



Research article

Malbec grape (*Vitis vinifera* L.) responses to the environment: Berry phenolics as influenced by solar UV-B, water deficit and sprayed abscisic acid



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ABSTRACT

High-altitude vineyards receive elevated solar ultraviolet-B (UV-B) levels so producing high quality berries for winemaking because of induction in the synthesis of phenolic compounds. Water deficit (D) after veraison, is a commonly used tool to regulate berry polyphenols concentration in red wine cultivars. Abscisic acid (ABA) plays a crucial role in the acclimation to environmental factors/signals (including UV-B and D). The aim of the present study was to evaluate independent and interactive effects of high-altitude solar UV-B, moderate water deficit and ABA applications on *Vitis vinifera* cv. Malbec berries. The experiment was conducted during two growing seasons with two treatments of UV-B (+UV-B and -UV-B), watering (+D and -D) and ABA (+ABA and -ABA), in a factorial design. Berry fresh weight, sugar content, fruit yield, phenolic compounds profile and antioxidant capacity (ORAC) were analyzed at harvest. Previous incidence of high UV-B prevented deleterious effects of water deficit, i.e. berry weight reduction and diminution of sugar accumulation. High UV-B increased total phenols (mainly astilbin, quercetin and kaempferol) and ORAC, irrespectively of the combination with other factors. Fruit yield was reduced by combining water deficit and high UV-B or water deficit and ABA. Two applications of ABA were enough to induced biochemical changes increasing total anthocyanins, especially those with higher antioxidant capacity.

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1. Introduction

Enhancement in the synthesis of secondary metabolites like phenolics is part of the plant's response to environment, but also phenolics in grape berries are important for wine quality (Cheynier et al., 1998; Ghaste et al., 2015). Phenolics include flavonoids such as anthocyanins, flavanols (quercetin, kaempferol),

dihydroxyflavonols (e.g. astilbin) and flavanols (catechins, epicatechins and tannins), as well as non-flavonoids, such as stilbenes (resveratrol), hydroxycinnamic and hydroxybenzoic acids (Garrido and Borges, 2013). Phenolics attract pollinators and seed dispersers, and protect plants from pathogens (Dixon and Paiva, 1995; Solovchenko and Schmitz-Eiberger, 2003; Braidot et al., 2008). Additionally, they accumulate in response to several biotic and abiotic signals, mainly as antioxidants so reducing oxidative damages (Dixon and Paiva, 1995; Braidot et al., 2008).

As altitude increases and the atmosphere thins more solar ultraviolet-B (UV-B) radiation (280–315 nm) reaches the surface (Frohnmeyer and Staiger, 2003). High-altitude vineyards in Mendoza, Argentina, receive higher levels of erythemally weighted UV-B irradiance than those at lower altitudes (Berli et al., 2010), producing high quality berries for winemaking by inducing synthesis of phenolics in the berry skin (Berli et al., 2011, 2015).

Previous reports have shown that berry composition in phenolics is characterized by a differential sensitivity to water stress

Abbreviations: ABA, abscisic acid; D, water deficit; DAF, days after flowering; EC, epicatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate; FW, fresh weight; GC, gallic acid; GCG, gallic acid gallate; HPLC-MWD, high performance liquid chromatography-multiple wavelength detector; K-3-glc, kaempferol-3-glucoside; LMWP, low molecular weight phenols; ORAC, oxygen radical absorbance capacity; Q-3-glc, quercetin-3-glucoside; UV-B, ultraviolet-B.

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that is dependent on the cultivar (Medrano et al., 2003; Koundouras et al., 2006; Niculcea et al., 2014). Although in varieties for red wines such as Cabernet Sauvignon, Syrah and Merlot the effects of water deficit on berry phenolics is widely studied (Lovisolo et al., 2010), there are few studies in Malbec (Shellie and King, 2013; Shellie and Bowen, 2014). Irrigation shortage in the vineyard is a tool that regulates skin polyphenols in red grapevines (Kennedy et al., 2002; Lovisolo et al., 2010), and some authors (Ojeda et al., 2002; Castellarin et al., 2007) have shown that such increase is consequence of decreases in berry size (which increases the pulp-skin ratio), but also through augments in the expression of genes for the synthesis. However, the effect of water stress in the berry is genotype dependent (Niculcea et al., 2014), and polyphenol reductions have been reported (Zarrouk et al., 2012).

