Influence of post-veraison water status on the phenolic composition of grapes and wines of cv. Agiorgitiko (*Vitis vinifera* L.) grown in Nemea.

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Abstract

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. A field trial was conducted during two consecutive years (2007-2008) in Nemea, Southern Greece. Three irrigation treatments were applied from veraison through harvest: irrigation at 70% of crop evapotranspiration (ETc) (IR), irrigation at 30% of ETc (DI) 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 IR 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 IR grapes had a higher tannin content than those made from NI grapes. 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

Introduction

It is generally accepted that a mild to moderate limitation of water availability may be beneficial for grape composition (van Leeuwen et al., 2009). The desirable effects of water deficit are due to reduced vegetative growth and more open canopies (Romero et al., 2010), improved carbohydrate partitioning (Intrigliolo and Castel, 2008), but also smaller berry size leading to increased skin to juice ratio and enhanced colour extraction during vinification (Kennedy et al., 2002).

The effect of water deficit depends on the timing, duration and intensity of the imposed water restriction. During the last two decades, regulated deficit irrigation (RDI) strategies have been implemented in vineyards with the aim to maintain vines at a variable degree of water deficit shortly after fruit set, in order to reduce shoot and berry growth (Santesteban et al., 2011). Normally, irrigation is resumed after veraison to avoid impaired leaf photosynthesis and assimilate translocation to the ripening berries (Intrigliolo and Castel, 2010). Neveretheless, preveraison water deficits are commonly associated with a significant loss in yield (Shellie, 2006) because water stress during the first period of berry enlargement has an irreversible negative effect on berry growth (Ojeda et al., 2001). On the contrary, postveraison water deficit has a less direct impact on berry size and final yield (Acevedo-Opazo et al., 2010).

In the Nemea area, a limitation in yield caused by deficit irrigation implementation is not desirable because neither grape nor wine prices are high enough to compensate for a reduction in yield. However, it has been proved that differences in the sensorial properties of Agiorgitiko wines are mostly due to the variation in water conditions among different sites (Koundouras et al., 2006). Therefore, the aim of the present work was to examine the efficacy of an irrigation regime based on manipulating water availability from veraison through harvest, as a means to control berry and wine composition with minimum effect on yield.

Materials and Methods

The experiment was conducted during two consecutive years (2007-2008) in a 7-year-old vineyard in Nemea, Southern Greece, planted with cv. Agiorgitiko (*Vitis vinifera* L.) onto 1103 Paulsen, at 4000 vines per ha, 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 (ETc) (IR), irrigation at 30% of ETc (DI) and non irrigated (NI). The three treatments were replicated four times in randomized blocks, with three rows per replication. 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. Vine water status was estimated by measurements of stem water potential (Ψs) using a pressure chamber, according to Choné et al. (2001). Measurements were performed at solar noon (12h30 to 13h30), on four cloudless days corresponding approximately to the pea size, bunch closure, veraison and mid-ripening stages.

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), soluble solids (^{o}Brix) by refractometry and total acidity by titration with 0.1 N NaOH were determined on a sample of 200 berries per plot. A second sub-sample of 200 berries per plot was analyzed for total anthocyanins and phenols according to Iland et al. (2000) and for tannins according to the method described by Harbertson et al. (2002). Determination of individual skin anthocyanins and seed flavan-3-ols was also performed by HPLC as previously described (Koundouras et al., 2009).

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) following the same procedure. After crushing and destemming, 60 mg/L SO_2 (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 SO_2 (as potassium metabisulfite), filtered and bottled until analysis, approximately 6 months after winemaking.

Alcoholic degree was determined according to the OIV methods (Office International de la Vigne et du Vin, 1990). Colour intensity was determined by absorbance measurements at 420, 520 and 620 nm under 1 mm optical way and total anthocyanins using the SO_2 bleaching method (Ribereau-Gayon and Stonestreet, 1965). For tannin estimation, sample preparation (dilution) and protein precipitation assay was conducted according to the method described by Harbertson and Spayd (2006) and catechins according to Swain and Hillis (1959). Total wine phenolics were determined using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). All analyses were performed in triplicate.

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

Stem water potential (Ψs) was maintained at values higher than -0.9 MPa prior to veraison, in both years. Post veraison water status was more limiting in 2008 (Ψs of -1.05 MPa on average compared to -0.96 MPa in 2007). 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). Ψs was affected by postveraison irrigation treatmetns in both years with lower values for NI vines. Mean postverasion Ψs values for NI, DI and IR treatments were -1.03, -0.96 and -0.91 MPa in 2007 and -1.13, -1.09 and -0.94 MPa in 2008, respectively. According to Ψs critical values (van Leeuwen et al., 2009), postveraison water deficit for the NI vines was weak to moderate in 2007 (minimum recorded Ψs = -1.04 MPa) and moderate to severe in 2008 (minimum recorded Ψs = -1.19 MPa). On the contrary, in IR, water restriction levels never reached values inferior to -1.0 MPa, a commonly accepted threshold for the onset of mild water stress (Choné et al., 2001; Intrigliolo and Castel, 2011).

Irrigation increased yield per vine in IR vines as compared to NI vines, but only in 2008 (Table 1). However, postveraison water regime had no significant effect on berry weight, skin and seed weight per berry. These results do not confirm other works reporting that water deficit during the postveraison period can decrease berry mass at harvest (Kennedy et al., 2002). In the conditions of this study, grape sugar accumulation was not influenced by postveraison water conditions, possibly because deficit was short and of moderate intensity (Santesteban et al., 2011). Titratable acidity was increased by irrigation only in 2008, in agreement with previous reports (Bravdo et al., 1985).

