High-Altitude Solar UV-B and Abscisic Acid Sprays Increase Grape Berry Antioxidant Capacity

Federico J. Berli,[1]* Rodrigo Alonso,[1,2] José Beltrano,[3] and Rubén Bottini[1]

Abstract: It has been proposed that ultraviolet-B (UV-B) radiation activates the antioxidant defense system in grapevines and that abscisic acid (ABA) acts downstream in the signaling pathway. Here, we studied the effects of solar UV-B and ABA sprays on berry quality indicators and fruit yield in *Vitis vinifera* L. cv. Malbec grown in a high-altitude vineyard, for five developmental stages and three growing seasons. Grapevines were exposed to elevated ambient solar UV-B (+UV-B) or to UV-B-filtered sunlight (–UV-B) from 15 days before flowering, combined with weekly sprays of 1 mM ABA (+ABA) or water (–ABA) from 27 days before veraison. The concentration of phenols (anthocyanins and total polyphenols) in berry skin was increased by +UV-B and +ABA, and there was a significant interaction between UV-B and ABA. For a given increase in sugar content, antioxidant capacity and phenolic contents were higher in berries in the +UV-B/+ABA treatment than in berries in the –UV-B/–ABA treatment. +UV-B and +ABA interacted to lessen the number of berries, possibly because of higher ethylene emissions, and additively reduced cluster weight without affecting sugar concentration at harvest. Antioxidant compounds, which are protective for plants, were triggered by +UV-B/+ABA at the expense of sugar accumulation, berry retention, and growth (fruit yield). Effects of UV-B and ABA on sugar accumulation and berry growth were dependent on the developmental stage. In high-altitude vineyards, solar UV-B and ABA application interact to increase quality indicators in red grape berries. The effects of UV-B and ABA on berry quality indicators are significant on a concentration basis (important from a winemaking standpoint) and additive on a per-berry basis.

Key words: ABA, ethylene, ORAC, phenols, UV-B, *Vitis vinifera* L.

Sunlight drives primary productivity via photosynthesis and supplies informational cues that are vital to plant development. As altitude increases, the atmosphere thins and more solar ultraviolet-B (UV-B) radiation (280 to 315 nm, the most energetic fraction of sunlight) reaches the ground. High-altitude vineyards in Mendoza, Argentina (~1500 m asl) receive elevated levels of UV-B, with irradiance up to 0.40 W/m^2 at noon in summertime (Berli et al. 2010). UV-B has direct and indirect photobiological effects on higher plants, some of which are related to the evoked damage and others as induced acclimation (Pontin et al. 2010). The effects of UV-B are influenced by other environmental variables such as photosynthetically active radiation (PAR); therefore, realistic balances between UV-B and PAR should be used in experiments (Caldwell et al. 2003).

Many physiological and biochemical acclimation processes, some of which are common to different stress conditions, are regulated by the phytohormone abscisic acid (ABA; Seki et al. 2002), so it is feasible to assume that ABA might regulate plant responses to UV-B. Few studies have addressed the relationship between UV-B and ABA in plants (Duan et al. 2008), but a promotive effect of UV-B on ABA biosynthesis was found in leaf tissues of *Arabidopsis* (Rakitin et al. 2008), maize (Tossi et al. 2009), and grapevine (Berli et al. 2010, Gil et al. 2012). However, UV-B was not responsible for ABA levels in grape berry skins, in which ABA increased at the onset of ripening (veraison) and declined at harvest (Berli et al. 2011). This finding, and the fact that application of ABA can hasten grape berry ripening (Berli et al. 2011, Jeong et al. 2004), suggests that ABA plays a fundamental role in regulating fruit maturation. Changes in ethylene production at veraison have also been described in grape berries (Tesniere et al. 2004). Ethylene regulates many aspects of plant growth and development, including senescence and abscission, and plays an important role in the response to stressors including UV-B (He et al. 2011).

Phenols are secondary metabolites with a variety of biological functions that include acting as attractants for pollinators and seed dispersers and as defense compounds against herbivores, pathogens, and environmental stress (Croteau et al. 2000). Phenols play a significant role in winemaking and

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contribute to wine quality by determining color, structure, mouthfeel, and antioxidant potential (Cheynier 2005). The phenolic composition (i.e., phenolic acids, stilbenes, anthocyanins, flavonols, and flavanols) of grape berries depends on the cultivar (genetic factors), ontogeny (berry development and maturation), environmental conditions, and management practices (Downey et al. 2006). In general, phenol biosynthesis is stimulated by stress.

Despite the importance of phenols in winemaking, relatively few studies have examined the response of phenolic compounds to UV-B in grape berries (Keller and Torres-Martinez 2004). We previously found that phenolic content increases in grape berries exposed to high levels of solar UV-B, and increases more when UV-B is combined with application of ABA (additive effects; Berli et al. 2011). In those experiments, we focused on phenolic profiles on a per-berry basis; the effects of UV-B and ABA on antioxidant capacity and fruit yield were not clarified. This paper reports independent and interactive effects of solar UV-B and ABA sprays on a field-grown red grapevine cultivar in a high-altitude vineyard during three consecutive growing seasons. Berry antioxidant capacity, anthocyanins, total polyphenols, sugar accumulation, and growth were evaluated as fruit quality indicators important to winemaking; berry number and cluster weight at harvest were measured to assess effects on fruit yield.

Materials and Methods

Plant material and experimental design. The experiment was carried out during three growing seasons (2009, 2010, and 2011) in a commercial high-altitude vineyard (Viñedo Adrianna, Catena Zapata; 1450 m asl, lat. 69°15′37″W; long. 33°23′51″S), Gualtallary, Mendoza, Argentina. The grapevines were a selected clone of *Vitis vinifera* L. cv. Malbec, planted in 1997 on their own roots. The vines were trained on a vertical trellis system arranged in north–south-oriented rows (2-m row spacing and 1.20 m between plants) and were maintained without soil water restriction using a drip irrigation system. The vines were cane-pruned and shoot-thinned to 12 shoots per vine when shoots reached 10-cm long; two clusters per shoot were left at flowering.

