Received: 9 May 2021 | Accepted: 26 July 2021 | Published: 30 August 2021
DOI:10.20870/oeno-one.2021.55.3.4706

OENO One
VINE AND WINE OPEN ACCESS JOURNAL

# Effects of post-veraison irrigation on the phenolic composition of *Vitis vinifera* L. cv. 'Xinomavro' grapes

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**ABSTRACT**

'Xinomavro' (the second planted red Greek variety behind 'Agiorgitiko') generally produces wines that are light in colour but with increased astringency, possibly related to grape flavonoid amount and composition; although irrigation is accepted as an effective means to enhance grape phenolic maturity, its role has not yet been sufficiently studied in the case of Xinomavro. This study aimed to determine the effect of post-veraison irrigation on berry anthocyanin and proanthocyanidin amount and composition, of field-grown Xinomavro vines (*Vitis vinifera* L.), under the typical summer conditions of Northern Greece. In a 10-year-old Xinomavro vineyard, two post-veraison watering regimes were applied—irrigation starting 20 days after veraison (mid-ripening irrigation, MRI) and irrigation starting immediately after veraison was completed (after veraison irrigation, AVI)—alongside non irrigated vines (NI), and vines irrigated continuously from berry set through harvest (continuous irrigation, CI). Proanthocyanidin composition was determined in both skins and seeds by employing phloroglucinolysis followed by HPLC-UV and MS detection (high-performance liquid chromatographic with tandem mass spectrometric and ultraviolet absorbance detection), and the anthocyanin profile was identified only in the skin extracts by HPLC-UV (high-performance liquid chromatographic with ultraviolet absorbance detection). Post-veraison irrigation increased yield parameters and reduced anthocyanin levels and the proportion of their stable forms (acylated vs. non-acylated, tri-oxygenated and methoxylated on the B-ring vs. di-oxygenated and hydroxylated), compared to NI vines; however, these effects were more pronounced in the case of early post-veraison irrigation (AVI) than late-season irrigation (MRI). Irrigation also increased the mean Degree of Polymerization (mDP) and prodelphinidin percentage of skin tannins and decreased mDP of seed tannins. In the light of the necessity to control the accelerated ripening under the increasingly hotter and drier climatic conditions, late irrigation (MRI) might provide a solution to avoid excessive sugar levels and allow a slightly higher yield without significant reductions in berry phenolic content. The results suggest that the optimisation of the timing of irrigation could provide an effective adaptation strategy to climate change in Mediterranean viticultural areas.

**KEYWORDS**

grapevine, water, anthocyanins, proanthocyanidins, mean Degree of Polymerization

## INTRODUCTION

Among grape phenolic compounds, anthocyanins (located in the skins of berries) and proanthocyanidins or 'condensed tannins' (polymers composed of flavan-3-ols subunits present in both skins and seeds) are important quality factors in red grapes as they determine, respectively, the red colour and the astringent and bitter sensation of the produced wines, while their interactions define wine colour stability and ageing potential (Kallithraka *et al.,* 1997). Although they both derive from the phenylpropanoid biosynthetic pathway, proanthocyanidins are produced during the first period of berry growth reaching a maximum around veraison, while anthocyanins start to accumulate at veraison increasing levels until harvest (Ollé *et al.,* 2011). Anthocyanin and tannin evolution during berry ripening is, therefore, an important parameter for the decision of the harvest date of red grapes. However, the assessment of 'phenolic maturity' is difficult, especially in the case of proanthocyanidins where, apart from their concentration, their structural composition (e.g., polymerisation), the interactions with berry cell wall proteins and polysaccharides, and their extractability, are also responsible for red wine mouthfeel and sensory properties (Koundouras, 2018).

The phenylpropanoid pathway is controlled by diverse biotic and abiotic factors, among which, water availability is known to significantly affect the levels and physicochemical properties of grape phenolics. Specifically, a moderate water deficit is reported to positively affect the accumulation of skin anthocyanins (Theodorou *et al.,* 2019) and proanthocyanidins (Cassasa *et al.,* 2015) either indirectly by improving within vine allocation of photosynthates or increasing the skin proportion of (generally smaller) berries (Triolo *et al.,* 2019), or by directly stimulating secondary metabolism by up-regulation of the specific genes of the phenylpropanoid pathway (Castellarin *et al.,* 2007b). In fact, many studies have reported that berry mass *per se* is unlikely to be the main driver of increased phenolic concentration in grapes and that the cultural practices that induce those small berries are responsible for a large part of the observed changes in berry chemistry (Roby *et al.,* 2004; Mirás-Avalos *et al.,* 2019). According to Kyraleou *et al.* (2017), water deficit also increased polymerisation of skin tannins in cv. Syrah. However, fewer studies have reported on the possible role of water restriction on the modulation of the structural properties of grape proanthocyanidins.

Since water deficits are considered beneficial for red wine quality, RDI (Regulated Deficit Irrigation) is established as a common irrigation strategy in winegrapes to efficiently balance crop productivity and grape quality, and also economise water resources, in semiarid areas. RDI aims at inducing a controlled water deficit, usually during the early berry growth (pre-veraison), to restrict berry mass and control vegetative growth (Keller *et al.,* 2008; Romero *et al.,* 2013). A relatively recent article by Mirás-Avalos and Intrigliolo (2017) reported a significant relationship between vine stem water potential in pre-veraison and the wine anthocyanin content. However, RDI is accompanied by significant reductions in yield; moreover, recent studies (Ollé *et al.,* 2011) have shown that pre-veraison water deficit did not affect proanthocyanidin accumulation and had limited effect on anthocyanins (particularly on the levels of malvidin-O-glucoside). Despite scientific evidence (Koundouras *et al.,* 2013), in Greece, post-veraison irrigation is rarely applied, considered as detrimental for berry and wine composition, especially for red winegrapes, due to the alleged decreases in berry sugar and phenolics concertation.

'Xinomavro' (*Vitis vinifera* L.) is the flagship red variety of Northern Greece, covering a significant part of the Greek vineyard with plantings of 1150 ha (3.90 % of the total area under winegrapes), making it the second planted red Greek variety behind 'Agiorgitiko' (2008 ha, 7.74 %). Grapes and wines of Xinomavro are generally light in colour (Kyraleou *et al.,* 2019) but with a high proportion of more stable (acylated and methoxylated) anthocyanins (Kyraleou *et al.,* 2020). On the contrary, Xinomavro grapes are rich in skin and seed tannins (Kallithraka *et al.,* 2006) leading to dry and astringent wines even though they confer a long ageing potential. In a recent comparative study of four red winegrapes, Xinomavro anthocyanin content was positively affected by water deficits imposed from early berry growth (Theodorou *et al.,* 2019). However, in a normal year, in most grape-growing areas of Northern Greece, Xinomavro is rarely subjected to water deficits before veraison. Moreover, the effect of water conditions on Xinomavro proanthocyanidin structural properties, which could possibly be related to the astringent character of its wines, has not yet been sufficiently studied. As Xinomavro is one of the varieties with the higher heat

requirements to achieve full ripeness in the world, expected to gain popularity in the next generation of grape growers in the frame of adaptation measures to climate change (Wolkovich *et al.,* 2018), its response to water availability is of great interest. Therefore, the present work aimed to investigate the effect of post-veraison irrigation regimes on berry anthocyanin and proanthocyanidin amount and composition, of field-grown Xinomavro vines under the typical summer conditions of Northern Greece.

## MATERIALS AND METHODS

### 1. Experimental conditions and vine measurements

A field trial located in Thessaloniki, Northern Greece was carried out for two consecutive years (2015 and 2016) in a 10-year-old vineyard, planted with *Vitis vinifera* L. cv. Xinomavro onto 1103 Paulsen (*V. rupestris* × *V. berlandieri*) at 4000 vines per ha (1.0 m × 2.5 m). The vineyard was located on a loamy-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 a standard of 16 buds per vine.

Treatments included: non irrigated vines (NI), irrigation starting 20 days after veraison (mid-ripening irrigation, MRI), irrigation starting immediately after veraison was completed (after veraison irrigation, AVI), and vines irrigated from berry set through harvest (continuous irrigation, CI). Each treatment was replicated three times in randomised blocks. In each block, treatments were applied on single rows, with 10 consecutive plants used for measurements and samplings, and separated by two buffer rows to avoid any border effects. Irrigation was equivalent to 70 % of crop evapotranspiration (ETc). ETc was estimated from the potential evapotranspiration data (calculated by the Penman-Monteith method) obtained from an on-site automatic weather station (iMETOS, Pessl Instruments GmbH, Weiz, Austria) and crop coefficients increasing from 0.50 to 0.75 from June to July, for the CI vines, and remaining at 0.75 until harvest.

