Skin Anthocyanins[a] (Milligrams per 100 g Berry Fresh Weight) in Sangiovese[b]

		Dp	Cy	Pt	Pn	Mv	MvC	MvA	3′-OH	3′,5′-OH	3′/3′,5′-OH ratio
2007		10.8	7.7	13.8	10.0	37.0 b	0.9		17.6	78.4	0.28
2008		8.8	7.0	13.0	12.0	48.1 a	2.1		19.0	78.9	0.27
2007											
	FR	10.8	6.8	13.2	11.3 a	31.8	0.1		14.4	55.9	0.26
	LR	12.2	8.9	15.1	11.0 b	38.0	0.6		19.9	65.9	0.29
	ND	9.4	7.3	13.1	7.6 b	41.1	2.0		18.6	65.7	0.28
2008											
	FR	11.6	9.0 a	16.6 a	13.0	54.1	2.7		22.0	85.1	0.26
	LR	7.8	6.0 b	11.7 b	11.4	47.1	1.7		17.4	68.4	0.26
	ND	7.0	5.9 b	10.6 b	11.7	43.1	1.8		17.5	62.4	0.29

[a]Dp, delphinidin-3-*O*-glucoside; Cy, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; MvC, malvidin 3-*O*-coumaroylglucoside; MvA, malvidin 3-*O*-acetylglucoside. [b]3′-OH, 3′-hydroxylated anthocyanins: all Cy and Pn derivatives. 3′,5′-OH, 3′,5′-hydroxylated anthocyanins: all Dp, Pt, and Mv derivatives. FR, full leaf removal in the cluster zone; LR, lateral shoot removal in the cluster zone; ND, nondefoliated. In the same column, statistically significant differences between years (n = 9) and leaf removal treatments within a year (n = 3) are indicated by different letters ($p < 0.05$).

Merlot (Table 3). Because malic acid is generally low under Greek climatic conditions,[41] and although individual concentrations of malic and tartaric acid were not measured, in can be hypothesized that the positive effect of defoliation on must acidity in Merlot was related to greater tartaric acid synthesis under increased exposure, as reported in previous works with Carignan[10] and Trebbiano.[14] Light exclusion in Shiraz clusters enclosed in boxes resulted in a significant reduction of tartaric

