INFLUENCE OF SOME VITICULTURAL PRACTICES ON THE POLYPHENOLIC CONTENT OF WINES PRODUCED FROM CV. AGIORGITIKO (*VITIS VINIFERA* L.)

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Abstract

Aims: Viticultural practices that improve light penetration and air circulation into the canopy can improve wine quality. The aim of this work is to investigate the consequences of some commonly applied viticultural practices on the polyphenolic content of cv. Agiorgitiko, an indigenous Greek grape variety.

Methods and results: Three viticultural practices (training system, leaf removal and shoot elongation) were applied on cv. Agiorgitiko in the Nemea wine region and the phenolic content of the wines produced was compared. Between the two training systems (Guyot vs. double Royat), no changes in polyphenolic compounds and yield components were observed. Shoot elongation and leaf removal caused a significant decrease in the polyphenolic content of the wines. Anthocyanin content was reduced significantly due to shoot elongation, while small differences were observed in yield components.

Conclusion: The study showed that increasing bunch sun exposure in warm viticultural areas may not be beneficial to the quality of the wine.

Significance and impact of the study: Climate and weather conditions should be taken into account before applying practices that increase bunch exposure, especially in warm wine regions where light could be detrimental to the polyphenolic content of the wines.

Key words: phenolic compounds, anthocyanins, Agiorgitiko, light penetration, vineyard practices

Résumé

Objectifs : Les pratiques viticoles qui améliorent la pénétration de la lumière et la circulation d'air dans la surface foliaire peuvent améliorer la qualité du vin. L'objectif de cette étude est de chercher les conséquences de certaines pratiques viticoles traditionnelles sur la teneur polyphénolique du cépage cv. Agiorgitiko, un cépage viticole indigène de la Grèce.

Méthodes et résultats : Trois pratiques viticoles (le système de conduite de la vigne, l'éclaircissement des feuilles et la longueur des sarments) ont été appliquées sur le cépage Agiorgitiko de la région viticole de Nemea et la teneur phénolique des vins produits a été comparée. Entre les deux systèmes de conduite (Guyot vs. double Royat), aucun changement a été observé sur les composés polyphénoliques et le rendement. La longueur des sarments et l'éclaircissement des feuilles ont diminué la teneur en anthocyanes et en tannins des vins produits. Des différences mineures ont été observées au niveau du rendement des grappes.

Conclusion : L'étude montre que dans des régions viticoles de climat chaud, l'augmentation de l'exposition des grappes au rayonnement solaire n'est peut-être pas bénéfique pour la qualité du vin.

Signification et impact de l'étude : Le climat et les conditions météorologiques doivent être pris en compte avant d'appliquer des pratiques qui augmentent l'exposition des grappes au soleil, en particulier dans des régions viticoles chaudes, où la lumière peut être nuisible à la teneur polyphénolique des vins.

Mots clés : composés phénoliques, anthocyanes, Agiorgitiko, pénétration de la lumière, pratiques viticoles

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INTRODUCTION

Grape phenolic compounds are very important constituents of red wines because, in addition to their antioxidant properties, they contribute to color, astringency and bitterness (Robichaud and Noble, 1990), oxidation reactions (Cheynier and Ricardo da Silva, 1991), interactions with proteins (Ricardo da Silva *et al*., 1991) and ageing behavior of wines (Haslam, 1980).

Several strategies for increasing the content of phenolic compounds in wine have been made during the process of wine making and grape growing. Most of the viticultural practices applied in the vineyards are targeting on the increase of light penetration and air circulation into the canopy due to the benefits that they present : reducing humidity (Kliewer and Lider, 1968 ; Rojas-Lara and Morrison, 1989 ; Haselgrove *et al*., 2000), reducing the risk of fungal and bacterial infection (Emmett *et al*., 1992), increasing berry temperature and possibly increasing vine development and metabolite accumulation (Hawker, 1982 ; Ebadi *et al*., 1995 ; Dokoozlian and Kliewer, 1996) and wine quality (Reynolds *et al*., 1996 ; Bordelon *et al*., 2008).