We used a randomized complete block design with a 2 × 2 factorial arrangement of treatments (UV-B and ABA) and five blocks (n = 5). The experimental unit consisted of four plants that were selected based on homogeneity from six consecutive plants in the row. In each experimental unit, two shoots were marked and used to determine cluster weight and number of berries per cluster at harvest (fruit yield); the remaining shoots were used for berry sampling at the different developmental stages. Repeated measurements were taken in each experimental unit at 52, 72 (veraison), 96, 112, and 131 (harvest) days after flowering (DAF) during each growing season.

UV-B and ABA treatments. Two radiation regimes were established for the grapevine canopy and were performed from 15 days before flowering (mid-November, stage 21 [Coombe 1995]) until harvest (131 DAF, early April). A minus-UV-B treatment (–UV-B) was provided by using a polyester cover that absorbed 78% of UV-B and transmitted 88% of PAR. A full-UV-B treatment (+UV-B) was established by covering the canopy with low-density polyethylene that transmitted 90% of UV-B and 87% of PAR and minimized environmental differences between the two treatments. Plastics were placed 2.5 m above ground level (~30 cm above the grapevines) covering the east- and west-facing sides of the canopy at a 45° angle to the soil, and were protected with anti-hail nets (Figure 1A). The transmittance spectral characteristics were reported previously (Berli et al. 2008, 2011). A LI-250 light meter with a LI-190SA quantum sensor (LI-COR, Lincoln, NE) and a PMA2200 radiometer with a PMA2102 UV-B detector (Solar Light Company, Glenside, PA), were used to measure PAR and UV-B, respectively. Mean values of UV-B and PAR above the canopy on a typical sunny summer day are shown in Figure 1B.

Two ABA treatments were administered weekly by spraying aerial parts (leaves and berries) of the plants, from 27 days before veraison (late January, stage 33 [Coombe 1995]) until harvest. We initiated a plus-ABA treatment (+ABA) using a 1 mM aqueous solution of ±-S-*cis*,*trans*-abscisic acid (90% purity; Kelinon Agrochemical, Beijing, China) containing 0.1% v/v Triton X-100 and a minimum amount of ethanol, according to previous work with grapevine (Quiroga et al.

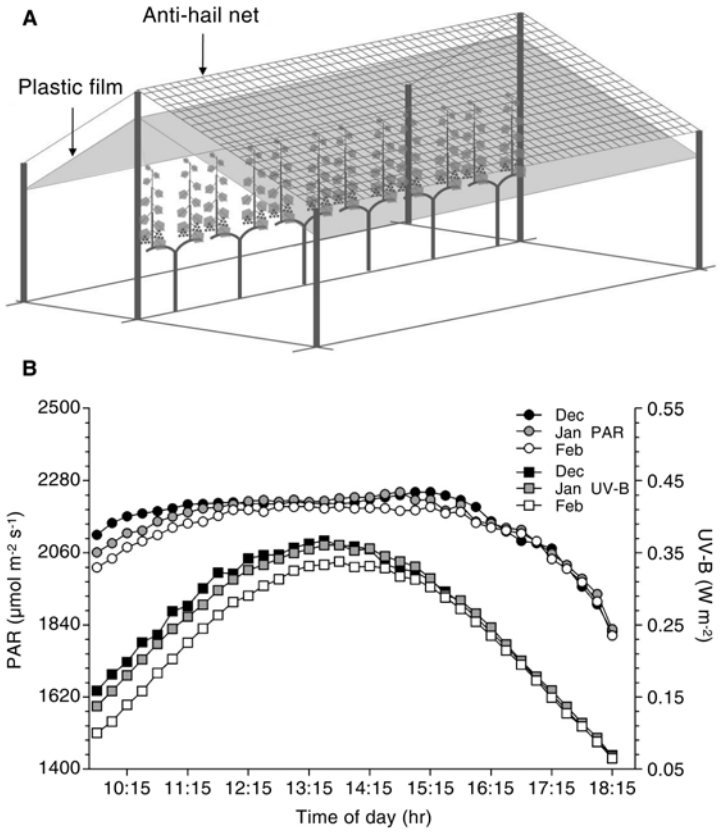

Figure 1 Schematic representation of an experimental unit in the -UV-B and +UV-B treatments (polyester and low-density polyethylene films, respectively) protected with anti-hail nets (**A**). Solar UV-B and photosynthetically active radiation (PAR) registered on a typical sunny summer day (December, January, and February) above the canopy at the experimental site. Values are means for 2009, 2010, and 2011 (**B**).

2009). A solution containing water, Triton X-100, and ethanol as described above was used as the minus-ABA treatment (–ABA).

Berry weight, sugars, phenols, and antioxidant capacity. Berry samples were taken at 52, 72, 96, 112, and 131 DAF (the maximum difference between dates for the three growing seasons was ± 5 days). Fifty berries were randomly collected from 10 clusters (five berries from each cluster: two top, two middle, and one bottom berry) in each experimental unit. The berries were collected into nylon bags and kept on dry ice to prevent enzyme degradation and dehydration, and were taken to the laboratory where berry fresh weight (FW) was determined before storage at –20°C. Then, berries were defrosted at room temperature (25 ± 2°C) and peeled by hand. The relative concentration of sugar (Brix) and sugar on a per berry basis (absolute amounts) were determined in pulp according to Berli et al. (2011).

Berry skins were extracted with 50 mL aqueous ethanolic solution (12% ethanol, 6 g/L tartaric acid, pH 3.2) at 70°C for 3 hr in darkness. The liquid fraction was separated by decanting, maintained for 24 hr at 4°C, and centrifuged for 10 min at 10,000 × *g* to eliminate tartrates and other sediments. Finally, the supernatant was collected and stored at –20°C. Anthocyanins and the total polyphenol index (TPI) were determined as described in Berli et al. (2008) and were calculated on a per-berry and a concentration basis.

