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Berry morphology and composition in irrigated and non-irrigated grapevine (*Vitis vinifera* L.)

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ABSTRACT

The present study was carried out in a 5-year-old vineyard (*Vitis vinifera* L., cv. Aglianico) located in Southern Italy. Half of the plants (IRR) were fully irrigated, whereas the other half were not irrigated (NIRR). In both of the treatments, plant water status, gas exchange, photosynthetic efficiency and productive performance were determined. The arid conditions resulted in significant decreases in stem water potential in NIRR (minimum values of −1.34 and −1.52 MPa in IRR and NIRR, respectively). The values of yield per plant, cluster weight and total berry weight were significantly higher in IRR. Grape berries were separated into four weight classes, and morphometric and microscopic analyses were carried out to measure and calculate berry skin characteristics. Irrigation determined a marked shift toward heavier (+23% in the class ≥ 1.25 g) and bigger (336.35 mm^3 vs 299.15 mm^3) berries, and induced significant changes in other morphometric berry parameters. No differences among berry weight classes and irrigation treatments were observed for berry skin thickness. In all of the berry weight classes, total anthocyanins extracted from berry skins were significantly higher in NIRR than in IRR (12301.53 and 9585.52 mg kg^{-1} fresh berry skin, respectively), and appeared to be positively related to berry weight, whereas total flavonols were not significantly different between the two treatments. Qualitative changes in the levels of single anthocyanin and flavonol compounds were detected between IRR and NIRR. In addition, iron, copper and zinc, whose high concentration can negatively affect wine quality, were significantly higher in the IRR treatment. The results highlighted that the absence of irrigation did not determine decreases in grape quality. Such data can be of primary importance in environments where water availability is by far the most important limiting factor for plant growth.

Introduction

Among flavonoids, anthocyanins are pigmented compounds responsible for the dark blue coloration of ripened grape berries. They are located in the vacuoles of the hypodermal cells of berry skins and leaf epidermis (Clarke and Bakker, 2004; Conde et al., 2007; Gambuti et al., 2007; Ristic et al., 2007). Flavonols, another class of flavonoids, are colorless berry skin constituents generally considered to act as UV protectants and free-radical scavengers, and to contribute to wine color as anthocyanin co-pigments (Downey et al., 2006; Terrier et al., 2009). The quantity and composition of anthocyanins and flavonols greatly depend on genetic factors (Boss et al., 1996), but their amount can be affected by environmental factors and cultural practices (Downey et al., 2006). In grapevine, water availability alters fruit chemical composition both directly, through the activation of specific metabolic pathways (Castellarin et al., 2007a,b), or indirectly through the control of berry size (Roby and Matthews, 2004; Roby et al., 2004) and other plant biochemical and physiological processes (Conde et al., 2007). At the same time, grapevine anthocyanin and flavonol content also depend on berry growth and berry skin characteristics, such as thickness and total surface per unit of volume, and the relative proportion with respect to seeds and pulp, which is turn affected by berry size (Hardie et al., 1996; Roby et al., 2004; Cadot et al., 2011).

Abbreviations: A_n, net photosynthesis; *E*, transpiration; ETc, cultural evapotranspiration; ETo, reference evapotranspiration; F_v/F_m, maximum quantum yield of PSII photochemistry; g_s, stomatic conductance; IRR, irrigated plants; NIRR, non-irrigated plants; PPFD, photosynthetic photon flux density; SSC, soluble solid content; VPD, leaf-to-air vapor pressure deficit; Φ_{PSII}, effective quantum yield of PSII; ψ_w, stem water potential.

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Other than phenolics, grape and wine chemistry at the elemental level ("ionomic"), including the content of all mineral nutrients and trace elements, are poorly studied. All of the inorganic cations are naturally present (not from winemaking equipment containing alloys nor from fertilizers and pesticides) at non-toxic concentrations in grape berries and wine, and some of them, such as iron and copper, play a major role in winemaking and wine quality (Ribéreau-Gayon et al., 2006). Furthermore, the metal composition of the grape berry is of concern not only to the viticulturist, but also to the oenologist due to the direct impact on juice and must composition, which in turn affect wine quality.

Based on this accepted knowledge, it was of interest to investigate anthocyanin, flavonol and metal composition within the grape berries of Aglianico, a high-quality and late-ripening grape variety of southern Italy, whose premium red wine is appreciated worldwide (Gambuti et al., 2007). The aims of this research were (1) to evaluate the effect of irrigation management on plant yield and physiological status, and on berry morphologic characteristics, polyphenol and metal composition, and (2) to establish whether the absence of irrigation can affect grape quality. This could provide useful information for improving vine cultivation, winemaking processes and final product quality.

Materials and methods

Experimental site and plant material

The experiment was carried out in 2008, from bud break to the harvest, in a 5-year-old vineyard (cv. 'Aglianico' clone VCR11 grafted on 1103 Paulsen) sited on a clay-lam soil in Montegiordano Marina (42°02′N, 16°35′E; Southern Italy). According to Winkler, it is a climatic region 5, classified as 'very hot', with a thermic summation of 2603 °C above the threshold of 10 °C between 1 April and 31 October. The experimental plot, of about 0.30 ha, consisted of ten rows of spur-pruned vines to a permanent horizontal unilateral cordon. Each vine, decked at 0.60 m above the ground, was characterized by about 8 spurs of 2–3 buds each. The distance between the vines was of 2.5 m × 1.0 m, with a final plant density of 4000 vines ha^{-1}. Rows were north-south oriented.

Half of the plants (IRR) were irrigated from 9 June to 1 August (from the early stages of fruit set to veraison) using a water amount equal to 100% of cultural evapotranspiration (ETc) (24 L per plant per each of ten irrigation turn at approximately 5-day intervals), whereas the other half were not irrigated (NIRR). The value of ETc was calculated using ETo × Kc, where ETo is the reference evapotranspiration calculated according to Hargreaves method, and Kc is the cultural coefficient during the experimental period, equal to 0.6 for grapevine, according to Allen et al. (1996). In the watered plot, irrigation started when the stem water potential (ψ_w) was lower than −0.8 MPa and ended at veraison. The seasonal irrigation volume was of 960 m^3 ha^{-1} (240 L $plant^{-1}$). Each vine was irrigated by two drip emitter per plant discharging 4 L h^{-1} each. Meteorological variables were monitored by a weather station placed within 50 m of the experimental plot. Measurements of temperature, rainfall, and photosynthetic photon flux density (PPFD) were taken throughout the experimental period. The values of PPFD were recorded at 1-h intervals, and daily integrated values were logged. Leaf-to-air vapor pressure deficit (VPD) was calculated according to Goudrian and Van Laar (1994).

Plant water status, gas exchange and chlorophyll fluorescence

The plant water status was determined throughout the experimental period on ten plants per treatment by measurements of stem water potential (ψ_w). Plants located in the central part of the row, where microclimatic conditions and soil physic-chemical characteristics were similar, were chosen. The values of ψ_w were measured around midday on 5 fully expanded leaves and well-lightened selected from each plant on fruiting shoots situated in the median zone of the plant using a pressure chamber (PMS Instrument Co., Corvallis, OR, USA, model 600). For the determination of ψ_w, leaves were covered with an aluminum foil and a polyethylene bag at least 2 h before each measurement for avoiding transpiration (Choné et al., 2001).

