

EFFECTS OF POSTVERAISON WATER REGIME ON THE PHENOLIC COMPOSITION OF GRAPES AND WINES OF CV. AGIORGITIKO (*VITIS VINIFERA* L.)

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Abstract

Aims: Vine water deficit is widely accepted as a powerful means to control grape and wine attributes. However, quality improvement is often achieved at the expense of a reduction in yield, especially when water deficit conditions are applied during the preveraison period. The aim of the present work was to test an irrigation regime based on manipulating water availability from veraison to harvest, as a means to control berry and wine composition with minimum effect on reproductive growth parameters.

Methods and results: A field trial was conducted during two consecutive years (2007-2008) in Nemea, Southern Greece. Three irrigation treatments were applied on seven-year-old, vertical shoot positioned and spur pruned Agiorgitiko vines (*Vitis vinifera* L.), from veraison through harvest: irrigation at 70 % of crop evapotranspiration (ET_c) (I70), irrigation at 30 % of ET_c (I30) and non irrigated (NI). Irrigation amount produced significant differences in postveraison midday stem water potential pattern, especially during the drier year 2008. Yield was increased by irrigation in 2008, whereas berry growth was unaffected in both seasons. Berries of NI vines achieved higher total skin anthocyanin content in 2007, although individual anthocyanin levels were not affected by water regime. Irrigation effect on skin tannins was inconsistent but seed tannins were higher in I70 vines, with increased levels of catechin and epicatechin monomers. Among wine attributes, tannin concentration, but not anthocyanin, was mostly responsive to water deficit-induced changes in berry phenolic composition. The wines made from I70 grapes had a higher tannin content than those made from NI grapes.

Conclusions: The results presented show that postveraison water regime had a significant effect on skin anthocyanins and, more markedly, on seed tannins, without altering berry growth parameters. Especially for seed tannins, this effect appears to predominate over variations in climatic conditions between years.

Significance and impact of the study: This trial suggests that Agiorgitiko vines grown on the loamy soils of Nemea perform better under non irrigated conditions during the postveraison period since rainfed vines had improved phenolic composition (higher colour with lower contribution of seed tannins) without significant loss in productivity.

Key words: irrigation, berry growth, skin anthocyanins, seed flavan-3-ols, wine tannins

Résumé

Objectifs: Le déficit hydrique chez la vigne est reconnu comme un moyen puissant de contrôle de la composition chimique des raisins et des vins. Néanmoins, l'amélioration de la qualité ainsi obtenue est souvent associée à une réduction des rendements, en particulier dans le cas où la contrainte hydrique se manifeste avant véraison. L'objectif de ce travail a été de tester un régime d'irrigation basé sur la manipulation des conditions hydriques après véraison, dans le but de contrôler la composition de la baie et du vin tout en minimisant les effets sur la croissance de l'appareil reproductif de la vigne.

Méthodes et résultats: L'expérimentation a été réalisée en 2007 et 2008 en Némée, Grèce. Trois niveaux d'irrigation ont été appliqués depuis la véraison jusqu'aux vendanges. Les trois modalités d'irrigation ont abouti à une évolution différente du potentiel tige à la mi-journée pendant la période post-véraison. Le rendement par souche a été augmenté par l'irrigation seulement en 2008 mais la croissance des tissus de la baie est restée invariable. Les pellicules des raisins de NI ont montré une quantité plus élevée d'anthocyanes en 2007. Le contenu tannique des pellicules n'a pas été affecté par l'irrigation mais les tanins des pépins ont été supérieurs dans les baies de I70, avec notamment des taux élevés des monomères de catéchine et d'épicatéchine. Parmi les caractéristiques chimiques des vins expérimentaux, les tanins ont été plus affectés que les anthocyanes par le régime hydrique de la vigne. Les vins élaborés à partir des raisins I70 ont présenté une teneur en tanins plus élevée que les vins NI.

Conclusions: Les résultats présentés montrent que le régime hydrique de la période post-véraison a eu un effet marqué sur le contenu anthocyanique des pellicules et, notamment, le contenu tannique des pépins, sans pour autant altérer les paramètres de croissance de la baie.

Signification et impact de l'étude: Ce travail a démontré que la performance du cépage Agiorgitiko sur les terrains argilo-limoneux de Némée a été supérieure en l'absence d'irrigation pendant la période post-véraison. Les vignes non irriguées ont présenté une meilleure composition phénolique de la baie (davantage de couleur avec une moindre contribution des tanins des pépins) sans diminution significative de la production.

Mots clés: irrigation, croissance de la baie, anthocyanes de la pellicule, flavan-3-ols des pépins, tanins du vin

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INTRODUCTION

In Greece, although irrigation of grapevines is still forbidden by law, vineyard area under irrigation has been steadily increasing during the last two decades. Moreover, since temperature and rainfall patterns are predicted to change, increasing the risk of drought in semiarid viticultural regions (Jones *et al.*, 2005), supplemental irrigation will greatly affect the ability of existing varieties to ripen fruit.

Although irrigation to ensure full evapotranspirational demand is detrimental to wine quality (Matthews *et al.*, 1990), it is acknowledged that a moderate restriction of water availability may be beneficial for berry and wine composition (Van Leeuwen *et al.*, 2009). The desirable effects of water deficit are mostly indirect: reduced vegetative growth leading to improved canopy microclimate (Romero *et al.*, 2010) and carbohydrate partitioning to ripening berries (Petrie *et al.*, 2004; Intrigliolo and Castel, 2008), and smaller berry size leading to higher relative amounts of skin and seed in harvested fruit (Kennedy *et al.*, 2002; Roby *et al.*, 2004), thereby positively affecting colour and flavour extraction into wine (Matthews *et al.*, 1990; Chalmers *et al.*, 2010). However, recent works have also demonstrated a direct effect of water deficit on grape metabolism, especially on the biosynthesis of sensorially important secondary metabolites such as grape flavonoid compounds (Castellarin *et al.*, 2007a and 2007b; Deluc *et al.*, 2009). Among those, anthocyanins have been previously reported to increase with water deficit especially when water limitation is applied prior to veraison (Bindon *et al.*, 2011). On the contrary, skin tannins have been shown to be less responsive to water availability conditions (Ojeda *et al.*, 2002; Kennedy *et al.*, 2002), while limited evidence exists on the effect of drought on seed proanthocyanidins (Kennedy *et al.*, 2000; Koundouras *et al.*, 2009). Due to the positive effect of water deficit on grape phenolics, water deficits are considered as particularly effective in controlling berry quality in red winegrapes. However, highly coloured red grapes do not always produce the most intensely coloured wines (Holt *et al.*, 2008), a fact that is probably related to the extractability of anthocyanins from skins to the must during fermentation (Ortega-Regules *et al.*, 2006).

