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Grape Metabolic Response to Postveraison Water Deficit Is Affected by Interseason Weather Variability

Jose C. Herrera,[*,†,#] Uri Hochberg,[†,⊥] Asfaw Degu,[‡] Paolo Sabbatini,[§] Naftali Lazarovitch,[‡] Simone D. Castellarin,[∥] Aaron Fait,[‡] Giorgio Alberti,[†] and Enrico Peterlunger[†]

[†]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, 33100 Udine, Italy

[‡]The French Associates Institute for Agriculture and Biotechnology of Drylands, Ben-Gurion University of the Negev, 849900 Sede Boqer Campus, Israel

[§]Department of Horticulture, Michigan State University, East Lansing, Michigan 48824, United States

[∥]Wine Research Centre, The University of British Columbia, V6T 1Z4 Vancouver, BC, Canada

[⊥]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138, United States

[#]Division of Viticulture and Pomology, University of Natural Resources and Life Sciences, Vienna, 3430 Tulln an der Donau, Austria

Supporting Information

ABSTRACT: Postveraison water deficit is a common strategy implemented to improve fruit composition in many wine-growing regions. However, contrasting results are often reported on fruit size and composition, a challenge for generalizing the positive impact of this technique. Our research investigated the effect of water deficit (WD) imposed at veraison on Merlot grapevines, during two experimental seasons (2014−2015). In both years WD resulted in reduced carbon assimilation rates and leaf shedding. However, the treatment effect on the analyzed berry parameters varied between seasons. Modification of skin metabolites was more evident in 2015 than in 2014, despite the similar soil water content and water stress physiological parameters (gas exchange, water potential) recorded in the two experimental years. Higher solar radiation and air temperature in 2015 than in 2014 hint for the involvement of atmospheric parameters in fulfilling the potential effect of WD. Our results suggest that the interaction between water availability and weather conditions plays a crucial role in modulating the grape berry composition.

KEYWORDS: *anthocyanins, primary metabolites, secondary metabolites, photosynthesis, Vitis vinifera, water potential, grapevine*

INTRODUCTION

Grapevine production and fruit quality are highly dependent on agro-climatic factors, among which soil water availability is one of the most important and certainly the most studied (see review of Medrano et al.[1]). In rain-fed Mediterranean viticulture such as in many wine premium appellation regions of Italy and France, where irrigation is traditionally not employed or in some cases not allowed, water stress can be particularly severe during the summer, especially if associated with dry winter and spring.[2] In recent years, unexpected erratic rainfall distribution combined with record-high temperatures increased the number of drought events, prompting the introduction of irrigation to sustain yields and desired fruit compositional traits (e.g., acidity, aroma). Having said that, grape berry chemical composition has a complex relationship with vine water status; thus predicting the outcome of water availability on fruit traits is not trivial. In contrast to many other crops, it is widely recognized that grapevine fruit quality benefits from moderate levels of water stress via two main mechanisms: increased skin-to-pulp ratio[3−5] and enhanced biosynthesis of secondary metabolites such as color[6−8] and aroma precursors in grapes and in subsequent wines.[9−11] However, water deficit can reduce photosynthesis and consequently sugar accumulation,[12] and negatively affect vine yield.[11,13−15] In the last decades, a large body of basic and applied research followed by practical applications have proposed the adoption of deficit irrigation strategies in vineyards.[16,17] However, the grapevine response to different levels and timings of water deficit in combination with specific soil and atmospheric conditions differs between cultivars, indicating a complex interaction between environment, genetics, metabolism, and berry development, not yet fully elucidated.[2]

In general, water deficit imposed during early stages of berry development (i.e., from fruit set to veraison) induces major modifications on grape berry metabolism. In particular, preveraison water deficit accelerates fruit pigmentation process[8,18] and increases the biosynthesis and concentration of anthocyanins in red varieties,[7,8,11,13,14,17,19−21] while reducing berry size and yield at harvest.[5,22,23] Contrarily, the above-mentioned effects showed inconsistencies with postveraison water deficit across experiments.[14,24−26] For example, no effect of postveraison water deficit (WD) on total soluble solids (TSS) was found at harvest in Merlot,[26,27] Agiorgitiko,[25] Tempranillo,[24] Crimson Seedless,[28] and Sangiovese,[29] while an

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increase was observed in Cabernet Sauvignon[8] and Shiraz.[14] In these last two cases[8,14] a reduction in berry size was also reported and used to explain the so-called "concentration" effect in TSS.

Differently from Mediterranean dry climates, the Friuli Venezia Giulia region (northeast Italy) usually benefits from intense spring rainfall (ca. 450 mm) and more than adequate summer rainfall (ca. 380 mm). However, water availability for grapevines is strongly influenced by the holding capacity of the soil. In fact, several soils within the region are characterized by coarse deposits with a high permeability and by gravel−sand deposits variously interspersed with clay and silt, determining a low soil water holding capacity. Therefore, in those years when summer rainfall cannot compensate for water losses by evapotranspiration and drainage, vineyards could experience severe water deficit during the period of berry ripening (i.e., veraison to harvest). The recent years were characterized by events of a combination of erratic rainfall distribution and record-high temperature likely related to climate change processes. In the present study, the effect of water deficit imposed from veraison-to-harvest on berry composition was evaluated for two consecutive years in potted 4-year-old grapevines. Given the large weather differences between the two growing seasons, we analyzed and discussed the results considering the two seasons separately with the aim to better understand possible climate-related effects of water deficit on crop size, berry development, and chemical composition.

MATERIALS AND METHODS

Plant Material and Site Description. The experiment was carried out in 2014 and 2015 at the University of Udine experimental station "A. Servadei" (46°02′ N, 13°13′ E; 88 m asl) on potted 4-year-old *Vitis vinifera* cv. Merlot (clone R3) grapevines grafted to SO4 rootstock. The experimental setup is described in detail by Hochberg et al.[30] Briefly, vines were planted in 2010 in 40 L pots filled with a mixture of soil (49.0% sand, 31.5% silt, and 19.5% clay) supplemented with 20% perlite, and cane pruned to a single Guyot with 8−10 nodes per cane and trained with a vertical shoot positioning system. The fruiting cane was 0.8 m from the ground, and the shoots were allotted in a trellising system consisting of three sets of catch wires placed at 30 cm intervals from the fruiting cane. A total of 32 pots were positioned under ground level to prevent excessive root heating, and arranged in two north−south oriented rows with 16 plants each, with a spacing of 1 m between the vines and 2.5 m between rows. Each row was divided into four plots with four consecutive vines, and irrigation treatments (described below) were assigned to each plot following a fully randomized design (two irrigation treatments × four replicates). Within each plot, one vine was positioned on a weighting lysimeter as described by Hochberg et al.[30] to compute evapotranspiration values (ET_{lys}). Hedging and other canopy management practices were not performed over the course of the experiments. The plants were provided with mineral nutrition (N−P−K Nitrophoska), and fungicide was applied as commonly practiced in the area. To prevent a rainfall effect, the trial was conducted under a transparent plastic (ethylene vinyl acetate) roof that covered the entire experimental plot while the sides of the framing structure remained open, as described by Herrera et al.[11] Water was supplied by a drip irrigation system with a set of two 2.0 L h^{-1} drip-emitters (PCJ, Netafim, Israel) per pot.