For each treatment, the same ten plants used for ψ_w measurements were chosen to measure gas exchange and chlorophyll fluorescence on five fully-expanded and well-lightened leaves selected from each plant on fruiting shoots situated in the median zone of the cordon. Gas exchange measurements were carried out on 5 August and 4 September using a portable Li-6400 photosynthesis system (Li-Cor, Lincoln, NE, USA) equipped with a 362-cm^2 wide leaf chamber. Light was provided by an artificial red LED source emitting at 670 nm, and an external bottled 12-g CO_2 source was used to infiltrate the leaf chamber with air at a constant 370 μmol mol^{-1} CO_2. A pulse modulated FMS1 fluorometer (Hansatech Instruments, Norfolk, UK) was used to carry out chlorophyll fluorescence measurements along the experimental period. The FMS1 fluorometer adopted a pulsed light source as a very weak exciting modulating (amber) light, peaked at 594 nm. The FMS1 was equipped with a halogen white lamp source to generate a super-saturating light pulse of 17,000 μmol m^{-2} s^{-1} applied for 0.7 s for fluorescence induction, and delivered to the leaf sample through an optical fiber probe inserted at 45° inclination into a closed black dome fitted over the leaf-clip. The basal fluorescence yield in dark-adapted leaves (F_o) or the steady state fluorescence yield under actinic light (F_s) were measured. The maximal fluorescence yield in the dark (F_m) and in light conditions (F'_m) were determined using white light pulses. The maximum quantum yield of PSII photochemistry in dark-adapted leaves was calculated as F_v/F_m, where the variable fluorescence (F_v) is the difference between F_m and F_o. Each leaf was dark-adapted for 30 min by means of a leaf clip before F_v/F_m measurement. In light-adapted leaves, the effective (or actual) quantum yield of PSII (Φ_{PSII}) was calculated as $(F'_m - F'_s)/F'_m$.

Yield and berry characteristics

At harvest, on 27 September 2008, the number of clusters and yield per plant, cluster weight, number of berries per cluster, and total berry weight per cluster, and the number of leaves per shoot were determined on 30 plants per treatment. From the same plants, three clusters per plant were randomly collected. All of the berries of these clusters were picked and separated into four weight classes: $x < 0.60$ g, $0.60 \text{ g} \leq x < 0.90$ g, $0.90 \text{ g} \leq x < 1.25$ g, and $x \geq 1.25$ g. The weight class frequency distribution (% of sample population) per each treatment was calculated. For each plant, 20 berries per each weight class were randomly chosen to determine berry fresh weight, berry equatorial diameter and berry polar diameter. Considering that berry shape was approximately spherical (mean equatorial/polar diameter ratio = 0.99 ± 0.08), surface, volume, surface/volume ratio, and skin specific surface (defined as the ratio berry surface/skin weight), and skin specific weight (defined as the ratio skin weight/berry weight) were calculated. Each berry was cut into two halves and the seeds and skins were separated from the pulp through a small metal spatula, washed in deionized water, dried with absorbent paper, and the fresh weights of skins, pulps and seeds were determined. The soluble solid content (SSC) of each berry was obtained by squeezing his pulp on a manual refractometer (model MT-032 ATC; Turoni & C., Forlì, Italy).

Anthocyanin and flavonol extraction and determination

At harvest, three clusters per plant were randomly sampled in the central and well-irradiated area of the canopy from three plants located in the central part of the rows, in order to minimize soil differences between the two treatments. Again, the berries of the four weight classes were detached from each cluster, peeled with a scalpel and the skin, rapidly frozen at −80 °C, and then stored for the analytical determinations. The berries of $x < 0.60$ g were discarded, as they were not always sufficient for a complete analysis in IRR and NIRR plants. Five grams of skin of frozen grape berries were collected and placed in a 100 mL methanol–HCl 0.75% (w/w) solution at 20 °C in the dark. The extraction was carried out for 24 h. The resulting extracts were filtered through 0.20 μm Minisart SFCA sterile filters (Sartorius Stedim Biotech GmbG, Goettingen, Germany), and immediately frozen at −80 °C. Berry extracts were analyzed by high-performance liquid chromatography for the anthocyanin determination. The anthocyanin contents of skin extracts were determined by high performance liquid chromatography (HP 1110, Agilent Technologies, Palo Alto, CA, USA). All berry skin extracts were filtered through 0.45-μm Whatman filters. The HPLC–MS analyses were conducted to confirm the identity of each peak using an HP 1100 MS system with a PDA UV-vis detector coupled to an Agilent 6110 Quadrupole LC/MS equipped with an ESI source (Agilent Technologies, Palo Alto, CA, USA), a Luna 5 μ-Phenyl-Hexyl column, 4.60 mm × 250 mm (Phenomenex Inc., Torrance, CA, USA) and injection valve (20 μL loop). An aqueous solution containing 0.1% TFA and MeCN was used as eluent. The column was eluted at room temperature using a consecutive isocratic gradient of 100% aqueous solution containing 0.1% TFA for 1 min, then a gradient of 0–40% MeCN for 39 min, 40–0% MeCN for 1 min, and 100% aqueous solution containing 0.1% TFA for 1 min at a flow rate of 1.0 mL min^{-1}. Mass scans were measured from m/z 100 up to m/z 800. Mass spectrometry data were acquired in the negative ionization mode. The quantification of both anthocyanins and flavonols were carried out using corresponding external standards (Extrasynthèse S.A.S., Genay, France).

Metal determination

Berry skins deriving from the same clusters used for anthocyanin and flavonol analysis were digested in a HNO_3:H_2O_2 solution (5:1, v/v) using a high performance microwave digestion unit (MLS-1200 Mega, Milestone Inc., CT, USA). The levels of iron, copper, zinc and calcium were determined by means of quadrupole inductively coupled plasma mass spectrometry, ICP-QMS (Elan DRC II, Perkin-Elmer SCIEX, CT, USA). Operational parameters were as follows: sample uptake rate, 1 mL min^{-1}; sample introduction, Meinhard nebulizer with cyclonic spray chamber; gas flow rates (L min^{-1}): plasma, 15; auxiliary, 1.0; nebulizer, 0.85; dwell time, 50 ms; interface, Pt cones; extraction lens voltage, optimized for maximum I (^{56}Fe). High purity He (99.9999%) and H_2 (99.9995%) were used, in order to minimize the potential problems caused by unidentified reactive contaminant species in the cell. The reference wavelengths for each metal were chosen avoiding interferences with the other elements analyzed. Before use, all glassware and plastic containers were cleaned by washing with 10% ultra-pure grade HNO_3 for at least 24 h, and then rinsed copiously with ultrapure water before use. The calibration solutions were prepared from multi-elemental standard stock solutions of 1000 mg L^{-1}, and the calibration curves were obtained by using at least 6 calibration solutions. Reagent blanks containing ultra-pure water were also analyzed to control the purity of the reagents and the laboratory equipment. Standards and blanks were subjected to the same treatment as berry skin samples.