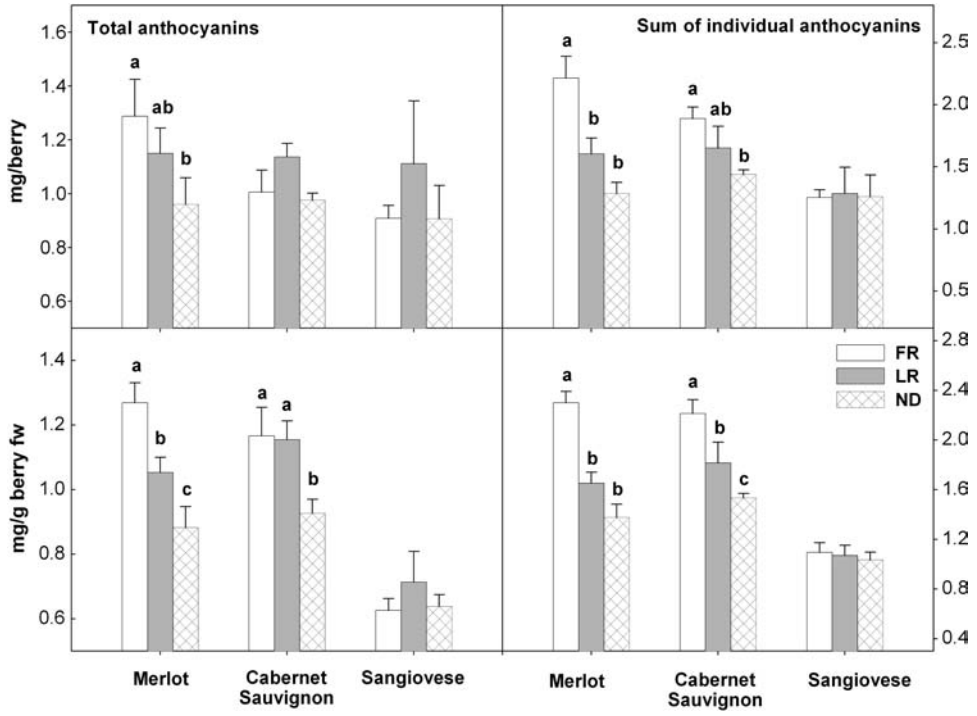

Figure 2. Effect of severity of leaf removal on total berry anthocyanin amount and sum of individual skin anthocyanidins at harvest. FR, full leaf removal in the cluster zone; LR, lateral shoot removal in the cluster zone; ND, nondefoliated; fw, fresh weight. Means are combined over years (n = 6). Vertical bars represent ± SE. Means labeled with a different letter within a variety are significantly different ($p < 0.05$).

acid and a slower degradation of malic acid as compared to light-exposed clusters.[42] The observation of a defoliation effect only in Merlot in contrast to the other cultivars was possibly related to the looser clusters of the defoliated shoots in this variety, allowing a better exposure of berries of the interior of the cluster to light.

Phenolic Compounds. Important differences in the content of anthocyanins were detected among varieties (Table 4). Mv was the prevalent anthocyanin determined especially in Cabernet Sauvignon,[43] in which it represented (together with its derivatives) 79% of the total skin anthocyanins (Table 4). Mv accounted for 65% of total anthocyanins in Merlot but only 51% in Sangiovese. Averaging years and treatments, Dp, Pt, and Pn were found in the highest amounts in Merlot, whereas Cy was the highest in Sangiovese (Table 4). However, Sangiovese presented the higher proportion of Dp, Cy, Pt and Pn (Table 4) and the higher 3′-hydroxylated/3′,5′ hydroxylated anthocyanin ratio. Merlot and Cabernet Sauvignon grapes were previously reported to contain high amounts of Mv 3-*O*-glucoside and low levels of Cy 3-*O*-glucoside,[44] whereas Sangiovese berry skins were found to contain high amounts of Cy and Pn 3-*O*-glucosides.[6]

Season affected differently the levels of Mv and its derivatives as compared to Dp, Cy, Pt, and Pn. Mv was found in higher amounts under the relatively cooler 2008 conditions in all varieties, whereas Dp, Cy, Pt, and Pn showed increased content in 2007 except for Sangiovese (Tables 5−7).

Leaf removal severity affected the concentration of all individual anthocyanins in Merlot (Table 5) and Cabernet Sauvignon (Table 6) skin tissues at harvest, with FR vines having the higher amounts as compared to ND with the exception of MvA, Cy (in 2007 for Merlot, both years in Cabernet Sauvignon), and Dp and Pn (in 2008 in Cabernet Sauvignon). LR generally presented intermediate values or values similar to ND with the exception of Mv in Cabernet Sauvignon, in 2008. Figure 2 shows the total amount of anthocyanins in the berries of all treatments measured both in the whole berry extract and as sum of individual anthocyanins, expressed as mg/g fresh berry weight and on a mg/berry basis. Total anthocyanins were higher in FR as compared to ND in Merlot and (only as sum of individual anthocyanins) in Cabernet Sauvignon (Figure 2). No differences in the individual anthocyanins were found among treatments in Sangiovese, with minor exceptions (Table 7), as well as for their total amount, irrespective of the method and expression used (Figure 2).

High light incidence on grapes is generally considered to promote greater anthocyanin accumulation in the skins.[5,45] According to recent papers,[46] the specific anthocyanin biosynthetic gene encoding UDP-glucose:flavonoid 3-*O*-glucosyltransferase was particularly enhanced under increased exposure to solar radiation in Cabernet Sauvignon grapes. However, other studies reported reduced anthocyanins in exposed clusters in Cabernet Sauvignon,[18] Merlot,[19] and Syrah[47] due to concomitant increases in berry temperature, especially under hot climate conditions. In a comparative study of light and temperature effects on the anthocyanin composition of Merlot grapes,[48] it was observed that in conditions of low light intensity and high berry temperatures skin anthocyanins decreased.