The plant growth regulator abscisic acid (ABA) plays a crucial role not only in fruit development and ripening, but also in the plant's adaptive responses to biotic and abiotic stresses (Leng et al., 2014; Cohen et al., 2015). In grapevine, it is well known that berry skin ABA levels increase during veraison (Wheeler et al., 2009; Berli et al., 2011), and also that ABA applications increase phenolics in grape berries (Koyama et al., 2010; Balint and Reynolds, 2013), even in the cv. Malbec (Berli et al., 2015).

The aim of the present study was to compare independent and interactive effects of high-altitude solar UV-B, moderate water deficit and ABA applications on berries of *V. vinifera* cv. Malbec, the most cultivated variety of Argentina. We hypothesize that i) phenols increase as defense mechanisms of tissues against UV-B and water deficit, which in turn enhance the quality for red wine-making; and ii) ABA is involved in signaling of these defense mechanisms, whereby ABA application increase phenols.

2. Materials and methods

2.1. Plant material and experimental design

The experiment was conducted during the 2011–2012 and 2012–2013 growing seasons using Malbec vines grown in a high altitude vineyard in Gualtallary, Mendoza, Argentina (69°15'37" W and 33°23'51" S) at 1450 m a.s.l., as it is described in Alonso et al. (2015). Briefly, a low UV-B treatment (-UV-B) was set by using a polyester cover that absorbed 78% of UV-B and 18% of ultraviolet-A radiation (UV-A); and a close to ambient UV-B treatment (+UV-B) was established with a low-density polyethylene that transmitted 90% of UV-B and 87% of UV-A. Even though for technical reasons we were not able to completely exclude UV-B in -UV-B treatment, and thus plants received a 22% of ambient solar UV-B, Pontin et al.

(2010) found that low UV-B irradiance induced grape plants morphogenic responses (purportedly modulated by UVR8; Jenkins, 2009) while high fluence rate UV-B doubled the number of genes modulated by low fluence UV-B (probably induce by ROS; Hideg et al., 2013). The UV-B treatments were given from 15 days after flowering (DAF) until harvest at 142 DAF. Vines were maintained with no soil water restriction until veraison (84 DAF), and then two irrigation regimes were set, a well-watered treatment (-D) and a moderate water deficit treatment (+D), maintaining stem water potentials at midday of -0.7 and -1.0 MPa, respectively. Additionally, the aerial part of plants was sprayed at veraison and repeated once 15 days after, with 1 mM ABA (+ABA; \pm -*cis*, *trans*-abscisic acid, Kelinon Agrochemical Co., Beijing, China) or water (-ABA) solutions containing 0.1% v/v of Triton X-100 as tensioactive. In summary, a total of 8 combined treatments were performed: (i) +UV-B/+D/+ABA; (ii) +UV-B/-D/+ABA; (iii) +UV-B/+D/-ABA; (iv) +UV-B/-D/-ABA; (v) -UV-B/+D/+ABA; (vi) -UV-B/-D/+ABA; (vii) -UV-B/+D/-ABA; and (viii) -UV-B/-D/-ABA.

A randomized complete block design with a $2 \times 2 \times 2$ factorial arrangement of treatment and 5 blocks was used, and the experimental unit consisted of two selected plants for the two consecutive seasons.

2.2. UV-B irradiance

Measurements of erythemal UV-B were performed in the vineyard (in a wide open area) on day 5 (± 2 days because clear days without cloud cover were chosen) of the months of December to April in seasons 2011–2012 and 2012–2013 (Fig. 1), with a radiometer and erythemally-weighted UV-B detector (PMA2200 and PMA2102, Solar Light Company Inc., Glenside, PA, USA).

2.3. Berry sampling, berry growth and fruit yield

At harvest (142 DAF), 30 berries per experimental unit were collected into nylon bags (5 berries per cluster; 2 from the top, 2 from the middle and 1 from the bottom). Samples were kept on dry ice to prevent enzyme degradation and dehydration, taken to the laboratory where berry fresh weight (FW) was determined before storage at -20 °C. After berry sampling, all clusters were harvested, weighed and yield per plant was calculated.

