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Irrigation and Rootstock Effects on the Phenolic Concentration and Aroma Potential of *Vitis vinifera* L. cv. Cabernet Sauvignon Grapes

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Compositional changes of skin and seed phenolic compounds and berry glycosylated aroma precursors were measured in *Vitis vinifera* L. cv. Cabernet Sauvignon onto 1103P and SO4 rootstocks, in three irrigation regimes (FI, 100% of evapotranspiration; DI, 50% of evapotranspiration; and NI, non-irrigated). The study was conducted in a commercial vineyard of central Greece, in a factorial experiment during two growing seasons (2005−2006). Grape samples were obtained at commercial harvest. The deficit water supply decreased berry size but did not affect the skin/pulp weight ratio. Water limitation, especially pre-veraison, caused a substantial increase of skin anthocyanin concentration, and this effect was independent of water deficit-induced reductions in berry size and vine vigor. Among individual anthocyanins, malvidin-3-*O*-glucoside was mostly affected by water supply. The rootstock genotype did not affect berry growth parameters and skin polyphenol concentrations. The irrigation regime (mainly post-veraison) and rootstock genotype affected total flavan-3-ol monomers in seed tissue, mainly as a result of variations in the catechin amount. The lower seed phenolic concentration was found in non-irrigated and SO4-grafted vines, probably as a result of the restriction of scion vigor caused by these treatments, thereby altering cluster exposure. Skin and seed tannins were not affected by either rootstock or irrigation. The limited water supply was associated with increased aroma potential at harvest.

KEYWORDS: Grapevine; water deficit; rootstock; vine vigor; phenolic compounds; aroma potential

INTRODUCTION

Grape-derived secondary metabolites play a critical role in grape composition and wine quality. Phenolic compounds of the skin and seeds are the principal sources of wine color and structural properties (*1*), while volatile metabolites are the major determinants of wine aroma and flavor (*2*).

Grape-based phenolic compounds are classified as nonflavonoid (benzoic and cinamic acids and stilbenes) and flavonoid (anthocyanins, flavonols, and tannins). Among the latter, anthocyanins are pigmented compounds located in the skins of grape berries in red cultivars (*1*), while tannins derive from both skins and seeds of berries and range from flavan-3-ol monomers, such as catechin and epicatechin, to polymeric proanthocyanidins, known as condensed tannins (*3*). Skin proanthocyanidins differ from those found in seeds in that skins contain a lower concentration of flavan-3-ol monomers and have a higher degree of polymerization (*4*). Anthocyanin accumulation commences at veraison and continues throughout ripening, with a possible decline late in berry development (*5*). Tannins are biosynthesized during the first phase of berry growth, with maximum levels around veraison (*6*).

Aroma compounds occur in grapes at low concentrations and are mainly found in the form of non-odorant glycosylated precursors. Among secondary metabolites with sensory significance, monoterpenes, C_{13}-norisoprenoids, and volatile phenols have increasing trends with grape maturity (*7*), rising more rapidly during the advanced stages of grape ripening (*8*). Although the total concentration of glycosylated aroma compounds is not directly related to wine organoleptic properties, it can provide an assessment of grape aroma potential (*9*).

It is well-documented that grape phenolic compounds vary greatly with vintage (*10*), site (*11*), maturity level (*12*), and viticultural techniques, which include management of vine canopy and fruit exposure, nutrient availability, and water status (*4*). Among cultural practices, irrigation management seems to be the

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largest and most controllable factor in determining grape and wine quality (*13*), especially in arid and semi-arid areas, with the primary focus on grape phenolic compounds (*5*, *14*). However, it remains largely unclear whether the influence of water conditions on grape phenolics arises directly from changes in the biosynthetic pathway of flavonoids or from water-availability-related modifications in berry growth and/or vine vigor and microclimate. Limited data also exist regarding the effect of vine water status on grape-derived volatile metabolites (*15*). Moreover, water effects on berry components are often contrasting, mainly because of different irrigation volumes and environmental conditions, leading to variations in water availability.

Rootstocks affect numerous vegetative and reproductive parameters of the scion, such as water and gas exchange status (*16*), canopy growth (*17*), and yield (*18*), most likely because of their role on root density (*19*). However, limited knowledge exists on the effect of specific rootstock genotypes on scion berry attributes in the field, especially under drought conditions.

The aim of the present work was to investigate the effect of irrigation water regimes and rootstock cultivar on berry phenolic and aroma components of field-grown *Vitis vinifera* cv. Cabernet Sauvignon vines. Emphasis was given in understanding whether differences were due to a direct impact on the biosynthetic pathway of these compounds or variations in berry and canopy growth.

MATERIALS AND METHODS

Experimental Conditions and Vine Parameters. The study was carried out during two growing seasons (2005−2006) in a 10-year-old commercial vineyard in Larissa, central Greece (39°48′ N, 22°27′ E, 190 m), planted with cv. Cabernet Sauvignon (*Vitis vinifera* L.) at 3200 vines/ha (1.3 × 2.4 m). The vineyard was located on a deep loamy soil (calcic cambisol) containing 44% sand, 31% silt, and 25% clay. The experiment was arranged as a 2 × 3 factorial design with two rootstocks [1103 Paulsen (*V. rupestris* × *V. berlandieri*) and SO4 (*V. riparia* × *V. berlandieri*)] and three irrigation regimes [full irrigation (FI), 100% of crop evapotranspiration (ET_c); deficit irrigation (DI), 50% ET_c; and non-irrigated (NI)]. Irrigation was scheduled on a weekly basis starting at berry set through harvest, according to climatic data recorded on a Vantage Pro2 automatic weather station (Davis Instruments Corp., Hayward, CA) located inside the vineyard. The total amount of applied water for the season was approximately 300 mm for the FI treatment and 150 mm for DI. The six treatments were replicated 3 times in randomized blocks, with three rows per replication. In each plot, only the central four vines of the middle row were used for measurements and the other rows served as borders.

Vine water status and physiology were estimated by midday measurements of stem water potential (Ψ_s) and stomatal conductance (g_s) on three cloudless days per season, corresponding to the growth stages of bunch closure (D1), veraison (D2), and harvest (D3). Vine vigor was assessed by leaf area index (LAI) determinations and the measurement of pruning weight per vine (PW). A detailed description of the experimental conditions and measurements of vine parameters is given by Koundouras et al. (*17*).