Many studies using artificial shading, defoliation and other techniques have shown alterations in the production of tannins and anthocyanins (Spayd *et al*., 2002 ; Bavaresco *et al*., 2008 ; Guidoni *et al*., 2008 ; Tarara *et al*., 2008). In cool climates, increased light penetration is related to enhanced anthocyanin and phenolic production (Cortell and Kennedy, 2006 ; Ristic *et al*., 2007). However, it is under consideration whether this practice can be beneficial to wine quality in warm to hot and sunny climates (Dry, 2009), since extended bunch exposure could sharply increase bunch temperature and thus reduce the anthocyanin content of grapes (Bergqvist *et al*., 2001 ; Mori *et al*., 2009 ; Lorrain *et al*., 2011).

Agiorgitiko (*Vitis vinifera*) is an indigenous Greek red grape variety that gives Denomination of Origin, deeply coloured red wines (Koundouras *et al*., 2006). It is a late-maturing cultivar, providing wines with blackcurrant and damson aromas (Manessis, 2000). The wines made from Agiorgitiko grapes are rich in total hydroxycinnamic acids (95.8 mg/l) and poor in stilbenes (0.74 mg/l). The average total flavonol, flavanol and anthocyanin composition of Agiorgitiko wines is 43.3, 114.5 and 612.5 mg/l, respectively (Kallithraka *et al*., 2007 ; Makris *et al*., 2006). The polyphenolic profile of this cultivar reflects to a great extent its genetic potential. However, it can also be influenced by environmental stimuli, which could play critical roles in regulating the activities of enzymes implicated in polyphenol biosynthesis.

This variety is cultivated almost exclusively in Nemea, the most important vine-growing region in southern Greece. The wine region of Nemea has a Mediterranean type climate, characterized by high temperatures and water deficiency during the summer season. To increase the tannin and anthocyanin concentration of Agiorgitiko wines, local grape growers are applying viticultural practices that, according to literature, are consider beneficial such as modifications in training systems (Vanden Heuvel *et al*., 2004 ; Reynolds and Vanden Heuvel, 2009), leaf removal (Bureau *et al*., 2000 ; Guidoni *et al*., 2008) and shoot elongation. However, there is a lack of information concerning the relationship between practical implications and wine quality due to the uniqueness of this grape variety and its limited cultivation. In this study, the above practices were applied in the vineyard and the quality of the produced wines was determined based on two quality parameters of red wines ; anthocyanin content and tannin composition.

MATERIALS AND METHODS

1. Vineyard description

The experiments were conducted in 2006 and 2007 in three vineyards in the Koutsi sub-region of Nemea, Greece at an altitude of 610 meters above sea level. The soil in the region is low in organic matter, sandy-loamy, slightly alkaline (pH 6.5-7.0) and poor in nutrients.

All the vineyards were planted with *Vitis vinifera* L. cv. Agiorgitiko grafted onto 41b rootstock. The row orientation was east-west and the training system was double Royat consisting of a 0.7 m high bilateral cordon (50 cm on each side), with 4 spurs per cordon and two shoots per spur with a set of catchwires at 1.7 m for maintaining vertical shoot positioning.

Vineyard 1 (2.0 ha) was established in 1980 at a density of 3 400 vines/ha and vine spacing of 1.20 x 2.20 m. Vineyards 2 and 3 (Vineyard 2: 1.8 ha, Vineyard 3: 2.0 ha) were both established in 2000 at a density of 5 200 vines/ha and vine spacing of 0.90 x 2.20 m. Since supplemental irrigation is usually required in this region, a drip irrigation system was established in all vineyards (water supplementation is optional). All viticultural practices (pruning, leaf removal, shoot positioning and harvest) were performed manually.