Microscopic analysis

Berries deriving from the same clusters used for anthocyanin, flavonol and metal analysis were used to measure skin thickness. Discs of berry fresh tissue, turgid and free from surface residues, were taken from 20 berries randomly chosen within each berry weight class by two parallel cuttings made on either sides of the berry equatorial diameter (2.5 mm above and 2.5 below). A microtome blade was used to obtain fine dissections, avoiding surface disruption. The skin sections were mounted on slides and observed at different magnifications using a compound optical microscope (Eclipse 80i; Nikon, Tokyo, Japan) under transmitted light, and then photographed (Digital Camera DS-Fi1; Nikon). The thickness of berry skin was measured by image analysis software (NIS-Elements Imaging Software; Nikon).

Statistical analysis

The data on ψ_w, gas exchange and chlorophyll fluorescence were represented as the means of ten separate measurements on ten different plants per treatment, with five replicates per plant ($n = 50$). The data on yield, berry morphometric parameters and SSC were represented as the means of 30 separate measurements on 30 different plants per treatment, with no replicates for yield measurements ($n = 30$), and 20 replicates per plant for morphometric parameters and SSC ($n = 600$). The data on anthocyanin, flavonol, metal levels and skin microscopic analysis were represented as the means of three separate measurements on three different plants per treatment, with nine replicates per plant for anthocyanin, flavonol and metal levels ($n = 27$), and 100 triplicates for microscopic analysis ($n = 100$).

Statistical analysis was performed by analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC, USA). Significant differences were determined at $P \leq 0.05$, according to Fisher's LSD test.

Results

Climatic variables

The experimental period was characterized by high temperatures and scarcity of rainfall. Maximum temperatures ranged between 15.3 and 38.5 °C, with maximum peaks occurring in the period from the end of July to the beginning of August, in correspondence to grape veraison. Minimum temperatures ranged between 12.3 and 29.1 °C. Average annual rainfall was 245 mm, but during the experimental period was particularly low, showing a value of 21.9 mm, with the most relevant rainfall (7.7 mm) on 28 August. Daily values of reference evapotranspiration (ETo) were often above 5 mm, and fluctuated between 1.06 and 6.82 mm, with the higher values in the first 10 days of July and the lower at the end of September. The mean value of daily crop evapotranspiration (ETc) during the experimental period was 3 mm. Total daily global radiation ranged between 25 and 30 MJ m^{-2}, showing the highest values before 14 September, followed by a sharp decrease after this date. The values of VPD reached the maximum between 26 June and 10 September (maximum of 3.59 kPa on 22 August) and then decreased after this period.

Plant water status gas exchange and chlorophyll fluorescence

The environmental arid conditions resulted in a rapid depletion of water reserves in the soil. At the beginning of the experiment, the values of stem water potential (ψ_w) in IRR and NIRR were −0.56

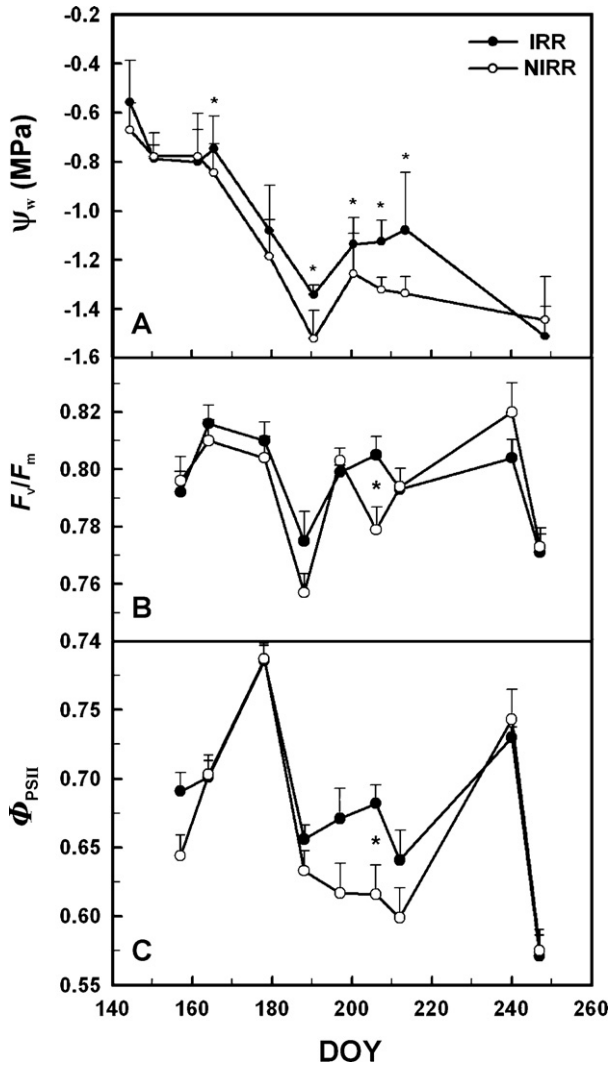

Fig. 1. (A) Stem water potential (ψ_w), (B) maximum quantum yield of PSII photochemistry in dark-adapted leaves (F_v/F_m) and (C) effective quantum yield of PSII (Φ_{PSII}) in irrigated (IRR, black circles) and non-irrigated (NIRR, white circles) vines. Mean values (±st. dev.) with the asterisk are significantly different between IRR and NIRR at $P \leq 0.05$, according to Fisher's LSD test. DOY = day of year.

and −0.67 MPa, respectively. They were close to −0.80 MPa near flowering (late May), decreased until 8 July (−1.34 and −1.52 MPa in IRR and NIRR, respectively), and then remained almost constant until the end of the experimental period. Significant differences of ψ_w between IRR and NIRR were found on some dates (Fig. 1A).

Net photosynthesis (A_n) in IRR and NIRR was 2.71 and 2.48 μmol CO_2 m^{-2} s^{-1} on 5 August, respectively, and 5.80 and 5.69 μmol CO_2 m^{-2} s^{-1} on 4 September. The mean A_n values were 4.26 and 4.00 μmol CO_2 m^{-2} s^{-1} in IRR and NIRR, respectively, and significant differences between the two treatments were not found. Transpiration (E) in IRR and NIRR was 1.89 and 2.11 mmol H_2O m^{-2} s^{-1}, respectively, on 5 August, and 3.60 and 3.86 mmol H_2O m^{-2} s^{-1}, respectively, on 4 September. The mean E values in IRR and NIRR were 2.75 and 2.98 mmol H_2O m^{-2} s^{-1}, respectively, without any statistical differences between the two treatments. The values of stomatic conductance (g_s) measured on 5 August were 0.03 mol H_2O m^{-2} s^{-1} in both IRR and NIRR, and 0.08 and 0.09 mol H_2O m^{-2} s^{-1} on 4 September in IRR and NIRR, respectively. Mean g_s in IRR and NIRR were 0.05 and 0.06 mol H_2O m^{-2} s^{-1}, respectively, and no significant differences between the two treatments were revealed.

In general, the maximum quantum yield of photosystem II (F_v/F_m) and the actual quantum yield of PSII reaction centers (Φ_{PSII}) in leaves were not affected by the irrigation treatment during the growing season (Fig. 1B and C). However, in NIRR vines F_v/F_m and Φ_{PSII} showed a significant lower value of 0.62 if compared to 0.68 in IRR vines during one of the hottest day of July. In both treatments, only on two dates (half July and beginning of September) were there some indications of permanent photoinhibition, as demonstrated by F_v/F_m values of about 0.77 (Fig. 1B).