Berry Sampling and Must Analysis. Grapes were harvested on August 31st, 2005 and August 30th, 2006, for all treatments, from the four chosen vines in each plot, and the total yield per plant was weighed. Individual berry fresh weight was determined on a sample of 200 berries per plot. The remaining berries per plot were pressed, and the must was analyzed for soluble solids (°Brix) by refractometry.

Analysis of Phenolic Compounds. *Skin Extractability Assay*. A subsample consisting of 200 berries of each plot was ground using a blender, and 50 mL were macerated for 4 h with pH 1 buffer solution according to the extractability assay described by Saint-Criq et al. (*20*). The total anthocyanin concentration was chemically assayed in the supernatant solution after bisulphite bleaching, by measuring the absorbance of the samples at 520 nm (*21*).

Skin and Seed Tannins and Total Phenols. Seed and skin tannin concentrations were evaluated using a protein precipitation assay. A sample preparation and protein precipitation assay was conducted according to the method described by Harbertson et al. (*22*). From each sample, three 20-berry samples were processed for tannin extraction. A standard curve was prepared using (+)-catechin in the range of 25−300 μg. Tannin values for skin and seed extracts were obtained from the standard curve; thus, values for tannin are reported in catechin equivalents. The skin and seed total phenols were determined using the Folin−Ciocalteau method (*23*) and were expressed as mg/L gallic acid (GAE). All analyses were performed in triplicate.

Determination of Individual Anthocyanins by High-Performance Liquid Chromatography (HPLC). A lot of 100 berries from each plot was weighted and manually skinned, and the skins were weighed and freeze-dried. The freeze-dried tissues were then extracted with 100 mL of 1% HCl in MeOH. Extraction was carried out under stirring for 48 h and repeated 3 times in triplicate. Extracts were pooled, and this mixture was used for further procedure analysis either immediately or after deep-freezing (−70 °C) for no longer than 4 days. Anthocyanin analysis was carried out according to Arnous et al. (*24*). Identification was based on comparing retention times of the peaks detected to those of original compounds and UV−vis online spectral data. Quantification was performed by establishing calibration curves for each compound determined, using the standards. Results were expressed as milligrams of malvidin per fresh skin weight and per berry. All analyses were performed in duplicate.

Determination of Individual Seed Polyphenols by HPLC. Berries of the same lot were manually deseeded, and the seeds were counted, weighed, frozen in liquid nitrogen, and stored in the freezer (−20 °C) until analyzed. A lot of 2 g of seeds was ground with a pestle and mortar and subsequently placed in a vial, and 8 mL of ethyl acetate was added. The mixture was vortexed for 3 min. The extract was centrifuged at 6000 rpm for 5 min at 4 °C, and this process was repeated twice more. The clear extracts were then pooled and taken to dryness in a rotary vacuum evaporator (35 °C), and the resulting residue was dissolved in 8 mL of MeOH, containing 5% (v/v) perchloric acid. 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 (*25*). Quantification was performed by establishing calibration curves for each compound determined, using the standards. Procyanidins are expressed as mg/L (+)-catechin, whereas the rest of the compounds are expressed against their own calibration curves. All analyses were performed in duplicate.

Glycosyl-glucose Assay. Standard glycoside used was *n*-octyl-β-D-glucopyranoside (≥99%, Fluka BioChemica, Germany). Absolute ethanol and methanol were from Riedel-de Haën (Seelze, Germany); sodium hydroxide was from Merck (Darmstadt, Germany); sulfuric acid (95−97%) was from Panreac Quimica S.A. (Spain); and 0.2 M triethanolamine buffer was purchased from Sigma (St. Louis, MO). Water used was obtained by a Milli-Q water system, with a minimum resistance of 18.2 MΩ cm. The glucose concentration of hydrolysates was determined with a HK/G-6-P-DH spectrophotometric assay kit (Boehringer Mannheim/R-Biopharm, Darmstadt, Germany).

Grape samples were homogenized using a BioSpec Products M133/1281-0 BioHomogenizer (BioSpec Products, Inc., Bartlessville, OK) and the hydroalcoholic extract separated with a SV11 Firlabo (Lyon, France) centrifuge. SPE was performed using a Supelco Visiprep SPE Vacuum manifold (12 places) (Germany), a Büchi B-169 vacuum pump (Switzerland), and Waters Oasis HLB 6 cm^3 (200 mg) cartridges (Ireland), while a Shimadzu UV-1601 spectrophotometer (Kyoto, Japan), accompanied with UVPC-1601 software, was used for all UV−vis absorbance measurements. Adjustment of pH was achieved using a Consort 5231 model portable pH-meter (Turnhout, Belgium).

Extraction and isolation of phenol-free glycosides was conducted according to the method of Iland et al. (*26*), as modified by Zoecklein et al. (*27*) and Whiton and Zoecklein (*28*). The phenol-free glycosides were eluted from the Oasis HLB cartridges using 2 mL of an ethanol/methanol mixture (90:10, v/v) and distilled water (ca. 4 mL) and then hydrolyzed as in Williams et al. (*29*). The D-glucose released in the hydrolysates was finally determined using a HK/G-6-P-DH enzyme assay kit.

Statistics. Data were subjected to three-factor (year, rootstock, and irrigation regime) analysis of variance (ANOVA), using SPSS software (version 16.0, SPSS, Inc., IL). Only the mean of the four measurements per plot was used in data analysis. A comparison of means was

performed using Duncan's multiple range test at $p < 0.05$. Linear regression analysis was also used to explore the relationship between measured parameters.

RESULTS AND DISCUSSION

Vine Water Status and Vegetative Growth. Differences between irrigation and rootstock treatments in vine water status, physiology, and vigor were reported in a previous work (*17*) and are summarized in **Table 1**. With regard to the 2 years, 2006 was characterized by a more favorable vine water status than 2005; all data combined, g_s in 2006 was higher than in 2005 (0.57 and 0.42 mol m^{-2} s^{-1}, respectively; $p < 0.001$), although no year effect on Ψ_s was found (data not shown).