Oxygen radical absorbance capacity (ORAC) was determined based on Prior et al. (2003) with modifications. Berry skin extraction solutions were diluted 1:750 v/v in 75 mM potassium phosphate buffer (pH 7.0). Aliquots (50 µL) of diluted samples and Trolox standards were added to a 96-well black plate. Then, 100 µL of fluorescein (20 nM solution) was added and the mixture was incubated at 37°C for 7 min before the addition of 50 µL of the peroxyl radical generator AAPH (2,2′-azobis[2-amidinopropane]dihydrochloride, 140 mM solution) (Sigma-Aldrich, St. Louis, MO). Fluorescence was monitored using 485-nm excitation and 538-nm emissions at 1-min intervals for 90 min on a microplate fluorometer (Fluoroskan Ascent FL; Thermo Fisher Scientific, Wilmington, DE). The area under the fluorescence decay curve (90 min) was calculated, and ORAC was expressed as mmol Trolox equivalents (TE) per berry skin and per g berry FW.

Cluster weight and number of berries per cluster (fruit yield). At harvest (131 DAF), clusters from the two selected shoots per experimental unit (i.e., those not used for berry sampling) were collected in nylon bags and weighed, and the number of berries per cluster was counted.

Berry ethylene emissions. In the 2011 growing season, one cluster per experimental unit (from shoots not used for berry sampling) was introduced into a 1-L nylon bag at 52, 72, 96, 112, and 131 DAF. The bags were tied in the peduncle, and ethylene was allowed to accumulate over a 2 hr period (from 1000 hr to 1200 hr). Afterwards, the bags were punctured with a syringe needle and 10 mL gas were extracted, the clusters were cut, the syringes were sealed with parafilm, and both samples were ice-cooled and transported to the laboratory. The number of berries per cluster was counted and berries were weighed. Ethylene in the gas samples was determined as described in Beltrano et al. (1994).

Statistical analysis. Repeated measurements multifactorial ANOVA was used to evaluate effects of UV-B, ABA, developmental stage, growing season, and their interactions under the randomized complete block design ($p \leq 0.05$). We implemented a mixed-model approach with random effect(s) to account for serial correlation among measures within plots at each development stage and season, using SAS Proc Mixed (SAS Institute, Inc., Cary, NC). Principal component analysis (PCA) was performed using biplot graphics and standardized (centered and variance-scaled) data (InfoStat version 2009; Grupo InfoStat, Córdoba, Argentina). Linear regression models were calculated to assess the relationships between berry sugar and ORAC, anthocyanins, and TPI in the +UV-B/+ABA and –UV-B/–ABA treatments; we used *t*-tests to compare the slopes of the regression lines (InfoStat).

Results

Results are expressed on a per-berry FW basis (concentration, dependent on berry size), and on a per-berry (absolute) basis. The former is important from a winemaking standpoint, and the latter enables understanding of physiological effects (biosynthesis or accumulation).

Berry skin antioxidant capacity and polyphenol content. The effects of UV-B and ABA on berry skin antioxidant capacity (assessed as ORAC), anthocyanins, and TPI are presented in Table 1. On a per-berry basis, ORAC increased by 75% from 52 to 112 DAF, but on a concentration (g FW) basis, ORAC was highest at 52 DAF. The +UV-B and +ABA treatments had additive effects on ORAC (UV-B x ABA interaction effects were not statistically significant). Anthocyanins increased significantly from 52 to 131 DAF; there was an additive increase in these compounds by +UV-B and +ABA treatment on a per-berry basis, and significant +UV-B/+ABA interaction effects occurred on a concentration basis. Similarly, TPI increased significantly from 72 to 131 DAF, with additive effects of +UV-B and +ABA on a per-berry basis and significant interaction effects on a concentration basis. On an absolute basis, the +UV-B/+ABA treatment produced TPI values that were 4.0% higher, ORAC values that were 13.0% higher, and anthocyanins 12.4% higher than those in the –UV-B/–ABA treatment; by concentration, ORAC was 36.3% higher, anthocyanins were 33.6% higher, and TPI was 17.9% higher in the +UV-B/+ABA treatment. The interactions were analyzed but were not included in the table, and most variables were significantly higher in 2009. UV-B ABA × YEAR interaction effects were not significant.

The PCA indicates that, considering all the berry developmental stages, ORAC, anthocyanins, and TPI were associated with +UV-B/+ABA treatment (Figure 2A). Considering only determinations at harvest, these variables were associated with +ABA treatments (+UV-B/+ABA when they were expressed in concentration and –UV-B/+ABA when they were in absolute amounts, Figure 2B).

Berry growth, sugar accumulation, ethylene emission, and yield. Berry FW increased by ~97.3% from 52 to 112

DAF and then remained constant until harvest (sigmoidal growth pattern, Figure 3A). The +UV-B and +ABA treatments additively reduced berry growth, especially near harvest (UV-B × DAF significant interaction). Sugar content in the pulp increased continuously until harvest and was reduced by +UV-B and +ABA (per-berry basis), especially near harvest (UV-B × DAY and ABA × DAF significant interactions, Figure 3B). Sugar concentration (Brix) was increased by +UV-B and +ABA significantly at veraison (~72 DAF; Figure 3C). Berries in +UV-B/+ABA markedly increased ethylene emission, especially at veraison and to a lesser extent at harvest (UV-B x ABA significant interaction, Figure 4). Table 2 shows that at harvest the number of berries per cluster were limited by +ABA only in combination with +UV-B (UV-B × ABA significant interaction). Cluster FW was additively affected by +UV-B and +ABA. The +UV-B/+ABA treatment was 23.3% lower in cluster FW and 15.7% lower in berry FW than –UV-B/–ABA. The PCA showed that, at harvest, cluster FW, berries per cluster, and berry FW were associated with –ABA treatment (+UV-B/–ABA and –UV-B/–ABA, Figure 2B). Growing season did not affect the number of berries per cluster, but berry growth, sugar accumulation, and cluster FW were significantly lower in 2011 (Table 2).

Relationships between berry sugar content and ORAC, anthocyanins, and polyphenols. Figure 5 presents linear regression models for berry sugar vs. ORAC (A), vs. anthocyanins (B), and vs. TPI (C) in +UV-B/+ABA and –UV-B/–ABA treatments, considering all the stages of berry development and growing seasons. More prominent slopes were obtained for +UV-B/+ABA than for –UV-B/–ABA treatment (increasing 35.8, 18.3, and 35.2% for sugar vs. ORAC, vs. anthocyanins, and vs. TPI, respectively). The slopes of all regressions were steeper for the +UV-B/+ABA than for –UV-B/–ABA.