The effect of water deficit in grapevines largely depends on the timing, duration and intensity of the stress. During the last two decades, deficit irrigation strategies have been successfully

implemented in vineyards with the aim to maintain vines at some degree of water restriction for a prescribed part of the season (Santesteban *et al.*, 2011). Commonly, a deficit period is applied shortly after fruit set in order to reduce shoot and berry growth. Irrigation is resumed after veraison to avoid extreme water limitation that may impair leaf photosynthesis and assimilate translocation to the ripening berries (Intrigliolo and Castel, 2010).

However, preveraison water deficits are commonly associated with a significant loss in yield (Shellie, 2006). A severe water restriction between flowering and setting can reduce the number of berries per bunch due to abscission and desiccation of flowers (Hardie and Considine, 1976) while water stress during the first period of berry enlargement has an irreversible negative effect on berry growth (Ojeda *et al.*, 2001). On the contrary, late season (i. e., postveraison) deficits have a less direct impact on berry size (McCarthy, 1997) and final yield (Acevedo-Opazo *et al.*, 2010).

While yield limitation is acceptable in many prestigious European areas producing “Appellation of Origin” wines, in many grape growing areas of Greece a severe limitation in yield is not desirable because neither grape nor wine prices are high enough to compensate for a marked reduction in productivity. Therefore, in Greece, irrigation cutoff is commonly applied after veraison. The cultivar Agiorgitiko originates from the area of Nemea in Peloponnesus (Southern Greece) and is the most widely cultivated indigenous cultivar for the production of red wines in Greece. In the Nemea area, Agiorgitiko is traditionally grown on deep loamy soils without supplemental irrigation, such that changes in vine water conditions, especially during ripening, are mostly responsible for the existing variation in the sensorial properties of these wines (Koundouras *et al.*, 2006). The aim of the present work was to test an irrigation regime based on manipulating water availability from veraison to harvest, as a means to control berry and wine composition with minimum effect on reproductive growth parameters.

MATERIALS AND METHODS

1. Vineyard site and experimental design

A field trial was conducted during two consecutive years (2007-2008) in a 7-year-old vineyard in Nemea, Southern Greece (37° 79' N, 22° 61' E, 280 m), planted with cv. Agiorgitiko (*Vitis vinifera* L.) onto 1103 Paulsen (*V. rupestris* × *V. berlandieri*) at 4000 vines per ha (1.0 m × 2.5 m). The vineyard

was located on a deep clay soil (30 % sand, 25 % silt and 45 % clay). Vines were trained on a vertical trellis with three fixed wires and spur-pruned on a bilateral cordon system to 16 nodes per vine.

Three irrigation treatments were applied, starting at veraison (29th July 2007 and 5th August 2008) through harvest: irrigation at 70 % of crop evapotranspiration (ET_c) (I70), irrigation at 30 % of ET_c (I30) and non irrigated (NI). The three treatments were replicated four times in randomized blocks, with three rows per replication. Only the central vines of the middle row were used for measurements. ET_c was estimated from the potential evapotranspiration data (calculated by the Penman-Monteith method) obtained from an on-site iMETOS automatic weather station (Pessl Instruments GmbH, Weiz, Austria). Water was supplied by a drip irrigation system with two 2.7 L/h emitters per vine on either side of the trunk, positioned at 33 cm intervals along the pipe. The total amount of water applied per season for I30 and I70 was 28 and 78 mm in 2007 and 35 and 98 mm in 2008, respectively.

2. Vine water status

Vine water status was estimated by measurements of stem water potential (Ψ_s) using a pressure chamber, according to Choné *et al.* (2001). In each measurement set, four leaves of the inside part of the canopy were enclosed in plastic bags and covered with aluminium foil for at least 90 min before measurement, to allow equilibration of Ψ_s . Measurement of Ψ_s was performed at solar noon (12h30 to 13h30), on four cloudless days corresponding approximately to the pea size, bunch closure, veraison and mid-ripening stages.

3. Berry sampling and must analysis

Grapes were harvested at commercial harvest (11th September 2007 and 14th September 2008) for all treatments, and total yield per plant was weighed. Individual berry fresh weight (fw) was determined on a sample of 200 berries per plot. A sub-sample of 200 berries per plot was pressed and the must was analyzed for soluble solids (°Brix) by refractometry and total acidity by titration with 0.1 N NaOH.

4. Analysis of grape phenolic compounds

Phenolic compounds were analyzed in whole berries by using the analytical protocol of Iland *et al.* (2000). Briefly, 50 berries from each plot were transferred into a 125 mL plastic beaker and

homogenized with a Polytron at 25.000 rpm for 30 sec. Then 1 g of homogenate (in triplicate) was transferred into 10-15 mL centrifuge tubes and 10 mL of 50 % v/v aqueous ethanol pH 2 were added to each tube 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 1M HCl and mixed thoroughly. After 3 h, absorbance at 520 nm and 280 nm were recorded in a 10 mm cell. Anthocyanins (expressed as mg anthocyanins per g berry) were calculated from the absorbance measurement at 520 nm. Total phenolics (expressed as absorbance units per g berry weight) were calculated from the measurement of absorbance at 280 nm.

Seed and skin tannin concentration was evaluated using a protein precipitation assay according to the method described by Harbertson *et al.* (2002). From each plot, a 20-berry sample was processed for tannin extraction. A standard curve was prepared using (+)-catechin in the range of 25 to 300 µg. Tannin values for skin and seed extracts were obtained from the standard curve, thus values for tannin are reported in catechin equivalents. All analyses were performed in triplicate.