The water quantities were applied by a drip irrigation system equipped with 4 L/h drip emitters positioned at 50 cm intervals along the pipe. The total amount of water applied in 2015 and 2016, respectively was 78 and 97 mm for MRI, 120 and 163 mm for AVI, 305 and 376 mm for CI). Regarding climatic conditions, 2015 and 2016 presented differences in terms of total growth season rainfall (301 mm against 213 mm, respectively, from April to September) but showed comparable mean temperatures (mean growing season temperature of 29.1 °C in 2015 against 29.5 °C in 2016), 2016 being an overall drier year.

Measurements of vine water status were made at predawn (between 3 h prior to, and at, dawn) and at midday, on five sample dates in 2015 (DOY 212, 220, 227, 240, 254) and six sample dates in 2016 (DOY 199, 211, 220, 226, 240, 256) with the use of a pressure chamber. Predawn water potential (Ψdawn) measurements were conducted according to Koundouras *et al.* (2006), while midday stem water potential (Ψstem) measurements according to Choné *et al.* (2001). In each measurement set, four mature leaves per plot from the central four vines of each plot were used. Cluster temperature (°C) was determined simultaneously to Ψstem on 8 clusters of the same vines per plot, using an HI 99551 infrared thermometer (Hanna Instruments®, Keysborough, Australia). Vine vigour was assessed by a non-destructive estimation of leaf area per vine according to the method of Lopes and Pinto (2000) on four vines per plot at harvest. On the same vines, measurements of pruning weight per vine (kg/vine) were obtained during the following winter period.

Four berry samplings took place after veraison in 2015 (DOY 212, 220, 240, 254) and 2016 (DOY 215, 227, 245, 263). Samples of 200 berries were collected randomly from the central four vines of each plot and weighed to determine the mean berry mass. Berries were pressed and the must was analysed for total soluble solids (°Brix) by refractometry, pH and titratable acidity (g/L tartaric acid). All grapes per plot were picked at commercial harvest (12 September 2015 and 20 September 2016), and total yield per plant (kg/vine) and average cluster weight (g) was estimated.

### 2. Analysis of anthocyanins and proanthocyanidins

Phenolic compounds were first analysed 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 homogenised 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 HC1 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 weight) 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. Finally, two subsamples of 100 berries were stored at –30 °C for subsequent analysis of anthocyanins and proanthocyanins by HPLC.

Berries of the first subsample were slowly de-frozen at 5 °C and skins were removed by hand, freeze-dried and grounded to a fine powder. Anthocyanins were extracted from 1 g of dried skin powder for three different time duration steps (4, 18 and 24 h) with 20 mL, 10 mL and 10 mL acidified methanol (1 mL/L in 0.012 mol/L HCl). After centrifugation, the supernatants were combined and analysed. The content of anthocyanins was determined by HPLC. The equipment used consisted of a Jasco AS-1555 Intelligent Sampler, a Jasco PU 2089 Plus Quaternary Gradient Pump, coupled to a UV-vis detector (Jasco MD- 910 Multiwavelength Detector) set at 520 nm and a Jasco LC-Net II/ ADC (Jasco Corporation, Tokyo, Japan). A Restek Pinnacle II C18 (Restek Corporation, Bellefonte, PA, USA) (250 mm × 4.6 mm, 4 µm) column was employed. Eluent A was 100 mL/L aqueous formic acid and eluent B was methanol at a flow rate of 1 mL/min. The elution was as follows: 90 % A for 1 min, then from 90 to 50 % A in 22 min, from 50 to 5 % A in 10 min and finally isocratic for a further 2 min. Identification of detected compounds was based on comparing retention times and UV spectra of the peaks with those of original compounds (standard) or based on previous observations reported in the literature (Kyraleou *et al.,* 2015). The following compounds were identified: delphinidin-3-*O*-monoglucoside, cyanidin-3-*O*-monoglucoside, petunidin-3-*O*-monoglucoside, peonidin-3-*O*-monoglucoside, malvidin-3-*O*-monoglucoside, malvidin-3-*O*-acetylglucoside and malvidin-3-(6-*O*-p-coumaroyl)glucoside. The concentration of anthocyanins was expressed as mg/g skin dry weight of malvidin-3-*O*-monoglucoside equivalents. All analyses were performed in triplicate.

Berries of the second subsample were slowly de-frozen at 5 °C and seeds and skins were removed by hand, freeze-dried and grounded to a fine powder. The extraction of skin and seed tannins was carried out according to previously reported methods (Chira *et al.,* 2009). An amount of 3 g of the powder (skins or seeds) was first extracted with 25 mL of 80 % acetone in water for 3 h and then with 25 mL of 60 % methanol in water for 2.5 h. The supernatants were combined and evaporated under reduced pressure at 30 °C to remove organic solvents. The residue was lyophilised, was weighted and re-dissolved in 5 % of ethanol and then the lipophilic material was removed by chloroform. The aqueous fractions were collected and lyophilised to obtain a dry powder. The final tannin extracts were weighed and dissolved in methanol (5 g/L). Acid-catalysed depolymerisation occurred in the presence of phloroglucinol (50 g/L phloroglucinol, 10 g/L ascorbic acid, 0.1 N HCl, in methanol) for 20 min at 50 °C. The reaction was terminated by the addition of 1 mL aqueous sodium acetate (40 mM). Reaction products were analysed on an LC-MS 2010A instrument coupled to a single quadrupole mass spectrometer equipped with an electrospray ion source (Shimadzu, Kyoto, Japan). The mass spectrometer was operated in positive-ion mode. The source's temperature was set at 70 °C, the capillary voltage at 3.5 kV and the cone voltage at −30 eV. The absorbance was recorded at 280 nm and mass spectra were recorded in the range of 50–1500 amu. Separation was performed on an XTerra RR C18 (100 × 4.6 mm, 3.5 µm) reversed-phase column (Waters, Milford, MA, USA) at a flow rate of 0.5 mL/min, using a 20 µL injection volume and the following elution program: eluent A from 80 % to 40 % in 20 min, which was kept isocratic for further 10 min and then from 40 % to 80 % in 2 min. Eluent A was 0.1 % aqueous acetic acid and eluent B methanol. All analyses were performed in triplicate. The calculation of mean polymerization degree (mDP), and the percentage of (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC) and (-)-epicatechin gallate (ECG) according to previously published methods (Kyraleou *et al.,* 2017).

### 3. Statistical analysis

All values were averaged per plot and only the mean value per plot was used in the statistical analysis. Data were expressed as means of three replicates (n = 3) and subjected to analysis of variance (ANOVA) with watering regimes as the main factor (Duncan's test $p < 0.05$).