Although berry temperature was not monitored in the conditions of our trial, ambient temperature remained relatively high during the study period (Figure 1), suggesting increased temperature for exposed clusters.[49] However, findings reported here suggest that cluster exposure had a positive effect on anthocyanin levels in two of the three varieties examined. It is therefore possible that the response of skin anthocyanins to the combined effects of light and temperature was probably

Table 8. Individual Seed Flavan-3-ol Monomer and Dimer[a] Concentrations and Proportion of Total Seed Flavan-3-ols Examined (TSF) across Varieties[b]

	mg/100 g berry fresh weight			% TSA		
	Merlot	Cabernet Sauvignon	Sangiovese	Merlot	Cabernet Sauvignon	Sangiovese
GA	0.6 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	2.0 ± 0.1
C	24.3 ± 1.8	21.4 ± 0.7	11.4 ± 0.7	35.3 ± 0.9	47.2 ± 0.4	28.6 ± 0.9
EC	27.5 ± 1.3	16.5 ± 0.7	17.6 ± 0.9	40.8 ± 0.9	36.4 ± 0.6	44.7 ± 1.0
ECG	9.0 ± 1.2	1.2 ± 0.1	5.0 ± 0.8	12.4 ± 1.1	2.6 ± 0.1	12.0 ± 1.6
EGCG	1.3 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	2.0 ± 0.2	2.6 ± 0.1	2.7 ± 0.3
EGC	1.8 ± 0.1	1.3 ± 0.2	1.8 ± 0.1	2.8 ± 0.2	2.7 ± 0.3	4.5 ± 0.3
B_1	1.9 ± 0.1	2.4 ± 0.2	1.3 ± 0.1	3.0 ± 0.2	5.4 ± 0.4	3.3 ± 0.3
B_2	1.4 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	2.2 ± 0.2	1.8 ± 0.3	2.2 ± 0.1
TSA	68.2 ± 4.1	45.3 ± 1.5	39.8 ± 2.3			

[a]GA, gallic acid; C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-*O*-gallate; EGCG, (−)-epigallocatechin-3-*O*-gallate; EGC, (−)-epigallocatechin; B_1, procyanidin B_1; B_2, procyanidin B_2. [b]Values are the mean ± standard error over years and treatments (n = 18).

Table 9. Year (n = 9) and Leaf Removal Severity (n = 6) Effects on Seed Flavan-3-ols[a] (Milligrams per 100 g Berry Fresh Weight)[b]

	GA	C	EC	ECG	EGCG	EGC	B_1	B_2
				Merlot				
2007	0.8	30.3 a	30.8 a	13.5 a	1.3	1.7	1.9	1.3
2008	1.1	18.3 b	24.1 b	4.6 b	1.4	2.1	2.0	1.6
FR	0.8	21.9 b	24.5 b	8.0 b	1.4	1.6	1.7	1.4
LR	0.9	23.2 b	28.5 a	9.6 a	1.1	1.9	2.0	1.4
ND	0.9	27.7 a	29.3 a	9.5 a	1.5	1.9	2.1	1.4
y × tr[c]	ns	ns	ns	ns	ns	*	ns	ns
				Cabernet Sauvignon				
2007	0.6	20.8	16.5	0.9 b	1.3	0.7 b	2.6	0.7
2008	0.5	21.9	16.6	1.4 a	1.1	1.8 a	2.2	0.9
FR	0.5 b	19.1 b	15.0 b	1.0	1.2	1.1	2.1 b	0.5 b
LR	0.6 a	21.1 ab	15.5 ab	1.0	1.1	1.2	3.0 a	1.1 a
ND	0.7 a	23.9 a	19.1 a	1.5	1.3	1.5	2.1 b	0.8 ab
y × tr	*	ns	ns	ns	ns	ns	ns	ns
				Sangiovese				
2007	0.6 b	11.4	19.1 a	7.9 a	0.8	1.6	1.2	0.8
2008	0.9 a	11.3	16.2 b	2.1 b	1.4	1.9	1.4	0.9
FR	0.8	11.0 ab	15.7 b	5.4 a	1.1	1.7	1.5 a	0.8
LR	0.8	13.6 a	20.2 a	6.3 a	1.3	1.9	1.7 a	1.0
ND	0.7	9.5 b	16.9 ab	3.4 b	0.8	1.7	0.8 b	0.7
y × tr	ns	ns	ns	ns	ns	*	ns	ns