Discussion

We observed positive effects of UV-B radiation and weekly ABA sprays (starting ~1 mo before veraison) on antioxidant capacity, anthocyanins, and total polyphenol accumulation in

Table 1 Oxygen radical absorbance capacity (ORAC), anthocyanin content, and total polyphenol index (TPI) of berry skins in UV-B and ABA treatments according to developmental stage (days after flowering, DAF) and growing season (year).

	ORAC (mmol TE/ berry skin)	ORAC (mmol TE/ g berry FW)	Anthocyanin (µg/berry)	Anthocyanin (mg/100 g berry FW)	TPI (TPI/berry)	TPI (TPI/100 g berry FW)
UV-B						
+UV-B	564.45 a[a]	527.12 a	923.84 a	70.71 a	68.57 a	6.02 a
–UV-B	545.08 a	458.85 b	873.22 a	60.74 b	65.22 a	5.32 b
ABA						
+ABA	578.48 a	534.19 a	927.09 a	70.59 a	66.56 a	5.80 a
–ABA	530.60 b	450.56 b	869.96 b	60.86 b	67.23 a	5.54 a
DAF						
52	384.23 d	543.26 a	17.38 e	1.52 e	36.82 d	5.40 c
72	424.61 c	458.85 b	198.06 d	19.91 d	36.70 d	4.14 d
96	620.78 b	477.61 b	1115.84 c	81.80 c	75.54 c	5.66 c
112	674.19 a	488.32 b	1433.86 b	101.41 b	87.33 b	6.14 b
131	670.69 a	495.91 b	1727.50 a	123.97 a	98.08 a	7.00 a
YEAR						
2009	773.11 a	601.66 a	1065.71 a	72.10 a	73.99 a	5.45 b
2010	454.74 b	339.57 c	942.22 b	60.65 b	70.51 a	5.09 b
2011	444.26 b	540.51 b	687.65 c	64.42 b	56.18 b	6.47 a
UV-B × ABA						
+UV-B/+ABA	581.37 a	568.42 a	975.15 a	78.45 a	69.22 a	6.38 a
+UV-B/–ABA	547.23 ab	485.11 bc	872.52 b	62.97 b	67.92 ab	5.67 b
–UV-B/+ABA	575.63 a	500.53 ab	879.03 b	62.74 b	63.89 b	5.22 b
–UV-B/–ABA	514.53 b	417.16 c	867.40 b	58.74 b	66.55 ab	5.41 b
ANOVA[b]						
$p_{(UV\text{-}B)}$	0.0480	0.0157	0.0631	0.0009	0.0612	0.0004
$p_{(ABA)}$	0.0056	0.0079	0.0398	0.0011	0.6547	0.0912
$p_{(DAF)}$	0.0001	0.0029	0.0001	0.0001	0.0001	0.0001
$p_{(YEAR)}$	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
$p_{(UV\text{-}B \times ABA)}$	0.1162	0.9010	0.0907	0.0259	0.2490	0.0099
$p_{(UV\text{-}B \times DAF)}$	0.3544	0.6834	0.3270	0.0774	0.4786	0.5674
$p_{(ABA \times DAF)}$	0.6235	0.7981	0.6059	0.0676	0.8270	0.7803
$p_{(UV\text{-}B \times ABA \times YEAR)}$	0.4474	0.4855	0.7199	0.3168	0.1580	0.0583

[a]Values are means for each factor. Different lowercase letters within columns indicate significant differences (Fisher's LSD, $p \leq 0.05$).
[b]$p_{(UV\text{-}B)}$, $p_{(ABA)}$, $p_{(DAF)}$, and $p_{(YEAR)}$: effects of UV-B, ABA, developmental stage, and growing season, respectively; $p_{(UV\text{-}B \times ABA)}$, $p_{(UV\text{-}B \times DAF)}$, $p_{(ABA \times DAF)}$, and $p_{(UV\text{-}B \times ABA \times YEAR)}$: interaction effects of factors.

berry skins in high-altitude vineyards. We previously reported additive effects of UV-B and ABA on berry skin phenols, but those results were evaluated on a per-berry basis with the objective of understanding effects on phenol biosynthesis and accumulation (Berli et al. 2011). The present work shows that the effects of UV-B and ABA are more pronounced in terms of concentration. Significant UV-B × ABA interactions were observed for anthocyanin and total polyphenol concentrations, meaning that ABA application was more effective under full exposure to UV-B. The effects of UV-B and ABA on anthocyanin and phenol concentration were important because these treatments also additively reduced berry size at harvest. Phenol concentrations in berries determine the composition of secondary metabolites in wine, and thus are critical from a winemaking standpoint. It is also important that the effects of UV-B and ABA were consistent across the three-year study, and that most of the variables related to plant defense were significantly higher in 2009 when higher total solar radiation and air temperatures occurred (Berli et al. 2013).

In grapevine leaves, key enzymes of the phenylpropanoid and flavonoid pathways are activated by UV-B (Pontin et al. 2010) and ABA (Jeong et al. 2004). Flavonoids are polyphenolic structures containing numerous double bonds and hydroxyl groups that can donate electrons through resonance to stabilize free radicals, and thus act as powerful antioxidants to protect plants against oxidative stress (Machlin and Bendich 1987). UV-B increases flavonol content in berry skins (Gregan et al.