Yield, cluster and berry characteristics

The mean numbers of clusters per plant showed no significant differences between IRR and NIRR plants. The yield per plant was 1.79 kg in IRR and 1.17 kg in NIRR, with an increase by 53% in IRR, mainly due to the higher cluster weight (+35%) (133.95 g) if compared to NIRR (99.34 g) (Table 1). The values of yield per plant, cluster weight and total berry weight were significantly different between the two treatments.

Class frequency distribution of berry weight in IRR and NIRR was statistically different, as irrigation determined a marked shift toward heavier berries (Table 2). In particular, irrigation significantly increased the frequency of grapes with a weight ≥ 1.25 (+20%) and reduced the frequency of berries with a weight < 0.6 g (−61%).

Irrigation significantly affected berry fresh weight (+9%) and skin fresh weight (−9%) (Table 2). The values of the berry surface/volume ratio were higher in smaller berries, but they were not significantly affected by the different watering regime in the four weight classes (Table 3). Irrigation had a significant effect on berry surface and volume, which was significantly higher in IRR (Table 3). With the exception of the weight < 0.60 g, skin fresh weights of NIRR berries were statistically higher than those observed in IRR, and this caused decreases in skin specific surface and increases in skin specific weight (mean value +9%) (Tables 2 and 3). In the two intermediate weight classes, significant differences in seed weight per berry were observed (Table 2), and this was due to differences in seed number per berry (1.41 ± 0.66 in NIRR and 1.21 ± 0.49 in IRR for the class 0.60 g ≤ x < 0.90 g; and 1.87 ± 0.66 in NIRR and 1.67 ± 0.74 in IRR for the class 0.90 g ≤ x < 1.25 g). With the exception of the weight < 0.60 g, skin fresh weights of NIRR berries were statistically higher than those observed in IRR, and this caused decreases in skin specific surface and increases in skin specific weight (mean value +9%) (Table 3). In the two intermediate weight classes, significant differences in seed weight per berry were observed (Table 2), and this was due to differences in seeds number per berry (1.41 ± 0.66 seeds/berry in NIRR and 1.21 ± 0.49 in IRR for the class 0.60 g ≤ x < 0.90 g; and 1.87 ± 0.66 in NIRR and 1.67 ± 0.74 in IRR for the class 0.90 g ≤ x < 1.25 g). The values of soluble solid content (SSC) were significantly higher in NIRR than in IRR for all the four berry weight classes considered (Table 2).

Anthocyanin and flavonol levels

The anthocyanins revealed in the berry skin samples are shown in Tables 4–6. Under our experimental conditions, free, di-substituted and tri-substituted anthocyanins were not observed in IRR or in NIRR. Total anthocyanins were significantly higher in NIRR than in IRR, in all the three weight classes, and appeared to be positively related to berry weight (Table 6). Malvidin-3-*O*-glucoside was found to be the major anthocyanin present along with its coumaroylated form, accounting for 77 and 60% of total content in anthocyanins in IRR and NIRR, respectively (Table 4). Significant differences were found in the levels of petunidin-3-*O*-acetylglucoside, peonidin-3-*O*-acetylglucoside

Table 1
Yield, number of clusters, cluster weight, number of berries per cluster, and total berry weight in irrigated (IRR) and non-irrigated (NIRR) grapes. Mean values (±st. dev.) followed by different letters are significantly different between IRR and NIRR at $P \leq 0.05$, according to Fisher's LSD test.

Treatment	Number of clusters (number plant^{-1})	Yield (kg plant^{-1})	Cluster weight (g cluster^{-1})	Number of berries (berries cluster^{-1})	Total berry weight (g cluster^{-1})	Number of leaves (number shoot^{-1})
IRR	15.3 ± 4.5 a	1.8 ± 0.9 a	133.95 ± 67.80 a	86.57 ± 39.87 a	127.27 ± 65.35 a	15 ± 0.49 a
NIRR	14.0 ± 4.1 a	1.2 ± 0.6 b	99.34 ± 46.81 b	86.14 ± 37.20 a	95.44 ± 45.23 b	8 ± 0.31 b

Table 2
Measured morphometric parameters and total soluble solids in berries of irrigated (IRR) and non-irrigated (NIRR) grapes. Mean values (±st. dev.) followed by different letters are significantly different (lowercase between IRR and NIRR, and uppercase between berry weight classes) at $P \leq 0.05$, according to Fisher's LSD test.

Treatment	Berry weight class (g)	Frequency distribution (%)	Berry fresh weight (g)	Skin fresh weight (g)	Skin thickness (μm)	Seed fresh weight (g)	Total soluble solids (°Brix)
IRR	$x < 0.60$	2.0 bC	0.53 ± 0.06 aD	0.13 ± 0.06 aB	111 ± 5 aA	0.049 ± 0.033 aC	21.96 ± 2.49 bA
	$0.60 \leq x < 0.90$	24.1 bB	0.78 ± 0.08 aC	0.19 ± 0.05 bB	104 ± 3 aA	0.045 ± 0.015 bC	21.41 ± 3.10 bA
	$0.90 \leq x < 1.25$	36.5 aA	1.05 ± 0.10 aB	0.22 ± 0.08 bA	118 ± 2 aA	0.064 ± 0.024 bB	21.46 ± 2.18 bA
	$x \geq 1.25$	37.4 aA	1.42 ± 0.19 aA	0.27 ± 0.10 bA	114 ± 3 aA	0.087 ± 0.031 bA	20.68 ± 2.10 bA
	Average	–	1.01 ± 0.34 a	0.22 ± 0.09 b	112 ± 6 a	0.063 ± 0.031 b	21.29 ± 2.52 b
NIRR	$x < 0.60$	12.5 aB	0.53 ± 0.06 aD	0.15 ± 0.05 aD	106 ± 3 aA	0.048 ± 0.021 aC	24.19 ± 3.55 aA
	$0.60 \leq x < 0.90$	38.9 aA	0.80 ± 0.08 aC	0.22 ± 0.07 aC	109 ± 1 aA	0.053 ± 0.024 aC	23.42 ± 3.34 aA
	$0.90 \leq x < 1.25$	34.2 aA	1.05 ± 0.09 aB	0.27 ± 0.08 aB	105 ± 3 aA	0.078 ± 0.091 aB	22.96 ± 2.43 aA
	$x \geq 1.25$	14.4 bB	1.38 ± 0.12 bA	0.34 ± 0.10 aA	109 ± 3 aA	0.093 ± 0.053 aA	23.01 ± 2.24 aA
	Average	–	0.92 ± 0.31 b	0.25 ± 0.10 a	107 ± 2 a	0.067 ± 0.058 a	23.39 ± 2.99 a

Table 3
Calculated morphometric parameters in berries of irrigated (IRR) and non-irrigated (NIRR) grapes. Mean values (±st. dev.) followed by different letters are significantly different (lowercase between IRR and NIRR, and uppercase between berry weight classes) at $P \leq 0.05$, according to Fisher's LSD test.