[a]GA, gallic acid; C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-*O*-gallate; EGCG, (−)-epigallocatechin-3-*O*-gallate; EGC, (−)-epigallocatechin; B_1, procyanidin B_1; B_2, procyanidin B_2. [b]FR, full leaf removal in the cluster zone; LR, lateral shoot removal in the cluster zone; ND, nondefoliated. In the same column, statistically significant differences between years (n = 9) and leaf removal treatments (n = 6) within varieties, are indicated by different letters ($p < 0.05$). [c]*, **, and *** represent significance of the year × treatment (y × tr) interaction at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns, not significant.

cultivar-dependent. In a previous work,[48] total concentrations of Dp, Cy, Pt, and Pn 3-*O*-glucosides decreased as berry temperature increased in Merlot clusters exposed to direct solar radiation, whereas Mv-based anthocyanins were unaffected. Because the anthocyanin composition of Sangiovese is less dominated by Mv (Table 4), defoliation-induced increases in berry temperature might have negative effects on skin anthocyanins in this cultivar. A positive response of skin anthocyanins to increased light in the fruit zone was previously reported for Sangiovese[15] but under more temperate conditions (latitude 44° 30′ N). It is possible that, under the typically warmer conditions of mainland Greece, the generally positive effect of light on anthocyanins was offset in this cultivar by elevated berry temperatures.

However, differences in berry composition among treatments related to carbohydrate partitioning cannot be excluded. In previous studies with both prebloom[11,14] and postbloom[35] leaf removal, leaf area-to-fruit ratios were reported to increase in defoliated vines due to both a reduction in yield and leaf area recovery after veraison. Moreover, because leaves removed are typically those of the base of the shoots which might undergo a substantial loss of assimilation capacity during the late stages of berry development, it is likely that the better chemical composition of the defoliated berries could be due to a more favorable composition of the leaf area by ripening (more median and apical leaves that are more active photosynthetically).[14] However, in a study under Mediterranean climate conditions in Spain,[35] postbloom defoliation did not alter the final leaf area. It is therefore possible that under the semiarid

conditions of the area of this trial and the timing of leaf removal (postflowering), compensatory growth in the defoliated treatments was not triggered, although leaf area development was not measured to allow definitive conclusions. However, the reduction in yield in FR in Merlot and Sangiovese might have exerted a positive effect on source-to-sink ratio of the defoliated vines during ripening. Improved must and phenolic composition due to leaf removal has also been attributed to a change in berry size and the skin-to-pulp ratio.[14] In this study, there were no effects of leaf removal on the relative skin weight (Table 3). This is in turn reflected in the similar results of skin anthocyanins between treatments, irrespective of the expression used (per single berry and per berry fresh weight).

The anthocyanin profile was altered by leaf removal in Merlot and Cabernet Sauvignon (Tables 5 and 6): FR had a greater proportion of 3′-hydroxylated anthocyanins (Cy and Pn), indicating a proportionally greater increase with light of these compounds as compared to the 3′,5′-hydroxylated ones (Dp, Pt, and Mv).[50] In previous studies,[51] Cy was most sensitive to light conditions, decreasing with increasing shade, whereas Mv was the least affected.