2.4. Berry skin phenolics extraction and sugar accumulation

Fifteen berries per experimental unit were defrosted at room temperature (25 ± 2 °C) and peeled by hand. Phenols extraction from berry skins, pulps relative concentration of sugars (°Brix) and

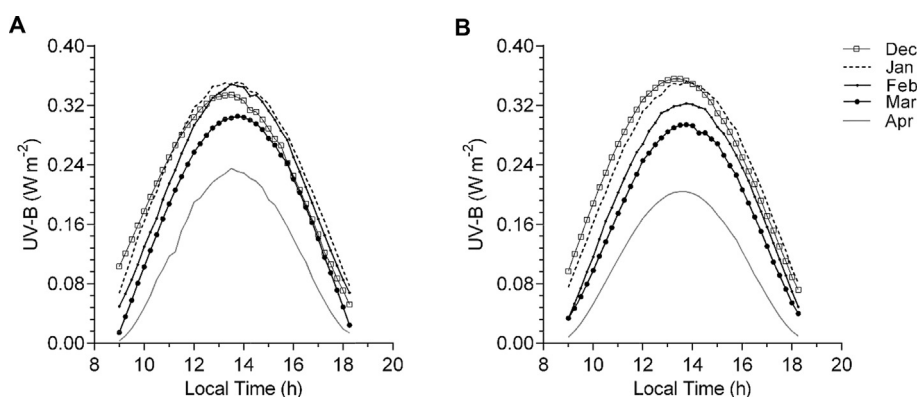


Fig. 1. Erythemal UV-B irradiance (W m^{-2}) measured in the vineyard on day 5th, from December to April, in season 2011–2012 (A) and season 2012–2013 (B).

Table 1

Oxygen radical absorbance capacity (ORAC; $\mu\text{mol Trolox Equivalent berry}^{-1}$) and low molecular weight phenolic (LMWP; $\mu\text{g berry}^{-1}$) determined at harvest in berries of cv. Malbec exposed to combined UV-B, water deficit and ABA treatments during two years.