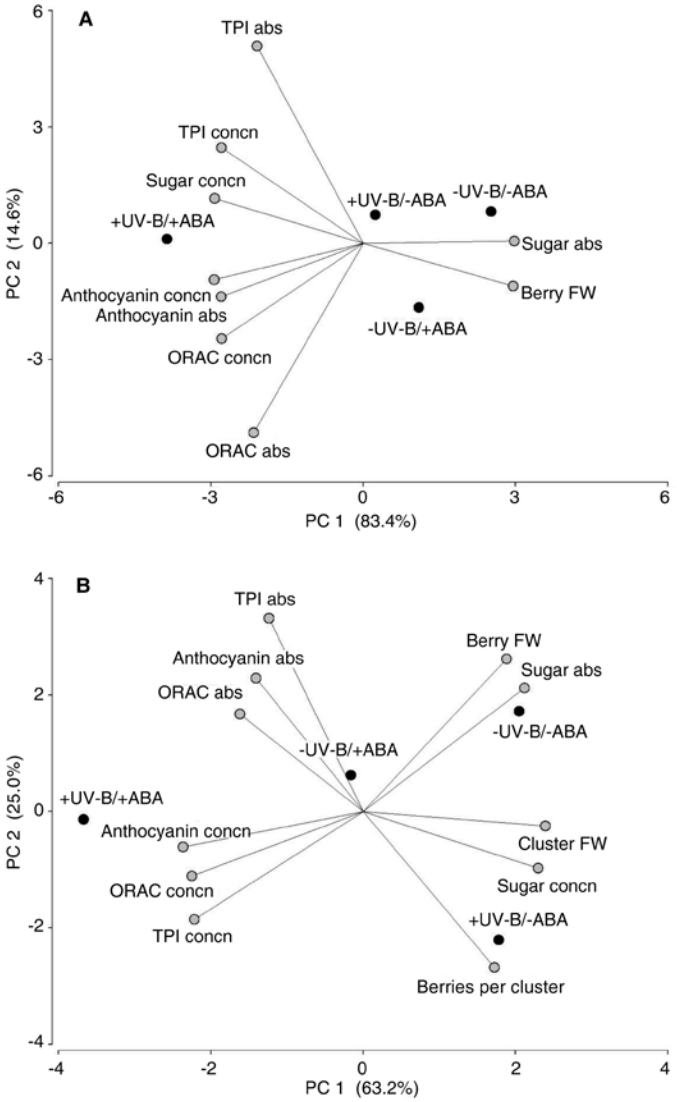

Figure 2 Biplot display of principal component analysis (PCA) of variables measured for three growing seasons in berries in +UV-B and –UV-B treatments in combination with +ABA and –ABA. Results are shown for all berry developmental stages (**A**) and for harvest stage only (**B**). The variables are expressed in terms of absolute quantities (abs) and concentration (concn).

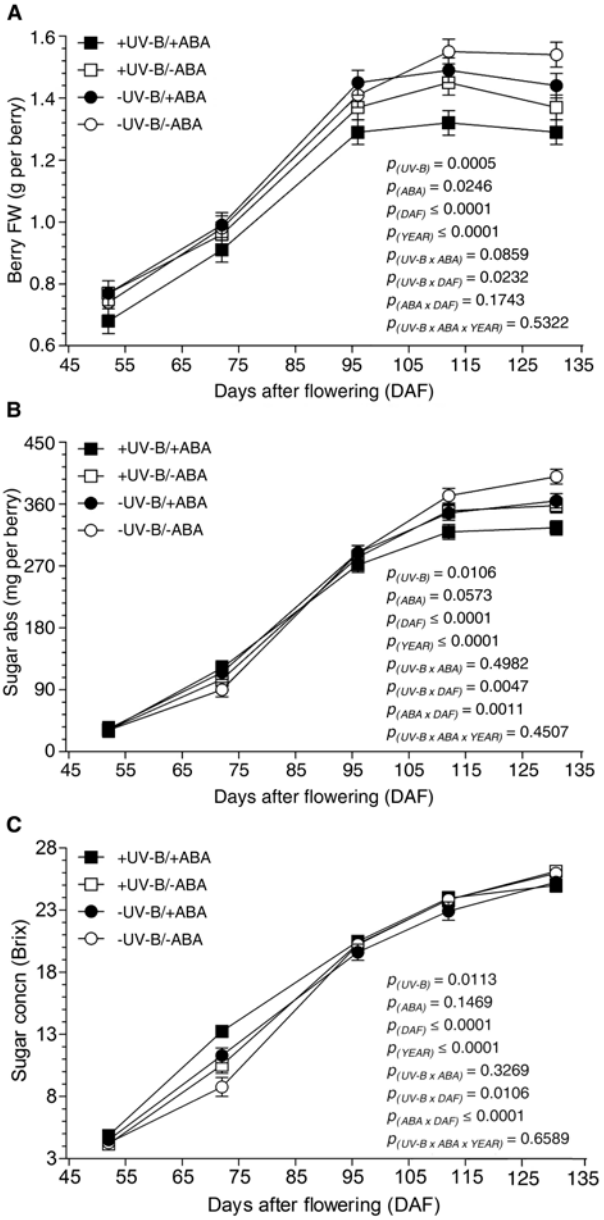

Figure 3 Berry characteristics according to developmental stage and treatment. Fresh weight (FW) (**A**); sugar, absolute quantity (abs) (**B**); and sugar, concentration (concn) (**C**). Values are means ± SE for 2009, 2010, and 2011 growing seasons. $p_{(UV-B)}$, $p_{(ABA)}$, $p_{(DAF)}$, and $p_{(YEAR)}$: effects of UV-B, ABA, developmental stage, and growing season, respectively; $p_{(UV-B \times ABA)}$, $p_{(UV-B \times DAF)}$, $p_{(ABA \times DAF)}$, and $p_{(UV-B \times ABA \times YEAR)}$: interaction effects of factors.

2012), and ABA increases flavonols and antioxidant capacity in berry skins (Sandhu et al. 2011) and wines (Xi et al. 2013). UV-B and ABA additively increase phenols, especially compounds with higher antioxidant capacity (e.g., dihydroxylated anthocyanidins such as cyanidin, and flavonols such as quercetin and kaempferol; Berli et al. 2011).