Treatment	Berry weight class (g)	Frequency distribution (%)	Berry surface (mm^2)	Berry volume (mm^3)	Surface/volume ratio (mm^{-1})	Skin specific surface (mm^2 g^{-1} skin)	Skin specific weight (g skin g^{-1} berry)
IRR	$x < 0.60$	2.0 bC	229.37 ± 59.69 aD	335.03 ± 128.63 aD	0.72 ± 0.11 aA	1679.02 ± 688.76 aA	0.29 ± 0.10 aA
	$0.60 \leq x < 0.90$	24.1 bB	288.68 ± 57.43 aC	468.66 ± 131.35 aC	0.64 ± 0.11 aA	1740.43 ± 780.84 aA	0.24 ± 0.06 aB
	$0.90 \leq x < 1.25$	36.5 aA	350.80 ± 66.21 aB	626.67 ± 169.81 aB	0.58 ± 0.06 aB	1709.93 ± 830.35 aA	0.23 ± 0.07 aB
	$x \geq 1.25$	37.4 aA	418.98 ± 78.37 aA	817.69 ± 226.37 aA	0.53 ± 0.05 aB	1795.14 ± 860.70 aA	0.19 ± 0.06 bC
	Average	–	336.35 ± 93.33 a	597.01 ± 242.11 a	0.60 ± 0.11 a	1739.05 ± 794.38 a	0.23 ± 0.08 a
NIRR	$x < 0.60$	12.5 aB	191.54 ± 55.47 bD	257.42 ± 106.79 aD	0.80 ± 0.17 aA	1423.36 ± 714.24 bA	0.29 ± 0.10 aA
	$0.60 \leq x < 0.90$	38.9 aA	278.40 ± 58.31 aC	444.38 ± 135.04 aC	0.65 ± 0.08 aB	1489.37 ± 738.57 bA	0.27 ± 0.09 aA
	$0.90 \leq x < 1.25$	34.2 aA	330.51 ± 72.67 aB	575.81 ± 182.81 bB	0.60 ± 0.08 aB	1360.26 ± 536.44 bA	0.26 ± 0.08 aA
	$x \geq 1.25$	14.4 bB	408.04 ± 70.62 bA	784.21 ± 204.07 bA	0.53 ± 0.05 aC	1341.82 ± 593.77 bA	0.25 ± 0.07 aB
	Average	–	299.15 ± 98.59 b	506.91 ± 241.87 b	0.65 ± 0.14 a	1407.92 ± 653.87 b	0.27 ± 0.09 a

Table 4
Anthocyanin-3-*O*-glucosides in berry skins of irrigated (IRR) and non-irrigated (NIRR) grapes. Mean values (±st. dev.) followed by different letters are significantly different (lowercase between IRR and NIRR, and uppercase between berry weight classes) at $P \leq 0.05$, according to Fisher's LSD test.

Treatment	Berry weight class (g)	Delphinidin-3-*O*-glucoside (mg kg^{-1} fresh berry skin)	Cyanidin-3-*O*-glucoside (mg kg^{-1} fresh berry skin)	Petunidin-3-*O*-glucoside (mg kg^{-1} fresh berry skin)	Peonidin-3-*O*-glucoside (mg kg^{-1} fresh berry skin)	Malvidin-3-*O*-glucoside (mg kg^{-1} fresh berry skin)
IRR	$0.60 \leq x < 0.90$	301.81 ± 24.89 aB	286.29 ± 56.72 aC	442.94 ± 29.97 aB	21.76 ± 5.50 aB	4066.14 ± 173.81 aC
	$0.90 \leq x < 1.25$	367.33 ± 41.02 aB	365.44 ± 65.81 aB	494.24 ± 39.86 bB	35.55 ± 6.25 aA	5686.92 ± 167.35 aB
	$x \geq 1.25$	416.84 ± 50.23 aA	413.56 ± 53.34 aA	556.10 ± 35.89 aA	39.32 ± 8.63 aA	6572.19 ± 161.93 aA
	Average	361.99 ± 57.70 a	355.10 ± 64.26 a	497.76 ± 56.66 a	32.21 ± 9.24 a	5441.75 ± 1270.89 a
NIRR	$0.60 \leq x < 0.90$	358.22 ± 60.61 aB	261.21 ± 39.81 aC	532.64 ± 98.49 aB	44.77 ± 11.22 aA	4735.71 ± 585.23 aB
	$0.90 \leq x < 1.25$	348.53 ± 53.53 aB	359.54 ± 74.67 aB	531.97 ± 72.92 aB	46.89 ± 10.48 aA	6033.29 ± 390.69 aA
	$x \geq 1.25$	482.58 ± 90.42 aA	480.16 ± 95.42 bA	715.91 ± 31.48 aA	33.61 ± 5.74 aA	6608.24 ± 211.83 aA
	Average	408.44 ± 65.54 a	366.97 ± 109.66 a	593.51 ± 106.01 a	41.76 ± 7.14 a	5792.41 ± 959.22 a

Table 5
Anthocyanin-3-*O*-acetylglucosides in berry skins of irrigated (IRR) and non-irrigated (NIRR) grapes. Mean values (±st. dev.) followed by different letters are significantly different (lowercase between IRR and NIRR, and uppercase between berry weight classes) at $P \leq 0.05$, according to Fisher's LSD test.

Treatment	Berry weight class (g)	Delphinidin-3-*O*-acetylglucoside (mg kg^{-1} fresh berry skin)	Cyanidin-3-*O*-acetylglucoside (mg kg^{-1} fresh berry skin)	Petunidin-3-*O*-acetylglucoside (mg kg^{-1} fresh berry skin)	Peonidin-3-*O*-acetylglucoside (mg kg^{-1} fresh berry skin)	Malvidin-3-*O*-acetylglucoside (mg kg^{-1} fresh berry skin)
IRR	$0.60 \leq x < 0.90$	168.78 ± 63.69 aB	92.86 ± 31.32 bB	39.44 ± 14.65 bC	55.56 ± 8.11 bB	55.27 ± 16.11 aA
	$0.90 \leq x < 1.25$	156.41 ± 36.79 aB	162.11 ± 64.26 bA	62.78 ± 16.18 bB	50.00 ± 6.60 bB	65.59 ± 15.96 aA
	$x \geq 1.25$	250.26 ± 39.70 bA	122.47 ± 41.47 bA	100.00 ± 12.50 bA	193.33 ± 26.00 bA	49.55 ± 14.69 bA
	Average	233.41 ± 122.82 a	125.81 ± 34.75 b	67.41 ± 30.54 b	99.63 ± 81.20 b	56.84 ± 8.18 b
NIRR	$0.60 \leq x < 0.90$	153.19 ± 57.47 aB	128.10 ± 33.39 aB	332.22 ± 45.81 aC	1127.78 ± 21.32 aB	59.94 ± 9.82 aA
	$0.90 \leq x < 1.25$	199.41 ± 36.21 aB	211.96 ± 67.13 aA	590.00 ± 73.50 aB	1456.67 ± 286.02 aB	70.44 ± 5.78 aA
	$x \geq 1.25$	429.33 ± 48.59 aA	296.13 ± 34.76 aA	867.22 ± 60.32 aA	2777.78 ± 466.02 aA	104.66 ± 21.86 bB
	Average	260.64 ± 147.91 a	212.06 ± 84.02 a	596.48 ± 267.56 a	1748.41 ± 873.31 a	78.35 ± 23.38 a

Table 6
Other anthocyanins and total anthocyanin concentration in berry skins of irrigated (IRR) and non-irrigated (NIRR) grapes. Mean values (±st. dev.) followed by different letters are significantly different (lowercase between IRR and NIRR, and uppercase between berry weight classes) at $P \leq 0.05$, according to Fisher's LSD test.