The most abundant polyphenol in the seeds of Merlot and Sangiovese was EC, accounting for approximately 40−45% of the total monomer concentration of seeds, followed by C and an important contribution (approximately 12%) of ECG (Table 8). On the contrary, seed flavanol monomers in Cabernet Sauvignon were dominated by C (47%), followed by EC and minor contributions of the other compounds analyzed (Table 8). Year did not affect polyphenol concentration of seeds in Cabernet Sauvignon (Table 9), but there was a tendency for higher EC and ECG seed content in 2007 for Merlot and Sangiovese. In a previous study, EC content was found to increase in Pinot noir seeds with decreasing vigor,[52] which could be the case during the hotter and drier experimental conditions of 2007 in our study.

C, EC, and ECG concentrations in Merlot seeds and C and EC concentrations in Cabernet Sauvignon were higher in ND vines without significant differences for the minor compounds (Table 9). The total free flavan-3-ol amount (calculated as the sum of individual polyphenols detected), expressed both as mg/100 g of berry fw and per berry, was also higher in ND vines as compared to FR ones in these cultivars, with intermediate values for LR (Figure 3). In Cabernet Sauvignon, the higher seed flavanols in shaded berries could be also related to the higher seed weight in ND vines.[26] A different trend was observed, however, in Sangiovese, in which the highest C and EC levels, as well as total seed polyphenols, were recorded in LR. The relative contribution of C and EC to the total pool of seed polyphenols analyzed was not altered by leaf removal in Merlot and Cabernet Sauvignon (data not shown), but it was higher in the ND vines in Sangiovese (49% compared to 41 and 43% in FR and LR, respectively; $p < 0.05$). Cortel et al.[52] have previously reported an increased proportion of EC in more open canopies of Pinot noir grapevines.

In the conditions of this study, defoliation in the fruit zone decreased the levels of free flavan-3-ol monomers and dimers in grape seeds in Merlot and Cabernet Sauvignon. Increased flavan-3-ols in grape seeds are often associated with higher levels of bitterness and astringency in the wine.[53] With regard to leaf removal effects on seed phenolics, reported data are not consistent. According to previous works conducted on Syrah[23] and Cabernet Sauvignon grapes,[54] cluster shading did not affect the levels of free seed flavan-3-ol monomers at harvest. On the contrary, in other leaf removal studies,[24,26] shaded fruit had increased seed tannins at ripeness. A significant influence of vine vigor on total flavan-3-ol monomers in seeds of Cabernet Sauvignon has also been reported[43] with higher levels in high vigor vines possibly because denser canopies increase shading in the fruit zone.

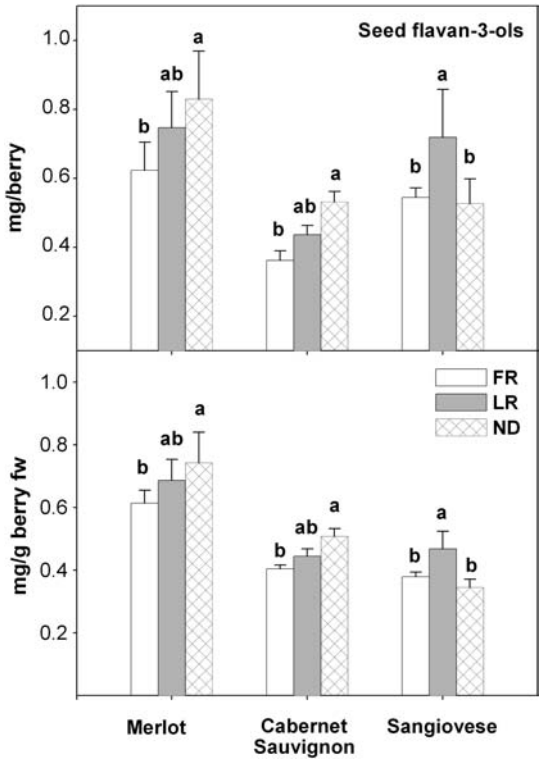

Figure 3. Effect of severity of leaf removal on total free seed flavan-3-ols at harvest. FR, full leaf removal in the cluster zone; LR, lateral shoot removal in the cluster zone; ND, nondefoliated; fw, fresh weight. Means are combined over years ($n = 6$). Vertical bars represent ± SE. Means labeled with a different letter within a variety are significantly different ($p < 0.05$).