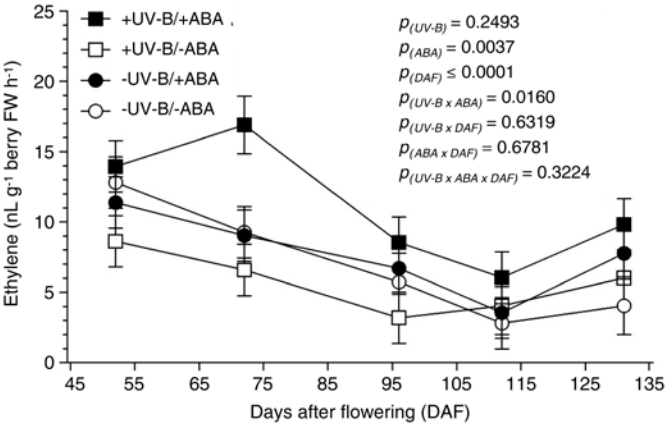

Figure 4 Berry ethylene emissions for UV-B and ABA treatments according to developmental stage. Values are means ± SE for the 2011 growing season. $p_{(UV-B)}$, $p_{(ABA)}$ and $p_{(DAF)}$: effects of UV-B, ABA, and developmental stage, respectively; $p_{(UV-B \times ABA)}$, $p_{(UV-B \times DAF)}$, $p_{(ABA \times DAF)}$, and $p_{(UV-B \times ABA \times DAF)}$: interaction effects of factors.

Table 2 Cluster fresh weight (FW), number of berries per cluster, and berry FW at harvest (131 DAF) for UV-B and ABA treatments in three growing seasons.

	Cluster FW (g/cluster)	Berries per cluster (n)	Berry FW (g/berry)
UV-B			
+UV-B	83.41 a[a]	64 a	1.33 b
–UV-B	91.46 a	62 a	1.49 a
ABA			
+ABA	80.70 a	59 a	1.36 b
–ABA	94.17 a	66 a	1.45 a
YEAR			
2009	89.24 ab	60 a	1.49 a
2010	99.19 a	63 a	1.58a
2011	73.87 b	66 a	1.15 b
UV-B × ABA			
+UV-B/+ABA	70.66 b	55 b	1.29 c
+UV-B/–ABA	96.17 a	73 a	1.37 bc
–UV-B/+ABA	90.75 a	64 ab	1.44 ab
–UV-B/–ABA	92.16 a	60 ab	1.53 a
ANOVA[b]			
$p_{(UV-B)}$	0.2377	0.6837	0.0010
$p_{(ABA)}$	0.0568	0.1593	0.0373
$p_{(YEAR)}$	0.0074	0.4903	0.0001
$p_{(UV-B \times ABA)}$	0.0848	0.0417	0.8036
$p_{(UV-B \times ABA \times YEAR)}$	0.4673	0.1018	0.7218

[a]Values are means for each factor. Different letters within columns indicate significant differences (Fisher's LSD, $p \leq 0.05$).

[b]$p_{(UV-B)}$, $p_{(ABA)}$, and $p_{(YEAR)}$: effects of UV-B, ABA, and growing season, respectively; $p_{(UV-B \times ABA)}$ and $p_{(UV-B \times ABA \times YEAR)}$: interaction effects of factors.

At the onset of berry maturation, before anthocyanins have accumulated in the skins, non-anthocyanin phenolics are responsible for the antioxidant capacity. At this early

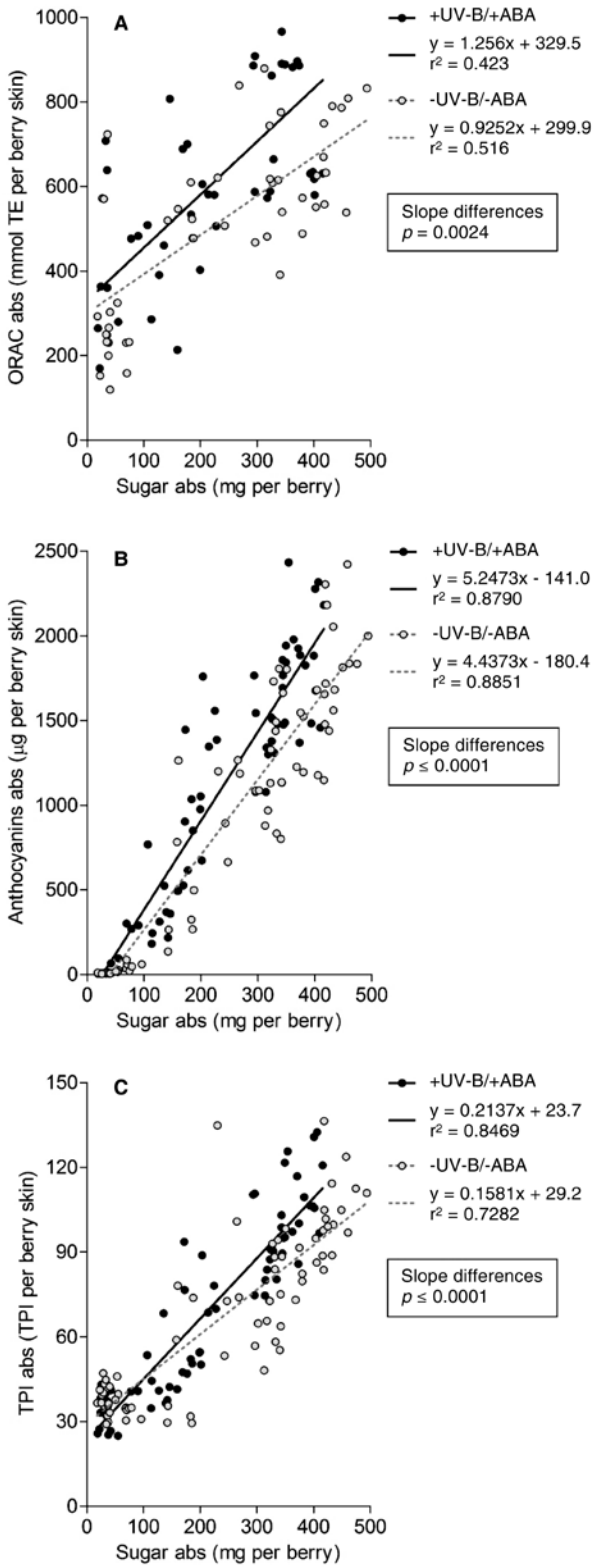

Figure 5 Linear regressions between berry sugar and: oxygen radical absorbance capacity (ORAC) (**A**); anthocyanins (**B**); and total polyphenolic index (TPI) (**C**). The regressions were performed for +UV-B/+ABA and –UV-B/–ABA treatments across all growing seasons and developmental stages. Variables are presented in absolute quantities (abs).