5. Determination of skin anthocyanins and seed flavan-3-ols by HPLC

A lot of 100 berries from each plot was weighed 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 three times in triplicate. Extracts were pooled, and this mixture was used for further analysis either immediately or after deep freezing in liquid nitrogen and storage at -70 °C not longer than 4 days. Anthocyanin analysis was carried out according to Arnous *et al.* (2002). Identification was based on comparing retention times of the peaks detected with those of original compounds and on UV – vis on-line spectral data. Quantification was performed by establishing calibration curves for each compound determined, using the standards. Results are expressed as mg malvidin per skin fw and per berry. All analyses were performed in duplicate.

Berries of the same lot were manually de-seeded, and the seeds were counted and weighed, frozen in liquid nitrogen and stored at -20 °C until analyzed. A lot of 2 g of seeds was ground with a pestle and a mortar and subsequently placed in a vial, 8 mL of ethyl acetate was added and 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 (Kallithraka *et al.*, 2006). 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.

6. Vinification

Winemaking trials were conducted in the Experimental Cellar of Fassoulis Nurseries, Leontio, Nemea during 2007 and 2008. Each treatment was performed in duplicate (200 kg of grapes in stainless steel tanks) to determine the repeatability of the differences among the three irrigation levels. After crushing and destemming, 60 mg/L SO₂ (as potassium metabisulfite), 3 g/hL pectolytic enzymes (Uvazym Couleur, Esseco, Italy), and 20 g/hL commercial lyophilized Vintage Red yeasts (Esseco, Italy), previously hydrated in water (15 min, 38 °C), were added. Beginning on the second day of fermentation, and for the following days, two punching downs per day were conducted. After 7 days of maceration, the wines were drained and transferred to other tanks and spontaneous malolactic fermentation was completed after approximately 3 weeks. The wines were racked, cold stabilized, supplemented with 50 mg/L SO₂ (as potassium metabisulfite), filtered and bottled until analysis, approximately 6 months after winemaking.

7. Wine analysis

Alcoholic degree and titratable acidity of the wines were determined according to the OIV methods (Office International de la Vigne et du Vin, 1990). Colour intensity and hue were determined by absorbance measurements at 420, 520 and 620 nm under 1 mm optical way, according to Glories (1984). Total anthocyanins were determined using the SO₂ bleaching method (Ribéreau-Gayon and Stonestreet, 1965) at 520 nm optical density in HCl media.

For tannin estimation, sample preparation (dilution) and protein precipitation assay was conducted according to the method described by Harbertson *et al.* (2002). A standard curve was prepared using (+)-catechin in the range of 25 to 300 µg in model wine (12 % v/v, tartaric acid 4 g/L, pH set at 3.5 using NaOH pellets). Tannin values in wines were obtained from the standard curve, thus values for tannin are reported in catechin equivalents.

Catechins were assessed according to the method proposed by Swain and Hillis (1959) as follows: vanillin was added to wine samples and then the absorbance was recorded at 500 nm. Quantification was performed by establishing a calibration curve by using various concentrations of catechin.

For gelatin index, 5 mL of cold soluble gelatin solution (70 g/L) were added to 50 mL of sample. After three days, the wine was centrifuged and total tannins (C) (g/L) were measured in the supernatant as described by Ribereau-Gayon and Stonestreet (1966). For the control sample (Co), 5 mL of water were added to the sample instead of the gelatin solution. The gelatin index was calculated as $100\frac{C-Co}{Co}$.

Total wine phenolics were determined using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). A 1 mL sample of red wine, diluted 1/10 with distilled water, was mixed with 5 mL of Folin-Ciocalteu reagent (previously diluted 10 times) and 20 mL of sodium carbonate solution (20 % w/v). After 120 min, the absorbance at 750 nm was measured in a 10 mm optical path. A calibration curve was plotted using solutions of gallic acid (0 - 50 - 100 - 250 - 500 - 1000 mg/L). All analyses were performed in triplicate.

8. Statistical analysis

Data were subjected to analysis of variance (ANOVA), using SPSS software (version 17.0, SPSS Inc., IL, USA). Only the mean of the 4 measurements per plot was used in data analysis. Comparison of means were 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

1. Vine water status

Monthly temperature was above the 1973-2005 average in both years, except for September 2007 and 2008 (Table 1). Mean temperatures for the

April to September period were 20.7°C and 20.4°C, respectively (Table 1), equivalent to a 6-month heat summation of 1964 and 1906 degree days, showing similar temperature conditions between the two years of the trial. However, total rainfall for the April to September period was 259 mm in 2007 (mostly due to an extremely rainy month of April), whereas 2008 was drier with only 93.2 mm during the same period.

Water deficit intensity depended on the climatic conditions of each year, as manifested by the evolution of midday Ψ_s (Figure 1). The higher spring rainfall in 2007 (together with the deep loamy soil of the experimental site) was probably responsible for a higher water replenishment of the soil profile, than during the drier 2008 season. As a consequence, soil water content was closer to field capacity at the beginning of measurements in 2007 (Ψ_s close to -0.6 MPa) than in 2008 (Figure 1). However, Ψ_s values during the preveraison period were maintained within the range of weak water deficit during both years (Van Leeuwen *et al.*, 2009) without significant differences among irrigation treatments (Figure 1).