2. Experimental design

a) Treatment 1 : Training system (Guyot vs. double Royat)

Two training systems, Guyot and double Royat, were applied in 2006 in Vineyards 2 and 3. Both training systems are the most commonly applied in the region of Nemea. The Guyot training system was applied to one fourth of each vineyard (approximately 0.5 ha) and double

Royat was applied to the remaining area of the vineyard. In the plots with Guyot training system, cane pruning with vertical shoot positioning and 10 shoots per meter of row was applied. Double Royat training system was applied as described in the previous section.

b) Treatment 2: Leaf removal

In the south side of Vineyard 1, during the year 2006, leaf removal was applied to 150 vines (approximately 0.5 ha). Two to four leaves per bunch were manually removed four days after the beginning of veraison, which reduced canopy surface per vine by 15%. No leaves were removed from the bunches of control vines. The training system was double Royat and no irrigation was applied.

c) Treatment 3: Extended shoot length

Vertical shoot length was allowed to exceed 1.3 m (extended shoot treatment), although it is normally 1.0 m in this region (control treatment). The treatment was applied for two consecutive years (2006-2007) in Vineyard 1 under controlled drip irrigated and non-irrigated conditions. Therefore, there were four treatments: 1) 1.0 m shoot length, irrigated, 2) 1.3 m shoot length, irrigated, 3) 1.0 m shoot length, not irrigated and 4) 1.3 m shoot length, not irrigated. The training system in all plots was double Royat and each experimental condition was applied to approximately 100 vines.

3. Vinification

The grapes from each plot were vinified separately each year. The grapes were destemmed, crushed and 70 mg/l SO_2 was added. The grape juice was inoculated with ADWY (*Saccharomyces cerevisiae*, Lallemand UV-299) and fermentation lasted for 10 days at 24-26 °C with two punch downs per day. The pomace was pressed in a pneumatic press with no fraction separation. Following pressing, malolactic fermentation was completed with addition of malolactic bacteria (*Oenococcus oeni*, Lalvin VP-41). Finally, the wines were sulphured with 60 mg/l SO_2.

4. HPLC determination of anthocyanins

Wine samples were filtered through 0.45 μm syringe filters prior to High Pressure Liquid Chromatographic (HPLC) analysis (Hewlett-Packard 1050) using a HP 1050 chromatography apparatus coupled to a diode array detector. Analyses were performed as in Kallithraka *et al*. (2005) on a Spherisorb ODS-2 column (particle size, 5 μm; 250 x 4 mm id), at a flow rate of 1 ml min^{-1}, using a 20 μl injection volume, detection at 520 nm, and the following elution programme: 95 % eluent A for 1 min, then from 95 to 50 % in 25 min, and finally from 50 to 5 % in 3 min, which was kept isocratic for another 3 min. Eluent A was 10 % aqueous formic acid and eluent B was MeOH (HPLC grade, Sigma). Identification was based on comparing retention times of the peaks detected with those of original compounds and on UV-vis on-line spectral data. Malvidin-3-O-glucose coumarate (MvCoum) and malvidin-3-O-glucose acetate (MvAcet) were tentatively identified based on previous observations (Arnous *et al*., 2002). All peaks were quantified as malvidin-3-O-glucose (Mv) (Extrasynthèse, France). Results were expressed as mg/l. All analyses were performed in triplicate.

5. HPLC determination of individual phenolics

The concentration of individual polyphenols was determined by HPLC, according to the method described by Kallithraka *et al*. (2001). A Hewlet-Packard 1050M Series II with an auto injector (25 μl injection volume) and a diode array detector, recording at 265, 280, 320 and 365 nm, was used to detect the phenolic compounds. A reversed phase ODS-2 Shperisorb column (250 x 4 mm id, particle size 5 μm) at 40 °C was used with a flow rate of 1 ml min^{-1}. Using 0.6% aqueous perchloric acid and methanol as eluents (HPLC grade, Sigma), the following linear gradient was used: from 5 to 80 % methanol in 55 min, hold for 15 min at 80 % methanol to wash the column and then return to the initial conditions to re-equilibrate for 10 min.