Midday stem water potential (Ψ_s) was affected by the irrigation regime, and differences were consistent between years (2005 and 2006) and rootstocks (SO4 and 1103P). Overall, vine water deficit was greater under NI conditions (more negative Ψ_s) than under DI or FI conditions. According to Ψ_s critical values (*30*), the water deficit was weak in FI, weak to moderate in DI, and moderate to severe in NI vines. Limited water availability reduced stomatal conductance (g_s), with the NI vines showing the lowest values for both years and rootstocks (**Table 1**). However, g_s did not reach mean values less than 0.05 mol m^{-2} s^{-1} reported previously as the onset of a more intense water deficit (*31*).

In pooled data, rootstock did not influence vine water status and physiological parameters (except for a significant effect on g_s in 2005) but altered vine vegetative growth (**Table 1**). A combination of data from irrigation treatments illustrated that 1103P vines had greater LAI in both years and higher winter pruning weights (PWs) in 2006 compared to SO4-grafted vines. LAI (both years) and PW (only 2006) declined with decreasing water availability only in vines grafted on 1103P, whereas no differences in vigor among irrigation treatments were detected for SO4 (**Table 1**). The higher canopy growth of 1103P-grafted vines is probably associated with its higher responsiveness to soil−water supply because of its denser root system compared to the shallower rooting SO4 (*32*). On the basis of this rational, 1103P is expected to provide the scion with higher amounts of water, especially under full irrigation, maximizing total biomass production (*17*).

Berry Growth. Differences between irrigation and rootstock treatments in reproductive growth parameters are presented in **Table 2**. The yield was similar between years (1.91 and 1.86 kg/vine for 2005 and 2006, respectively; $p = 0.849$) and rootstocks (1.81 and 1.95 kg/vine for SO4 and 1103P, respectively; $p = 0.586$). The yield per vine decreased with water limitation, although no differences were observed among irrigation treatments (respectively for NI and FI, 1.46 and 2.07 kg/vine in SO4; $p = 0.077$, and 1.39 and 2.36 kg/vine in 1103P; $p = 0.083$). No differences were recorded for individual cluster weight and average cluster number per vine for year, rootstock, or irrigation treatment (data not shown). Variation in the cluster size mainly accounted for differences in yield; in pooled data ($n = 18$), the yield per vine was positively correlated with the cluster weight on both rootstocks (SO4, $r = 0.923$, $p < 0.001$; and 1103P, $r = 0.955$, $p < 0.001$). The yield and cluster weight were linearly related to the intensity of the water deficit (expressed by the decrease in Ψ_s) only in the 1103P-grafted vines (**Table 3**), especially when regressed on Ψ_s measured at veraison (D2) or harvest (D3). Vegetative growth, yield, and cluster weight were less sensitive to the irrigation regime in SO4-grafted vines (except for a significant correlation of the cluster weight with Ψ_s in D3).

The year affected berry growth parameters, with 2005 showing higher berry, skin, and seed mass than 2006 (**Table 2**). Berry growth was not affected by rootstock cultivar but was controlled by the water regime in both rootstocks, being lowest under NI and highest under FI (**Table 2**). Previous works have reported the effect of the water deficit on berry growth, especially when applied before veraison (*33*, *34*), even though differences between the effects of pre- and post-veraison water deficits on berry growth were not always observed (*35*). In this study, berry size in the 1103P-grafted vines was highly dependent upon both pre- and post-verasion water deficits (D1, D2, and D3 in **Table 3**). In contrast, upon SO4, a significant correlation between berry weight and water-deficit intensity was evident only for D3. The higher sensitivity of berry growth to water availability in 1103P-grafted vines is probably associated with the higher responsiveness of 1103P to soil−water supply compared to SO4.

According to the distribution of fresh mass in mature berries, skin consisted of approximately 20%, while seeds consisted of 4% of the whole berry mass, on both rootstocks (calculated from data of **Table 2**). Skin mass followed variations in berry size as shown by a positive linear correlation between these parameters, on both rootstocks (SO4, $r = 0.622$; and 1103P, $r = 0.646$; $p < 0.01$; $n = 18$), confirming previous reports of a coordination between skin and pulp growth (*36*). Moreover, the skin/pulp ratio varied

Table 1. Rootstock (R) and Irrigation (I) Effects on Water Status and Vegetative Growth of Cabernet Sauvignon, in 2005 and 2006[a]

	2005				2006			
	Ψ_s (MPa)	g_s (mol m^{-2} s^{-1})	LAI (m^2/m^2)	PW (kg/vine)	Ψ_s (MPa)	g_s (mol m^{-2} s^{-1})	LAI (m^2/m^2)	PW (kg/vine)
				R ($n = 27$)				
SO4	−0.96	0.46 a	2.83 b	0.95	−0.93	0.57	3.11 b	0.94 b
1103P	−1.04	0.36 b	3.22 a	1.07	−0.97	0.57	3.69 a	1.55 a
				R × I ($n = 9$)				
SO4								
NI	−1.28 c	0.16 b	2.76	0.85	−1.13 b	0.40 b	2.94	0.84
DI	−0.98 b	0.54 a	2.84	0.85	−0.91 a	0.63 a	3.16	0.97
FI	−0.63 a	0.68 a	2.88	1.15	−0.74 a	0.68 a	3.16	1.00
1103P								
NI	−1.39 b	0.12 b	2.99 b	0.90	−1.24 b	0.32 b	3.28 b	1.04 b
DI	−0.93 a	0.49 a	3.19 ab	1.10	−0.91 a	0.67 a	3.79 ab	1.88 a
FI	−0.80 a	0.54 a	3.47 a	1.21	−0.77 a	0.70 a	4.01 a	1.73 a

[a] Adapted from ref *17*. In the same column, statistically significant differences between rootstocks ($n = 27$) and irrigation treatments within rootstocks ($n = 9$) are indicated by different letters ($p < 0.05$). $n = 9$ and 3, respectively. Ψ_s, midday stem water potential; g_s, midday stomatal conductance; LAI, leaf area index; PW, pruning weight; NI, non-irrigated; DI, deficit irrigated; FI, full irrigated. Data are combined over three samplings per year, except for PW.