Midday Ψ_s was affected by postveraison irrigation regime in both years. However, a steeper increase of water deficit was observed for NI and I30 vines during the ripening period of 2008, with Ψ_s values approximately 0.2 MPa lower than in I70 vines (Figure 1). Differences in water status between irrigated and non irrigated vines were generally smaller during 2007, probably because the higher recharge of the soil water capacity did not allow for a stronger differentiation among irrigation treatments. However, NI vines had lower Ψ_s values than the irrigated treatments during the last measurement of 2007 (Figure 1). According to Ψ_s critical values (Van Leeuwen *et al.*, 2009),

postveraison water deficit for the NI vines was weak to moderate in 2007 (minimum recorded $\Psi_s = -1.04$ MPa) and moderate to severe in 2008 (minimum recorded $\Psi_s = -1.19$ MPa). On the contrary, in I70, water restriction levels never

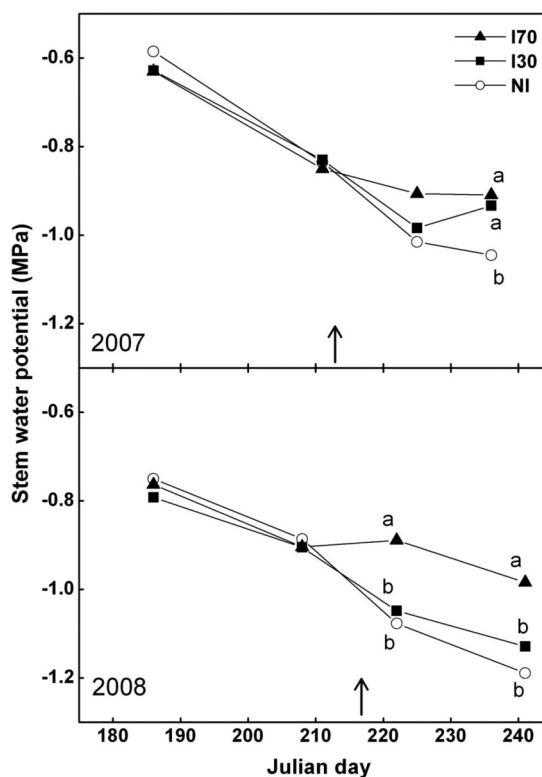


Figure 1 - Seasonal pattern of midday stem water potential of Agiorgitiko grapevines for the three irrigation treatments (NI, non irrigated; I30, irrigated at 30% ET_c ; I70, irrigated at 70% ET_c), during the growth seasons of 2007 and 2008. Each point represents the mean of four replicates. Statistically significant differences among irrigation treatments are indicated by different letters. Arrows at abscissa indicate veraison.

Table 1 - Monthly mean temperature (°C) and monthly total rainfall (mm) recorded from April to September during the two seasons of study (2007 and 2008).

Month	2007		2008		Average 1973-2005	
	temperature	rainfall	temperature	rainfall	temperature	rainfall
April	12.9	134.0	14.3	19.0	12.3	59.0
May	17.9	74.0	17.5	16.0	17.4	33.0
June	23.4	26.0	22.9	4.0	21.6	11.0
July	25.6	0.0	25.3	0.2	24.8	11.0
August	25.0	0.0	24.4	14.0	23.3	14.0
September	19.4	25.0	17.9	40.0	19.8	26.0
Mean or Total	20.7	259.0	20.4	93.2	19.9	154.0

Table 2 - Irrigation effects on yield components and must composition of Agiorgitiko grapes at ripeness stage, over two growth seasons; NI, non irrigated; I30, irrigated at 30% ET_c; I70, irrigated at 70% ET_c.

	Yield (kg/vine)	Berry weight (g)	Berry Skin weight (g)	Relative Skin weight (%)	Berry Seed weight (mg)	Seeds per berry	Relative Seed weight (%)	Total Soluble Solids (%)	Titrateable Acidity (g tartaric acid/L)	
2007	NI	2.08	1.27	0.37	0.29	64	1.60	0.050	21.30	7.35
	I30	2.00	1.32	0.43	0.33	63	1.66	0.048	21.10	7.39
	I70	2.11	1.41	0.43	0.31	68	1.54	0.048	21.40	7.56
2008	NI	1.79 b	1.63	0.32	0.20	66	1.68	0.041	23.90	5.51 b
	I30	2.02 ab	1.65	0.31	0.19	67	1.80	0.041	23.20	5.48 b
	I70	2.14 a	1.69	0.30	0.18	66	1.67	0.039	23.80	5.87 a
Y	ns	***	***	***	ns	*	***	***	***	
Y × I	*	ns	ns	ns	ns	ns	ns	ns	ns	

In each column, statistically significant differences between irrigation treatments within a year are indicated by different letters ($p < 0.05$). * and *** represent significance of the year (Y) effect and the year x irrigation (Y x I) interaction at $p < 0.05$ and $p < 0.001$, respectively; ns, not significant.

reached values inferior to -1.0 MPa, accepted as a threshold for the onset of mild water stress (Choné *et al.*, 2001; Intrigliolo and Castel, 2011).

2. Berry growth and must composition at harvest

Yield was similar among seasons with average values of 2.06 and 1.98 kg/vine for 2007 and 2008, respectively (corresponding to 8.24 and 7.92 T/ha). A significant season effect was observed for berry and skin weight since berry size was lower in 2007 than in 2008 with a higher relative contribution of skin and seed mass (Table 2), despite the overall higher water availability conditions in 2007.

Postveraison irrigation increased yield per vine in I70 as compared to NI vines only in the drier 2008 season (Table 2). However, postveraison water regime had no significant effect on berry growth. Similarly, skin weight per berry, total seed weight per berry and relative skin and seed weights (i. e., the ratios of skin/seed to berry weight) were unaffected by irrigation (Bowen *et al.*, 2011). Moreover, the absence of year x treatment interaction for all berry growth parameters showed that the limited effect of postveraison water restriction on berry growth was consistent, despite the slight differences in water availability conditions between years (Acevedo-Opazo *et al.*, 2010; Blanco *et al.*, 2010).

Berry growth can be effectively controlled by preveraison water stress (McCarthy, 1997). However, water deficit during the postveraison period has also been reported to decrease berry mass at harvest while increasing skin mass accumulation (Kennedy *et al.*, 2002). Recent work with Merlot (Bucchetti *et al.*, 2011) reported

reduced berry weight (but not skin mass) at harvest as a result of postveraison water deficit of similar magnitude, suggesting that the different response of Agiorgitiko berry weight to postveraison water stress could be due to cultivar differences.

Grapes had higher soluble solids and lower acidity during the drier 2008 season (Table 2). However, grape sugar accumulation was not influenced by postveraison water conditions in both years (Ojeda *et al.*, 2002). According to Sipiora and Gutierrez (1998), increased irrigation can delay soluble solids accumulation, mainly as a result of increased competition for carbon resources. These authors reported midday values of leaf water potential close to -1.6 MPa immediately after veraison. The water restriction imposed on NI vines in this study did not affect sugar accumulation in ripening berries, possibly because deficit was short and of moderate intensity (Santesteban *et al.*, 2011).