	ORAC	Total LMWP	Hydroxybenzoic acids		Procyanidins			Prodelphinidins			Hydroxycinnamic acids		Dihydroflavonol	Flavonols		
			Syringic	Gallic	Catechin	EC	ECG	GC	EGCG	GCG	Cafeic	Ferulic	Astilbin	Quercetin	Q-3-Glc	K-3-Glc
UV-B																
+UV-B	544.9 ^a ±33a	81.7 ± 3.2a	14.03 ± 0.54	3.24 ± 0.15	7.48 ± 0.39	0.45 ± 0.06	7.15 ± 0.49a	2.16 ± 0.14	1.20 ± 0.06a	0.335 ± 0.016	4.29 ± 0.36	0.231 ± 0.021	26.5 ± 1.6a	2.06 ± 0.12a	9.18 ± 0.80a	3.56 ± 0.21a
-UV-B	469.9 ± 26b	62.1 ± 4.1b	14.30 ± 0.46	3.05 ± 0.19	7.17 ± 0.34	0.37 ± 0.05	5.68 ± 0.42b	1.79 ± 0.16	0.98 ± 0.07b	0.324 ± 0.021	3.69 ± 0.37	0.240 ± 0.017	19.1 ± 1.5b	1.40 ± 0.05b	4.09 ± 0.44b	1.65 ± 0.14b
ABA																
+ABA	516.4 ± 32	74.3 ± 4.5	14.64 ± 0.46	3.37 ± 0.17a	7.43 ± 0.41	0.43 ± 0.06	6.82 ± 0.50	2.20 ± 0.17a	1.09 ± 0.07	0.329 ± 0.018	4.29 ± 0.38	0.243 ± 0.021	23.6 ± 1.6	1.78 ± 0.12	7.19 ± 0.85	2.64 ± 0.25
-ABA	498.4 ± 28	69.7 ± 3.6	13.69 ± 0.52	2.92 ± 0.16b	7.22 ± 0.32	0.39 ± 0.05	6.01 ± 0.44	1.75 ± 0.13b	1.08 ± 0.07	0.330 ± 0.019	3.69 ± 0.35	0.228 ± 0.017	22.0 ± 1.6	1.69 ± 0.11	6.08 ± 0.72	2.57 ± 0.25
D																
+D	519.7 ± 33	67.2 ± 4.1	14.02 ± 0.53	2.94 ± 0.17	7.35 ± 0.45	0.37 ± 0.05	5.75 ± 0.37b	1.86 ± 0.16	1.09 ± 0.08	0.328 ± 0.020	3.58 ± 0.36	0.228 ± 0.019	21.7 ± 1.6	1.63 ± 0.09b	5.68 ± 0.66b	2.43 ± 0.23
-D	495.1 ± 28	76.8 ± 3.9	14.31 ± 0.47	3.34 ± 0.16	7.30 ± 0.25	0.45 ± 0.06	7.08 ± 0.53a	2.09 ± 0.15	1.08 ± 0.06	0.332 ± 0.017	4.41 ± 0.36	0.243 ± 0.019	23.9 ± 1.6	1.84 ± 0.13a	7.59 ± 0.87a	2.78 ± 0.27
Treatments																
+UV-B																
+D/+ABA	541.0 ± 69	73.9 ± 5.1	14.16 ± 1.11	3.05 ± 0.26	7.75 ± 1.19	0.34 ± 0.06	5.91 ± 0.52	2.14 ± 0.27	1.14 ± 0.16	0.313 ± 0.039	3.63 ± 0.60	0.205 ± 0.044	22.5 ± 2.7	1.87 ± 0.21	7.77 ± 1.26	3.15 ± 0.29
-D/+ABA	631.4 ± 64	92.9 ± 7.6	14.73 ± 0.68	3.70 ± 0.38	7.96 ± 0.56	0.58 ± 0.19	9.01 ± 1.23	2.59 ± 0.36	1.24 ± 0.10	0.320 ± 0.020	5.10 ± 0.81	0.275 ± 0.055	29.9 ± 2.8	2.33 ± 0.29	11.46 ± 2.11	3.70 ± 0.64
+D/-ABA	576.8 ± 87	83.1 ± 5.1	13.74 ± 1.23	2.97 ± 0.23	7.64 ± 0.77	0.52 ± 0.12	6.40 ± 0.59	2.11 ± 0.28	1.33 ± 0.10	0.396 ± 0.029	4.66 ± 0.92	0.230 ± 0.029	28.8 ± 1.9	1.93 ± 0.16	8.60 ± 1.22	3.71 ± 0.34