Table 1. Post-veraison irrigation effects on yield and must components of Agiorgitiko grapes at ripeness stage, in 2007 and 2008; NI, non irrigated; DI, irrigated at 30% ET_c; IR, irrigated at 70% ET_c.

		Yield (kg/vine)	Berry weight (g)	Berry Skin weight (g)	Berry Seed weight (mg)	Total Soluble Solids (%)	Titratable Acidity (g/L)
2007	NI	2.08	1.27	0.37	64	21.30	7.35
	DI	2.00	1.32	0.43	63	21.10	7.39
	IR	2.11	1.41	0.43	68	21.40	7.56
2008	NI	1.79 b	1.63	0.32	66	23.90	5.51 b
	DI	2.02 ab	1.65	0.31	67	23.20	5.48 b
	IR	2.14 a	1.69	0.30	66	23.80	5.87 a

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

Postveraison water regime did not affect the concentration of individual anthocyanidins or their total amount in skin tissues at harvest (Table 2). However, the total amount of anthocyanins was higher in NI and DI vines compared to IR vines during the more limiting conditions of 2008 (Iland et al., 2000). Anthocyanin content was negatively correlated to mean postveraison Ψs for the 2008 season ($r=-0.691$, $p<0.05$). Since growth of berry tissues was not affected by irrigation in the present study, our findings suggest that moderate postveraison water deficits exert a direct positive effect on anthocyanin content of Agiorgitiko grapes, independently to berry size (Roby and Matthews, 2004).

Skin tannins were affected by irrigation only in 2007 (Table 2), with a higher amount in DI vines. The accumulation of tannin is essentially completed by veraison, thus postveraison water deficits may have a limited effect on tannin biosynthesis (Kennedy et al., 2002). Irrigation regime during the ripening period significantly affected individual flavan-3-ol levels, with IR vines showing increased values of catechin (data not shown) resulting in a higher total flavan-3-ol amount as compared to NI (both years) and to DI (only 2008). No irrigation effects on seed tannins were observed. For total berry polyphenols, differences were small and, only in 2007, NI vines showed slightly higher levels than IR ones (Table 2).

Table 2. Post-veraison irrigation effects on skin and seed phenolic compounds of Agiorgitiko grapes at ripeness stage, in 2007 and 2008; NI, non irrigated; DI, irrigated at 30% ET_c; IR, irrigated at 70% ET_c.

		Total anthocyanins (mg/g berry fw)	Sum of anthocyanidins (mg/g skin fw)	Total skin tannins (mg/g berry fw)	Total seed tannins (mg/g berry fw)	Sum of seed flavan-3-ols (mg/g seed fw)	Total berry phenolics (au*/g berry fw)
2007	NI	0.84	1.72	0.65 b	1.20	2.16 b	1.39 a
	DI	0.79	1.48	0.98 a	1.28	2.36 ab	1.28 b
	IR	0.81	1.51	0.61 b	1.17	3.54 a	1.31 ab
2008	NI	0.95 a	3.00	0.48	1.06	4.09 b	1.46
	DI	0.93 a	3.10	0.48	1.06	4.00 b	1.47
	IR	0.74 b	2.82	0.50	1.11	5.50 a	1.38

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

Decreasing Ψs values (higher water deficit) during the ripening period were strongly associated with lower seed tannins on both years ($r=0.844$, $p<0.001$ and $r=0.664$, $p<0.05$ for 2007 and 2008, respectively) confirming previous evidence that water deficits during berry ripening significantly increase the rate of loss of flavan-3-ol monomers in grape seeds 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 oligomers are mainly responsible for the bitterness and astringency of red wines (Brossaud et al., 2001).

The wines from both 2007 and 2008 seasons had similar values in alcohol concentration (Table 3) following must soluble solids content at harvest. However, despite the higher levels of skin anthocyanins in NI vines in 2008, wine anthocyanins did not differ among treatments, although colour intensity was higher in NI and DI wines of 2008. It is possible that anthocyanin levels are influenced by other factors such as maceration time, fermentation temperature and pH, yeast strain and skin to flesh ratio (Chalmers et al., 2010) but mainly by anthocyanin extractability as affected by berry 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 due to a tighter skin cell-wall structure at harvest (Sivilotti et al., 2005). Contrary to anthocyanins, tannin and catechin levels increased with irrigation in 2007 while no differences were observed in 2008. Total wine polyphenols were higher in DI wines in 2008.

Table 3. Post-veraison irrigation effects on the phenolic composition of Agiorgitiko wines, in 2007 and 2008; NI, non irrigated; DI, irrigated at 30% ET_c; IR, irrigated at 70% ET_c.

		Colour Intensity	Total Anthocyanins (mg/L)	Tannins (g/L)	Catechins (mg/L)	Total Polyphenols (g/L)
2007	NI	8.2	431	0.84 b	284 b	1.39
	DI	8.3	411	1.14 ab	284 b	1.47
	IR	8.2	429	1.33 a	323 a	1.50
2008	NI	8.8 a	449	0.78	273	0.75 c
	DI	9.0 a	437	0.79	254	1.38 a
	IR	7.2 b	424	0.75	263	1.19 b

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

Conclusions

These results suggest that an irrigation cutoff after veraison is generally beneficial for Agiorgitiko vines grown in Nemea, leading to the production of berries with 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.

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