developmental stage (50 DAF), flavonols, flavanols, dihydroflavonols, and hydroxycinnamic acids represent approximately 41, 36, 13, and 10% of total phenols, respectively, among which the primary compound is quercetin-3-glucoside (Berli et al. 2011). In the present experiment, solar UV-B reached a maximum in December and January and then decreased towards harvest in early April. Elicitation of flavonoids by UV-B can depend on the developmental stage; although grape berries are exposed to seasonally higher UV-B levels prior to veraison, anthocyanins only appear after veraison (Gregan et al. 2012).

The effects of +UV-B and +ABA on sugar contents in fruit are different at veraison than at harvest, when the accumulation of sugar per berry decreases but sugar concentration is not affected (+UV-B/+ABA also reduces berry size significantly near harvest). These results confirm our previously reported findings (Berli et al. 2011). ABA was reported to stimulate production of invertases, hexose transporters, and enzymes that soften the cell wall (Koyama et al. 2010), and to increase hexose (glucose and fructose) accumulation in berries up to veraison (Moreno et al. 2011).

The lower sugar accumulation and berry growth after veraison in the +UV-B/+ABA treatment might be a result of decreased elasticity of berry cell walls (Gambetta et al. 2010), or of degradation of vacuolar invertases that regulate hexose accumulation in berries (Giribaldi et al. 2010). Berries on +UV-B/+ABA-treated plants may approach this limit earlier because of ABA-induced advancement of ripening. The increases in berry skin phenols promoted by UV-B and ABA might influence auxin levels by inhibiting auxin transport (Jacobs and Rubery 1988), thus affecting the extensibility of cell walls and limiting berry growth (Lüthen et al. 1990). The lower sugar accumulation and berry growth could be related to diminished carbon fixation in the +UV-B treatment caused by reduced leaf area and gas exchange (Berli et al. 2013). Sugar reductions may also be a response attributable to the cost of forming secondary metabolites to provide protection to plants (Berli et al. 2010), especially phenolics (Berli et al. 2008, 2011) and terpenes (Gil et al. 2012, 2013).

The reduced cluster FW at harvest by +ABA, especially in combination with +UV-B, was a result of diminished berry size and number. Because cluster number was regulated at the beginning of the experiment, cluster FW is a measure of fruit yield. Hilt and Bessis (2003) correlated high levels of ethylene with abscission of young grape berries and suggested that interactions between ABA and ethylene regulated fruit abscission. In addition, Zhang and Zhang (2009) found that grape clusters treated with ABA had increased hydrolase activity, especially of cellulose and polygalacturonase in pedicel abscission zones, and had accelerated rates of berry abscission. The effect of ABA on berry number (through berry retention) depends on the phenological stage (Quiroga et al. 2009).

Conclusion

In high-altitude vineyards, solar UV-B and ABA sprays interact to increase quality indicators in red grape berries. The effects of UV-B and ABA on berry quality indicators are significant on a concentration basis (important from a winemaking standpoint) and additive on a per-berry basis. The quality of berries for winemaking is a function of various characteristics; for red wines, especially those to be aged, berry quality is strongly correlated with phenolic content and antioxidant capacity (nutraceutical value). Antioxidant compounds, which have protective effects for plants, are triggered by UV-B in combination with ABA, at the expense of sugar accumulation, berry retention (possibly because of higher ethylene emissions), and growth (fruit yield). The effects of UV-B and ABA on sugar accumulation and berry growth depend on the developmental stage.

Literature Cited

Beltrano, J., M.G. Ronco, E.R. Montaldi, and A. Carbone. 1998. Senescence of flag leaves and ears of wheat hastened by methyl jasmonate. J. Plant Growth Regul. 17:53-57.

Berli, F., J. D'Angelo, B. Cavagnaro, R. Bottini, R. Wuilloud, and M.F. Silva. 2008. Phenolic composition in grape (*Vitis vinifera* L. cv. Malbec) ripened with different solar UV-B radiation levels by capillary zone electrophoresis. J. Agric. Food Chem. 56:2892-2898.

Berli, F.J., D. Moreno, P. Piccoli, L. Hespanhol-Viana, M.F. Silva, R. Bressan-Smith, J.B. Cavagnaro, and R. Bottini. 2010. Abscisic acid is involved in the response of grape (*Vitis vinifera* L.) cv. Malbec leaf tissues to ultraviolet-B radiation by enhancing ultraviolet-absorbing compounds, antioxidant enzymes and membrane sterols. Plant Cell Environ. 33:1-10.

Berli, F.J., M. Fanzone, P. Piccoli, and R. Bottini. 2011. Solar UV-B and ABA are involved in phenol metabolism of *Vitis vinifera* L. increasing biosynthesis of berry skin polyphenols. J. Agric. Food Chem. 59:4874-4884.

Berli, F.J., R. Alonso, R. Bressan-Smith, and R. Bottini. 2013. UV-B impairs growth and gas exchange in grapevines grown in high altitude. Physiol. Plantarum 149:127-140.

Caldwell, M.M., C.L. Ballare, J.F. Bornman, S.D. Flint, L.O. Björn, A.H. Teramura, G. Kulandaivelu, and M. Tevini. 2003. Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climatic change factors. Photoch. Photobiol. Sci. 2:29-38.

Coombe, B.G. 1995. Growth stages of the grapevine: Adoption of a system for identifying grapevine growth stages. Aust. J. Grape Wine. Res. 1:104-110.

Croteau, R., T.M. Kutchan, and N.G. Lewis. 2000. Natural products (secondary metabolites). *In* Biochemistry and Molecular Biology of Plants. B. Buchanan et al. (eds.), pp. 1250-1268. American Society of Plant Biologists, Rockville, MD.