-D/-ABA	469.6 ± 44	77.6 ± 6.8	13.49 ± 1.32	3.23 ± 0.26	6.55 ± 0.37	0.36 ± 0.06	7.28 ± 1.16	1.81 ± 0.19	1.07 ± 0.14	0.313 ± 0.034	3.77 ± 0.45	0.212 ± 0.040	24.8 ± 2.8	2.13 ± 0.31	8.91 ± 1.64	3.67 ± 0.41
-UV-B																
+D/+ABA	416.0 ± 57	54.3 ± 10.7	14.41 ± 1.06	3.13 ± 0.43	6.48 ± 0.85	0.28 ± 0.10	5.58 ± 0.98	1.68 ± 0.39	0.95 ± 0.19	0.321 ± 0.047	3.28 ± 0.80	0.251 ± 0.043	19.4 ± 3.3	1.37 ± 0.11	3.44 ± 0.67	1.48 ± 0.19
-D/+ABA	461.3 ± 47	76.1 ± 7.1	15.02 ± 0.97	3.64 ± 0.28	7.46 ± 0.59	0.51 ± 0.10	6.85 ± 0.77	2.39 ± 0.30	1.03 ± 0.13	0.362 ± 0.041	5.20 ± 0.67	0.254 ± 0.030	23.3 ± 2.9	1.59 ± 0.13	6.26 ± 1.04	2.25 ± 0.37
+D/-ABA	477.8 ± 47	57.5 ± 7.3	13.53 ± 1.00	2.67 ± 0.47	7.46 ± 0.85	0.32 ± 0.09	5.18 ± 0.88	1.49 ± 0.31	0.94 ± 0.17	0.278 ± 0.041	2.78 ± 0.49	0.239 ± 0.041	16.8 ± 3.3	1.37 ± 0.11	3.08 ± 0.63	1.40 ± 0.23
-D/-ABA	516.5 ± 57	60.5 ± 6.0	14.00 ± 0.73	2.81 ± 0.29	7.22 ± 0.46	0.36 ± 0.11	5.17 ± 0.71	1.59 ± 0.26	0.99 ± 0.13	0.333 ± 0.039	3.56 ± 0.79	0.230 ± 0.027	17.6 ± 2.5	1.32 ± 0.04	3.74 ± 0.66	1.49 ± 0.17
ANOVA^b																
$P_{(UV-B)}$	0.0419	0.0024	0.5898	0.4269	0.2757	0.5502	0.0111	0.0603	0.0001	0.5074	0.2255	0.6553	0.0066	0.0004	0.0019	0.0002
$P_{(ABA)}$	0.8246	0.3288	0.0887	0.0373	0.6589	0.5582	0.1567	0.0320	0.9054	0.9831	0.2019	0.3774	0.3259	0.3623	0.2167	0.7683
$P_{(D)}$	0.5957	0.0876	0.5413	0.0793	0.9196	0.2475	0.0230	0.1940	0.9649	0.8535	0.1020	0.4781	0.2385	0.0353	0.0482	0.2323
$P_{(UV-B \times ABA)}$	0.0605	0.7422	0.8144	0.4279	0.2858	0.7598	0.7539	0.7898	0.7981	0.1199	0.3436	0.8544	0.2108	0.8769	0.7782	0.2124
$P_{(UV-B \times D)}$	0.6402	0.5514	0.8073	0.8020	0.4486	0.6006	0.2598	0.4506	0.4509	0.0920	0.2844	0.5873	0.7947	0.3272	0.9078	0.7614
$P_{(ABA \times D)}$	0.2368	0.0263	0.7218	0.3513	0.2190	0.0571	0.1348	0.1119	0.2899	0.4443	0.0755	0.1771	0.0403	0.2355	0.1405	0.2531
$P_{(UV-B \times ABA \times D)}$	0.2541	0.7681	0.6535	0.9700	0.9487	0.4469	0.7163	0.8466	0.3741	0.2758	0.5515	0.4599	0.2818	0.9138	0.7804	0.9316