**TABLE 1**. Irrigation effects on vegetative and reproductive growth parameters

| Parameter | Treatment | 2015 | 2016 | Average | Year | T *x* year |
|---|---|---|---|---|---|---|
| Main Leaf Area ($m^2$/vine) | NI | 2.4 b* | 2.2 c | 2.3 b | 0.815** | 0.079 |
| | MRI | 2.8 ab | 2.5 bc | 2.6 b | | |
| | AVI | 2.2 b | 2.9 ab | 2.6 b | | |
| | CI | 3.3 a | 3.2 a | 3.2 a | | |
| Lateral Leaf Area ($m^2$/vine) | NI | 1.0 b | 1.3 b | 1.1 b | 0.021 | 0.532 |
| | MRI | 1.4 ab | 2.1 b | 1.7 b | | |
| | AVI | 1.7 ab | 1.9 b | 1.8 b | | |
| | CI | 2.2 a | 3.2 a | 2.7 a | | |
| Pruning Wood Weight (kg/vine) | NI | 1.1 d | 1.1 d | | <0.001 | <0.001 |
| | MRI | 1.4 c | 1.3 c | | | |
| | AVI | 1.8 b | 1.4 b | | | |
| | CI | 2.7 a | 1.8 a | | | |
| Cane Weight (g) | NI | 53 d | 54 d | | <0.001 | <0.001 |
| | MRI | 71 c | 60 c | | | |
| | AVI | 83 b | 65 b | | | |
| | CI | 117 a | 100 a | | | |
| Berry Temperature (°C) | NI | 29.9 a | 28.4 a | 29.2 a | 0.002 | 0.051 |
| | MRI | 29.5 a | 27.1 a | 28.3 b | | |
| | AVI | 27.9 b | 27.6 a | 27.8 bc | | |
| | CI | 27.3 b | 27.2 a | 27.2 c | | |
| Yield (kg/vine) | NI | 2.6 d | 1.2 d | | <0.001 | <0.001 |
| | MRI | 3.0 c | 2.4 c | | | |
| | AVI | 4.1 b | 4.0 b | | | |
| | CI | 5.3 a | 6.3 a | | | |
| Cluster Weight (g) | NI | 82 d | 65 c | 74 d | 0.137 | 0.025 |
| | MRI | 93 c | 78 c | 86 c | | |
| | AVI | 127 b | 141 b | 134 b | | |
| | CI | 183 a | 178 a | 181 a | | |
| Skin/berry weight (%) | NI | 6.08 a | 8.86 ab | 7.47 ab | 0.195 | 0.080 |
| | MRI | 5.20 b | 10.47 a | 7.83 a | | |
| | AVI | 5.03 b | 9.44 ab | 7.24 ab | | |
| | CI | 4.67 b | 8.38 b | 6.53 b | | |
| Seed/berry weight (%) | NI | 3.39 a | 4.26 a | 3.83 a | 0.261 | 0.141 |
| | MRI | 2.63 b | 4.40 a | 3.51 ab | | |
| | AVI | 2.35 b | 4.05 a | 3.20 b | | |
| | CI | 2.05 b | 3.08 a | 2.57 c | | |

Measurements were conducted at harvest except for pruning and cane weight. Irrigation treatments: non irrigated vines (NI), irrigation starting at mid-ripening (MRI), irrigation starting immediately after veraison (AVI), and continuous irrigation from berry set through harvest (CI).

*Within each column and parameter, means followed by a different letter are significantly different at $P < 0.05$ based on Duncan test.

** p-values for the effect of year and the interaction between treatment and year.

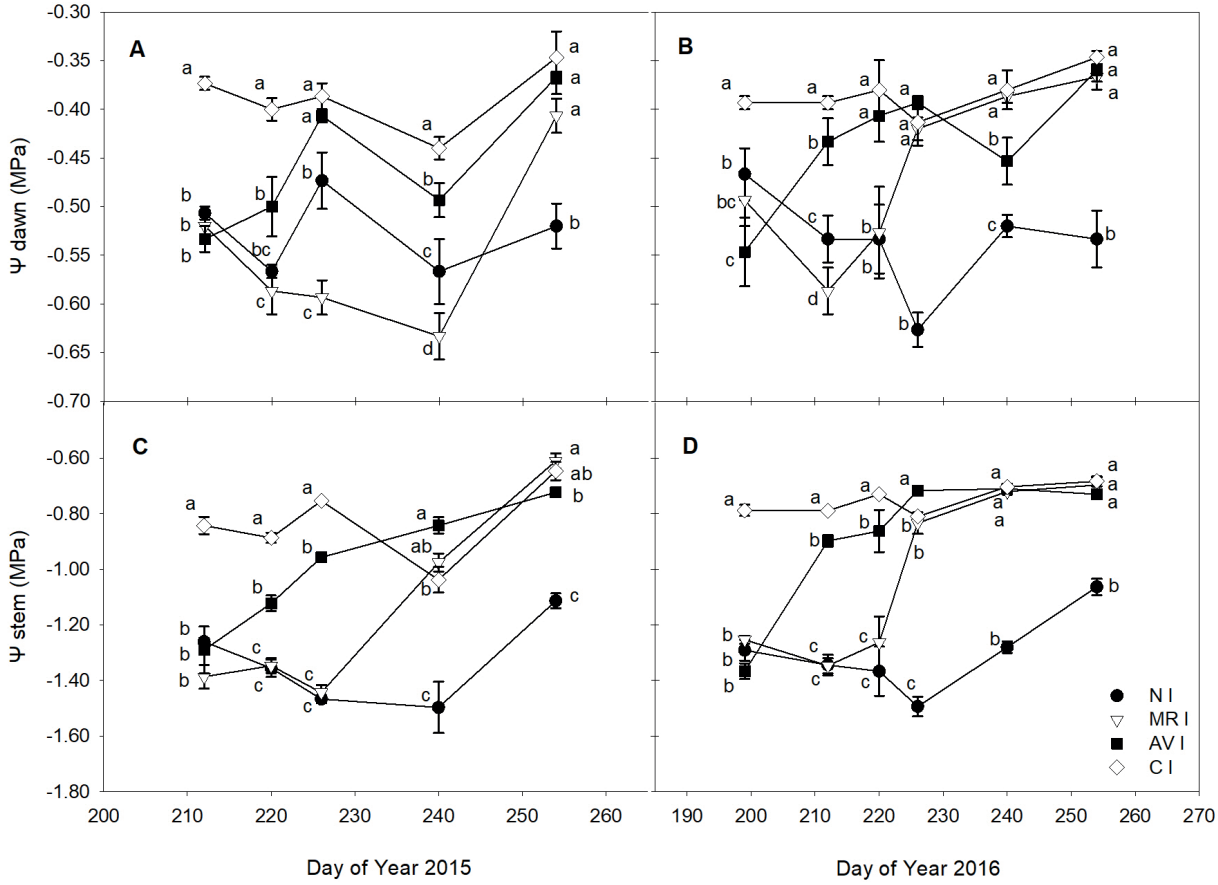


**FIGURE 1.** Predawn leaf (Ψdawn, A, B) and midday stem water potential (Ψstem, C, D) post-veraison pattern, in 2015 and 2016.

* Irrigation treatments: non irrigated vines (NI), irrigation starting at mid-ripening (MRI), irrigation starting immediately after veraison (AVI), and continuous irrigation from berry set through harvest (CI). Bars indicate ± S.E. of the mean value. Significant differences among treatments within samplings and years are indicated by different letters (Duncan's test, $p < 0.05$)

Principal components analysis (PCA) was applied on the correlation matrix of nine variables measured at harvest (midday berry temperature, yield per vine, berry mass, must total soluble solids, pH and titratable acidity, sum of anthocyanins, mDP of skin and seed tannins) together with the mean midday stem water potential for the post veraison period, to study the groupings, similarities, and differences between treatments. A single PCA was performed combining years (2015 and 2016), and the corresponding biplot was graphed. IBM SPSS Statistics for Windows, Version 22.0. (Armonk, NY: IBM Corp.) was used for the analysis.

## RESULTS

### 1. Vine measurements

The non-irrigated (NI) and the continuously irrigated (CI) vines showed a constant difference of approximately 0.2 to 0.3 MPa in Ψdawn and 0.5 to 0.6 MPa in midday Ψstem during both 2015 and 2016 ripening periods, while the two post-veraison irrigated treatments (AVI and MRI) responded to the timing of irrigation increasing values of water potential after the onset of water application, starting at values comparable to NI to both reach values similar to CI at the moment of harvest (Figure 1A–1D).

Leaf area growth was higher for CI, compared to the other treatments in both years; on the contrary, AVI and MRI vines showed similar values to NI, for both main and lateral leaf area, on average across seasons (Table 1). However, pruning wood weight was increased by post-veraison water application compared to NI, in both seasons. Except for two cases, there was no year × irrigation interaction for vigour parameters, showing a consistent effect of water conditions on vine vegetative growth. As expected, midday berry temperature was higher in the non-irrigated vines and lower in CI, but not significant in 2016, although it was a hotter and drier year (Table 1).