Table 2. Year (Y), Rootstock (R), and Irrigation (I) Effects on Reproductive Growth Parameters of Cabernet Sauvignon at the Ripeness Stage[a]

	berry fresh weight (g)	skin fresh weight (g)	skin/berry weight ratio	seeds fresh weight per berry (mg)	seeds/berry weight ratio	total soluble solids (g/L)
			Y (n = 18)			
2005	1.08 a	0.22 a	20.6	75 a	7.1	271
2006	0.92 b	0.19 b	21.1	62 b	6.9	274
			R (n = 18)			
SO4	0.99	0.21	21.4	67	6.8	280 a
1103P	1.01	0.20	20.3	71	7.1	265 b
			R × I (n = 6)			
SO4						
NI	0.89 b	0.18	20.7	63	7.1 a	294 a
DI	0.98 ab	0.22	23.0	71	7.4 a	277 ab
FI	1.09 a	0.22	20.4	66	6.1 b	270 b
1103P						
NI	0.84 b	0.16 b	19.8	65 b	7.9 a	273
DI	1.04 a	0.20 ab	19.7	69 b	6.7 b	263
FI	1.15 a	0.24 a	21.4	78 a	6.8 b	259

[a] NI, non-irrigated; DI, deficit irrigated; FI, full irrigated. In the same column, statistically significant differences between years (n = 18), rootstocks (n = 18), and irrigation treatments within rootstocks (n = 6) are indicated by different letters ($p < 0.05$).

Table 3. Linear Regression Coefficients between Stem Water Potential and Reproductive Growth Parameters of Cabernet Sauvignon at the Ripeness Stage[a]

	D1		D2		D3	
parameter	SO4	1103P	SO4	1103P	SO4	1103P
yield (kg)	0.29	0.51[b]	0.25	0.67[c]	0.41	0.63[c]
cluster fresh weight (g)	0.41	0.45	0.42	0.62[c]	0.55[b]	0.51[b]
berry fresh weight (g)	0.43	0.81[d]	0.32	0.65[c]	0.57[b]	0.76[d]
skin fresh weight (g)	0.47	0.71[d]	0.32	0.45	0.41	0.53[b]
skin/berry weight ratio	0.12	0.10	0.07	0.04	−0.09	−0.06
skin/pulp weight ratio	0.11	0.06	0.05	0.08	−0.11	−0.11
seeds fresh weight per berry (g)	0.21	0.57[b]	0.10	0.24	0.26	0.23
seeds/berry weight ratio	−0.27	−0.40	−0.26	−0.52[b]	−0.40	−0.65[c]
total soluble solids (g/L)	−0.48[b]	−0.32	−0.54[b]	−0.34	−0.53[b]	−0.45

[a] D1, bunch closure; D2, veraison; D3, harvest. Measurements were taken during 2005 and 2006 (n = 18). [b] Significance of the regression line at $p < 0.05$. [c] Significance of the regression line at $p < 0.01$. [d] Significance of the regression line at $p < 0.001$.

independently to the berry weight (SO4, $r = -0.310$, $p = 0.210$; and 1103P, $r = -0.210$, $p = 0.402$; $n = 18$; data not shown), although an effect of berry size on the skin/pulp ratio has previously been reported in other studies (*37*).

Skin growth was affected by the irrigation regime only in the 1103P-grafted vines, with increasing values from NI to FI (**Table 2**). Upon 1103P, the skin fresh weight was highly sensitive to variations of pre-veraison water deficit, while no correlation was found in SO4 on any stage (**Table 3**). Although the water deficit is often associated with an increase in the skin proportion per berry, in this study, the skin/berry weight ratio was not affected by irrigation on any rootstock (**Table 2**), in agreement with the lack of correlation between this parameter and berry weight.

Although a strong correlation between berry size and average seed number per berry has been reported (*36*), our data showed that berry growth depended more upon total seed mass per berry (SO4, $r = 0.641$; and 1103P, $r = 0.595$; $p < 0.01$; $n = 18$) than on average seed number (SO4, $r = 0.481$, $p < 0.05$; and 1103P, $r = 0.428$, $p = 0.076$; $n = 18$), as previously reported in Cabernet Sauvignon (*37*).

The seed number per berry was similar among irrigation regimes (respectively for NI and FI, 1.68 and 1.61 in SO4; $p = 0.366$, and 1.85 and 1.84 in 1103P; $p = 0.317$), while it differed between rootstocks, being higher in 1103P (1.67 and 1.82 for SO4 and 1103P, respectively; $p < 0.05$). The seed number per berry is determined at set (*38*), thus, before the onset of irrigation treatments. However, differences in soil−water accessibility between rootstocks could be responsible for the higher seed number in 1103P-grafted vines. In agreement with skin mass, the total seed mass per berry was affected by water limitation only in 1103P, with FI vines having the highest values (*39*), whereas no differences between irrigation regimes were evident for SO4 (**Table 2**). Moreover, upon 1103P, the total seed mass per berry was primarily determined by pre-veraison (D1) water conditions (**Table 3**), showing increasing values with increasing Ψ_s (i.e., larger seeds under higher water availability). This is in agreement with the fact that seed growth is largely completed at or soon after veraison (*6*). However, the proportion of seeds in total berry mass decreased with irrigation, with higher values in the NI vines on both rootstocks (**Table 2**). The seed/berry weight ratio was mostly controlled by late-season water deficit (D3) but only on 1103P (**Table 3**), presumably as a result of the reduced growth of mesocarp tissues under drought conditions, because seed growth is practically insensitive to a late-season water deficit (*37*).

Averaged over years and irrigation regimes, soluble solids at harvest were higher in the SO4-grafted vines (**Table 2**). Moreover, differences in total soluble solids among irrigation treatments were significant only in SO4, with NI berries showing the highest levels (*40*). On this rootstock, a negative correlation was found between must soluble solids at harvest and Ψ_s in all stages (**Table 3**). The accumulation of soluble solids decreased linearly with berry size in both rootstocks (SO4, $r = -0.579$; and 1103P, $r = -0.479$; $p < 0.05$; $n = 18$). However, the higher soluble solids in SO4 vines, despite the similar berry size with 1103P ones, suggests that a non-size-related effect of water deficit on must sugar may also exist, probably associated with a more favorable partitioning of photosynthates to developing berries (*41*).