Irrigation Treatments. Two irrigation treatments were established at veraison (50% of berry color change), on the 205th and 212th days of the year (DOY) in 2014 and 2015, respectively: (i) well-watered (WW), where daily irrigation was equivalent to 120% of the evapotranspiration measured by the lysimeter (ET_{lys}), and (ii) water deficit (WD), where daily irrigation was equivalent to 35% of the WW treatment's ET_{lys}. ET_{lys} was calculated as the mean (n = 4) daily mass loss in WW lysimeters. Irrigation treatments for each week were calculated according to the average ET_C of the previous week. Irrigation was applied daily and during hours of minimal transpiration (at 21:00 in 2014 and at 00:00 in 2015) to minimize significant effects (ET during the irrigation time was not considered) on the daily ET_{lys} calculation.

Physiological Measurements. Vine response to the irrigation treatments was assessed by midday measurements of stem water potential (Ψ_s), stomatal conductance (g_s), and net assimilation (A_N) on all the lysimeter vines (four replicates per treatment). Ψ_s was measured using the Scholander pressure chamber (Soil Moisture Co., Santa Barbara, CA, USA) as described by Hochberg et al.[30] Leaf gas exchange (g_s and A_N) were measured at midday (12:30−1.30 p.m.) using an infrared gas analyzer (LI-6400, LiCor Inc., USA). The measurements were taken on clear days under constant light intensity (1000 μmol m^{-2} s^{-1}) and CO_2 concentration (400 μmol mol^{-1}).

Leaf Area and Yield Components. Leaf area (LA) was assessed 6 and 9 times over the course of the season in 2014 and 2015, respectively, only on the eight lysimeter-grown vines, based on the number of leaves and their average area as described by Hochberg et al.[30] All vines were hand-harvested the same day, when sugar accumulation plateaued in WW vines (around 23°Brix). Yield and number of clusters per vine were recorded at harvest. Mean cluster mass was calculated as yield/cluster number per vine.

Berry Sampling and Juice Analysis. Berry samples were collected every 15 days starting the day before the irrigation treatment application. At each sampling date, two sets of 20 berries each were collected from every plot. Samples were immediately stored in an insulated cooler and transported to the laboratory. The first set was used to measure juice total soluble solids (TSS), pH, and titratable acidity (TA); the second set was stored at −80 °C until analysis of anthocyanins and metabolite profiling. Berries for juice measurement were weighed to calculate mean berry mass and then manually pressed at room temperature. The juice was used to determine TSS (°Brix) using a digital refractometer (PR-100, Atago, Tokyo, Japan), the pH by a pH meter (HI2211, Hanna Instruments, Woonsocket, RI, USA), and the TA (expressed as g/L tartaric acid equivalents) by titration with NaOH 0.1 N until pH 8.2.

Anthocyanin Accumulation during Ripening. Anthocyanin accumulation during berry ripening was measured using high-performance liquid chromatography (HPLC) as described by Sivilotti et al.[31] Briefly, skin tissue was separated from the frozen berries using a scalpel and immediately dropped into liquid nitrogen and weighed. Skin tissue was then grounded to a fine powder under liquid nitrogen using a mill (A11B, IKA, Königswinter, Germany). An aliquot of 1.8 mL of methanol:water 1:1 was added to 0.18 g of skin powder in a 2 mL microtube. The extraction was performed at room temperature in an ultrasonic bath for 1 h. Samples were then centrifuged at 15 000 rpm for 15 min, diluted, and filtered using regenerated cellulose membranes with pore size of 0.2 μm (15 mm syringe filter, Phenomenex, CA, USA). Anthocyanin concentration and profile were determined with an HPLC (LC-20AT, Shimadzu, Japan) equipped with a diode array detector (SPD-M 20 A, Shimadzu, Japan). Separation was performed using a C-18 column (LiChroCART 250-4, Merck, Germany) maintained at 25 °C. Solvent A was methanol and solvent B was perchloric acid (0.3%) in water. The gradient of mobile phase A was as follows: 0−32 min, 27%; 32−45 min, 67.5%; 45−50 min, 100%; 50−60 min, 27%. Individual anthocyanins (3-monoglucoside, 3-acetyl-glucocide, and 3-*p*-coumaroyl-glucoside anthocyanins) were identified by comparing the retention time of each chromatographic peak with available data in the literature.[32] The concentration of individual anthocyanins was determined with a standard curve constructed by using an external standard of oenin chloride (Extrasynthese, Genay, France) and expressed as oenin chloride equivalents in mg/g of fresh berry (FW).

Skin Metabolite Profiling at Harvest. Both primary metabolite profiling and secondary metabolite profiling were performed only for the berry skin tissue sampled at harvest. During sampling, the skin tissue was carefully separated from the pulp, snap-frozen in liquid nitrogen, and kept at −80 °C until further analysis. Prior to extraction, samples were freeze-dried in a lyophilizer, ground using prechilled

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holders and grinding beads (Tissuelyser Qiagen, Retsch Gmbh, Haan, Germany). Frozen tissue powder of 30 mg was transferred to a 2 mL tube, and metabolite extractions for both liquid chromatography−mass spectrometry (LC−MS) and gas chromatography−mass spectrometry (GC−MS) analysis were extracted in a prechilled methanol/chloroform/water extraction solution (2.5/1/1 v/v/v). All the extraction steps were performed as described by Degu et al.[33] From the last step of the extraction, the upper water/methanol phase was transferred to UPLC vials for LC−MS analysis while 100 μL of the extract was dried in a vacuum concentrator (Eppendorf Concentrator Plus) for derivatization[34] for GC−MS analysis.