EC: epicatechin; ECG: epicatechin gallate; GC: gallic catechin; EGCG: epigallocatechin gallate; GCG: gallic catechin gallate; Q-3-glc: quercetin-3-glucoside; K-3-glc: kaempferol-3-glucoside.

^a Values are means for each factor ($n = 5$) \pm SEM. Different lowercase letters within columns indicate significant differences (Fisher's LSD, $P \leq 0.05$).

^b $P_{(UV-B)}$, $P_{(ABA)}$ and $P_{(D)}$: effects of UV-B, ABA and water deficit, respectively; $P_{(UV-B \times ABA)}$, $P_{(UV-B \times D)}$, $P_{(ABA \times D)}$, and $P_{(UV-B \times ABA \times D)}$: interaction effects of factors.

Table 2
Anthocyanin content ($\mu\text{g berry}^{-1}$) determined at harvest in berries of cv. Malbec exposed to combined UV-B, water deficit and ABA treatments during two growing seasons.

	Total Antho	Dihydroxylated		Trihydroxylated			Trihydroxylated relative %	Methoxylated relative %	Nonacylated	Acetylated	p-coumarylated
		Cyanidin	Peonidin	Delphinidin	Petunidin	Malvidin					
UV-B											
+UV-B	926.9 ^a ±38	24.6 ± 0.85	73.37 ± 3.8	94.74 ± 6.3	135.15 ± 7.5	599.0 ± 24	89.33 ± 0.35	87.27 ± 0.41	682.9 ± 23	123.1 ± 4.4	120.8 ± 5.9
–UV-B	960.6 ± 45	25.7 ± 1.19	76.57 ± 5.3	96.80 ± 8.6	138.38 ± 9.7	623.2 ± 25	89.43 ± 0.32	87.60 ± 0.44	706.1 ± 37	127.6 ± 5.0	126.9 ± 5.8
ABA											
+ABA	1009.0 ± 42a	26.9 ± 0.95a	85.75 ± 4.4a	105.47 ± 6.9a	148.4 ± 8.0a	642.5 ± 27	88.66 ± 0.39b	86.95 ± 0.40b	746.3 ± 32a	131.9 ± 5.0	130.8 ± 7.1
–ABA	878.4 ± 38b	23.4 ± 1.00b	64.20 ± 3.8b	86.07 ± 7.5b	125.1 ± 8.6b	579.7 ± 20	90.08 ± 0.20a	87.90 ± 0.43a	642.7 ± 32b	118.8 ± 4.1	116.9 ± 4.0
D											
+D	905.7 ± 46	24.5 ± 1.01	63.71 ± 4.4	87.96 ± 7.9	126.9 ± 8.9	594.0 ± 28	89.16 ± 0.35	87.78 ± 0.44a	659.2 ± 36	122.6 ± 5.4	123.9 ± 7.0
–D	981.8 ± 35	25.8 ± 1.05	81.72 ± 4.7	103.58 ± 6.9	146.7 ± 8.0	628.2 ± 19	89.59 ± 0.32	87.09 ± 0.40b	729.9 ± 30	128.1 ± 3.8	123.8 ± 4.6
Treatments											
+UV-B											
+D/	940.5 ± 104	25.8 ± 1.86	80.77 ± 7.5	97.88 ± 17.1	136.1 ± 19.3	600.0 ± 75	88.10 ± 1.03	86.93 ± 1.13	687.3 ± 76	126.3 ± 13.7	126.9 ± 20.3
+ABA	1061.6 ± 59	27.4 ± 1.62	88.48 ± 7.8	115.56 ± 10.7	161.8 ± 12.6	668.4 ± 34	89.13 ± 0.63	86.67 ± 0.67	796.9 ± 49	134.2 ± 6.4	130.5 ± 7.9
–D/	826.9 ± 68	22.2 ± 1.49	59.60 ± 6.2	74.69 ± 10.9	113.6 ± 13.9	556.8 ± 41	90.09 ± 0.41	88.45 ± 0.75	596.2 ± 55	115.0 ± 7.5	115.7 ± 8.1
–ABA	878.5 ± 44	23.1 ± 1.45	64.64 ± 4.6	90.84 ± 7.9	129.1 ± 9.3	570.7 ± 29	90.00 ± 0.43	87.05 ± 0.64	651.3 ± 37	116.9 ± 4.0	110.2 ± 6.0
–UV-B											
+D/	1009.4 ± 110	27.2 ± 1.96	86.44 ± 9.0	101.80 ± 15.7	144.8 ± 17.8	649.2 ± 67	88.62 ± 0.50	87.38 ± 0.39	741.4 ± 81	133.2 ± 12.6	134.8 ± 16.6
+ABA	1030.7 ± 65	28.0 ± 2.41	89.53 ± 11.9	109.90 ± 13.3	154.7 ± 15.2	648.6 ± 36	88.81 ± 0.92	86.86 ± 0.88	767.3 ± 51	134.0 ± 7.4	129.4 ± 12.6
–D/	851.9 ± 90	23.5 ± 2.51	64.73 ± 10.1	80.73 ± 18.9	116.8 ± 20.3	566.1 ± 45	89.78 ± 0.45	88.31 ± 1.05	619.4 ± 76	115.8 ± 9.7	116.6 ± 9.5
–ABA	956.5 ± 100	24.7 ± 2.58	67.81 ± 9.2	98.02 ± 20.6	140.9 ± 23.8	625.1 ± 47	90.44 ± 0.37	87.81 ± 1.02	704.0 ± 85	127.3 ± 10.9	125.2 ± 9.0
ANOVA^b											
$P_{(UV-B)}$	0.3164	0.3916	0.4312	0.7128	0.6401	0.1795	0.7719	0.6031	0.3489	0.4812	0.1530
$P_{(ABA)}$	0.0257	0.0038	0.0002	0.0411	0.0300	0.0703	0.0005	0.0341	0.0206	0.0566	0.1067
$P_{(D)}$	0.3130	0.3637	0.5031	0.1662	0.1723	0.4120	0.2873	0.0305	0.2490	0.4718	0.9555
$P_{(UV-B \times ABA)}$	0.7474	0.7793	0.8612	0.6350	0.6894	0.8048	0.8546	0.8558	0.7288	0.8552	0.8097
$P_{(UV-B \times D)}$	0.8697	0.9972	0.8386	0.8992	0.9432	0.8499	0.8453	0.8397	0.8006	0.9147	0.9008
$P_{(ABA \times D)}$	0.9825	0.8539	0.8205	0.9133	0.9973	0.9595	0.7689	0.6285	0.9762	0.8728	0.8534
$P_{(UV-B \times ABA \times D)}$	0.5259	0.9364	0.9849	0.8489	0.6362	0.3824	0.2292	0.4088	0.5528	0.5418	0.4525

^a Values are means for each factor ($n = 5$) \pm SEM. Different lowercase letters within columns indicate significant differences (Fisher's LSD, $P \leq 0.05$).