Peaks were identified by comparison of retention times and ultraviolet (UV) spectra with commercial standards: gallic acid, protocatechuic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, (+)-catechin, (-)-epicatechin, myricetin, quercetin, kaempferol, kaempferol-3-O-glucoside, isorhamnetin-3-O-glucoside, rutin (all from Sigma), and procyanidins B1, B2 and C1 (gift from Dr. A.G.H. Lea, Reading, U.K.). Procyanidins and p-coumaric acid were expressed as mg/l (+)-catechin and coumaric acid, respectively. Caftaric and 2-S-glutathionylcaftaric acids were identified according to Makris *et al*. (2003) and expressed as mg/l caffeic acid, whereas the remaining compounds were expressed against their own calibration curves. All analyses were performed in triplicate.

6. Statistical analysis

All analyses were performed in triplicate unless otherwise specified, and the values were averaged. The standard deviation (SD) was also calculated. Comparison of samples was based on Student's T- test.

RESULTS

1. Weather conditions

During the first year of the experiment (2006), the weather conditions were normal for the region. No

extreme heat waves occurred and a few showers in June and early July caused limited fungal infection. There were no rainfall in August and only two rainfalls in mid September (20 mm each) which delayed harvesting. Drip irrigation was applied twice to each plot defined as irrigated in 2006: 1) on 24th August, 25 mm and 2) on 27th September, 35 mm. Veraison began on 2nd August and grape harvest started on 24th September.

The second year (2007) was exceptionally warm with daily summer temperatures exceeding 40 °C. Three extreme heat waves with temperatures reaching 44 °C occurred on 26th June, on 20th July and on 10th August (each lasted 2-3 days). These warm and dry conditions lasted up to the harvest period, cooling slightly only by the north winds in mid-August. From bud-burst up to harvest, rainfall occurred only in late May, and there were no rainfalls at all during summer and early autumn. Drip irrigation was applied three times to each plot defined as irrigated in 2007: 1) on 27th August, 25 mm, 2) on 9th September, 35 mm and 3) on 27th September, 25 mm. Veraison began on 20th July and grape harvest started on 22nd September.

In both years of the experiment, determination of the plant water potential was performed with the Scholander pressure chamber technique as described by Scholander *et al.* (1965). Measurements were carried out weekly at mid-day, between 1 200 to 1 400 hr (solar time) (results not shown).

2. Training system : Guyot vs. double Royat

When comparing grape composition between the two training systems, no important differences were observed in yield, titratable acidity, pH and total soluble solids (results not shown) ; however, small differences were evident in the case of anthocyanins and the studied phenolic compounds (Tables 1 and 2). In both Vineyards 2 and 3, Guyot trained vines produced wines with higher contents of total hydrocinnamate acids (THA) ; total phenolic acids (TPA) and flavonols showed variable results and flavonols were the less affected phenolic group. Concerning anthocyanins, the wines produced from double Royat trained vines had increased concentrations of 3-O-glucosides of delphinidin, cyanidin, petunidin and peonidin, and MvCoum, with these differences being more evident in Vineyard 3. By contrast, the concentration of malvidin-3-O-glucoside and MvAcet was higher in wines from Guyot trained vines. However, total anthocyanin concentration was not significantly different between the two training systems.

3. Leaf removal

Titratable acidity (expressed as tartaric acid) was the only grape juice parameter affected by leaf removal. Leaf removal treated vines showed significantly lower titratable acidity (5.7 mg/l) compared with the control (6.7 mg/l), while there was no significant difference in their pH levels (3.80 versus 3.82, respectively). As it can be seen in Table 1, all the classes of phenolic compounds showed an important decrease after leaves were removed. Total phenolic acids and flavanols were the most affected (showed a decrease by up to 25 %), while certain individual compounds were even decreased by up to 50 % (protocatechuic acid). Anthocyanins showed variable results, with malvidin-3-O-glucoside and its esters being

Table 1 - Polyphenolic concentration (mg/l) of wines produced under two viticultural practices : training system and leaf removal. Values represent means of triplicate determinations ± standard deviation.