Titrateable acidity was increased by irrigation only in 2008 (Table 2), in agreement with previous reports (Bravdo *et al.*, 1985). Although individual concentrations of malic and tartaric acid were not measured, it can be hypothesized that the positive effect of irrigation on must acidity was related to lower malic acid respiration rates (Intrigliolo and Castel, 2008; Koundouras *et al.*, 2006).

3. Berry phenolic composition at harvest

The analytical anthocyanin composition of skin extracts is presented in Table 3. Six different anthocyanins (3-*O*-monoglucosides of delphinidin Dp, petunidin Pt, peonidin Pn, malvidin Mv, malvidin 3-*O*-coumarate-glucoside MvC, and malvidin 3-*O*-acetate-glucoside MvA) were determined, levels of cyanidin 3-*O*-mono-glucoside

Table 3 - Irrigation effects on skin anthocyanin (Dp, delphinidin-3-O-glucoside; Pt, petunidin-3-O-glucoside; Pn, peonidin-3-O-glucoside; Mv, malvidin-3-O-glucoside; MvC, malvidin 3-O-coumarate-glucoside; MvA, malvidin 3-O-acetate-glucoside) concentration (mg/100 g skin fresh weight) of Agiorgitiko grapes at ripeness stage, over two growth seasons; NI, non irrigated; I30, irrigated at 30% ET_c; I70, irrigated at 70% ET_c.

		Dp	Pt	Pn	Mv	MvC	MvA	Total
2007	NI	6.2	4.4	3.1	111.3	44.0	3.5	172.5
	I30	5.4	5.1	7.1	98.1	30.2	2.7	148.6
	I70	5.5	3.1	2.8	97.4	39.1	3.1	151.0
2008	NI	7.7	20.1	22.9	171.8	62.4	14.9	300.0
	I30	7.8	20.2	23.0	178.8	65.1	15.9	310.8
	I70	8.4	18.3	21.6	161.5	55.4	17.5	282.7
Y		**	***	***	***	**	***	***
Y × I		ns	ns	ns	ns	ns	ns	ns

In each column, statistically significant differences between irrigation treatments within a year are indicated by different letters (p<0.05). ** and *** represent significance of the year (Y) effect and the year × irrigation (Y × I) interaction at p<0.01 and p<0.001, respectively; ns, not significant.

Table 4 - Linear regression coefficients between average postveraison stem water potential and phenolic composition parameters of Agiorgitiko grapes at ripeness stage, over two growth seasons (n=12).

Parameter	2007	2008
Anthocyanins (mg/berry)	0.287	-0.661 *
Anthocyanins (mg/g berry fw)	0.061	-0.691 *
Sum of individual Anthocyanins (mg/g skin fw)	-0.242	-0.114
Skin tannins (mg/berry)	0.364	0.549
Skin tannins (mg/g berry fw)	0.287	0.232
Seed tannins (mg/berry)	0.844 ***	0.664 *
Seed tannins (mg/g berry fw)	0.881 ***	0.163
Sum of individual seed flavan-3-ols (mg/g seed fw)	0.217	0.802 **
Total berry phenolics (au/berry)	0.497	0.124
Total berry phenolics (au/g berry fw)	0.246	-0.412

*, ** and *** represent significance at p<0.05, p<0.01 and p<0.001, respectively; fw, fresh weight; au, absorbance units.

being too low to quantify. Malvidin was the major anthocyanin determined (Kallithraka *et al.*, 2005), representing on average (together with its coumarate and acetate derivatives) 91 % and 83 % of the total skin anthocyanins in 2007 and 2008, respectively. The amount of skin anthocyanin was affected by year, with 2008 showing an increased overall concentration compared to 2007 (Table 3), possibly because of the higher water deficit conditions. Previous works have reported that differences in the anthocyanin composition of Agiorgitiko grapes and wines among years are mostly mediated by variations in water availability conditions (Koundouras *et al.*, 2006).

Postveraison water regime did not affect the concentration of individual anthocyanins or their total amount in skin tissues at harvest (Table 3), with no significant year × treatment effect, although values tended to be lower in irrigated vines in both years. However, the total amount of anthocyanins measured by the extractability assay (Iland *et al.*, 2000) was higher in NI and I30 vines compared to I70 vines during the water limiting conditions of 2008, expressed both as mg/g berry fw and as mg/berry (Figure 2). When regressed on Ψ_s average (Table 4), anthocyanin content was negatively correlated to mean postveraison Ψ_s for the 2008 season ($r = -0.661$, $p < 0.05$ and $r = -0.691$, $p < 0.05$ for mg/berry and mg/g berry fw, respectively) as previously reported (Basile *et al.*,

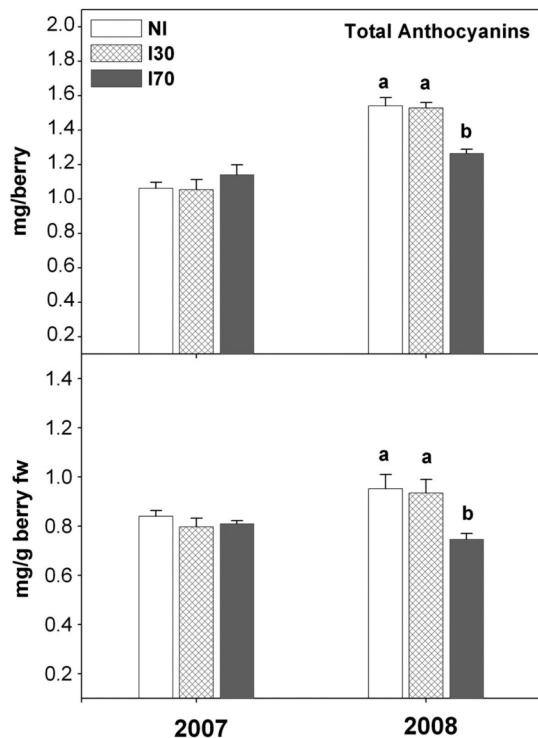


Figure 2 - Effect of postveraison irrigation on total anthocyanins of Agiorgitiko grapes at ripeness stage, over two growth seasons; NI, non irrigated; I30, irrigated at 30% ET_c; I70, irrigated at 70% ET_c. Vertical bars represent ± S.E. Means labelled with a different letter within a year are significantly different (p<0.05).