Treatment	Berry weight class (g)	Petunidin-(6-*O*-caffeoyl) glucoside (mg kg^{-1} fresh berry skin)	Malvidin-(6-*O*-caffeoyl) glucoside (mg kg^{-1} fresh berry skin)	Cyanidin-(6-*O*-coumaryoyl) glucoside (*trans* isomer) (mg kg^{-1} fresh berry skin)	Petunidin-(6-*O*-coumaryoyl) glucoside (*trans* isomer) (mg kg^{-1} fresh berry skin)	Peonidin-3-(6-*O*-coumaryoyl) glucoside (*trans* isomer) (mg kg^{-1} fresh berry skin)	Malvidin-3-(6-*O*-coumaryoyl) glucoside (*trans* isomer) (mg kg^{-1} fresh berry skin)	Total anthocyanins (mg kg^{-1} fresh berry skin)
IRR	$0.60 \leq x < 0.90$	17.10 ± 4.18 bA	42.98 ± 14.17 bB	54.31 ± 11.16 bA	109.20 ± 38.02 bB	78.24 ± 22.56 bB	1573.73 ± 328.13 aC	7406.41 bC
	$0.90 \leq x < 1.25$	4.04 ± 0.30 bB	58.47 ± 12.31 bB	55.80 ± 7.11 bA	96.32 ± 22.66 bB	80.32 ± 14.59 bB	1801.65 ± 452.17 aB	9542.97 bB
	$x \geq 1.25$	1.30 ± 0.11 bB	213.01 ± 51.82 aA	78.00 ± 21.14 bA	206.72 ± 36.63 aA	151.35 ± 39.65 aA	2443.17 ± 516.37 aA	11807.17 bA
	Average	7.48 ± 8.44 a	104.82 ± 94.01 b	62.70 ± 13.27 b	137.41 ± 60.37 b	103.30 ± 41.62 a	1939.52 ± 450.82 a	9585.52 ± 2200.69 b
NIRR	$0.60 \leq x < 0.90$	77.29 ± 16.28 aA	94.52 ± 22.73 a	78.58 ± 10.07 aA	193.25 ± 32.11 aA	102.31 ± 32.82 aA	1194.98 ± 307.11 bC	9474.71 aC
	$0.90 \leq x < 1.25$	55.13 ± 15.49 aB	136.37 ± 27.37 a	71.90 ± 15.64 aA	179.77 ± 45.68 aA	155.73 ± 42.42 aA	1624.10 ± 36.06 bB	12071.70 aB
	$x \geq 1.25$	39.82 ± 8.97 aC	202.07 ± 72.17 a	92.95 ± 15.82 aA	187.96 ± 33.95 aA	90.27 ± 23.04 bA	1949.49 ± 329.90 bA	15358.18 aA
	Average	57.41 ± 18.84 a	144.32 ± 54.22 a	81.15 ± 10.76 a	186.99 ± 6.79 a	116.10 ± 34.84 a	1589.53 ± 378.44 b	12301.53 ± 2948.46 a

and petunidin-(6-*O*-caffeoyl)glucoside, that were higher in NIRR (9-, 18-, and 10-times higher, respectively) (Tables 5 and 6). Excluding some cases (e.g., petunidin-(6-*O*-caffeoyl)glucoside), the content of single anthocyanins generally increased with decreasing berry weight (Tables 4–6). Interestingly, IRR berries presented a significantly lower ratio of acetylated anthocyanins/coumaroylated anthocyanins (0.24 in IRR and 1.49 in NIRR).

With respect to flavonols, quercetin-3-*O*-glucoside, and to a lesser extent, quercetin-3-*O*-glucuronide and myricetin-3-*O*-glucoside, were the most abundant both in IRR and NIRR (Table 7). The levels of total flavonols were not significantly different between NIRR and IRR plants, considering the average values and the values in the three berry weight classes. With some exceptions, the levels of the single flavonols in the heavier berries were significantly higher (Table 7). Kaempferol, myricetin and isorhamnetin were present as the corresponding 3-*O*-glucosides or 3-*O*-galacosides forms (Table 7).

Metal levels

Irrigation significantly affected metal distribution in all the three weight classes (Table 8). Iron, copper and zinc levels were significantly higher in the IRR treatment (Table 8). Calcium levels were not statistically different between the two irrigation treatments. Generally, metal levels significantly decreased with increasing berry weight (Table 8).

Morphological analysis of berry skin

From the analysis of transverse berry sections including the epidermis and a small amount of fleshy tissue, no differences among berries from different classes and/or treatments were observed for berry skin thickness, whose values among the treatments ranged from 96 to 118 μm (Table 2). The exocarp structure of the grape berries presented two outer epidermal cell layers, covered by a thick cuticle with surface waxes, and 6 or 8 inner collenchymatous hypodermal cell layers (Fig. 2). Furthermore, a high content of vacuolar polyphenols and anthocyanins was observed, as refractive droplets, in the skin cells (Fig. 2). It was evident, as the berries were mature, that the cell walls of the outer mesocarp, beneath the hypodermis, underwent a process of partial degradation and loosening (Fig. 2). No significant changes were detected in the number of skin cell layers and thickness, excluding epicuticular wax, and integument thickness (Table 3 and Fig. 2).

Discussion

The growing season in which this study was carried out was particularly hot and dry. Unfortunately, during the period when irrigation was performed, midday stem water potential (ψ_w) values in IRR plants (Fig. 1A) were comparable to those found by other authors (Salón et al., 2005; Van Leeuwen et al., 2009; Romero et al., 2010) in plants subjected to moderate-severe water stress (ψ_w range between −1.1 and −1.4 MPa), while those of NIRR plants (Fig. 1A) were comparable to a severe water stress situation ($\psi_w < -1.4$ MPa). At the values of stem water potential achieved by our plants (Fig. 1A), gas exchange is usually reduced due to the down-regulation of photosynthesis (Flexas et al., 2004), associated with phenomena of photoinhibition, and foliar photo-damage, chlorosis and subsequent necrosis (Palliotti et al., 2009), as confirmed by the significantly lower values of F_v/F_m and Φ_{PSII} in NIRR (Fig. 1B and C).

The greater foliar necrosis and subsequent abscission in NIRR plants (Table 1) certainly influenced the micro-climate at the cluster level (e.g., higher exposure to direct solar radiation), subjecting the cluster to multiple stresses (drought, high temperature and

Table 7
Flavonols in berry skins of irrigated (IRR) and non-irrigated (NIRR) grapes. Mean values (±st. dev.) followed by different letters are significantly different (lowercase between IRR and NIRR, and uppercase between berry weight classes) at $P \leq 0.05$, according to Fisher's LSD test.