Treatment		TPA	THA	Flavanols	Flavonols
		(mg/l)	(mg/l)	(mg/l)	(mg/l)
Training system	***Vineyard 2***				
	Guyot	72.4±0.44	223.2±0.42	367.7±1.80	25.3±0.01
	Double Royat	72.5±0.47	199.5±0.43	316.7±0.43	22.3±0.01
	Vineyard 3				
	Guyot	95.8±0.47	222.4±0.76	417.2±1.59	37.4±0.40
	Double Royat	84.3±0.20	193.8±0.29	467.2±0.16	35.4±0.10
Leaf removal	***Vineyard 1***				
	No leaves removed	168.0±0.84	207.5±0.98	596.3±2.77	36.4±0.04
	Leaves removed	117.5±0.47	194.8±6.35	425.5±1.07	34.6±0.21

Abbreviations : TPA : Total Phenolic Acids, THA : Total Hydrocinnamate Acids.

positively influenced by leaf removal and the other anthocyanins showing the reverse trend (Table 2).

4. Extended shoot length

In both years, the shoot elongation treatment (1.3 m) increased the berry weight as well as the bunch weight compared with the control treatment (1.0 m), although the results concerning total yield were variable (Table 3). In 2007, a significant increase of yield (kg grapes/vine) was observed under all experimental conditions, compared with 2006. During the same year, irrigated vines showed advanced maturity level as indicated by their lower acidity (TA) and elevated Baume (oBe) levels (Table 3).

In 2006, shoot elongation treatment had a negative impact on phenolic content (Figure 1). The percentage of decrease compared with the control was as follows: total phenolic acids 10 % under irrigated conditions and 35 % under non-irrigated conditions; flavanols 22 % under both irrigated and non-irrigated conditions; flavonols 15 % under irrigated conditions and 35 % under non-irrigated conditions. More specifically, the shoot elongation treatment caused a considerable decrease in gallic acid content by 35 % under non-irrigated conditions and in procyanidin B1 and procyanidin C1 (flavanols) by 50 % under both irrigated and non-irrigated conditions (results not shown). Hydrocinnamate acid contents of wines were not significantly affected by the treatment and only a slight decrease was observed.

In 2007, the content of flavonols was decreased by the shoot elongation treatment: by 30 % under irrigated conditions and 20 % under non-irrigated conditions. Hydrocinnamate acids also decreased by 10 % and 28 % under irrigated and non-irrigated conditions, respectively. However, the content of monomeric and oligomeric flavanols was increased by 15 % under irrigated conditions. Finally, the content of phenolic acids showed variable results.

As shown in Figure 2, shoot elongation did not enhance wine anthocyanin content. The concentrations of glucosides of delphinidin, cyanidin, petunidin and

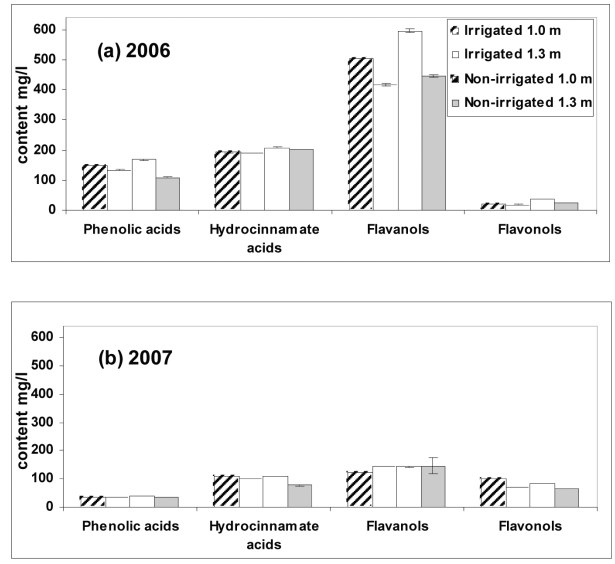

Figure 1 - Phenolic acid, hydrocinnamate acid, flavanol and flavonol concentrations (mg/l) of wines produced under normal shoot length (1.0 m) and extended shoot length (1.30 m), under two irrigation regimes for two consecutive years: (a) 2006 and (b) 2007.
Values are the means of triplicate determinations. Error bars indicate standard deviation and in most cases are too small to be seen.

Table 2 - Anthocyanin concentration (mg/l) of wines produced under two viticultural practices: training system and leaf removal. Values represent means of triplicate determinations ± standard deviation.