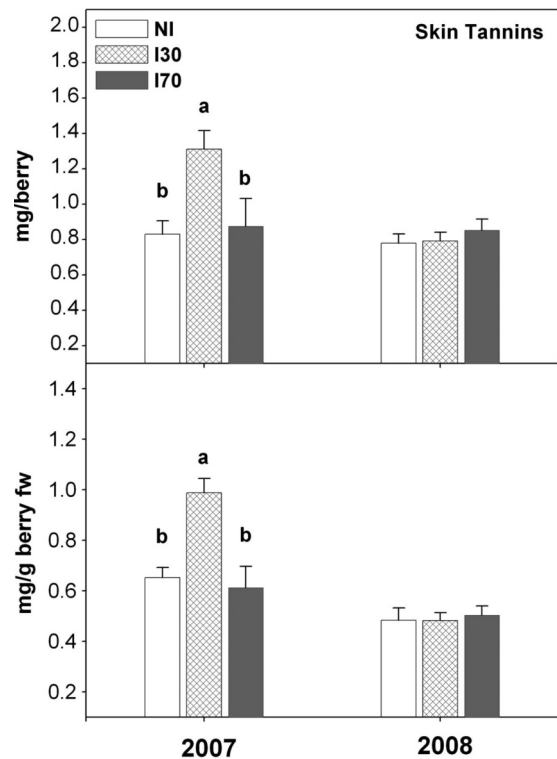


Figure 3 - Effect of postveraison irrigation on skin tannins of Agiorgitiko grapes at ripeness stage, over two growth seasons; NI, non irrigated; I30, irrigated at 30% ET_c; I70, irrigated at 70% ET_c. Vertical bars represent ± S.E. Means labelled with a different letter within a year are significantly different (p<0.05).

2011; Koundouras *et al.*, 2009). On the contrary, no correlation was observed between mean Ψ_s and the sum of individual skin anthocyanins (Table 4).

Previous studies have also highlighted that a water deficit period shortly after veraison, when most anthocyanin synthesis occurs, resulted in higher levels of skin anthocyanins at harvest (Santesteban *et al.*, 2011). A negative effect of water restriction on berry anthocyanins has been reported in the case of a severe water stress during the same period, possibly because of carbon source limitations (Girona *et al.*, 2009), but such conditions were not presented in our experiment.

The positive impact of moderate water deficit on anthocyanin concentration in grape berries is often attributed to the smaller berry size of the stressed vines (Esteban *et al.*, 2001; Roby *et al.*, 2004). Since growth of berry tissues was not affected by irrigation in the present study, our findings suggest that moderate postveraison water

deficits ($\Psi_s < -1.1$ MPa) might exert a direct positive effect on anthocyanin content of Agiorgitiko grapes, independently to berry size (Ojeda *et al.*, 2002; Roby and Matthews, 2004). Recent work by Castellarin *et al.* (2007a; 2007b) showed that water deficits imposed both prior and after veraison resulted in increased expression of key genes of the flavonoid biosynthetic pathway. However, the higher concentration of anthocyanins in the grapes of NI and I30 in 2008 might also be the result of water deficit-induced changes in cluster microclimate or assimilate translocation to the grapes due to reduced intra-plant competition (Smart *et al.*, 1985).

Skin tannins were affected by irrigation only in 2007 (Figure 3), with a higher amount in I30 vines. Moreover, skin tannins were not correlated to water deficit intensity (Table 4). In a previous study, late water deficit effects on Merlot skin tannins were few and inconsistent (Bucchetti *et al.*, 2011). The accumulation of tannin is essentially completed by

Table 5 - Irrigation effects on seed flavan-3-ol monomers and dimers (GA, gallic acid; C, (+)-catechin; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-O-gallate; EGCG, (-)-epigallocatechin-3-O-gallate; EGC, (-)-epigallocatechin; B₁, procyanidin B₁; B₂, procyanidin B₂) concentration (mg/100 g seed fresh weight) of Agiorgitiko grapes at ripeness stage, over two growth seasons; NI, non irrigated; I30, irrigated at 30% ET_c; I70, irrigated at 70% ET_c.

		GA	C	EC	ECG	EGCG	EGC	B1	B2	Total
2007	NI	2.0	76.0 b	52.7 b	5.9 ab	15.6 b	1.9	51.2	10.9	216.3 b
	I30	1.9	83.7 b	58.5 ab	5.6 b	19.9 b	2.5	20.1	14.6	236.7 ab
	I70	2.6	120.7 a	88.7 a	11.2 a	42.6 a	2.2	64.6	22.1	354.8 a
2008	NI	7.0	138.5 b	105.2	12.1	43.1	3.0 b	78.2 ab	22.4	409.4 b
	I30	6.2	135.7 b	102.5	14.8	48.7	2.6 b	69.5 b	20.1	400.2 b
	I70	8.0	203.5 a	138.6	14.1	61.0	4.1 a	96.8 a	24.4	550.4 a
Y		***	***	***	**	**	***	***	*	***
Y×I		ns	ns	ns	ns	ns	*	ns	ns	ns

In each column, statistically significant differences between irrigation treatments within a year are indicated by different letters ($p < 0.05$). *, ** and *** represent significance of the year (Y) effect and the year×irrigation (Y × I) interaction at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; ns, not significant.

Table 6 - Irrigation effects on the phenolic composition of Agiorgitiko wines, over two growth seasons; NI, non irrigated; I30, irrigated at 30% ET_c; I70, irrigated at 70% ET_c.