Skin Phenolic Concentration. The analytical anthocyanin composition of skin extracts is presented in **Table 4**. Five different anthocyanins [3-*O*-monoglucosides of delphinidin (Dp), petunidin (Pt), paeonidin (Pn), and malvidin (Mv) and malvidin 3-*O*-coumarateglucoside (MvC)] were determined, with levels of cyaniding 3-*O*-monoglucoside being too low to quantify (*12*).

Mv was the major anthocyanin determined (*42*), which also contained an important amount of its coumarate derivative, representing 75 and 10% of the total anthocyanin concentration,

Table 4. Year (Y), Rootstock (R), and Irrigation (I) Effects on Skin Anthocyanins (mg/g Skin Fresh Weight) of Cabernet Sauvignon Berries at the Ripeness Stage

	Dp	Pt	Pn	Mv	MvC
			Y (n = 18)		
2005	0.20	0.20	0.12 b	2.52 b	0.13 b
2006	0.26	0.24	0.19 a	3.03 a	0.48 a
			R (n = 18)		
SO4	0.23	0.22	0.15	2.66	0.15 b
1103P	0.23	0.22	0.16	2.89	0.46 a
			R × I (n = 6)		
SO4					
NI	0.23	0.23	0.14	3.19 a	0.17
DI	0.26	0.23	0.17	2.58 ab	0.14
FI	0.20	0.19	0.15	2.22 b	0.13
1103P					
NI	0.28	0.27	0.16	3.69 a	0.24 b
DI	0.27	0.25	0.18	2.68 b	0.67 a
FI	0.15	0.15	0.15	2.31 b	0.48 ab

Dp, delphinidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin- 3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; MvC, malvidin 3-*O*-coumarateglucoside; NI, non-irrigated; DI, deficit irrigated; FI, full irrigated. In the same column, statistically significant differences between years (n = 18), rootstocks (n = 18), and irrigation treatments within rootstocks (n = 6) are indicated by different letters ($p < 0.05$).

respectively. Dp, Pt, and Pn were present in lower amounts, not exceeding 300 mg/g of skin fresh weight, and together accounted for approximately 15% of the total anthocyanin concentration. Skin anthocyanins accounted for the major part of the variation of total skin polyphenols ($r = 0.794$; $p < 0.001$; $n = 36$).

The season affected Mv and MvC amount in skins, with 2005 showing lower overall concentrations than 2006 (**Table 4**). For most of the compounds detected, the rootstock did not impact anthocyanin concentrations. The exception was a lower amount of MvC in the SO4-grafted vines (**Table 4**).

Water availability affected the Mv concentration in skin tissues (**Table 4**), with NI vines having the higher amounts in both rootstocks (*14*). On the contrary, the irrigation regime did not affect the least abundant anthocyanins (Dp, Pt, and Pn). In pooled data over years and rootstocks ($n = 12$), the total anthocyanin concentration, calculated as the sum of individual compounds, was higher in NI vines (4.29 mg/g skin fresh weight compared to 3.07 mg/g in FI; $p < 0.05$). On the contrary, the total anthocyanin amount per berry was similar among irrigation treatments (0.71, 0.77, and 0.70 mg/berry, respectively, in NI, DI, and FI; $p = 0.484$). This result is most likely related to the higher skin weight of berries in irrigated vines (**Table 2**), compensating for their lower anthocyanin concentrations. According to the extractability assay (**Figure 1**), the anthocyanin concentration of the must was higher in NI vines compared to FI ones in both rootstocks. Because the skin/berry ratio was not affected by irrigation (**Table 2**), the higher anthocyanin concentration of the must in NI was entirely due to the higher anthocyanin concentration of the skins compared to DI and FI.

Our findings suggest that the water deficit exerts a direct positive effect on anthocyanin biosynthesis and especially on Mv. This is supported by recent evidence of an upregulation of the specific anthocyanin biosynthetic gene *UFGT* (UDP-glucose: flavonoid 3-*O*-glucosyltransferase) as well as genes coding for flavonoid 3′,5′ hydroxylase (*F3′5′H*) and *O*-methyl transferase (*OMT*), both involved in Mv biosynthesis (*43*). The anthocyanin concentration in skin tissues was strongly correlated to Ψ_s at early

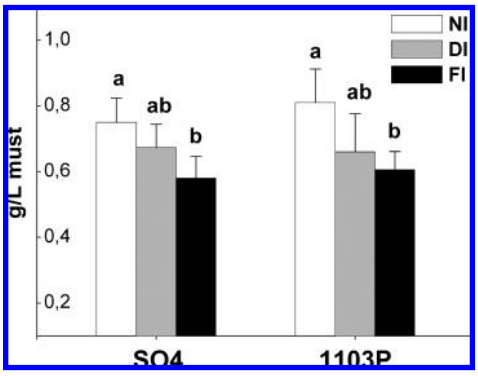

Figure 1. Effect of irrigation on total anthocyanins of Cabernet Sauvignon grafted onto SO4 and 1103P, estimated by the extractability assay; NI, non-irrigated; DI, deficit irrigated; FI, full irrigated. Means are combined over years (n = 6). Means labeled with a different letter within a rootstock are significantly different ($p < 0.05$).

Table 5. Linear Regression Coefficients between the Stem Water Potential and Phenolic Concentration of Cabernet Sauvignon Skins and Seeds at the Ripeness Stage[a]

	D1		D2		D3	
	SO4	1103P	SO4	1103P	SO4	1103P
skin (mg/g of skin fresh weight)						
anthocyanins	−0.59[b]	−0.53[c]	−0.45	−0.16	−0.45	−0.37
tannins	−0.38	−0.33	−0.22	−0.07	−0.15	−0.10
total phenolics	−0.32	−0.51[c]	−0.26	−0.16	−0.34	−0.35
seed (mg/g of seed fresh weight)						
flavan-3-ol monomers	0.41	0.37	0.54[c]	0.57[b]	0.44	0.70[b]
tannins	0.39	0.12	0.32	0.00	0.29	0.09
total phenolics	0.40	0.39	0.50[c]	0.59[b]	0.38	0.72[d]

[a] D1, bunch closure; D2, veraison; D3, harvest. Measurements were taken during 2005 and 2006 (n = 18). [b] Significance of the regression line at $p < 0.01$. [c] Significance of the regression line at $p < 0.05$. [d] Significance of the regression line at $p < 0.001$.

water deficits (**Table 5**) on both rootstocks (*11*). According to Castellarin et al. (*44*), the expression of genes of the flavonoid pathway was triggered earlier in Cabernet Sauvignon grapevines submitted to an early water deficit (i.e., after berry set) compared to those submitted to a late one (post-veraison), thus accelerating the onset of anthocyanin biosynthesis.