Treatment		Dp (mg/l)	Cy (mg/l)	Pt (mg/l)	Pe (mg/l)	Mv (mg/l)	MvAcet (mg/l)	MvCoum (mg/l)	Total (mg/l)
Training system	***Vineyard 2***								
	Guyot	13.1±0.01	*ND	29.1±0.09	24.2±0.13	815.8±1.38	76.5±0.14	95.7±0.13	1056.0±1.87
	Double Royat	14.2±0.10	*ND	30.8±0.01	23.2±0.03	791.5±4.57	75.4±0.32	101.1±0.45	1032.1±4.43
	Vineyard 3								
	Guyot	27.3±0.20	1.0±0.02	47.4±0.19	53.6±0.40	809.2±3.65	52.1±0.23	82.8±0.23	1078.1±4.90
	Double Royat	29.7±0.15	1.6±0.05	50.4±0.27	61.2±0.24	784.5±1.15	47.0±0.04	105.9±0.20	1084.2±3.95
Leaf removal	***Vineyard 1***								
	No leaves removed	35.7±0.17	3.0±0.04	44.7±0.17	60.8±0.16	652.7±1.27	38.4±0.14	62.8±0.18	899.2±0.99
	Leaves removed	34.0±0.27	1.9±0.05	47.6±0.04	53.9±0.17	725.7±1.55	43.3±0.06	86.7±0.30	992.8±0.67

*ND: not detected
Abbreviations: Dp, Cy, Pt, Pe and Mv stand for 3-O-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin, respectively. MvAcet: malvidin-3-O-glucose acetate. MvCoum: malvidin-3-O-glucose coumarate.

Table 3 - Yield components and fruit composition of Agiorgitiko with normal shoot length (1.0 m) and extended shoot length (1.3 m), under two irrigation regimes for two consecutive years (2006 and 2007).

Shoot length	Water status	Yield (kg grapes/vine	Bunch weight (gr)*	Berry weight gr. for 100 berries*	Baume (°Be)	TA**	pH
2006							
1.0 m	Non-irrigated	2.39	236.30±46.77	191.60±7.44	13.75	6.7	3.82
	Irrigated	2.08	296.30±9.92	217.90±5.86	14.02	5.4	3.98
1.3 m	Non-irrigated	2.16	366.30±21.34	259.00±3.82	14.05	5.0	3.98
	Irrigated	2.28	303.80±47.25	223.70±7.55	13.83	6.0	3.88
2007							
1.0 m	Non-irrigated	3.26	260.00±64.05	201.00±9.65	13.30	6.8	3.70
	Irrigated	3.50	270.30±17.04	190.20±10.09	14.10	5.3	3.96
1.3 m	Non-irrigated	3.31	279.50±37.96	210.00±11.08	13.30	5.6	3.88
	Irrigated	3.33	293.60±68.01	200.20±10.00	14.00	5.0	3.88

*Values are the means of triplicate determinations, **expressed as mg/l tartaric acid, TA ; titratable acidity

peonidin were decreased by the application of this treatment under both irrigated and non-irrigated conditions in both years of the experiment. Malvidin-3-O-glucoside showed variable results in 2006, while in 2007, shoot elongated vines had lower malvidin-3-O-glucoside content than control vines under both conditions (793 mg/l versus 756 mg/l under irrigated conditions ; 923 mg/l versus 758 mg/l under non-irrigated conditions). Regarding MvAcet, in the first year shoot elongation slightly increased its content under both irrigation conditions while in the second experimental year, it increased the content of the irrigated and decreased that of the non-irrigated vines. MvCoum content was also not affected similarly by shoot elongation in the two experimental years : the first year, the irrigated shoot elongated vines contained lower contents while the non-irrigated vines contained higher contents than control vines. In the second year, shoot elongation resulted in lower contents under both irrigation conditions.