		Alcohol (%)	Titrateable Acidity (g/L)	pH	Colour Intensity	Hue	Total Anthocyanins (mg/L)	Tannins (g/L)	Catechins (mg/L)	Gelatin Index	Total Polyphenols (g/L)
2007	NI	11.6	4.0	3.63	8.2	0.71	431	0.84 b	284 b	21.5 b	1.39
	I30	12.0	4.4	3.62	8.3	0.70	411	1.14 ab	284 b	26.4 ab	1.47
	I70	11.9	4.4	3.74	8.2	0.72	429	1.33 a	323 a	30.0 a	1.50
2008	NI	13.2	5.1	4.05	8.8 a	0.63	449	0.78	273	22.0 b	0.75 c
	I30	13.0	5.0	4.05	9.0 a	0.63	437	0.79	254	23.3 b	1.38 a
	I70	13.1	4.9	4.10	7.2 b	0.70	424	0.75	263	32.7 a	1.19 b
Y		***	***	***	ns	*	ns	***	***	ns	***
Y×I		ns	ns	ns	ns	ns	ns	*	ns	ns	***

In each column, statistically significant differences between irrigation treatments within a year are indicated by different letters ($p < 0.05$). * and *** represent significance of the year (Y) effect and the year×irrigation (Y × I) interaction at $p < 0.05$ and $p < 0.001$, respectively; ns, not significant.

veraison, thus postveraison water deficits may have a limited effect on tannin biosynthesis. However, proanthocyanidin levels and degree of polymerization were found to increase under strong postveraison water deficit in other cultivars (Ojeda *et al.*, 2002; Kennedy *et al.*, 2002). It is possible that different responses may also be related to cultivar characteristics since tannins represent a different portion of total polyphenols in each variety. Moreover, measured values greatly depend on the extraction method applied (Seddon and Downey, 2008).

For the examination of grape seed extracts, eight representative polyphenols were chosen and their contents were determined: gallic acid (GA), the flavanol monomers catechin (C), epigallocatechin (EGC), epigallocatechin 3-O-gallate (EGCG), epicatechin (EC) and epicatechin 3-O-gallate (ECG), and the dimers procyanidins B1 and B2. The most abundant polyphenol was C, accounting for approximately 35 % of the total monomer concentration of seeds, followed by EC (approximately 25 %) and an important contribution (approximately 18 %) of B1 (Table 5).

Concentrations of individual flavan-3-ols were on average higher in 2008.

Irrigation regime during the ripening period significantly affected individual flavan-3-ol levels (Table 5), with I70 showing higher values for C (both years), EC, ECG, EGCG (only 2007), EGC and B1 (only 2008) expressed as mg/100g seed fw. The total flavan-3-ol amount per seed fw (calculated as the sum of free individual monomers and dimers) was also higher in I70 vines compared to NI (both years) and to I30 (only 2008). The year \times irrigation interaction was not significant, except for EGC (Table 5). For seed tannins, differences were small and only in 2008 did I70 vines show slightly higher levels per berry than I30 and NI vines (Figure 4).

Decreasing Ψ_s values (higher water deficit) during the ripening period were strongly associated with lower seed tannins (mg/berry) on both years ($r = 0.844$, $p < 0.001$ and $r = 0.664$, $p < 0.05$ for

2007 and 2008, respectively) while total seed flavan-3-ols were best correlated with 2008 postveraison water status ($r = 0.802$, $p < 0.01$) (Table 4). These results confirm previous evidence that water deficits during berry ripening significantly affect the amounts of flavan-3-ol monomers and condensed tannins in grape seeds (except for those with more than 30 subunits), by increasing their rate of loss during fruit maturation (Kennedy *et al.*, 2000). This finding may indicate a positive response of Agiorgitiko grapes to postveraison water deficit since flavan-3-ol monomers and oligo-polymers are mainly responsible for the bitterness and astringency of red wines (Brossaud *et al.*, 2001).

In seeds, tannin concentration was not correlated with total flavanol-3-ol concentration ($r = 0.241$, $p = 0.405$ for 2007 and $r = 0.271$, $p = 0.394$ for 2008; data not shown). The lack of correlation between tannins and flavanol-3-ols in the seeds is probably related to the fact that the analytical

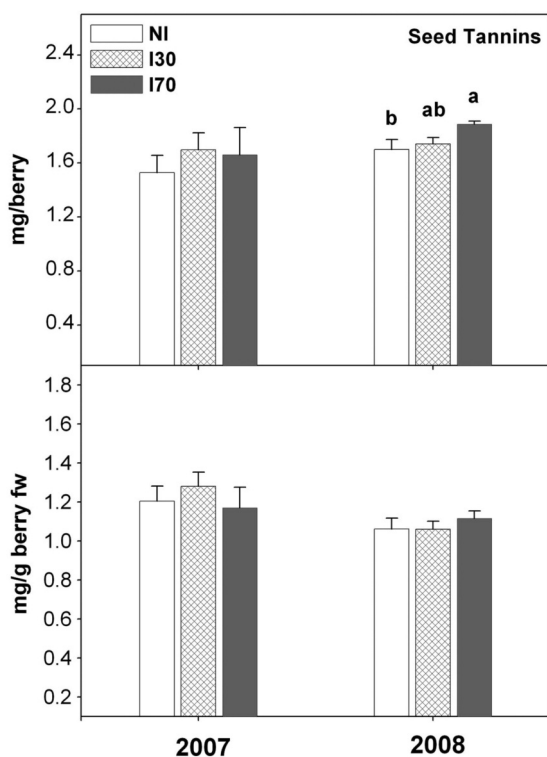


Figure 4 - Effect of postveraison irrigation on seed tannins of Agiorgitiko grapes at ripeness stage, over two growth seasons; NI, non irrigated; I30, irrigated at 30% ET_c; I70, irrigated at 70% ET_c. Vertical bars represent \pm S.E. Means labelled with a different letter within a year are significantly different ($p < 0.05$).