  

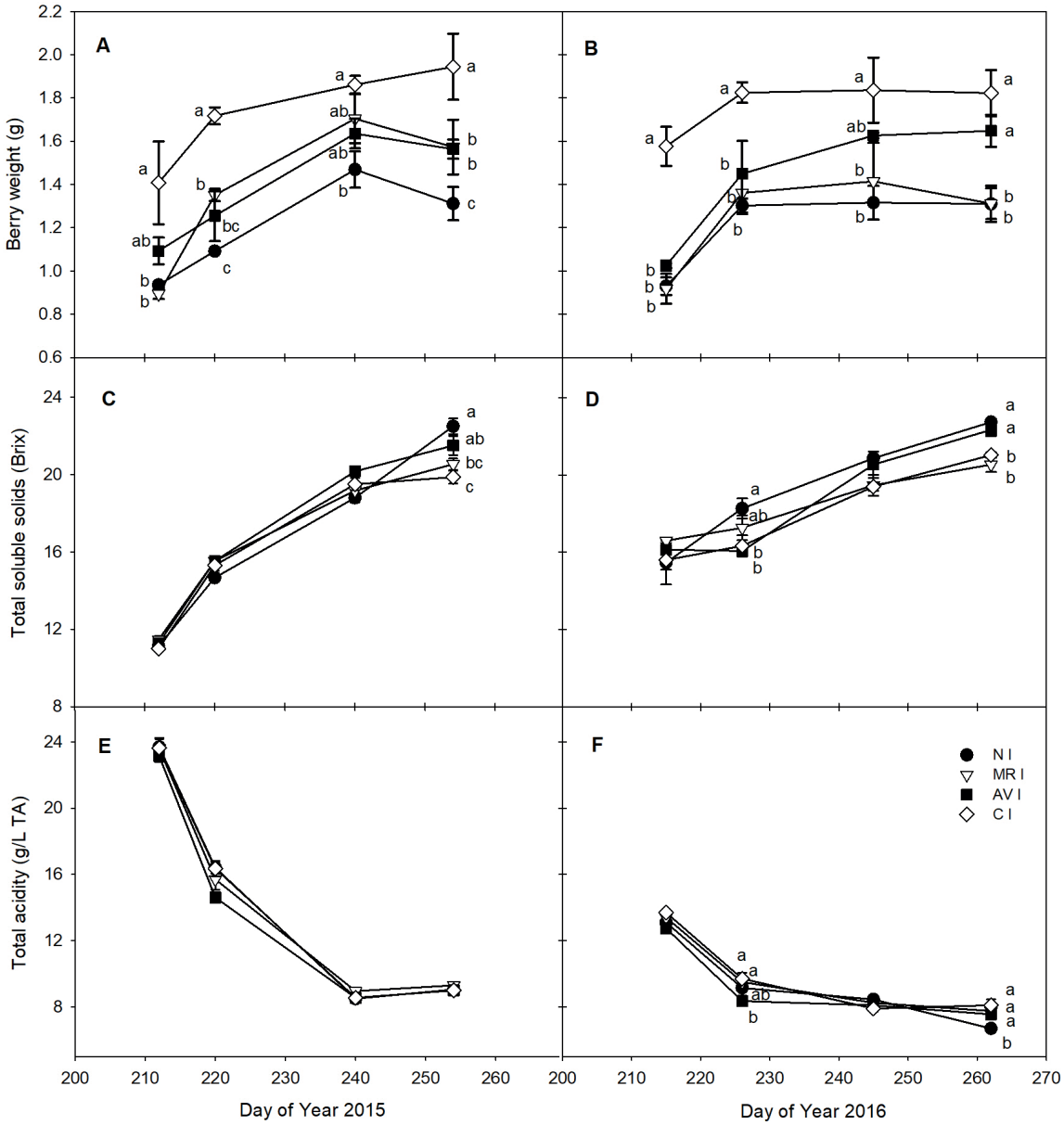


**FIGURE 2.** Evolution of berry weight (A, B), total soluble solids (C, D) and titratable acidity (E, F) during berry ripening, in 2015 and 2016.

* Irrigation treatments: non irrigated vines (NI), irrigation starting at mid-ripening (MRI), irrigation starting immediately after veraison (AVI), and continuous irrigation from berry set through harvest (CI). Bars indicate ± S.E. of the mean value. Significant differences among treatments within samplings and years are indicated by different letters (Duncan's test, $p < 0.05$)

Yield per vine and cluster weight were increased proportionally with the amount of water (Table 1) in the order CI > AVI > MRI > NI, with significant differences among all treatments in both seasons. In the conditions of our work, a significantly higher berry mass was shown for CI and lower for NI (Figure 2A, 2B); AVI (both years) and MRI (only 2015) berries had intermediate size (MRI had similar values to NI in 2016). Skin-to-berry and seed-to-berry weight ratios were different between CI and NI only in 2015, with higher values for the non-irrigated vines, AVI and MRI post-veraison irrigation treatments showing (despite an increasing trend) similar values to CI. The reduced skin-to-berry weight ratio in the two post-veraison irrigation treatments compared to NI was mostly the result of an increased berry volume rather than a decreased thickness of berry skin (data not shown). Regarding must chemistry, NI seemed to result in lower acidity only in 2016, compared to the irrigated treatments (Figure 2E, 2F) while AVI showed total soluble solids similar to NI in both years (Figure 2C, 2D).

## 2. Phenolic content and composition

The accumulation of total anthocyanins measured in whole berries and the sum of individual anthocyanins measured by HPLC [delphinidin-3-*O*-glucoside (Dp), cyanidin-3-*O*-glucoside (Cy), petunidin-3-*O*-glucoside (Pt), peonidin-3-*O*-glucoside (Pn), malvidin-3-*O*-glucoside (Mv), malvidin-3-*O*-acetylglucoside (MvA) and malvidin-3-(6-*O*-p-coumaroyl) glucoside (MvC)] did not follow a consistent pattern during ripening among years and treatments (Figure 3). An upward trend was observed for NI and MRI in 2015 but followed by a decrease before harvest, whereas the concentration of total anthocyanins in AVI and CI showed little variation during the season; a slight increasing pattern was observed for all treatments in 2016. A year effect was observed, with higher values in grapes from the drier 2016 vintage, compared to the same treatments in 2015 (mostly for NI and MRI grapes).

The predominant glucoside was Mv, followed by its coumaroyl derivative (MvC); the sum of Mv, MvC and MvA represented 58.3 % and 56.7 % of the total pool of anthocyanins, in 2015 and 2016 respectively (Figure 4). Acylated anthocyanins (MvA and MvC) accounted for 39.9 % and 34.3 % of total anthocyanins, respectively in 2015 and 2016. Moreover, tri-oxygenated derivatives (Dp, Pt, Mv, MvA, MvC) had a higher proportion than the di-oxygenated ones (Cy and Pn) in 2015 (94.5 %) and 2016 (96.3 %). Finally, the proportion of methoxylated anthocyanins on the B ring (Mv MvA, MvC and Pn) was higher than that of hydroxylated ones (Dp, Cy, Pt) representing 97.2 % in 2015 and 96.7 % in 2016.

NI berries had the highest and CI the lowest anthocyanin content expressed as both total content per g of berry and sum of individual anthocyanins per g of skin (Figure 3) in both seasons.