Cheynier, V. 2005. Polyphenols in foods are more complex than often thought. Am. J. Clin. Nutr. 81:223S-229S.

Downey, M.O., N.K. Dokoozlian, and M.P. Krstic. 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. Am. J. Enol. Vitic. 57:257-268.

Duan, B., Z. Xuan, X. Zhang, H. Korpelainen, and C. Li. 2008. Interactions between drought, ABA application and supplemental UV-B in *Populus yunnanensis*. Physiol. Plantarum 134:257-269.

Gambetta, G.A., M.A. Matthews, T.H. Shaghasi, A.J. McElrone, and S.D. Castellarin. 2010. Sugar and abscisic acid signaling orthologs are activated at the onset of ripening in grape. Planta 232:219-234.

Gil, M., M. Pontin, F. Berli, R. Bottini, and P. Piccoli. 2012. Metabolism of terpenes in the response of grape (*Vitis vinifera* L.) leaf tissues to UV-B radiation. Phytochem. 77:89-98.

Gil, M., R. Bottini, F. Berli, M. Pontin, M.F. Silva, and P. Piccoli. 2013. Volatile organic compounds characterized from grapevine (*Vitis vinifera* L. cv. Malbec) berries increase at pre-harvest and in response to UV-B radiation. Phytochem. 96:148-157.

Giribaldi, M., L. Gény, S. Delrot, and A. Schubert. 2010. Proteomic analysis of the effects of ABA treatments on ripening *Vitis vinifera* berries. J. Exp. Bot. 61:2447-2458.

Gregan, S.M., J.J. Wargent, L. Liu, J. Shinkle, R. Hofmann, C. Winefield, M. Trought, and B. Jordan. 2012. Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon blanc grapes. Aust. J. Grape Wine. Res. 18:227-238.

He, J., X. Yue, R. Wang, and Y. Zhang. 2011. Ethylene mediates UV-B-induced stomatal closure via peroxidase-dependent hydrogen peroxide synthesis in *Vicia faba* L. J. Exp. Bot. 62:2657-2666.

Hilt, C., and R. Bessis. 2003. Abscission of grapevine fruitlets in relation to ethylene biosynthesis. Vitis 42:1-3.

Jacobs, M., and P.H. Rubery. 1988. Naturally occurring auxin transport regulators. Science 241:346-349.

Jeong, S.T., N. Goto-Yamamoto, S. Kobayashi, and M. Esaka. 2004. Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. Plant Sci. 167:247-252.

Keller, M., and N. Torres-Martinez. 2004. Does UV radiation affect winegrape composition? Acta Hortic. 640:313-319.

Koyama, K., K. Sadamatsu, and N. Goto-Yamamoto. 2010. Abscisic acid stimulated ripening and gene expression in berry skins of the Cabernet Sauvignon grape. Funct. Integr. Genomics 10:367-381.

Lüthen, H., M. Bigdon, and M. Böttger. 1990. Reexamination of the acid growth theory of auxin action. Plant Physiol. 93:931-939.

Machlin, L.J., and A. Bendich. 1987. Free radical tissue damage: Protective role of antioxidant nutrients. FASEB J. 1:441-445.

Moreno, D., F.J. Berli, P.N. Piccoli, and R. Bottini. 2011. Gibberellins and abscisic acid promote carbon allocation in roots and berries of grapevines. J. Plant Growth Regul. 30:220-228.

Pontin, M.A., P.N. Piccoli, R. Francisco, R. Bottini, J.M. Martinez-Zapater, and D. Lijavetzky. 2010. Transcriptome changes in grapevine (*Vitis vinifera* L.) cv. Malbec leaves induced by ultraviolet-B radiation. BMC Plant Biol. 10:224.

Prior, R.L., et al. 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of plasma and other biological and food samples. J. Agric. Food Chem. 51:3273-3279.

Quiroga, A.M., F.J. Berli, D. Moreno, J.B. Cavagnaro, and R. Bottini. 2009. Abscisic acid sprays significantly increase yield per plant in vineyard-grown wine grape (*Vitis vinifera* L.) cv. Cabernet Sauvignon through increased berry set with no negative effects on anthocyanin content and total polyphenol index of both juice and wine. J. Plant Growth Regul. 28:28-35.

Rakitin, V.Y., V.V. Karyagin, T.Y. Rakitina, O.N. Prudnikova, and P.V. Vlasov. 2008. UV-B stress-induced ABA production in *Arabidopsis thaliana* mutants defective in ethylene signal transduction pathway. Russ. J. Plant Physiol. 55:854-856.

Sandhu, A.K., D.J. Gray, J. Lu, and L. Gu. 2011. Effects of exogenous abscisic acid on antioxidant capacities, anthocyanins, and flavonol contents of muscadine grape (*Vitis rotundifolia*) skins. Food Chem. 126:982-988.

Seki, M., et al. 2002. Monitoring the expression pattern of around 7,000 *Arabidopsis* genes under ABA treatments using a full-length cDNA microarray. Funct. Integr. Genomics 2:282-291.

Tesniere, C., M. Pradal, A. El-Kereamy, L. Torregrosa, P. Chatelet, J.P. Roustan, and C. Chervin. 2004. Involvement of ethylene signalling in a non-climacteric fruit: New elements regarding the regulation of *ADH* expression in grapevine. J. Exp. Bot. 55:2235-2240.

Tossi, V., L. Lamattina, and R. Cassia. 2009. An increase in the concentration of abscisic acid is critical for nitric oxide-mediated plant adaptive responses to UV-B irradiation. New Phytol. 181:871-879.

Xi, Z.M., J.F. Meng, S.S. Huo, L.Y. Luan, L.N. Ma, and Z.W. Zhang. 2013. Exogenously applied abscisic acid to Yan73 (*V. vinifera*) grapes enhances phenolic content and antioxidant capacity of its wine. Int. J. Food Sci. Nutr. 64:444-451.

Zhang, Y.L., and R.G. Zhang. 2009. Effects of ABA content on the development of abscission zone and berry falling after harvesting of grapes. Agric. Sci. China 8:59-67.