LC−MS Analysis. A 2 mL volume of extract was injected into an ultraperformance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-QTOF-MS) system equipped with an ESI interface (Waters QTOF Xevo; Waters MS Technologies, Manchester, U.K.). During the running, the column and autosampler were maintained at 40 and 10 °C, respectively. The different solvents for each running were maintained as described by Degu et al.[35] Leucine enkephalin lock mass calibration was used to verify the accuracy and reproducibility of the run. The MS conditions were set as described by Hochberg et al.[36]

Raw data acquisition was processed using MarkerLynx application manager (Waters) essentially as described previously.[36] Metabolite identification was performed as described in detail by Degu et al.[37] Each metabolite marker identified with the Waters MarkerLynx software was then normalized to the internal standards and initial tissue weight.

GC−MS Derivatization and Data Processing. The dried GC−MS samples were derivatized as described by Hochberg et al.[38] with a similar retention time standard mix. The sample set also included a reference quality control of authentic metabolite standards (1 mg mL^{-1}, each). Volumes of 1 μL were then injected into a 30-m VF-5ms GC column with 0.25 mm i.d., film thickness of 0.25 μm (Agilent, Santa Clara, CA, USA), and +10 m EZ-Guard (Agilent, Santa Clara, CA, USA) in splitless and split mode (32:1), allowing a more accurate comparison of highly abundant metabolites (e.g., sugars). The GC−MS system and the parameters of the machine were set exactly as described by Degu et al.[35] Spectral searching was performed by consulting the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) algorithm incorporated in the Xcalibur data software (version 2.0.7) against retention index (RI) libraries from the Max Planck Institute for Plant Physiology in Golm, Germany (http://www.mpimp-golm.mpg.de).

Statistical Analysis. Results were analyzed separately by year using analysis of variance (ANOVA) appropriate for a fully randomized design using JMP 7 (SAS Institute Inc., Cary, NC, USA). Data were tested for normality and homogeneity of variance prior to being subjected to the *F*-test ($P < 0.05$). Seasonal variations of berry mass, TSS, and anthocyanins are shown as means ± standard error ($n = 4$).

RESULTS

The two experimental years experienced different weather conditions (Figure S1). The 2014 growing season was characterized by several rain events and multiple cloudy days during berry ripening. Rainfall did not affect directly the irrigation trials because the vines were sheltered. However, the 2014 season was characterized by lower mean temperatures (monthly average temperatures in 2014 were 4.3 and 3.2 °C lower than those in 2015 in July and August, respectively) and reduced solar radiation. The cumulated incoming solar radiation in the experimental plot was 14.5 MJ m^{-2} lower in 2014 than in 2015 between July 20 and August 31 (Figure S1). These climatic conditions resulted in different atmospheric water demand and water consumption per vine in both seasons (Figure 1); in 2015 ET_{lys} of WW and WD vines were higher than in 2014 following the atmospheric conditions during the experiment.

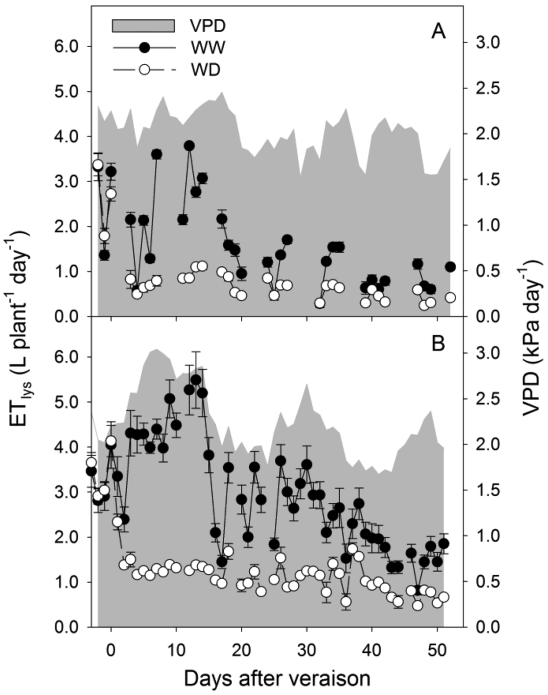

Figure 1. Grapevine evapotranspiration measured with lysimeters (ET_{lys}; L plant^{-1} day^{-1}) and daily vapor pressure deficit (VPD; kPa day^{-1}) during the experiment in (A) 2014 and (B) 2015. Vertical bars are standard error ($n = 4$).

In both years, the WD irrigation treatment resulted in significant differences compared to the WW control vines in midday stem water potential (Ψ_s), net assimilation (A_N), and stomatal conductance (g_s) already 2 days after the imposition of the treatment (Figure 2). Ψ_s in WW remained constant around −0.6 MPa in both experimental years, while Ψ_s in WD reached minimum values of −1.47 and −1.4 MPa in 2014 and 2015, respectively (Figure 2A). A_N and g_s in WW were always above 9 μmol m^{-2} s^{-1} and 0.1 mol m^{-2} s^{-1}, respectively, in both seasons (except in the last measurements in 2014, where lower values were observed), while in WD A_N and g_s reached minimum values of 1.5 μmol m^{-2} s^{-1} and 0.02 mol m^{-2} s^{-1}, respectively, in both seasons (Figure 2B,C). Toward the end of the growing season, Ψ_s measurements in WD gradually increased up to −0.9 MPa in both years, although irrigation amounts remained constant (35% ET_{lys} of WW). Concomitant with the increase in Ψ_s, but only in 2015, A_N and g_s increased to 6 μmol m^{-2} s^{-1} and 0.09 mol m^{-2} s^{-1}, respectively. The behavior of A_N was tightly correlated ($r^2 = 0.97$) with g_s (Figure S2) through a polynomial quadratic curve ($y = 2.6 + 59.9x - 78.9x^2$), reporting no differences between years (2014 and 2015) or treatments (WW and WD).