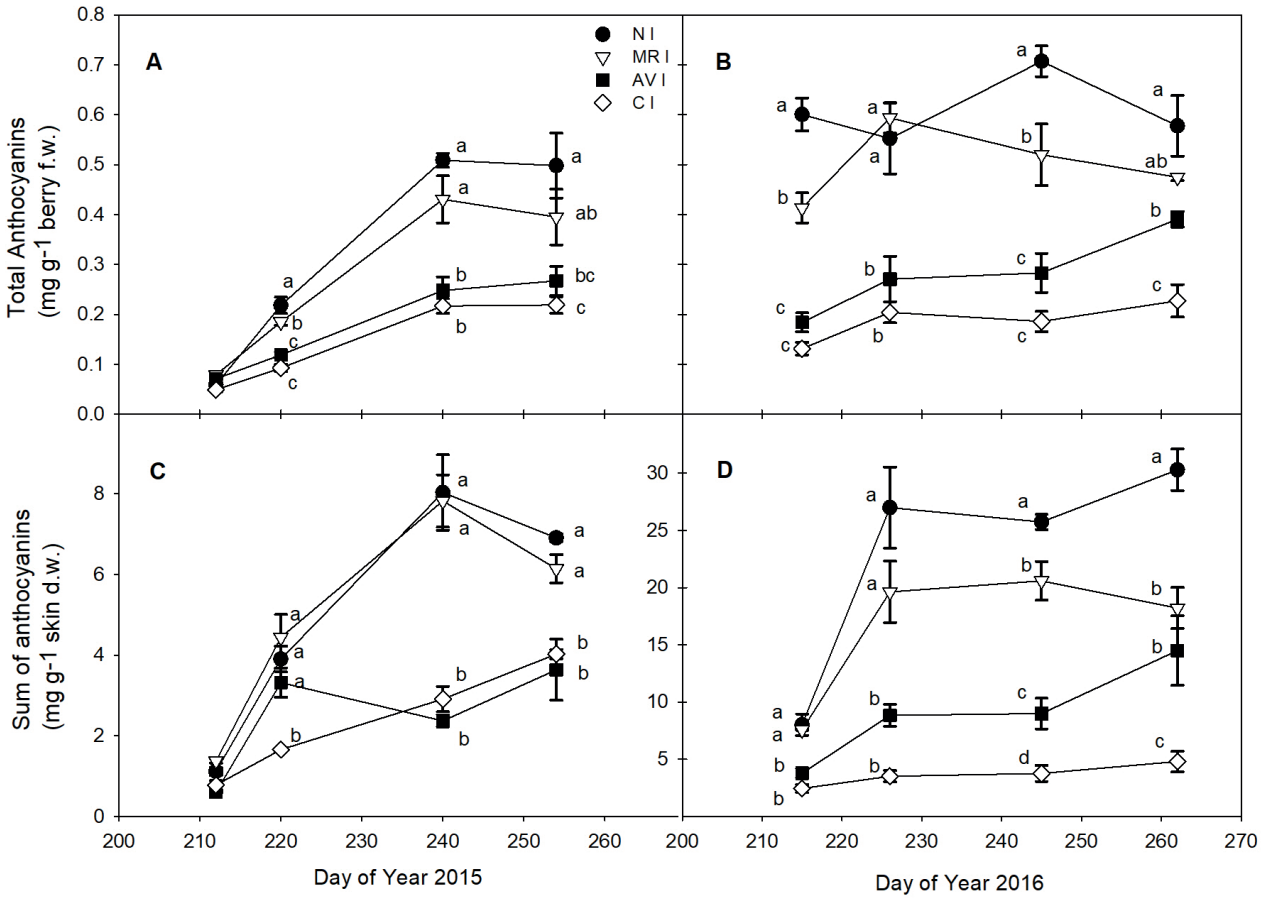


**FIGURE 3.** Evolution of total anthocyanin concentration (A, B) and sum of individual anthocyanins (C, D) during berry ripening, in 2015 and 2016.

* Irrigation treatments: non irrigated vines (NI), irrigation starting at mid-ripening (MRI), irrigation starting immediately after veraison (AVI), and continuous irrigation from berry set through harvest (CI). Bars indicate ± S.E. of the mean value. Significant differences among treatments within samplings and years are indicated by different letters (Duncan's test, $p < 0.05$)

  

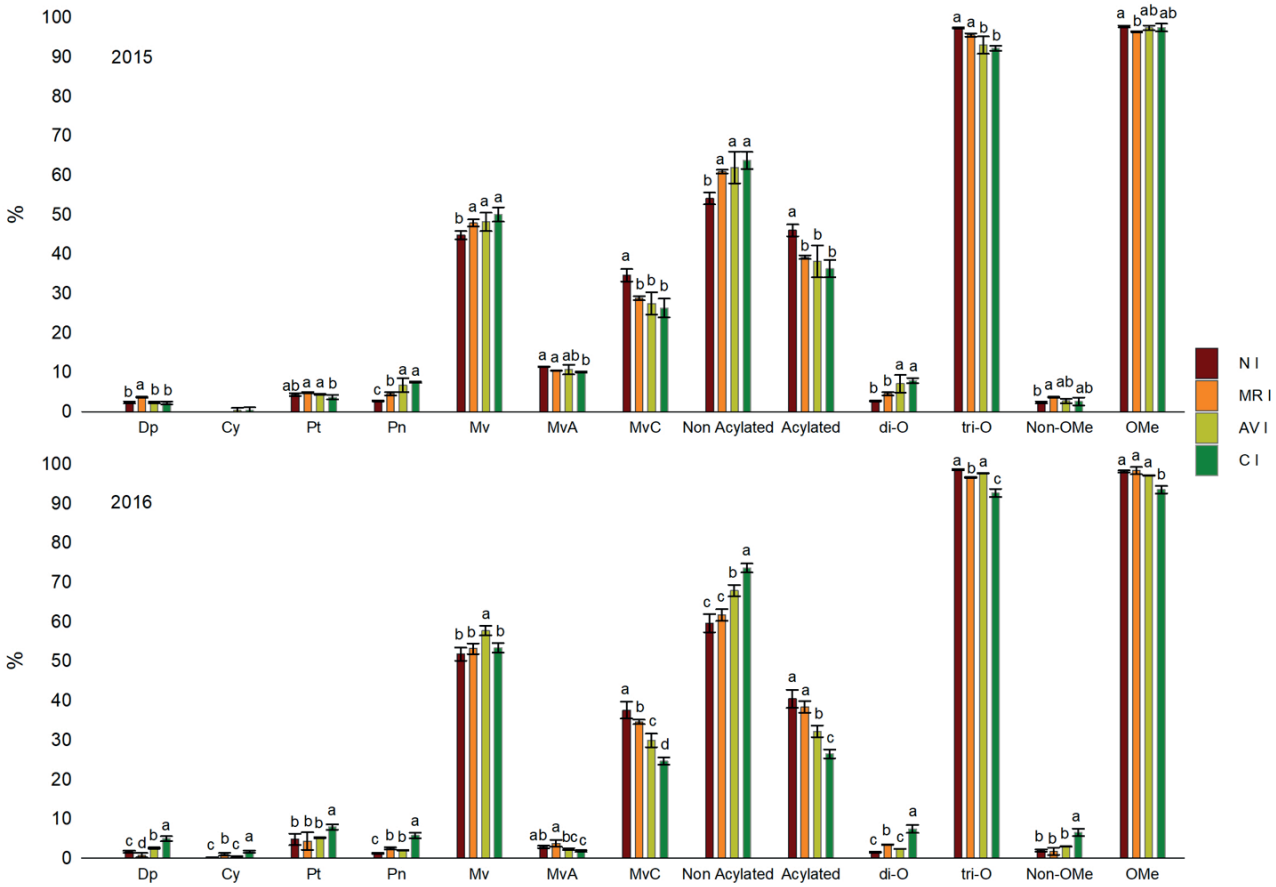


**FIGURE 4.** Influence of irrigation on anthocyanin composition (% total anthocyanin content in mg/g skin d.w.) at harvest of 2015 and 2016.

*Anthocyanins: delphinidin-3-O-glucoside (Dp), cyanidin-3-O-glucoside (Cy), petunidin-3-O-glucoside (Pt), peonidin-3-O-glucoside (Pn), malvidin-3-O-glucoside (Mv), malvidin-3-O-acetylglucoside (MvA) and malvidin-3-(6-O-p-coumaroyl) glucoside (MvC). Anthocyanin groups: Non acylated (Sum of Dp, Cy, Pt, Pn, Mv); acylated (Sum of MvA and MvC); di-O (Sum of Cy and Pn); tri-O (Sum of Dp, Pt, Mv, MvA, MvC); Non-OMe (Sum of Cy and Dp); OMe (Sum of Pn, Pt, Mv, MvA, MvC). Irrigation treatments: non irrigated vines (NI), irrigation starting at mid-ripening (MRI), irrigation starting immediately after veraison (AVI), and continuous irrigation from berry set through harvest (CI). Bars indicate ± S.E. of the mean value. Significant differences among treatments are indicated by different letters (Duncan's test, $p < 0.05$)

Regarding the evolution of anthocyanins, in 2015 (Figure 3A, 3C), MRI showed values close to NI, while AVI showed values close to CI; in 2016, anthocyanin levels increased in the order NI > MRI > AVI > CI (Figure 3B, 3D). The higher levels of anthocyanins in NI berries were mostly the result of an enhanced synthesis of Mv and its p-coumaroylated derivative, MvC (data not shown). Acylated anthocyanins (MvC) were increased in non-irrigated vines compared to non-acylated forms (Dp, Cy, Pt, Pn, Mv) in both years (Figure 4). NI berry skins also showed an increased proportion of tri-oxygenated anthocyanins over di-oxygenated as well as of methoxylated on the B ring (Mv and esters, and Pn) over hydroxylated ones (Dp, Cy, Pt) (the latter only in 2016).

Total phenol levels of whole Xinomavro berries (Figure 5A, 5B) did not show a clear evolution trend in 2015 in all treatments, while a declining pattern towards harvest was evident in 2016. Comparing vintages, 2016 berries were richer in phenolic compounds than in 2015, similarly to total anthocyanins.