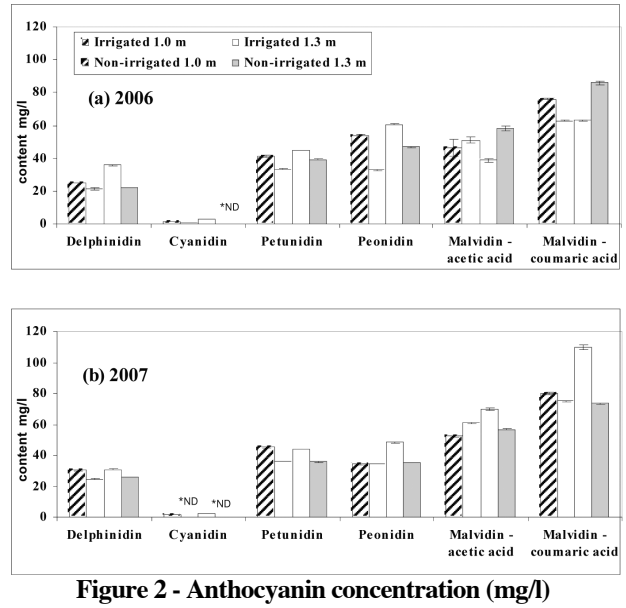

Figure 2 - Anthocyanin concentration (mg/l) of wines produced under normal shoot length (1.0 m) and extended shoot length (1.3 m), under two irrigation regimes for two consecutive years : (a) 2006 and (b) 2007.
Values are the means of triplicate determinations. Error bars indicate standard deviation and in most cases are too small to be seen.
*ND : not detected

DISCUSSION

1. Phenolic content

Although training system is an important parameter affecting the chemical composition of grapes (Reynolds and Vanden Heuvel, 2009), we found no significant differences in polyphenolic content between the two training systems Guyot and double Royat (Table 1). In that respect, Peterlunger *et al.* (2002) showed that vines under similar training systems do not present noticeable differences in their phenolic content.

It has been proposed that the decrease in the concentration of condensed tannins (i.e., polymeric flavonoid compounds) towards harvesting is not due to degradation but to decreased extractability into grape juice as they become less soluble in aqueous solutions (Saint-Cricq de Gaulejac *et al.*, 1997). According to a study conducted with Shiraz grapes, it was shown that the decrease in tannin extractability was greater in exposed than in shaded grapes, and as a result, at harvest, there were no significant differences between shaded and non-shaded grapes (Downey *et al.*, 2003). In our study, the polyphenolic content in wines made from grapes with greater exposure was decreased, possibly due to the decreased extractability of polyphenols into the grape juice. However, in order to be able to justify this relationship, it is required to monitor tannin content in both shaded and non-shaded grapes from veraison to harvest.

It has been known that the expression of genes involved in the regulation of flavonol synthesis is stimulated by sunlight (Spayd *et al*., 2002 ; Downey *et al*., 2004 ; Ristic *et al*., 2007) and that flavonol content in sun-exposed grapes is normally increased (Cortell and Kennedy, 2006 ; Ristic *et al*., 2007). However, in our study, the leaf removal treatment did not cause any significant change and the shoot elongation treatment even decreased the content of flavonols, even though both practices increased sun exposure. Although berry temperature has little or no effect on the total flavonol concentration in grapes (Spayd *et al*., 2002), it might affect total phenolic concentration (Bergqvist *et al*., 2001 ; Chira *et al*., 2011). It has been reported that berry temperature increases linearly with increasing exposure to sunlight (Smart and Sinclair, 1976), showing that differences in temperature between shaded and non-shaded berries could reach more than 10 °C (Kliewer and Lider, 1968 ; Spayd *et al*., 2002 ; Bergqvist *et al*., 2001). Although we did not measure berry temperature, it is possible that the intense sun exposure has elevated berry temperature to a level capable of causing a decline in polyphenol concentration.

Matus *et al*. (2009) observed that leaf removal decreased flavonol concentration, but the decline was not related to sunlight intensity. Furthermore, it was shown that the practice of leaf removal at veraison had a negative effect on flavonol accumulation. In our study, leaf removal slightly decreased flavonol content (although not significantly), most likely due to the practice of organ (leaf) removal rather than enhanced light penetration. Since the climate in Nemea is characterized as hot and dry with intense sunlight exposure, even the shaded grapes might have had enough sun exposure. Therefore, light intensity was not the limiting factor for flavonol accumulation.