The WD treatment led to a similar reduction of leaf area (LA) in both years. Thirty days after water deficit application, WD vines had 0.6 m^2 less LA (−33%) than WW ones (Figure 3). Moreover, WD significantly reduced yield per vine only during the first experimental year of about 30% (1.97 and 2.77 kg vine^{-1} in WD and WW, respectively; Table 1), as well as cluster mass (116 and 125 g cluster^{-1} in WD and WW, respectively) and berry mass (1.20 and 1.49 g berry^{-1} in WD and WW, respectively). Surprisingly, no significant effect of WD was detected for these three parameters in 2015. Specifically, WD berries were smaller than WW berries from 15 days after veraison (DAV) until harvest in 2014 (Figure 4A),

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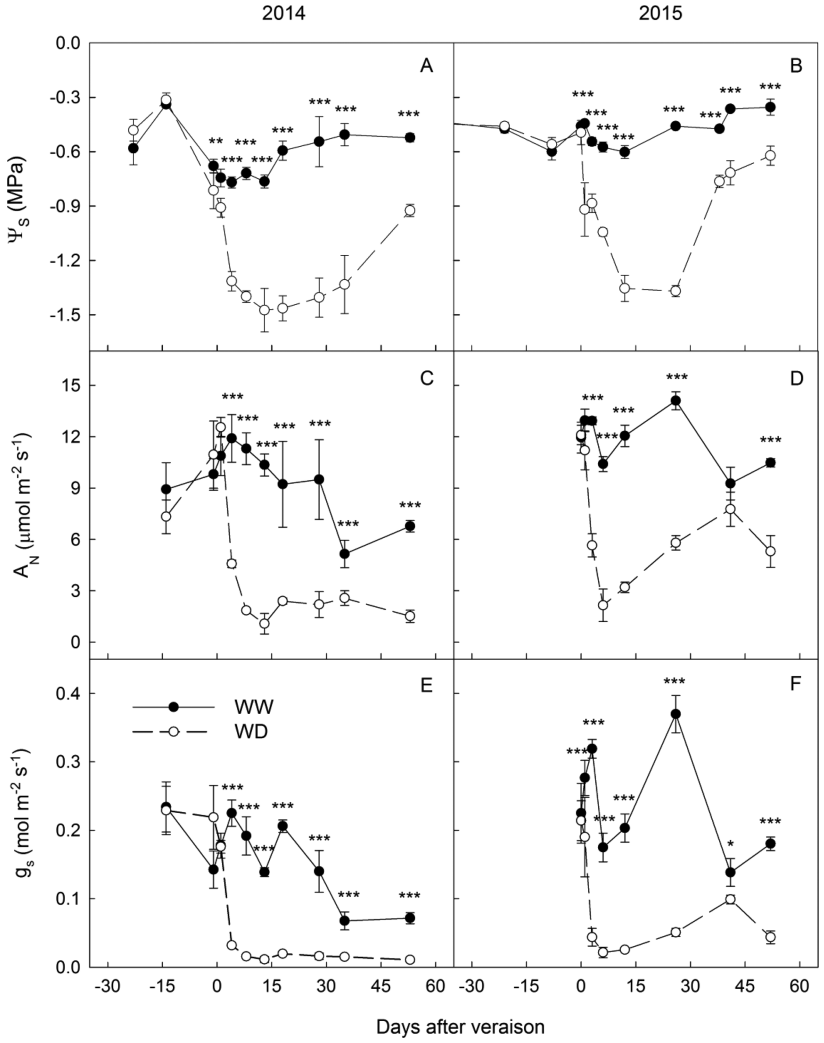

Figure 2. Midday stem water potential (Ψ_s, MPa) in 2014 (A) and 2015 (B), net assimilation (A_N, μmol CO_2 m^{-2} s^{-1}) in 2014 (C) and 2015 (D), and stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) in 2014 (E) and 2015 (F), measured in well-watered (WW; closed symbols) and water deficit (WD; open symbols) Merlot grapevines. Vertical bars are standard error (n = 4). Level of significance: ∗, ∗∗, ∗∗∗, significant at P < 0.05, 0.01, 0.001, respectively.

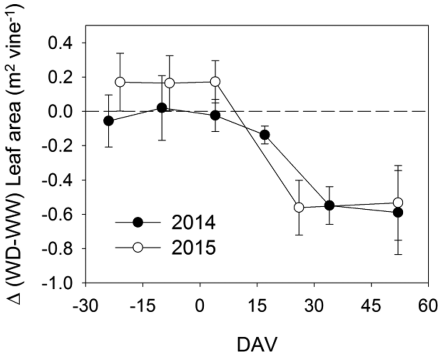

Figure 3. Leaf area (m^2 vine^{-1}), expressed as the difference between the leaf area of water deficit (WD) and well-watered (WW) treatment (n = 4), in 2014 (closed symbols) and 2015 (open symbols). Vertical bars are standard error (n = 4).

while they had similar mass increase dynamics throughout the 2015 growing season (Figure 4B). The leaf area/yield ratio was significantly lower in WD vines than in the WW treatment only in 2015 (1.08 vs 1.48 m^2 kg^{-1} in WD and WW, respectively;

Table 1. Yield and Berry Components in Well-Watered (WW) and Water Deficit (WD) Merlot Berries at Harvest in 2014 and 2015

	2014			2015		
	WW	WD	sign.[a]	WW	WD	sign.[a]
yield (kg/vine)	2.77	1.97	∗	1.66	1.52	ns
leaf area (m^2/vine)	1.98	1.53	∗	2.14	1.74	∗
leaf area/yield (m^2/kg)	0.78	0.78	ns	1.48	1.08	∗
clusters per vine	17	15	ns	19	17	ns
cluster mass (g)	125	116	∗	88	87	ns
berry mass (g)	1.49	1.20	∗	1.61	1.59	ns
skin mass (g/100 g of berry)	9.05	9.65	ns	8.47	10.2	ns
TSS (Brix)	20.8	19.5	∗	25.0	23.0	∗∗
TA (g/L)	6.75	6.36	ns	5.67	6.19	ns
pH	3.36	3.43	ns	3.47	3.42	ns
anthocyanins (mg/g)	1.11	1.24	ns	1.28	1.56	ns

[a]Level of significance: ∗, ∗∗, or ns, significant at P < 0.05, 0.01, or not significant, respectively.