^b $P_{(UV-B)}$, $P_{(ABA)}$ and $P_{(D)}$: effects of UV-B, ABA and water deficit, respectively; $P_{(UV-B \times ABA)}$, $P_{(UV-B \times D)}$, $P_{(ABA \times D)}$, and $P_{(UV-B \times ABA \times D)}$: interaction effects of factors.

malvidin-derived) and methoxylated (peonidin-, petunidin- and malvidin-derived) anthocyanins (Table 2). As well, D treatment only affected methoxylated anthocyanins relative abundance, being increased 1% in +D, while UV-B treatments did not affect anthocyanins (Table 2).

4. Discussion

Application of moderate water restriction to grapevines from veraison to harvest only affected berry growth and sugar accumulation in plants under –UV-B treatment. So, the implication is that high erythemally-weighted UV-B irradiance from flowering acclimated plants to stressful situations like water hindrance. Several authors have reported reductions of berry size in red varieties such as Cabernet Sauvignon, Syrah, Tempranillo and Malbec subjected to water restriction (Castellarin et al., 2007; Santesteban et al., 2011; Song et al., 2012; Shellie and Bowen, 2014). Berry size is susceptible to water deficit, especially when it occurs in the early stages of its development, i.e. before veraison (Ojeda et al., 2001, 2002). Other than the phenological stage, the intensity of a water deficit is important to evaluate and discuss its effects. In the present work the stem water potential at midday was maintained at ca. –1 MPa, which is considered a moderate water restriction (Castellarin et al., 2007; Leeuwen et al., 2009; Shellie and Bowen, 2014).

Ojeda et al. (2002) submitted Syrah plants to water stress after

veraison, and found a reduction of total sugar content per berry, but not in sugar concentration as °Brix (berry size dependent). In the present work, previous incidence of high UV-B prevented berry weight reduction (and diminution of sugar accumulation) by water deficit. Some authors have reported that certain responses of plant defense against high levels of UV-B can mitigate the detrimental effects of a subsequent water deficit (Bandurska et al., 2013). Drilias et al. (1997) show that high levels of UV-B on the leaves of *Nerium oleander* L. mitigated the following incidence of water deficits, mainly through increments of cuticle thickness and reductions in transpiration.

Fruit yield was reduced by combining water deficit and high UV-B levels or water deficit and ABA. Alonso et al. (2015) found that gas exchange and photosynthesis were reduced by water deficit, and highly impaired in the UV-B and water deficit combined treatment, possibly being one of the causes that explain the lower fruit production.

ABA sprays induce anticipating in the accumulation of sugars (Matsushima et al., 1989; Koyama et al., 2010; Moreno et al., 2011) and anthocyanins in berries, i.e. berry ripening (Berli et al., 2011). Exogenous ABA triggered the expression of genes involved in sugars biosynthetic pathways and transport (Lecourieux et al., 2014). Major effects of ABA are found at veraison and differences are reduced at harvest (Wheeler et al., 2009; Berli et al., 2011). Apparently, applying ABA more frequently, as in previous

experiments is necessary to affects sugars (in the present study ABA was sprayed only at veraison and 15 days later).