In the skin extracts, Epicatechin (EC) was the main subunit, accounting (on average across treatments) for 50.8 % and 49.4 % of total tannin subunits at harvest of 2015 and 2016 respectively (Figure 6). Epigallocatechin (EGC) was the second more abundant (42.6 % of total in 2015 and 37.8 % in 2016). Catechin (C) proportion in skins was <15 % of total subunits while Epicatechin-3-O-gallate (ECG) had the smallest contribution, especially in 2016.

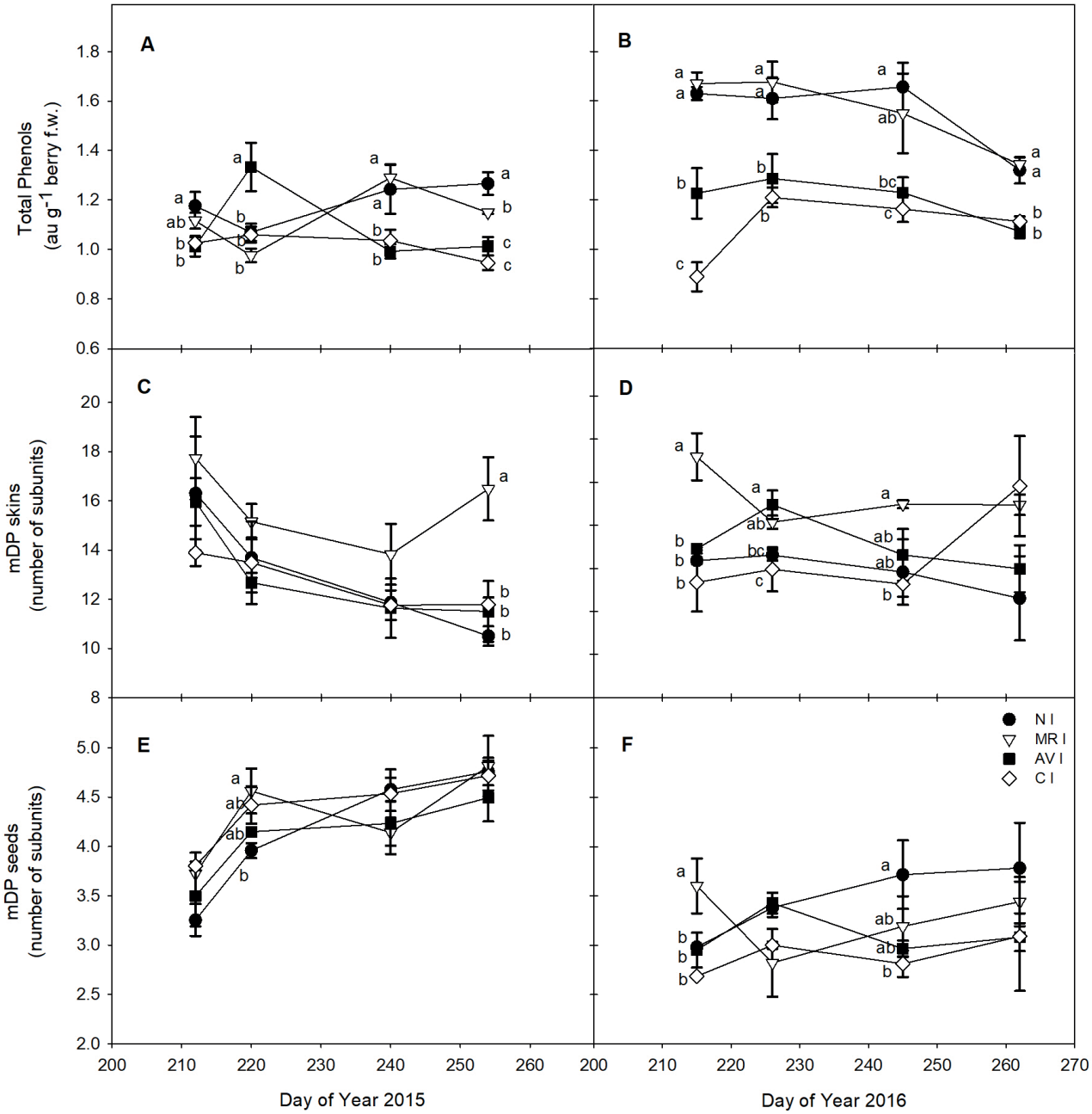


**FIGURE 5.** Evolution of berry total phenolic content (A, B) and of the mean degree of polymerisation (mDP) of skin (C, D) and seed (E, F) proanthocyanidins during ripening, in 2015 and 2016.

* Irrigation treatments: non irrigated vines (NI), irrigation starting at mid-ripening (MRI), irrigation starting immediately after veraison (AVI), and continuous irrigation from berry set through harvest (CI). Bars indicate ± S.E. of the mean value. Significant differences among treatments within samplings and years are indicated by different letters (Duncan's test, $p < 0.05$)

In the seeds, EC represented, on average, 67.8 % and 68.2 % of subunits at harvest of 2015 and 2016, respectively. Catechin (C) had the second-highest contribution, representing at harvest (on average across treatments) 20.7 % (in 2015) and 25.9 % (in 2016) of the total subunits, followed by ECG; Epigallocatechin (EGC) was not detected in the seeds. Irrigation affected the amount of total phenolics per g of berry with NI and MRI presenting the highest levels while CI and AVI the lowest in both years at harvest (Figure 5A, 5B). The irrigation regime did not alter the subunit composition of seeds (both years) and skins (in 2015); however, in 2016, skins from all irrigation treatments (CI, AVI and MRI) were enriched in EGC compared to non-irrigated vines, NI presenting the highest proportion of EC (Figure 6).

  

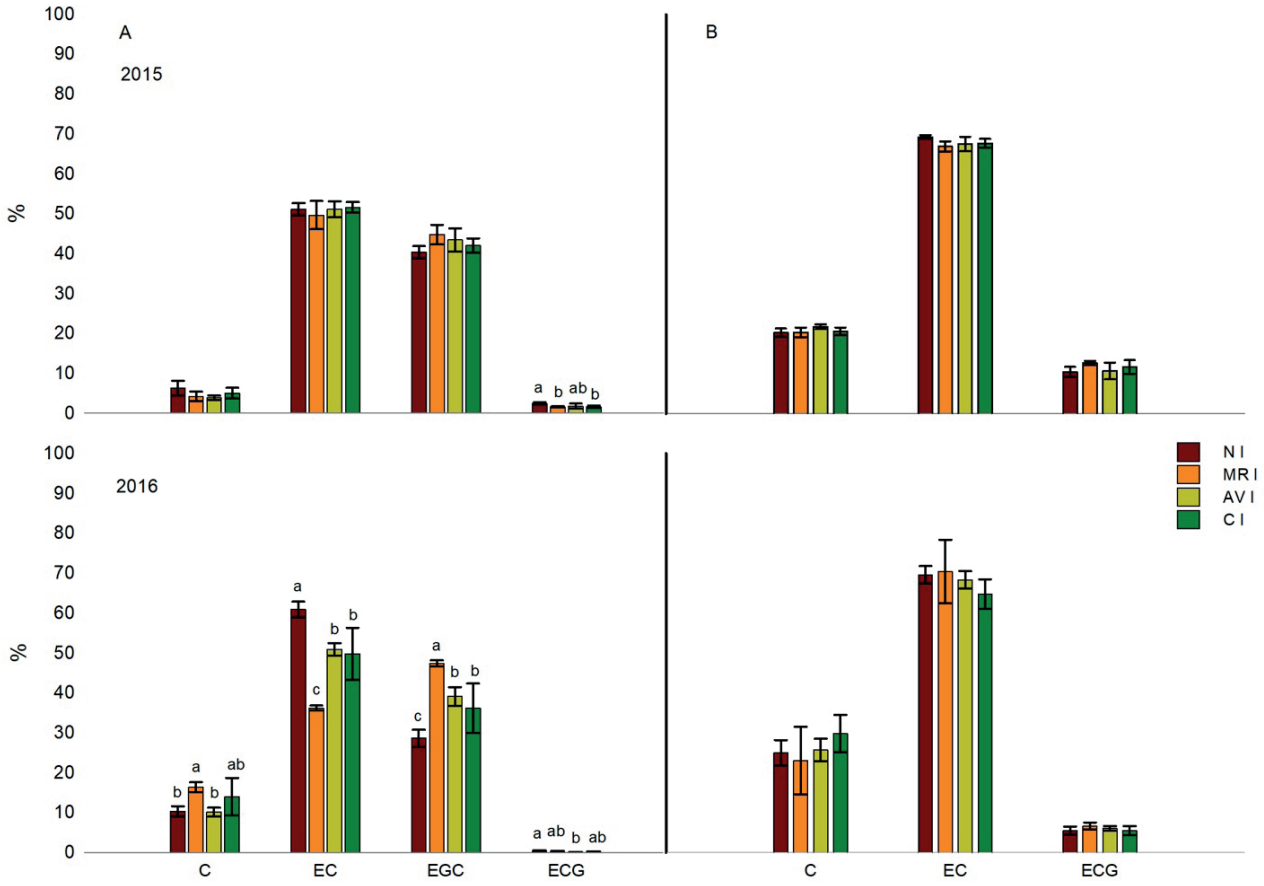


**FIGURE 6.** Influence of irrigation on proanthocyanidin composition (in the percentage of subunits %) of A. skins and B. seeds, at harvest of 2015 and 2016.