Tannins explained a smaller although significant part ($r = 0.540$; $p < 0.01$; $n = 36$) of skin total phenolics variation. Skin tannins were not affected by either rootstock (3.88 and 4.28 mg/g of skin fresh weight for SO4 and 1103P, respectively; $p = 0.276$) or irrigation (**Figure 2**), except for a lower amount per berry in NI vines. Moreover, tannins were not correlated to the water-deficit intensity on either rootstock (**Table 5**). Contrary to our results, a decreasing water supply has been reported to increase skin proanthocyanidins in Cabernet Sauvignon berries (*6*) by altering berry size but not flavonoid biosynthesis (*4*). In Shiraz, proanthocyanidin biosynthesis was stimulated under late water deficit, with an increase in the degree of tannin polymerization (*14*). However, tannins represent a different portion of total polyphenols in each cultivar and greatly depend upon the extraction method applied (*45*).

The levels of total skin polyphenols were not affected by rootstock cultivar (26.9 and 26.9 mg/g of skin fresh weight for SO4 and 1103P, respectively; $p = 0.985$). On the contrary, the total polyphenol concentration of skin tissues was higher in NI vines compared to DI and FI ones, only in 1103P-grafted vines (**Figure 2**). For this rootstock, a negative correlation was found

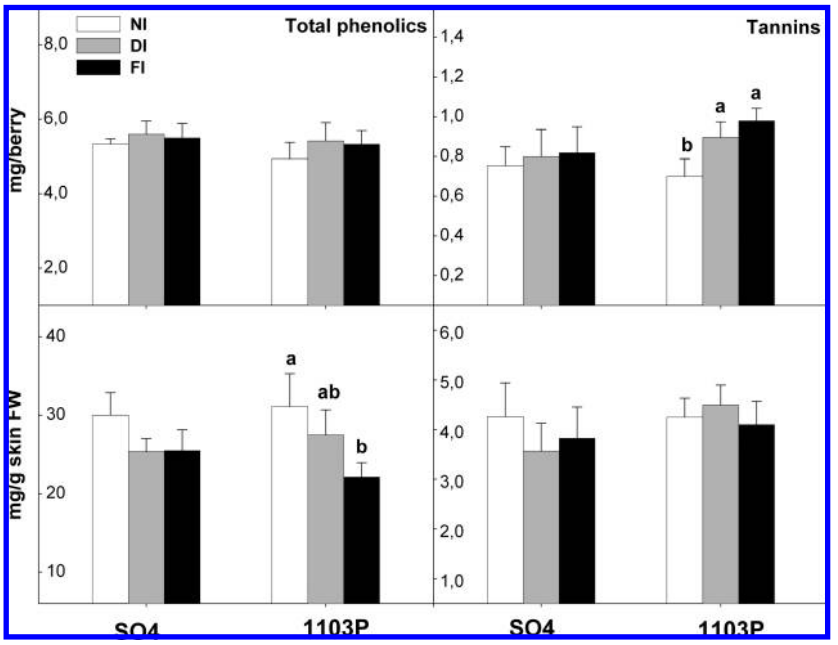

Figure 2. Effect of irrigation on skin total phenolics and tannins of Cabernet Sauvignon, grafted onto SO4 and 1103P; NI, non-irrigated; DI, deficit irrigated; FI, full irrigated. Means are combined over years ($n = 6$). Means labeled with a different letter within a rootstock are significantly different ($p < 0.05$).

with an early water deficit (D1) similarly to anthocyanins (**Table 5**). The total per berry polyphenols did not differ among irrigation treatments (**Figure 2**).

Variation in the phenolic concentration of berry skins is often interpreted from changes in the exposure environment of grapes, affecting light conditions. Sunlight exposure of grapes was reported to promote the accumulation of anthocyanins [and particularly that of Mv (*46*)] and skin procyanidins (*47*). Moreover, modification of the grape microclimate is often associated with differences in vine vigor (*4*), with medium-vigor zones tending to have berries with higher anthocyanin (*10*) and procyanidin (*48*) levels.

In our work, both rootstock (SO4 < 1103P) and irrigation (NI < DI < FI) treatments strongly differed in vegetative vigor parameters (**Table 1**) but differences in anthocyanin and total phenolic concentration of skins were observed only among irrigation regimes. Moreover, skin anthocyanins, tannins, and total phenolics were not correlated with mean season LAI or PW (**Table 6**). Therefore, vine vigor is less likely to explain the variation in skin flavonoids, although it is difficult to assess whether canopy structure effects on berry phenolic composition are related to a modification of the grape microclimate or to other factors (e.g., nutrient or carbon partitioning). It is also possible that, under the elevated midday temperatures of the study area, the effects of light on phenolic composition might be greatly undermined by extreme berry temperature (*49*).

Seed Phenolic Concentration. For the examination of grape seed extracts, five representative flavan-3-ol monomers were chosen (**Table 7**): catechin (C), epicatechin (EC), epicatechin 3-*O*-gallate (ECG), epigallocatechin 3-*O*-gallate (EGCG), and epigallocatechin (EGC). The most abundant polyphenol was C, accounting for approximately 40% of the total monomer concentration of seeds, followed by EC (30%) and ECG (25%) and a minor contribution of EGCG and EGC (*39*).

Total flavan-3-ol monomers explained the major part of the variation of total seed polyphenols ($r = 0.854$; $p < 0.001$; $n = 36$). Contrary to skin tannins, seed tannins were not correlated with the total polyphenol concentration of seeds ($r = 0.089$; $p = 0.607$; $n = 36$). The lack of correlation between tannins and total phenolics in the seeds is probably related to the analytical method used in this work. The potein precipitation is only measuring a subset of total tannins, notably those with more than four subunits (*50*). Hence, in berry seeds, where polymerized procyanidins represent a lower proportion of total phenolic compounds compared to the skins, tannins are expected to explain a smaller part of the variation of the total phenolic concentration.