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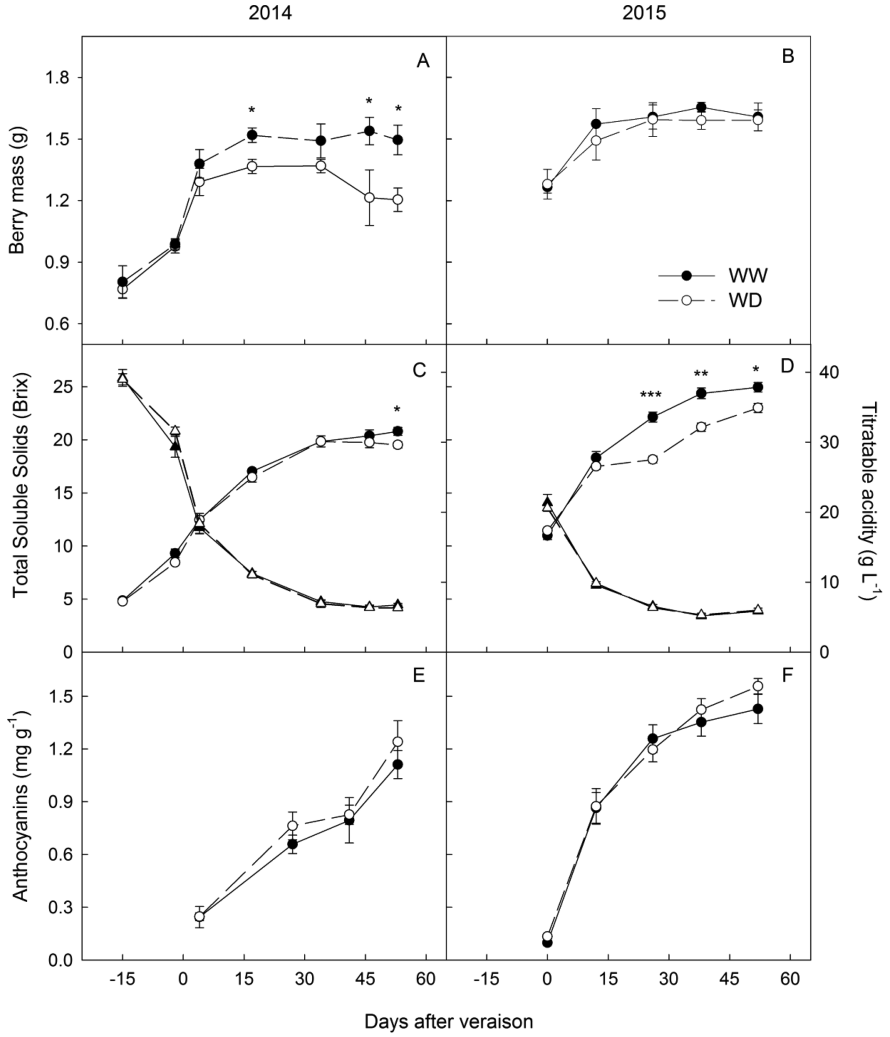

Figure 4. Berry growth in (A) 2014 and (B) 2015, total soluble solids (Brix) and titratable acidity (g L^{-1}, triangles) in (C) 2014 and (D) 2015, and total anthocyanin concentration (mg g^{-1} berry) in (E) 2014 and (F) 2015. Vertical bars are standard error ($n = 4$). Level of significance: *, **, ***, significant at $P < 0.05$, 0.01, 0.001, respectively.

Table 1). WD had no effect on the berry relative skin mass in both experimental years (Table 1).

Berry total soluble solids (TSS, °Brix) was reduced by the WD treatment during ripening in both years (Figure 4C,D), showing a more pronounced effect in 2015, where TSS was 3°Brix lower in WD than in WW starting from 26 DAV (Figure 4D). At harvest, the TSS was 20.8 and 19.5°Brix in WW and WD in 2014, respectively, and 25.0 and 23°Brix in WW and WD in 2015, respectively. When the differences were expressed as per-berry basis (TSS $berry^{-1}$), the reduced sugar accumulation in WD was more evident during the entire ripening period (Figure 5) and more pronounced in 2015 when WW berries had 0.1 g $berry^{-1}$ more sugar than WD from 26 DAV until harvest (Figure 5B) in agreement with the calculated rate of sugar accumulation (Figure 5D). On the contrary, titratable acidity (TA; Figure 4C,D) and pH (Table 1) during the ripening period and at harvest were not affected by WD treatment in the two consecutive experimental years. Sugar accumulation was 4°Brix (average of WW and WD) lower in 2014 when compared to 2015 (Table S1).

Regarding the total anthocyanin accumulation (mg/g berry), no effect of the deficit irrigation treatment was observed in both years during ripening (Figure 4E,F) and at harvest (Table 1). The cloudy 2014 season led to lower total anthocyanin concentration (−20%) when compared to the concentration measured in the berries of the 2015 experimental season (Table S1). However, WD did impact the anthocyanin profile, mainly during the late phases of ripening, although also in this case seasonal variability accounted for inconsistencies among years. A general trend of lower concentration of 3′,4′ OH anthocyanins was observed in WD, although statistical differences were significant only at harvest in 2014 (0.25 vs 0.20 mg/berry in WW and WD, respectively; Figure 6A); on the other hand 3′,4′,5′ OH anthocyanins were increased ($P < 0.05$) by WD only in 2015 at 38 DAV (1.04 vs 1.13 mg/berry in WW and WD, respectively; Figure 6B) and at harvest (1.03 vs 1.25 mg/berry in WW and WD, respectively; Figure 6B). In both years, malvidin 3-*O*-(6-*p*-coumaroyl-glucoside) and malvidin 3-*O*-(6-caffeoyl-glucoside) showed a significantly higher relative abundance (2 year average +43% and +150%, respectively) in WD than in WW, while cyanidin 3-*O*-(6-*p*-coumaroyl-glucoside) was significantly reduced (2 year average −30%) (Table 2).

Grape berry skin metabolite profiling at harvest showed a different modulation of primary and secondary metabolism due to WD in the two seasons (Table 2). In general, a higher

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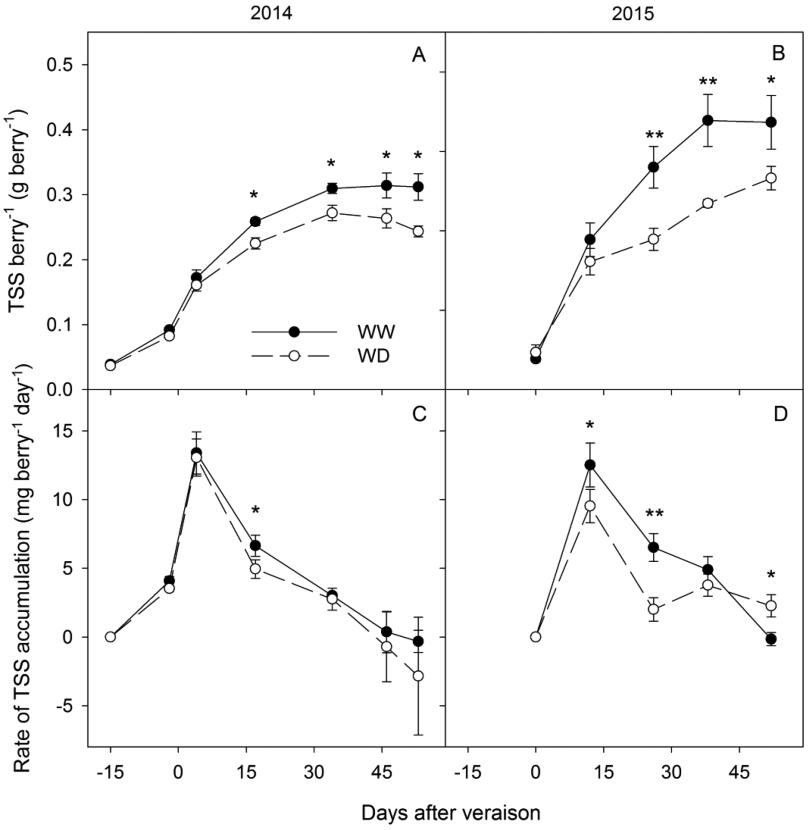