Treatment	Berry weight class (g)	Myricetin-3-*O*-glucoside (mg kg^{-1} fresh berry skin)	Quercetin-3-*O*-glucuronide (mg kg^{-1} fresh berry skin)	Quercetin-3-*O*-glucoside (mg kg^{-1} fresh berry skin)	Laricitrin-3-*O*-galactoside (mg kg^{-1} fresh berry skin)	Laricitrin-3-*O*-rhamnose-7-*O*-trihydroxycinnamic acid (mg kg^{-1} fresh berry skin)	Kaempherol-3-*O*-glucoside (mg kg^{-1} fresh berry skin)	Kaempferol-3-*O*-caffeoylate (mg kg^{-1} fresh berry skin)	Isorhamnetin-3-*O*-glucoside (mg kg^{-1} fresh berry skin)	Syringetin-3-*O*-galactoside (mg kg^{-1} fresh berry skin)	Total flavonols (mg kg^{-1} fresh berry skin)
IRR	$0.60 \leq x < 0.90$	121.39 ± 36.88 aB	124.22 ± 19.46 aB	349.88 ± 25.50 aB	31.06 ± 5.38 aB	55.07 ± 13.88 aB	21.15 ± 9.35 aB	26.26 ± 16.43 aB	48.55 ± 17.04 aC	16.77 ± 1.83 aC	794.35 aC
	$0.90 \leq x < 1.25$	139.18 ± 38.00 aB	138.90 ± 29.40 aB	428.60 ± 70.06 aB	38.88 ± 7.71 aB	40.34 ± 10.75 aB	19.40 ± 9.74 aB	36.37 ± 18.39 aB	64.49 ± 31.97 aB	39.77 ± 8.57 aB	945.93 aB
	$x \geq 1.25$	158.83 ± 52.51 aA	233.11 ± 50.41 aA	514.85 ± 61.42 aA	43.72 ± 9.89 aA	72.56 ± 27.36 aA	39.04 ± 14.75 aA	70.14 ± 23.39 aA	81.75 ± 27.59 aA	75.02 ± 10.21 aA	1289.02 aA
	Average	139.80 ± 18.73 a	165.41 ± 59.09 a	431.11 ± 82.51 a	37.88 ± 6.39 a	55.99 ± 16.13 a	26.53 ± 10.87 a	44.26 ± 22.98 a	64.93 ± 16.61 a	43.85 ± 29.34 a	1009.77 ± 253.44 a
NIRR	$0.60 \leq x < 0.90$	123.49 ± 11.89 aB	136.77 ± 22.45 aB	336.51 ± 48.88 aB	19.61 ± 1.60 bB	48.76 ± 10.95 aB	8.51 ± 2.37 bB	25.01 ± 6.01 aB	60.58 ± 11.21 aB	25.21 ± 5.45 aB	784.45 aB
	$0.90 \leq x < 1.25$	77.82 ± 19.11 bB	121.54 ± 43.01 aB	379.17 ± 16.01 aB	8.61 ± 1.46 bC	30.15 ± 6.59 aB	2.65 ± 1.67 bB	31.55 ± 6.88 aB	72.30 ± 23.27 aA	46.04 ± 13.80 aA	769.83 aB
	$x \geq 1.25$	197.09 ± 27.99 aA	205.49 ± 63.38 aA	489.24 ± 56.23 aA	30.18 ± 6.37 aA	62.92 ± 15.40 aA	37.07 ± 8.01 aA	49.53 ± 10.92 bA	85.89 ± 18.45 aA	46.81 ± 10.41 bA	1204.22 aA
	Average	132.80 ± 60.18 a	154.60 ± 44.72 a	401.64 ± 78.80 a	18.57 ± 10.88 a	47.27 ± 16.44 a	16.08 ± 18.41 a	35.36 ± 12.70 a	72.93 ± 12.67 a	39.35 ± 12.25 a	919.50 ± 246.68 a

irradiance). These stresses likely affected, directly or indirectly, berry size and chemical composition (Keller, 2010). Some authors (Roby and Matthews, 2004; Shellie, 2006) have highlighted growth inhibition of the berry at an average value of leaf water potential measured at midday equal or lower than −1.2 MPa. This can explain the shift of berry frequency distribution toward lighter weight classes in the absence of irrigation (NIRR), and the decrease in average berry weight in these plants (−9% if compared to IRR) (Table 2). This result is in accordance with the increases between 20 and 25% of seed weight due to water deficit observed by Roby and Matthews (2004) and Shellie (2010). In particular, the former have subjected Cabernet Sauvignon grapes to water deficit during the post-veraison phase, while the latter applied it on Merlot at fruit-set.

The values of skin weight were found to be positively related to the berry fresh weight in both treatments (Table 2). In IRR plants, skin weight increased by about 2-folds (1.9 times) with increasing berry weight by almost 3-folds (2.7 times), while in the NIRR treatment it increased by 2.2 times for a berry weight increase similar to that observed for IRR (2.6 times). In IRR, this caused a decrease in skin specific weight with increasing berry weight (Table 3). By contrary, changes of these two parameters in NIRR plants were smaller and not always significant (Tables 2 and 3). This different behavior could be explained by stress evolution during the season and growth dynamics by cell divisions of berry exocarp. Indeed, in grape seeded varieties, exocarp cell divisions usually end approximately 38 days after flowering, while those occurring in the mesocarp 21–28 days after flowering. In our conditions, the vines of both the treatments reached a ψ_w value of −0.8 MPa 15 days after full flowering, and of −1.0 MPa 15 days later (Fig. 1A). Successively, the flowers that anticipated flowering could have benefited from a more favorable water status for mesocarp growth, if compared to exocarp.

The higher content of total anthocyanins detected in NIRR treatment (Table 7) represents a positive characteristic, as these compounds, together with tannins and flavonols, are involved in the long-term color stability of red wines (Boulton, 2001; Zimman and Waterhouse, 2005). Furthermore, interactions of anthocyanins with other non-phenolic wine components (aldehydes, SO_2, polysaccharides) can affect positively sensory impressions in terms of wine balance, harshness and smoothness (Clarke and Bakker, 2004). Anthocyan qualitative profiles in NIRR and IRR were significantly different for some compounds (Tables 4–6). In particular, in NIRR plants, the very high levels of petunidin-3-*O*-acetylglucoside and peonidin-3-*O*-acetylglucoside, that confer a deep red color when transferred from grape to the corresponding wine and represent a positive sensory parameter (Santos-Buelga and de Freitas, 2009), can be related to the up-regulation of the genes involved both in the leucocyanidin and leucodelphinidin flavonoid pathway, as found in Cabernet Sauvignon by Castellarin et al. (2007a). Furthermore, the higher level in NIRR of total acetylated anthocyans (2895.94 and 583.10 mg kg^{-1} fresh berry skin in NIRR and IRR, respectively) and the higher acetilated/coumarated anthocyanin ratio (1.47 and 0.26 mg kg^{-1} fresh berry skin in NIRR and IRR, respectively) constitute other positive qualitative indexes (Santos-Buelga and de Freitas, 2009) (Tables 5 and 6). Regarding flavonols, the main compounds detected in berry skins were quercetin (3-*O*-glucoside and 3-*O*-glucuronide) and myricetin (3-*O*-glucoside), but also other compounds such as kaempferol and laricitrin derivatives were found (Table 7). The significant effect of the absence of irrigation on anthocyanin levels than on flavonols (Tables 6 and 7) is consistent with the results of Roby et al. (2004). Furthermore, anthocyanins seem to show little turnover once formed and so they are accumulated in the skin at low water content, while flavonols are used for the synthesis of other compounds and are easily degraded (Adams, 2006).