In addition, no differences were observed between the two vineyards even in irrigated and non-irrigated conditions, although it has been reported that in Nemea water deficit is related to an increased anthocyanin and polyphenolic content (Koundouras *et al*., 2006).

The results of leaf removal are variable and are influenced by many parameters such as training system, fruit load, vine age, fertility, cultivar, rootstock, irrigation practice and macroclimate (Main and Morris, 2004). The results reported in this study suggest that the extreme hot and dry conditions may have a greater effect on grape phenolic content than any other viticultural practices, and therefore, viticultural practices increasing light exposure of grape bunches are not beneficial for the wine quality.

2. Anthocyanins

In accordance with the results of wine polyphenolic content, training system did not significantly affect the anthocyanin content of the experimental wines. The content of all individual anthocyanins was similar in the wines produced from the vines in both training systems and in both vineyards, suggesting that small differences between training systems do not affect the anthocyanin composition of the wines.

It is known that temperature can influence the accumulation of anthocyanins in berry skins (Spayd *et al*., 2002 ; Yamane *et al*., 2006). However, exposing the vines to high temperatures especially after veraison can be detrimental to the color of wines (Yamane *et al*., 2006 ; Mori *et al*., 2007 ; Tarara *et al*., 2008 ; Lorrain *et al*., 2011). Mori *et al*. (2007) applied two different temperature regimes to Cabernet-Sauvignon vines one week after veraison (max. 25 °C and max. 35 °C) and showed that the elevated temperature regime reduced total anthocyanin content by 50 %, suggesting that high temperature might result in anthocyanin degradation. In our study, the content of malvidin-3-O-glucoside and its derivatives was increased after leaf removal while that of the other anthocyanins was decreased. Shoot elongation in 2006 caused a decrease in the content of 3-O-glucosides of delphinidin, cyanidin, petunidin and peonidin, while the content of malvidin-3-O-glucoside and its derivatives showed variable results. It has been reported that the accumulation of malvidin-3-O-glucoside and its derivatives is not decreased by high temperatures (Pereira *et al*., 2006 ; Cortell *et al*., 2007 ; Tarara *et al*., 2008) because of their stable chemical structure called acylated form (i.e., malvidin 3-coumaroyl-glucoside and malvidin 3-acetyl-glucoside), although the concentration of the other anthocyanins might decrease (Tarara *et al*., 2008). The results reported in the present study for the year 2006 are consistent with their observations.

Leaf removal was conducted only in 2006 while shoot elongation was applied for two consecutive years (2006 and 2007). In 2007, shoot elongation resulted in a decrease in the content of all anthocyanins with the exception of acetic acid ester of malvidin-3-O-glucoside, under irrigated conditions. The weather conditions of summer 2007 were extremely hot and dry compared with 2006, which was a year without extreme weather conditions. Hence, in 2007 the temperature of berries on shoot elongated vines was possibly already too high, and the treatment further reduced the synthesis and increased the degradation of all anthocyanins. Similar results showing that anthocyanin concentration is decreased at extremely high temperatures (above 40 °C) were reported by Tarara *et al*. (2008) and recently confirmed by Lorrain *et al*. (2011).

CONCLUSION

This is the first report evaluating the impact of sunlight exposure on Agiorgitiko grapes in the wine region of

Nemea (Greece). Leaf removal and shoot elongation are two practices commonly used to improve wine composition by increasing light exposure of grape bunches.

However, in our study, we showed that viticultural practices that increase light penetration into the canopy are not always beneficial for the phenolic and anthocyanin content of the wines in that region, and should be set under consideration. This is probably related to the weather conditions, dry and high temperatures in summer, naturally occurring in the region. Possibly the increased light exposure elevated the grape temperature, resulting in a decrease in their polyphenolic and anthocyanin content. Furthermore, it is possible that higher amounts of phenolic compounds are synthesized in non-shaded than in shaded vines ; however, this cannot be confirmed due to their reduced extractability.

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