Figure 5. Sugar accumulation in per-berry basis (TSS berry^{-1}) calculated from the TSS and berry mass values (Figure 4) in 2014 (A) and 2015 (B), and the rate of sugar accumulation per berry (mg berry^{-1} day^{-1}) in 2014 (C) and 2015 (D), for well-watered (WW; closed symbols) and water deficit (WD, open symbols) Merlot berries. Vertical bars are standard error (n = 4). Level of significance: ∗, ∗∗, significant at P < 0.05, 0.01, respectively.

impact of WD on metabolite level was observed in 2015 than in 2014 (28 and 18 significantly modulated metabolites out of 69 in 2015 and 2014, respectively), consistent with the seasonal weather differences previously described. Among secondary metabolites only a few showed consistent statistical differences (P < 0.05) in both seasons: myricetin 3-*O*-glucoside showed a significant increase in relative abundance in the skins of WD berries (2 year average +30%), while procyanidin dimer B3 (2 year average −25%) and epicathechin (2 year average −40%) were significantly reduced (Table 2). Abscisic acid (ABA) increased (2 year average +130%) in WD berries in both years (Table 2). Among the skin primary metabolites, the only organic acids significantly affected (P < 0.05) by the WD at harvest were dehydroascorbate and galactonate acids (reduced by 40 and 75% in 2014, respectively), and gluconate and malonate acids (reduced by −60 and −25% in 2015, respectively). On the contrary, amino acids had substantially higher values in response to WD in 2015 (e.g., Ser, Pro, and Trp were increased by 9-, 2-, and 15-fold, respectively), while no difference between the two treatments in any of the amino acid values were reported in 2014 (Table 2).

DISCUSSION

The large variability in the deficit irrigation effects between the two growing seasons on berry composition suggest that careful regulation of the soil water availability is not sufficient in order to shape berry chemical traits during ripening and at harvest. Notably, shortly after deficit irrigation was imposed, the vines developed similar physiological responses to water stress condition in the two experimental years, as indicated by reduced values of g_s, A_N, and Ψ_s. However, large differences between the 2014 and 2015 seasons were shown for berry developmental traits and ripening parameters, as well as for primary and secondary metabolites in the skins. These results highlight the complex interaction existing between climatic conditions, irrigation, and berry development. In particular, a strong effect of season-specific environmental factors can be observed in the lower level of sugars and total anthocyanin concentration in 2014 compared to 2015 and regardless of the treatment (Table 1, Table S1). It is possible that lower temperatures and light interception (Figure S1) jeopardized the expected WD effects in 2014. A previous research carried out in climatically different vintages reported larger influence of the climatic factors than the irrigation treatments.[39] In general, higher air temperatures lead to higher total soluble solids concentration.[40,41] We observed a good correlation between growing degree days (GDD) accumulated from the first of April and final TSS in the berries (Figure 7A); contrarily, when considering only GDD accumulation from veraison to harvest (Figure 7B), the results did not explain the differences between seasons. These lines of evidence suggest that heat accumulation is important throughout the whole growing season and not only during the ripening period. Similarly, anthocyanin biosynthesis is stimulated by visible light; however, above values of 100 mmol m^{-2} s^{-1} photon flux light intensity, temperature becomes the dominant factor in berry coloration and anthocyanin production increases up to an optimum berry temperature of 30 °C,[42] a temperature that was more frequently

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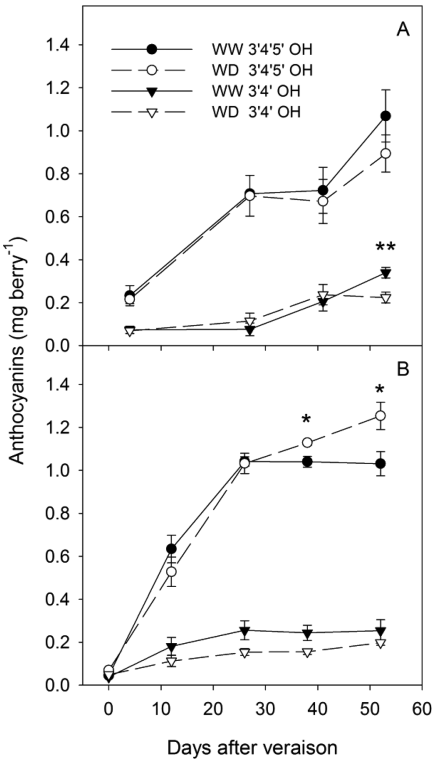

Figure 6. Skin 3′,4′,5′ and 3′,4′ hydroxylated anthocyanins (expressed in mg berry^{-1}) in well-watered (WW) and water deficit (WD) Merlot grapevines in 2014 (A) and 2015 (B). Vertical bars are standard error (n = 4). Level of significance: ∗, ∗∗, significant at P < 0.05, 0.01, respectively.

achieved in 2015 and seldom in the cooler 2014 season. Our data and other evidence for an interactive and additive effect of high temperatures and drought on the chemical composition of berries[39,43−45] prompt the need to experimentally separate the effects of these factors to better understand the plant response.