Table 6. Linear Regression Coefficients between Mean Season Leaf Area Index (LAI) and Pruning Weight (PW) and Phenolic Concentration of Cabernet Sauvignon Skins and Seeds at the Ripeness Stage[a]

	LAI (m^2/m^2)	PW (kg/vine)
skin (mg/g skin fresh weight)		
anthocyanins	−0.04	−0.10
tannins	0.03	0.02
total phenolics	−0.07	−0.09
seed (mg/g fresh weight)		
flavan-3-ol monomers	0.51[b]	0.63[b]
tannins	0.11	0.17
total phenolics	0.53[b]	0.65[b]

[a] Measurements were taken during 2005 and 2006 ($n = 36$). [b] Significance of the regression line at $p < 0.001$.

The year did not affect the polyphenol concentration of seeds (**Table 7**). On the contrary, rootstock cultivar affected individual flavan-3-ol levels, with 1103P showing higher values, except for C (**Table 7**). Vines grafted on 1103P also showed higher total seed phenolics (9.49 mg/g of seed fresh weight compared to 7.94 mg/g for SO4; $p < 0.05$) but similar tannins to SO4 ones (19.5 and 18.4 mg/g of seed fresh weight for SO4 and 1103P, respectively; $p = 0.337$).

The C concentration in seeds was higher in the irrigated vines on both rootstocks, while the other monomers had similar amounts among irrigation treatments (**Table 7**). The total flavan-3-ol amount per seed fresh weight (calculated as the sum of individual monomers) was also higher in FI vines compared to NI ones, on both rootstocks (**Figure 3**). A similar trend was observed for the total flavan-3-ol amount and total seed phenolics when results were calculated per berry, despite the fact that seed contribution to total berry weight was higher in non-irrigated

vines (**Table 2**). Decreasing Ψ_s values (higher water deficit) during the ripening period (D3) were strongly associated with lower total flavan-3-ol monomers and total phenolics per seed FW (**Table 5**), and this effect was more pronounced in 1103P-grafted vines. In agreement with our findings, Kennedy et al. (*6*) reported that flavan-3-ol monomers of Cabernet Sauvignon seeds declined drastically during berry ripening and their rate of loss increased under minimal water availability. On the contrary, seed tannin composition showed an irregular pattern in relation to water status (**Figure 3**), while no correlation with the water deficit intensity was found for either rootstock [**Table 5**; (*39*)].

Table 7. Year (Y), Rootstock (R), and Irrigation (I) Effects on Seed Flavan-3-ol Monomers (mg/g of Seed Fresh Weight) of Cabernet Sauvignon Berries at the Ripeness Stage[a]

	C	EC	ECG	EGCG	EGC
			Y (n = 18)		
2005	1.24	1.00	0.81	0.10	0.03
2006	1.45	1.14	0.90	0.11	0.02
			R (n = 18)		
SO4	1.21	0.91 b	0.75 b	0.08 b	0.02 b
1103P	1.48	1.23 a	0.96 a	0.13 a	0.03 a
			R × I (n = 6)		
SO4					
NI	0.80 b	0.68	0.59	0.07	0.02
DI	1.24 ab	1.00	0.83	0.08	0.02
FI	1.59 a	1.04	0.83	0.09	0.02
1103P					
NI	1.13 b	1.05	0.86	0.11	0.03
DI	1.54 ab	1.26	0.96	0.11	0.03
FI	1.77 a	1.37	1.05	0.11	0.03

[a] C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-*O*-gallate; EGCG, (−)-epigallocatechin-3-*O*-gallate; EGC, (−)-epigallocatechin; NI, non-irrigated; DI, deficit irrigated; FI, full irrigated. In the same column, statistically significant differences between years (n = 18), rootstocks (n = 18), and irrigation treatments within rootstocks (n = 6) are indicated by different letters (p < 0.05).

In the conditions of this study, vine vegetative growth affected the levels of total flavan-3-ol monomers and total seed polyphenols at harvest, as manifested by the highly significant positive correlation of both variables with mean season LAI and PW (**Table 6**), possibly by modifying cluster microclimate (*4*, *10*). In contrast, seed tannins were not affected by vine vigor. With regard to cluster microclimate effects on seed phenolics, reported data are variable. In a recent work, bunch shading did not affect the levels of seed flavan-3-ol monomers at harvest (*46*), possibly because of the lower sensitivity to light exposure of the specific flavanol biosynthesis genes, anthocyanin reductase (*ANR*) and leucoanthocyanin reducatase (*LAR*), in the seeds as compared to the skins (*47*). On the contrary, Ristic et al. (*51*) reported that shaded fruit had increased seed tannins at ripeness but mainly as a result of increased seed weight. However, our findings are in agreement with previous studies reporting a higher amount of total flavan-3-ol monomers in seeds of high vigor vines (*52*). Our results suggest that rootstock and irrigation effects on seed polyphenols (mainly monomeric flavan-3-ols) were probably mediated through their respective effect on canopy growth and microclimate, with increased levels in the more vigorous treatments (1103P compared to SO4 and FI compared to NI).