Table 8
Metal levels in berry skins of irrigated (IRR) and non-irrigated (NIRR) grapes. Mean values (±st. dev.) followed by different letters are significantly different (lowercase between IRR and NIRR, and uppercase between berry weight classes) at $P \leq 0.05$, according to Fisher's LSD test.

Treatment	Berry weight class (g)	Iron (μg kg^{-1} dry berry skin)	Copper (μg kg^{-1} dry berry skin)	Zinc (μg kg^{-1} dry berry skin)	Calcium (μg kg^{-1} dry berry skin)
IRR	$0.60 \leq x < 0.90$	426.41 ± 63.96 aA	324.74 ± 70.47 aA	578.97 ± 78.32 aA	3926.85 ± 278.32 aA
	$0.90 \leq x < 1.25$	150.76 ± 40.90 aB	251.81 ± 14.07 aB	453.84 ± 43.44 aA	3091.84 ± 403.44 aB
	$x \geq 1.25$	145.48 ± 22.16 aB	138.04 ± 21.11 aC	380.58 ± 33.89 aB	2970.95 ± 313.89 aB
	Average	240.88 ± 38.03 a	238.20 ± 94.09 a	471.13 ± 100.32 a	3329.88 ± 520.51 a
NIRR	$0.60 \leq x < 0.90$	81.14 ± 23.96 bA	152.06 ± 45.46 bA	335.49 ± 38.40 bA	3946.33 ± 323.49 aA
	$0.90 \leq x < 1.25$	80.56 ± 20.90 bA	126.35 ± 44.94 bA	272.94 ± 13.42 bA	2513.50 ± 223.42 aB
	$x \geq 1.25$	30.62 ± 12.16 bB	59.12 ± 12.88 bB	209.43 ± 13.49 bB	2587.70 ± 488.40 aB
	Average	64.11 ± 29.00 b	112.51 ± 47.99 b	238.20 ± 94.09 b	3015.84 ± 806.68 a

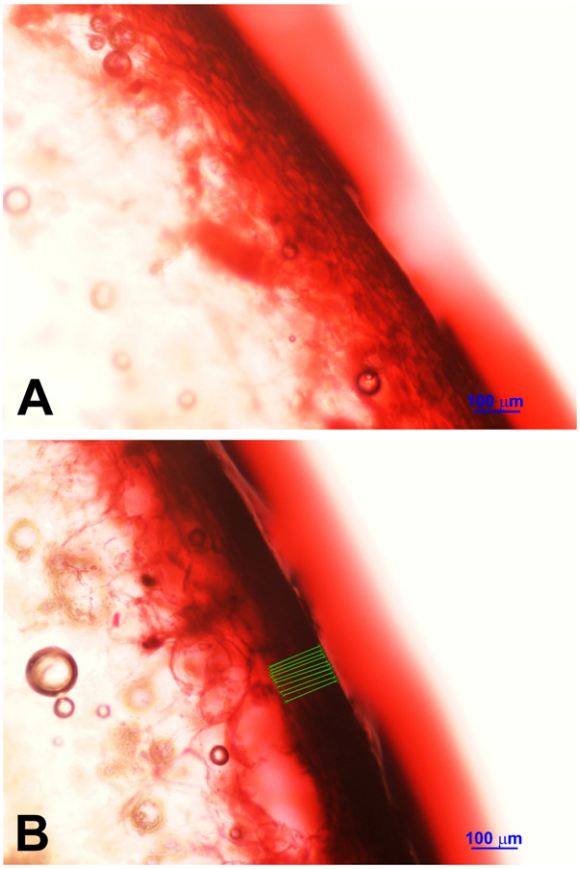

Fig. 2. Section of grape berry from (A) irrigated and (B) non-irrigated vines observed from the equatorial axis by optical light photo-microscopy. The green segments indicate examples of skin thickness measurements. Magnification = 100×; scale bar = 100 μm.

The thickness of berry skin ranged from 104 to 118 μm, confirming the data of Alleweldt et al. (1981), and this parameter was not influenced by the irrigation type (Table 2 and Fig. 2). Indeed, cell division within the dermal tissue of the grape berry is completed during the first of the two postanthesis growth cycles, which are separated by a phase of slow or nil growth (Coombe, 1980). During these early cycles, the field conditions that grapes experienced could not be so severe to cause berry skin anatomic modifications. The limited variations observed, not statistically significant, should be due just due to cell hydration (Considine and Knox, 1979), as evidenced by the lower values of skin specific surface in NIRR (Table 3). Therefore, the explanation of the increase in total anthocyanins in NIRR plants (Table 6) resides in other factors than berry skin thickness (Table 2) or volume (calculated as berry surface × berry thickness), and probably depends on the metabolic biosynthetic patterns induced by the lower plant water content (Castellarin et al., 2007a,b).

Generally, metal levels significantly decreased with increasing berry weight (Table 8). The perception of a negative metallic olfactory sensation is occasionally found in red wines, and it can be induced by Fe, Cu and Zn ions already present in the berries (Jackson, 2000). Furthermore, it is known that both Fe and Cu excess determines wine turbidity, can delay fermentation during winemaking, and are significant causes of instability, as in the case of the formation of an unstable colloid resulting from a reaction between these two cations, proteins and phosphoric acid (ferric and copper casse). In addition, ferric iron reacts with anthocyanins, producing a soluble complex that leads to a color intensity that is too high (darker, more purplish hue). In NIRR plants, Fe, Cu and Zn levels in berry skins were significantly lower than those found in the IRR treatment (Table 8), confirming that lower irrigation can increase grape (and wine) quality. In red grapes and wines, the poorly soluble Ca is involved in colloid flocculation and salt precipitation, being responsible for wine turbidity (Ribéreau-Gayon et al., 2006). In our experiment, no significant changes in Ca content were observed between the two treatments (Table 8), confirming that reducing irrigation water did not influence skin Ca content. The dilution effects accompanied by no further accumulation after the first phase of berry growth can be the cause of the lower metal levels in the heaviest berries (Table 8). As there is not much information about the extractability of metals from grape berries to must during fermentation, our data could be used to predict wine quality during the following processes.

In conclusion, the results suggest that the absence of irrigation caused lower yield and reduction in berry size without affecting grape quality, in terms of flavonoids and metals extracted from the skins. Some of the observed modifications can be attributable to the specific action of lower water availability, while others are related to the amount of skin per total berry weight. Noteworthy, small differences in plant water potential caused significant differences in quality characteristics of the grapes. Our data can be of primary importance for understanding how grapevine responds in environments where water availability is by far the most important factor in quality control of grapes and wine. Indeed, careful irrigation management has a key role in producing the optimum quality grapes that the winemakers require.

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