Flavonols quercetin and kaempferol are photoprotective compounds with high antioxidant capacity that respond to high UV-B in several species (Agati and Tattini, 2010), even in leaves and berries of grapevine (Berli et al., 2010, 2011). The levels of dihydroflavonols, direct precursors of flavonols, are also responsive to high UV-B (Berli et al., 2011), and the dihydroflavonol astilbin (dihydroquercetin-3-O-rhamnoside) was correlated with Malbec wines from high altitude vineyards (Fanzone et al., 2010). Astilbin can acts against *Botrytis cinerea* (Landraut et al., 2002) and have a high antioxidant activity (Closa et al., 1997; Diao et al., 2014). Therefore, the ORAC results obtained for berry skins are in correspondence with the LMWP with higher antioxidant activities (i.e. quercetin, kaempferol and astilbin). ABA increased ORAC of berries only in plants under +UV-B (Fig. 2G) while Berli et al. (2015) obtained major effects applying ABA weekly. Only the application of ABA increased gallic acid, without affecting the most abundant hydroxybenzoic acid, the syringic acid, or the hydroxycinnamic acids, in correspondence with Berli et al. (2011). Additionally, the effect of +ABA on total LMWP and the dihydroxyflavonol astilbin are dependent on water status of vines; i.e. ABA applications increased total LMWP and astilbin only in those plants well watered (+ABA/-D combined treatment). Koyama et al. (2010) and Lacampagne et al. (2010) found that the application of ABA activate the expression of genes involved in the synthesis of anthocyanins.

The degree of hydroxylation and methoxylation of anthocyanins in grape berries are modified by environment signals, including UV-B and water status of grapevines. Trihydroxylated and methoxylated are the most oxidized anthocyanins, while dihydroxylated and nonacylated forms have high antioxidant power (Wang et al., 1997). Bindon et al. (2008) found a relative decrease in the proportion of methoxylated anthocyanins in grape berries in response to the partial rootzone drying (PRD), management practice that increase endogenous ABA levels (Stoll et al., 2000). We previously found that +UV-B and +ABA additively reduced the relative abundance of total trihydroxylated anthocyanins, whereas +ABA decreased the proportion of total methoxylated anthocyanins (Berli et al., 2011). In the present work, +ABA increased nonacylated anthocyanins, and in concordance with Berli et al. (2011), reduced the proportion of total methoxylated and total trihydroxylated anthocyanins (i.e. increased dihydroxylated anthocyanins), possibly due to ABA reduced the activity of O-methyltransferase and flavonoid 3′5′-hydroxylase, key enzymes in the biosynthesis of methoxylated and trihydroxylated anthocyanins, respectively. In the present, anthocyanins were not affected by UV-B, and the differences may be due to delays in the harvest time, e.g. it was two weeks later than in Berli et al. (2011). It was observed in *Vitis vinifera* cv. Touriga Francesa and *Vitis vinifera* cv. Cabernet Sauvignon that the concentration of some anthocyanins decreased at the end of maturation (Bindon et al., 2008; Jordao et al., 1998).

In Malbec there are few reports of water deficit effects, and our results are in concordance with those of Shellie and King (2013) where water deficit did not change the anthocyanin content. Shellie and Bowen (2014) reported increments in monomeric anthocyanins, but possibly by an indirect effect (reduction in the size of the berry so increasing the skin to pulp ratio) as the results should be expressed in absolute amounts per berry and not as concentration basis. We also found increments by +D in the relative abundance of methoxylated anthocyanins (most oxidized forms). Total LMWP was not significantly reduced by +D, but the procyanidin ECG, and the flavonols quercetin and Q-3-glc were diminished. Flavonoid 3-hydroxylase (F3′H) is responsible for quercetin precursors, and Castellarin et al. (2007) and Martinez-Luscher et al. (2014) observed that water deficit was associated with higher ratios

of flavonoid 3′5′ hydroxylase (F3′5′H) to F3′H transcription activity. However, astilbin, a quercetin precursor, was not affected by +D meaning that other factors may be implicated.

5. Conclusions

The environmental factors/signals assayed (UV-B and D) markedly affect the phenolics profile and sugar content of cv. Malbec berries at harvest, and only two applications of ABA (in veraison and 15 days after) induce biochemical changes that are maintained until harvest. The perception of high solar UV-B, irrespectively of other factors, markedly increased total LMWP in the berry skins, especially those with higher ORAC. Water restriction after veraison affected the accumulation of sugars in the berries, without affecting the majority of the polyphenols (LMWP and anthocyanins). ABA applications increased anthocyanins (irrespective of other factors) and also certain LMWP (only in plants without water deficit).

Author's contribution

R. Alonso and F.J. Berli conducted the experiment and carried out the physiological, biochemical and statistical analysis. A. Fontana participated in HPLC-MWD analysis. R. Bottini and P. Piccoli collaborated in experiment design and in decided strategies. R. Alonso and F.J. Berli wrote the body of the paper and all authors reviewed, edited and approved the final version of the manuscript.

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