The similar g_s, A_N, and Ψ_s values (Figure 2) measured in the WD vines in 2014 and 2015 indicate a similar stress degree in both years. This can be expected considering the similar soil water content and the atmospheric demand during the days in which water potential and gas exchange were measured. However, our results suggest that attention should be paid to the commonly adopted protocol (also used here) to measure physiological parameters only on clear sunny days, which were less frequent in 2014 (as shown in Figure S1). Accordingly, the values measured under such conditions could be considered as an indication of high/maximum levels of vine water stress, because they were taken under environmental conditions capable of maximizing the effects of the deficit irrigation treatments. Previous research investigated the close correlation of VPD and physiological measurements such as gas exchange and leaf water potential.[46,47] However, the plant stress degree is defined by both supply and demand of water, and thus it is probable that the humid and cooler summer of 2014 buffered some of the drought stress imposed on the vines (significantly higher potential evapotranspiration in 2015 during this experiment is presented by Hochberg et al.[30]). Berry metabolism is likely a function of the stress integral (rather than the maximum stress degree), and it is possible that the milder metabolic modification observed in 2014 originated from the cooler summer that characterized that year. For example, the increase of amino acids is a common cellular stress response reported in grapes,[6,36,48−50] but this was observed only in 2015 (Table 2). Also, organic acids were not modified by WD although previous research reported a 40% reduction in most TCA metabolites when preveraison stress was applied to both Shiraz and Cabernet Sauvignon.[51] Additionally, the modification of phenolic compounds (i.e., flavanols, flavonols, and hydroxycinnamic acids; Table 2) that is expected under water deficit conditions[7,50,51] was less evident in 2014. Taken together, these results suggest that monitoring plant physiology only on clear days could be potentially misleading, particularly in regions with climates similar to the one in this study. Atmospheric water demand is a critical factor as the soil water availability in order to better evaluate the stress degree of the vines.

Even if the water stress integral during both seasons was different, deficit irrigation regime consistently reduced berry sugar accumulation during ripening and consequently at harvest in both years (Figure 1). Some studies have shown different dynamics of sugar accumulation under deficit irrigation, often explained by the interaction between cultivar, environmental conditions, and water stress intensity.[52,53] In particular, TSS was shown to be alternatively increased,[8,14,54] not affected,[24−29] or decreased[15,22,23,55] by postveraison water deficit. We observed reduced sugar accumulation in WD vines in both seasons, although a similar leaf area/yield ratio between treatments was recorded in 2014. Besides modifying the size of the canopy and hence leaf area/yield ratio, water deficit also affects several other physiological parameters that contribute to sugar concentration, such as leaf photosynthetic activity,[56] rate of import of sugar into the berry,[57] and berry water budget.[58] Similarly to our results, reduced sugar accumulation was related to a lower photosynthetic rate induced by water stress.[15,22,23,55] Moreover, water deficit in our experiment was imposed after the canopy was fully developed; therefore, no potential sink competition should have occurred during berry ripening between the vegetative and reproductive organs of the vine. In fact, the reduction in leaf area in WD treatment (Table 1) was not attributable to restricted lateral growth, commonly observed in preveraison water deficit experiments,[11] but to primary leaf shedding in agreement with Merli et al.[29]

Phenolic compounds are responsible for most of the grape and wine quality,[59] and their accumulation in response to water stress is well documented at both the metabolic and transcriptional levels.[6,8,51] Among phenolics, increased anthocyanin concentration is a common effect associated with water deficit and related to modification in grape berry morphology (i.e., reduced berry size, increased skin:berry mass ratio[4,60]) and enhanced biosynthesis through the upregulation of related genes.[7,8,50] However, our results showed no impact of WD on the total anthocyanin concentration during ripening or at harvest in both seasons. In fact, skin:berry mass ratio was not affected by WD, even in the season (2014) when berry mass was reduced by WD. Significant increases of the skin:berry mass ratio are often[5,15] but not always[13,61] associated with WD-induced reduction of the whole berry mass, and possibly the decrease in the berry weight observed in this study (−13% in WD compared to WW) was not large enough to determine changes in the ratio. Nevertheless, WD did modify the anthocyanin profile during the last stages of ripening (Figure 6) and also in this case climate variability influenced the impact of WD: the concentration of 3′,4′,5′ OH anthocyanins increased under WD in 2015 but not in 2014, while a similar trend of reduced accumulation of 3′,4′ OH anthocyanins was

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Table 2. Merlot Berry Skin Primary and Secondary Metabolite Profiling in Well-Watered (WW) and Water Deficit (WD) Vines at Harvest in 2014 and 2015[a]

Primary metabolites	Analytical method	2014	2015
Amino acids			
Alanine	GC-MS	0.09	**0.30**
Leucine	GC-MS		**1.30**
Valine	GC-MS		**0.70**
Isoleucine	GC-MS		**0.98**
Serine	GC-MS	0.14	**0.95**
Proline	GC-MS	-0.01	**0.27**
Pyroglutamic	GC-MS	-0.03	**0.30**
Ethanolamine	GC-MS	-0.05	**0.12**
Phenylalanine	LC-MS	-0.01	**0.72**
Tryptophan	LC-MS	0.32	**1.20**
Organic acids			
Maleic	GC-MS	-0.12	0.00
Fumaric	GC-MS	-0.11	0.00
Malonic	GC-MS	-0.17	**-0.12**
Malic	GC-MS	-0.08	-0.09
Tartaric	GC-MS	-0.09	-0.09
Citric	GC-MS	-0.17	0.00
Dehydroascorbic	GC-MS	**-0.23**	
Galic	GC-MS	-0.05	-0.05
Gluconic	GC-MS		**-0.40**
Galactonic	GC-MS	**-0.61**	
Sugars			
Fructose	GC-MS	**-0.07**	
Glucose	GC-MS	**-0.15**	0.00
Myo-Inositol	GC-MS	-0.11	**0.11**
Sucrose	GC-MS	-0.07	0.01
Galactinol	GC-MS	-0.40	0.12
Rhamnose	GC-MS	**-0.18**	0.00
Raffinose	GC-MS	-0.12	
Erythritol	GC-MS		**0.11**
Gentiobiose	GC-MS		**-0.09**
Hormones			
Abscisic Acid [MH-H2O]+	LC-MS	**0.43**	**0.28**