*Proanthocyanins subunits: C: (+)-catechin; EC: (-)-epicatechin; ECG: (-)-epicatechin gallate; EGC: (-) – epigallocatechin. Irrigation treatments: non irrigated vines (NI), irrigation starting at mid-ripening (MRI), irrigation starting immediately after veraison (AVI), and continuous irrigation from berry set through harvest (CI). Bars indicate ± S.E. of the mean value. Significant differences among treatments are indicated by different letters (Duncan's test, $p < 0.05$)

Skin mDP varied between 10 and 18 in both years while in seeds between 2 and 5 with higher values in the more humid 2015 (Figure 5C, 5D). The mDP of skins showed a slight decreasing trend during ripening in 2015 while in 2016 it showed few fluctuations. In contrast to skin tannins, the mDP of seeds (Figure 5E, 5F) showed a slight increasing tendency in both years during the ripening season. MRI was characterised by the highest skin mDP in both years. Seed mDP did not show any consistent dependence on irrigation in 2015; however, in 2016, NI seeds had a higher mDP than the irrigated treatments.

Ten parameters related to water status, yield and berry composition were used to generate the PCA of Figure 7. Only measurements taken at harvest time of 2015 and 2016 were used to generate the PCA graph (except for Ψstem where the mean of the ripening period was used for both years). PCA revealed two components with eigenvalues greater than 1 (Kaizer's criterion) which accounted for 74.68 % of the total variance with PC1 explaining 42.94 % and PC2 explaining 31.74 %. Figure 7 shows the projection of the variables and the irrigation treatments onto the first two principal components (PC). Eight of ten variables had significant loadings (>0.500 in absolute value) on PC1 while TSS and TA loaded significantly on PC2. The scores (in triplicates) of the four irrigation treatments (NI, AVI, MRI and CI) displayed a similar separation within a year but also showed a clear separation of the two vintages (2015 and 2016). PC1 was positively associated with water potential and distinguished the non-irrigated vines (NI 2015 and 2016) as well as the MRI 2015 and AVI 2015 from the continuously irrigated vines (CI 2015 and 2016) while MRI 2016 and AVI 2016 fell on the positive side of PC1; PC2 separated the two vintages, 2015 and 2016

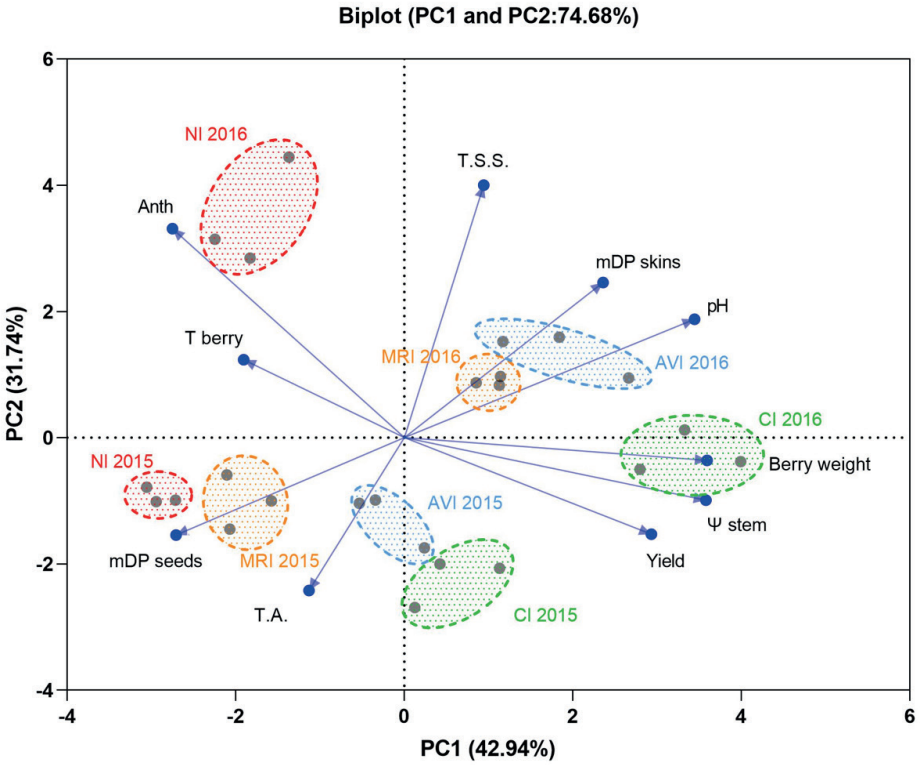


**FIGURE 7.** Bi-plot of the Principal component analysis (PCA) of cv. Xinomavro water status, yield and berry composition parameters from the four irrigation treatments.

* Parameter abbreviations: Ψstem, midday stem water potential; T berry, midday berry temperature; TSS, must total soluble solids; TA, must titratable acidity; Anth, sum of skin anthocyanins; mDP, mean degree of polymerization of skin and seed proanthocyanidins. Irrigation treatments: non irrigated vines (NI), irrigation starting at mid-ripening (MRI), irrigation starting immediately after veraison (AVI), and continuous irrigation from berry set through harvest (CI).

(effect of year), except for CI 2016. PC1 was also positively associated with total yield per vine and mean berry weight and negatively associated with berry temperature (resulting from a more "open" canopy at a lower water supply). Regarding berry polyphenol composition, mDP of seeds, and, to a lesser extent, total skin anthocyanins loaded negatively on PC1 while mDP of skins loaded positively on PC1. Furthermore, the year effect seemed to be stronger than the irrigation effect regarding total soluble solids and titratable acidity which loaded significantly on PC2.

## DISCUSSION

In the conditions of this trial, post veraison irrigation did not seem to affect canopy growth (Table 1). This is probably related to the fact that water in AVI and MRI was applied after shoot growth was nearly or completely ceased. According to Munitz *et al.* (2016), water availability at the beginning of the growth period is more effective in controlling canopy size than water availability during the ripening period. The earliness of shoot growth cessation is positively correlated with the earliness and intensity of water stress, Deloire *et al.* (2005) defined a threshold value of a predawn leaf water potential of –0.5 MPa for shoot growth inhibition although this threshold is highly dependent on the grapevine cultivar. According to our results, AVI and MRI had values of Ψdawn < –0.5 MPa and Ψstem < –1.2 MPa at veraison in both years which suggests that canopy size had been defined by the time irrigation began. On the contrary, post-veraison water application was effective in altering pruning wood weight, possibly by favouring a better partitioning of carbohydrates to shoots and leaves. According to Gomez del Campo *et al.* (2005), dry matter allocation to shoots and leaves was significantly higher in watered potted vine plants than in the stressed ones, especially during the period from veraison through harvest, possibly due to continued photosynthesis and nutrient uptake. Higher canopy growth in leaf surface can explain the lower temperature of berries in CI vines by a lower exposure to sunlight under warm climate conditions. However, even in the stressed vines (NI), berry temperature was

maintained below 30 °C, considered as a threshold for optimum primary and secondary metabolism of berries (Poni *et al.,* 2017).