Berry Aroma Potential. The season affected the total aroma glycosides of berries, estimated by the phenol-free glycosyl-glucose (PFGG), with higher levels per gram of fresh fruit in 2006 (**Table 8**). Where the data are pooled, both concentration and amount per berry of glycosylated volatile compounds were higher in grapes of 1103P-grafted vines (**Table 8**). The water deficit increased the PFGG per fresh weight, but no differences were found on a per berry basis (**Table 8**), probably because of the compensating effect of the higher berry size in the irrigated treatments. Our results are consistent with previous reports of increases in volatile secondary metabolites because of decreased

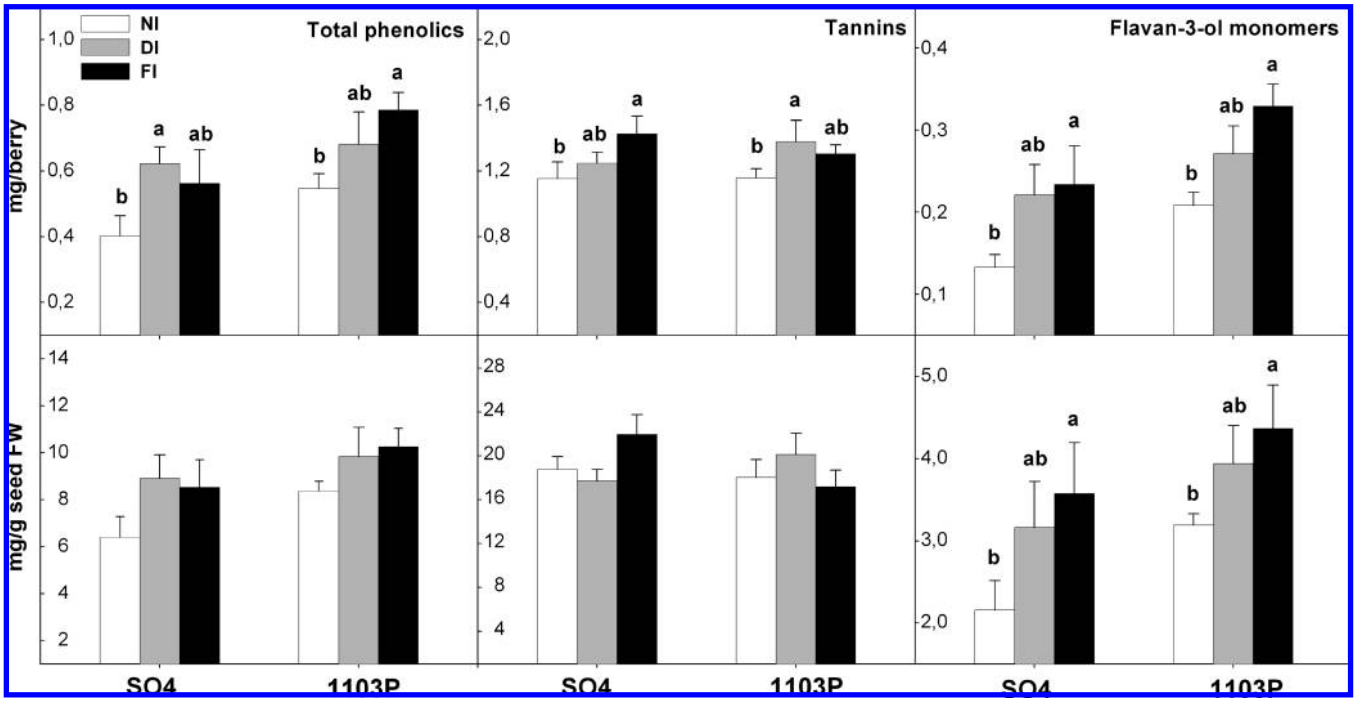

Figure 3. Effect of irrigation on seed total phenolics, tannins, and flavan-3-ol monomers of Cabernet Sauvignon, grafted on SO4 and 1103P; NI, non-irrigated; DI, deficit irrigated; FI, full irrigated. Means are combined over years (n = 6). Means labeled with a different letter within a rootstock are significantly different (p < 0.05).

Table 8. Year (Y), Rootstock (R), and Irrigation (I) Effects on PFGG of Cabernet Sauvignon Berries at the Ripeness Stage[a]

	PFGG	
	nmol/g of FW	nmol/berry
	Y (n = 18)	
2005	130.6 b	140.2
2006	161.5 a	146.3
	R (n = 18)	
SO4	134.8 b	132.9 b
1103P	157.3 a	153.6 a
	R × I (n = 6)	
SO4		
NI	164.8 a	149.9
DI	125.4 b	122.0
FI	114.2 b	123.0
1103P		
NI	177.2 a	144.4
DI	167.3 a	172.6
FI	127.4 b	144.8

[a] NI, non-irrigated; DI, deficit irrigated; FI, full irrigated; FW, fresh weight; PFGG, Phenol-Free Glycosyl-Glucose. In the same column, statistically significant differences between years (n = 18), rootstocks (n = 18), and irrigation treatments within rootstocks (n = 6) are indicated by different letters ($p < 0.05$).

water availability (*11*). According to Bindon et al. (*53*), vine water deficits in Cabernet Sauvignon increased total C_{13}-norisoprenoids, independent of water-deficit-induced changes in berry size. Modification of the canopy microclimate has been reported to increase the levels of grape glycoconjugates in several cultivars (*2*, *9*). However, it is unlikely that the higher levels of PFGG observed under limited water supply in this study are related to higher cluster exposure as a result of reduced vine vigor because an adverse trend was observed regarding rootstocks, with higher levels on the more vigorous 1103P. The total pool of glycoconjugates representing potential varietal aroma consists of a number of diverse volatile compounds within grape secondary metabolites (*8*). It is possible that observed differences among irrigation and rootstock treatments arose from differential responses of their specific metabolic pathways to water-related factors.

This 2 year experimentation demonstrated that moderate water deficit improved the phenolic and aroma potential of Cabernet Sauvignon grapes, under the semi-arid conditions of central Greece. Limited water availability was associated with higher levels of skin anthocyanins and a lower contribution of the seeds to the total pool of berry tannins. The effect of water availability on skin anthocyanins seemed to be mostly related to a positive effect of water deficit (especially pre-veraison) on anthocyanin biosynthesis rather than to an indirect effect of low water uptake on vine vigor or berry size. On the contrary, irrigation effects on seed monomeric flavan-3-ols were most likely related to changes in canopy development and microclimate, affecting the rate of flavan-3-ol decline during berry ripening. Results presented here are also important in evaluating rootstocks for dry-land conditions. Rootstock cultivars inducing higher vigor to the scion can result in a lower phenolic ripeness of the seeds at harvest, thereby affecting red wine quality. Additional knowledge is required to elucidate the dependence of each category of grape secondary metabolites upon water status and define more accurately whether water-deficit effects on grape attributes are mostly due to a direct impact on berry metabolism or an indirect effect related to alterations of vine vegetative and reproductive growth.

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