Secondary metabolites	Analytical method	2014	2015
Anthocyanins			
Cya-3-glu	LC-MS	**-0.19**	-0.15
Del 3-O-glu	LC-MS	-0.09	-0.04
Peo 3-O-glu	LC-MS	-0.05	-0.07
Pet 3-O-glu	LC-MS	-0.06	-0.03
Mal-3-O-glu	LC-MS	0.05	**0.08**
Cya 3-O-(6"-acetyl)-glu	LC-MS	**-0.2**	-0.19
Del 3-O-(6"-acetyl)-glu	LC-MS	-0.06	-0.09
Peo 3-O-(6"-acetyl)-glu	LC-MS	-0.02	-0.12
Pet 3-O-(6"-acetyl)-glu	LC-MS	-0.02	-0.03
Mal 3-O-(6"-acetyl)-glu	LC-MS	0.1	**0.12**
Cya 3-O-(6"-p-coumaroyl-glu)	LC-MS	**-0.11**	**-0.19**
Del 3-O-(6"-p-coumaroyl-glu)	LC-MS	0.02	-0.06
Peo 3-O-(6"-p-coumaroyl-glu)	LC-MS	0.03	-0.11
Pet 3-O-(6"-p-coumaroyl-glu)	LC-MS	0.07	0.02
Mal 3-O-(6"-p-coumaroyl-glu)	LC-MS	**0.12**	**0.18**
Mal 3-O-(6"-caffeoyl-glu)	LC-MS	**0.48**	**0.3**
Flavanols			
Procyanidin B1	LC-MS	-0.09	-0.02
Procyanidin B2	LC-MS	-0.06	0.06
Procyanidin B3	LC-MS	**-0.1**	**-0.13**
Catechin	GC-MS	-0.05	**-0.19**
Epicathechin	GC-MS	**-0.27**	**-0.15**
Epigallocatechin	GC-MS	-0.11	-0.03
Flavonols			
Quercetin	LC-MS	**-0.21**	-0.06
Kaempferol 3-O-glu	LC-MS	0.03	-0.07
Quercetin 3-O-glu	LC-MS	-0.06	**-0.14**
Myrecitin 3-O-glu	LC-MS	**0.16**	**0.07**
Hydroxycinnamic acids			
Coumarate	LC-MS	-0.04	**-0.06**
Coumaroyltartarate	LC-MS	-0.05	**-0.09**
Caffeic	LC-MS	-0.26	0.03
Stilbenes			
Resveratrol	LC-MS	**-0.13**	0.18
Piceid	LC-MS	**0.08**	0.24
δ-Viniferin	LC-MS	**0.38**	-0.08

[a]Bold numbers represent significant difference between irrigated and water deficit treatments as tested by the Student's *t*-test ($P < 0.05$; $n = 4$). Different colors represent the increase (red) or decrease (blue) in metabolites. Values are logarithmic transformed fold change (WD/WW).

observed under WD in both years (but proved statistically significant only in 2014) in agreement with previous research.[62] In the literature is extensively reported the upregulation of the flavonoid 3′,5′-hydroxylase (F3′5′H) by water stress,[7,35] in

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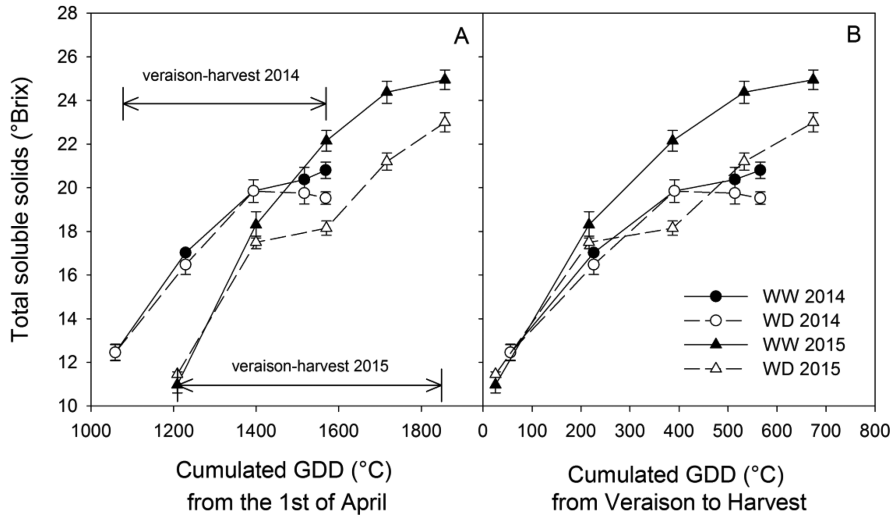

Figure 7. Berry total soluble solids (°Brix) accumulation during ripening in relation to heat accumulation (growing day degrees, GDD, °C) calculated from April 1 (A) or from veraison to harvest (B).

agreement with our results in 2015; however, the same effect was not observed in 2014 reinforcing the hypothesis that the interaction between water availability and climatic conditions could play a crucial role in modulating grape berry metabolism. The relative abundance of other phenolic compounds (i.e., flavanols, flavonols, hydroxycinnamic acids, and stilbenoids) were in general lower under WD in both seasons, although in most of them statistical differences ($P < 0.05$) were not ascertained. These results emphasize the difficulty of establishing a common phenolic response to water stress in grapevine.[51] For instance, statistically significant modification in the stilbene metabolism was observed only in 2014, when WD increased piceid (the glycosylated form of resveratrol) and δ-viniferin, but reduced accumulation of resveratrol. The regulation of stilbene metabolism under stress was investigated in several studies, but no clear picture has emerged: increase, reduction, mix response, or no response was reported[6,51,63,64] and it appears that the genotype plays a pivotal role.

The findings of the current study suggest that deficit irrigation can induce changes in the metabolism and thus in the composition of grape berries, but that the extent of these modifications are closely related to the climatic conditions. This is likely among the reasons for inconsistencies existing in the literature regarding the effects of postveraison deficit irrigation together with other factors such as soil characteristic and genotypes. Since berry composition is determined by the interplay between the soil water availability and the atmospheric conditions, it is reasonable to assume that in order to target desired berry traits the irrigation scheduling should be dynamic.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b01466.

Information regarding temperatures in the experimental site, relationship between photosynthetic rate and stomatal conductance, and table with vegeto-productive and compositional berry parameters analyzed using two-way ANOVA (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jose.herrera@boku.ac.at. Tel.: +43-(0)1-47654-95812.

ORCID

Jose C. Herrera: 0000-0001-9532-5809

Author Contributions

A.F., N.L., E.P., and S.D.C. conceived the IRRIGATE project and acquired the funds for this study. J.C.H. and U.H. designed and executed the research with the help of A.D. and G.A. A.D. sampled and performed metabolite profiling under A.F.'s supervision. E.P. coordinated all the experiment activities. J.C.H. and U.H. analyzed the data and wrote the manuscript. P.S. critically revised the draft and edited the English. All authors discussed the results and revised the manuscript. J.C.H. and U.H. contributed equally to this study.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

Ψ_s, midday stem water potential; A_N, net assimilation; g_s, stomatal conductance; DAV, days after veraison; ET_{lys}, evapotranspiration measured by lysimeter; GDD, growing day degrees; TSS, total soluble solids; TA, titratable acidity; VPD, vapor pressure deficit; WW, well-watered; WD, water deficit

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