Yield parameters were increased by post-veraison irrigation (Table 1). According to Intrigliolo *et al.* (2015), post-veraison irrigation water application significantly improved yield in comparison to rainfed vines, mainly by increasing berry weight. However, in our experiment, even full water supply after veraison (AVI) did not lead to a full recovery of berry growth from earlier water limitations. Post-veraison berry growth is commonly reported to be less sensitive to vine water status than pre-veraison berry growth (McCarthy, 1997) possibly related to a transition from a xylem-based flow of water into berries pre-veraison to a phloem-based after veraison (Bondada *et al.,* 2005). Must titratable acidity did not respond strongly to water conditions; regarding sugar content, irrigation starting immediately after veraison (AVI) seemed to maximise must total soluble solids rather than late-season irrigation (Figure 2C, 2D). Post-veraison irrigation helps maintain sugar transport through the phloem and sugar accumulation in the berries to sustain the ripening process and this effect is more pronounced if water is supplied as soon as veraison is complete since berry sugar content increases rapidly over the first 2–3 weeks after the onset of ripening (Božović *et al.,* 2019). Intrigliolo *et al.* (2012), also highlighted that a reduced water supply post-veraison might impair sugar accumulation into berries as a result of a reduced assimilation rate. In the light of the general trend in favour of lower alcohol in wines as well as the necessity to control the accelerated ripening under the increasingly hotter and drier climatic conditions of most viticultural areas, late irrigation (MRI) might provide a solution to avoid excessive sugar levels (Figure 2C, 2D) and allow a slightly higher yield (Table 1).

Mv was the major anthocyanin in all samples followed by MvC (Figure 4). These results are in agreement with a recent comparative study of Greek red cultivars (Kyraleou *et al.,* 2020) where Xinomavro had the lowest contribution of Dp, Cy, Pt and Pn as compared to cvs. Kotsifali, Mandilaria, Mavrotragano and Agiorgitiko. In the same study, Xinomavro had the highest percentage of esterified anthocyanins among the studied varieties. Regarding irrigation effects on anthocyanin levels (Figure 3), values were maximised under non irrigated conditions as was also reported for the same variety by Theodorou *et al.* (2019). Regarding the two post-veraison irrigation treatments, irrigation starting closer to harvest (MRI) did not seem to inhibit anthocyanin accumulation, especially in 2015.

Irrigation also affected the profile of anthocyanins (Figure 4). Irrigation amount was associated with a proportional decrease of acylated anthocyanins (MvC) compared to non-acylated forms (Dp, Cy, Pt, Pn, Mv) in both years. An increase in the content of the p-coumaroylated forms of anthocyanins under post-veraison water deficit was reported in cv. Syrah by Ollé *et al.* (2011) and similar results for Xinomavro were reported by Theodorou *et al.* (2019). Acylated anthocyanins are reported to be more stable under berry exposure to light and to high temperatures (Downey *et al.,* 2004), which might partly explain the positive effect of water deficits on the proportion of acylated forms. Irrigation amount was also associated with a proportional decrease of tri-oxygenated anthocyanins over di-oxygenated as well as of methoxylated on the B ring (Mv and esters, and Pn) over hydroxylated ones (Dp, Cy, Pt) (the latter only in 2016). Cook *et al.* (2015) reported higher tri-oxygenated anthocyanins over di-oxygenated ones in Merlot skins under increased water deficit which might be related to an up-regulation of the genes coding for flavonoid 3',5'-hydroxylase (Castellarin and Di Gaspero, 2007). Tri-oxygenated forms of anthocyanins are also reported as more stable over di-oxygenated ones to environmental conditions (Guidoni *et al.,* 2008). Thus, according to our results, post-veraison irrigation would negatively affect the colour stability of Xinomavro wines.

Total phenols changed little from veraison to harvest in all treatments, except for a declining pattern towards harvest in 2016 (Figure 5B). Berry total phenolic content is mainly determined by the levels of tannins of skins and seeds, often reported to follow opposite trends after veraison: a decreasing trend for seed tannins (Kennedy *et al.,* 2002) and a usually stable one for skin tannins (Harbertson *et al.,* 2002). Epicatechin (EC) was the dominant flavan-3-ol, in both skins and seeds (Figure 6); previous studies involving different varieties confirm our results that EC is the main subunit in skin and seed tannins (Kyraleou *et al.,* 2017). Epigallocatechin (EGC) was the second most abundant in skin extracts while Catechin (C) was in the seeds. Epicatechin-3-O-gallate (ECG) was almost entirely absent from skins while Epigallocatechin (EGC) was not detected in the seeds as also reported in other varieties (Kyraleou *et al.,* 2017).

Xinomavro berry phenolic content was sensitive to early post-veraison water conditions responding negatively to the water supply at the onset of ripening (AVI) (Figure 5A, 5B). This is further supported by recent evidence that skin proanthocyanidins resume synthesis after veraison, stimulated by post-veraison water deficit (Cáceres-Mella *et al.,* 2018). Moreover, both early season and continuous water deficit in Cabernet-Sauvignon grapes have been related previously to increased amounts of seed flavan-3-ols at harvest (Casassa *et al.,* 2015). The irrigation regime did not alter the subunit composition of seeds but increased the EGC to EC ratio in the skins, in 2016 (Figure 6). These results might imply a decrease in the perception of astringency in wines of the irrigated vines since prodelphinidins (proanthocyanidins containing flavanols with trihydroxylation on the B-ring, namely EGC) are considered as less astringent than C, EC and mostly ECG (Quijada-Morin *et al.,* 2012).

The mDP values of skin and seed proanthocyanidins were generally low (Figure 5C–5F) and varied within a range previously reported for this variety by Kyraleou *et al.* (2020); in the same work, four other Greek red cultivars showed similar mDP of their skin and seed tannins, which were lower compared to several international varieties as Merlot (Chira *et al.,* 2009) or Cabernet-Sauvignon (Bordiga *et al.,* 2011). The mDP of seeds were affected by harvest season with higher values in the more humid 2015 (Ćurko *et al.,* 2014) while in the skin samples, mDP was similar, on average, between years (Figure 5C, 5D). The mDP of skins and seeds did not present a consistent pattern during ripening although a slight increasing tendency could be observed for seed tannins contrary to most previous works reporting a decreasing trend in seed mDP during berry ripening (Kennedy *et al.,* 2000). Regarding the effect of irrigation on the polymerisation of tannins, late-season irrigation (MRI) had the highest mDP in berry skins at harvest 2015 (and a similar but not significantly confirmed trend in 2016) which suggests that late-season irrigation might increase the astringency potential of Xinomavro. Although it is generally accepted that proanthocyanidin degree of polymerisation is positively correlated with wine astringency intensity (Chira *et al.,* 2015), the skin extracts from non-irrigated Syrah vines were perceived as less astringent by a trained panel (Kyraleou *et al.,* 2016) possibly due to increased associations formed between skin tannins and cellular macromolecules (mostly acidic polysaccharides) which reduce tannin capacity to interact with salivary proteins (Vidal *et al.,* 2003).

To visualise the effects of the post-veraison irrigation regime on Xinomavro berry composition, principal component analysis (PCA) was performed (Figure 7). NI was associated with lower total yield per vine and berry weight and higher anthocyanins and seed mDP in both years, while the opposite was observed for CI, suggesting that continuous water supply was associated with a decrease in skin colour, and an increase in seed bitterness (low mDP of seeds). Regarding the post-irrigation treatments, late-season irrigation (MRI) effects on Xinomavro yield and composition were similar to NI in 2015 but not in 2016.

## CONCLUSION

Under the summer conditions of Northern Greece, supplemental irrigation during the post-veraison period has the potential to effectively control vine yield, must composition and phenolic compound content, profile and structural characteristics in Xinomavro grapes. Possible detrimental effects of post-veraison irrigation could be the increased berry size, lower skin colour amount and stability and higher bitter sensation in the produced wines. However, these non-desirable effects are mostly caused when irrigation is applied at the onset of ripening and could be offset if irrigation is applied closer to harvest. Late season water application could provide an effective adaptation strategy to climate change in Mediterranean viticultural areas. Under our experimental conditions, it led to lower sugar levels in the must and slightly higher yield without significant reductions in berry phenolic content (and possibly reducing wine astringency due to increased EGC%. However, if the aim is the maximisation of wine colour, mouthfeel quality and ageing potential of Xinomavro wines, soil water availability should remain within the range of a moderate to high water restriction during post-veraison. Nevertheless, any suggestion should be made with caution as final wine sensorial properties are influenced by water status in many ways apart from the phenolic composition, most importantly those related to aroma intensity and quality.

**Acknowledgements:** The authors would like to express their gratitude to the staff of the American Farm School of Thessaloniki, Greece for the field management of the experimental vineyard

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