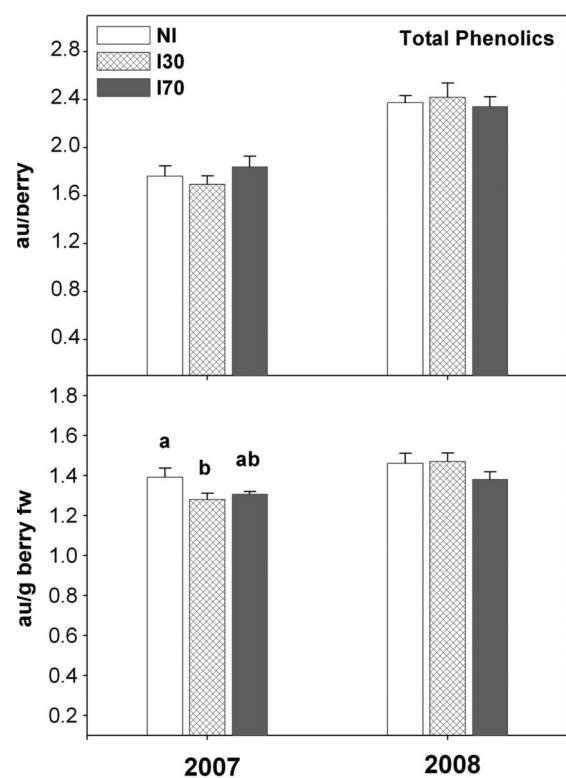


Figure 5 - Effect of postveraison irrigation on total phenolics of Agiorgitiko grapes at ripeness stage, over two growth seasons; NI, non irrigated; I30, irrigated at 30% ET_c; I70, irrigated at 70% ET_c. Vertical bars represent \pm S.E. Means labelled with a different letter within a year are significantly different ($p < 0.05$). au, absorbance units.

method used in this work for tannin estimation measures a subset of total tannins, notably those with more than 4 subunits (Adams and Harbertson, 1999).

Treatment effects on total berry polyphenols were inconsistent and detected only in 2007 (NI > I30) when expressed as concentration per berry fw (Figure 5). Moreover, total berry phenolics were not correlated to the intensity of postveraison water deficit (Table 4). Although measures of total phenolics have been systematically used as an indicator of tannin levels in grapes and wine (Harbertson and Spayd, 2006), in a comparative study over 36 grape cultivars, no correlation was found between the total berry phenolics and tannins by any of the methods employed (Seddon and Downey, 2008).

4. Wine phenolic composition

The wines from both 2007 and 2008 seasons showed similar values in alcohol concentration (Table 6), in agreement to must soluble solids content at harvest. Titrable acidity and pH were not significantly different between treatments, despite the higher titratable acidity observed in I70 grapes in 2008 (see Table 2), a fact that may be due to tartrate precipitation during fermentation.

Despite the higher levels of skin anthocyanins in NI vines in 2007 (see Table 3), the analysis of wine anthocyanins did not reveal differences among treatments (Table 6), although colour intensity was higher in NI and I30 wines in 2008. No differences were recorded in wine hue among treatments. Year effect on colour intensity and total anthocyanins was not significant but wine hue was higher in 2007, possibly because of the lower wine pH. No year x treatment effect was observed for either colour (intensity and hue) parameters (Table 6).

According to previous reports, the translation of phenolic composition from grape to wine is not always linear (Bindon *et al.*, 2011). Wine anthocyanin levels are influenced by many additional factors such as maceration time, fermentation temperature and pH, yeast strain and skin to flesh ratio (Chalmers *et al.*, 2010). The possibility that differences in wine phenolics in our study were due to the above factors can be excluded since all fermentations were made under similar conditions. Moreover, berry growth and wine pH were shown to be unaffected by irrigation.

However, phenolic extraction from grape skins during fermentation is greatly affected by skin cell-

wall composition and integrity (Ortega-Regules *et al.*, 2006). Previous reports have shown that grapes produced under water deficit conditions are characterized by lower anthocyanin extractability (Valdes *et al.*, 2009) due to a tighter skin cell-wall structure at harvest (Sivilotti *et al.*, 2005). Furthermore, wine colour formation is greatly affected by anthocyanin copigmentation with other phenolics, which in turn depends on their relative proportions in wine (Schwarz *et al.*, 2005). It is possible that in the case of I70 wines, the higher levels of seed phenols contributed to a faster stabilization of anthocyanin pigments during fermentation, leading to similar anthocyanin levels with NI wines.

Contrary to anthocyanins, wine tannin concentration was better related to tannin variations in berry tissues. As a general trend, tannin and catechin levels increased with irrigation in 2007 while no differences were observed in 2008. Since skin tannin variation was not consistent, this result could be attributed to the increased amount of extractable flavan-3-ol monomers and condensed tannins in the seeds of irrigated vines. Total wine polyphenols were higher in the irrigated treatments in 2008, but with higher values for I30 wines. Irrigation also increased the gelatin index of wines, suggesting a higher astringency of wines from irrigated treatments (Valdes *et al.*, 2009). Previously, tannin estimation by the protein precipitation method, used in this work, has been strongly correlated with perceived wine astringency by a trained sensory panel (Kennedy *et al.*, 2006).

CONCLUSIONS

The present work aimed at investigating the effects of postveraison water regime on multiple aspects of both berry and wine phenolic composition in cv. Agiorgitiko. Our results showed that, under the semiarid climate of Nemea, water conditions after veraison can affect the relative concentration of berry phenolic compounds at harvest maturity, without major effects on reproductive growth parameters and skin to flesh ratio. Postveraison irrigation resulted in lower levels of skin anthocyanins at harvest, especially during the drier season, while increasing the levels of flavan-3-ol monomers and condensed tannins at harvest. Seed flavan-3-ol monomers were particularly sensitive to the variation of vine water status during berry ripening, independently to differences in climatic conditions between years. The higher relative amount of seed tannins in the irrigated vines was

also translated to a higher tannin concentration and astringency of the experimental wines.

These results suggest that Agiorgitiko vines grown on the loamy soils of Nemea perform better under non irrigated conditions during the postveraison period since non irrigated vines had improved phenolic composition (higher colour with lower contribution of seed tannins) without significant loss in productivity. However, the role of supplemental irrigation during berry ripening remains to be confirmed under stronger water deficits, typical of the Mediterranean area. It would also be important for future studies to assess, apart from the amount of phenolic compounds present in fruit, the effect of vineyard conditions and management practices on tannin degree of polymerization and extractability into wine across sites, seasons and cultivars to better understand the relations between grape phenolic maturity and wine quality.

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