In Merlot and Cabernet Sauvignon, total berry polyphenols were higher in FR berries when expressed as concentration per fresh berry weight, but there were no differences on a per berry basis (Figure 4). In Sangiovese, total polyphenols per berry increased in FR and LR as compared to ND. A positive effect of grape exposure on berry phenolic content has been reported in many cultivars.[10,15,18,55] Berry total phenolics are determined by both skin and seed flavonoid levels. Our results have shown that exposed fruit had higher anthocyanins but lower seed flavan-3-ol monomers, especially in Merlot and Cabernet Sauvignon. Although skin proanthocyanidins were not measured in this study, other authors have reported significant increases in skin proanthcyanidins with cluster exposure,[24,26,54] particularly in condensed tannins.[23] Therefore, the higher phenolic content of FR berries in our study is probably related to the positive effect of leaf removal on the total amount of phenolic compounds in the skin rather than the seeds.

In summary, this two-year leaf removal trial across three red *V. vinifera* L. cultivars under the semiarid climate of mainland Greece showed that post-flowering leaf removal improved the overall berry composition in Merlot and Cabernet Sauvignon but had limited effect in Sangiovese. Increased severity of defoliation was associated with higher levels of skin anthocyanins and a lower contribution of the seeds to the

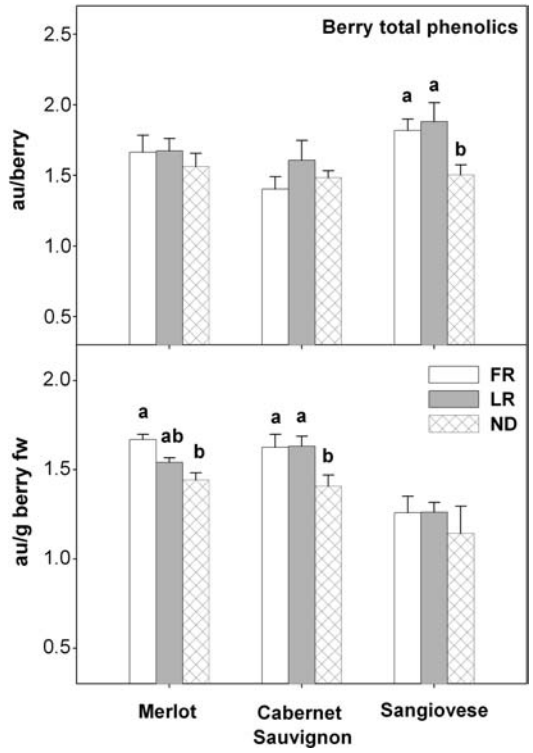

Figure 4. Effect of severity of leaf removal on berry total phenolics at harvest. FR, full leaf removal in the cluster zone; LR, lateral shoot removal in the cluster zone; ND, nondefoliated; fw, fresh weight; au, absorbance units. Means are combined over years ($n = 6$). Vertical bars represent ± SE. Means labeled with a different letter within a variety are significantly different ($p < 0.05$).

total pool of berry tannins in Merlot and Cabernet Sauvignon. These effects were also largely independent of any developmental variation in berry mass. It is also important that this was not achieved at the expense of a reduction in acidity or an undesirable increase in potential alcohol levels in the must. In Sangiovese, the impact of berry exposure to light was possibly undermined by its higher sensitivity to elevated berry temperature. Additional knowledge is required to elucidate the dependence of each category of grape phenolic compounds on the light environment of grapes across different varieties and climatic conditions.

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Notes

The authors declare no competing financial interest.

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