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Research Article

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]

[1]Laboratorio de Bioquímica Vegetal, Instituto de Biología Agrícola de Mendoza (IBAM), Facultad de Ciencias Agrarias, CONICET-Universidad Nacional de Cuyo, Almirante Brown 500, M5528AHB, Chacras de Coria, Mendoza, Argentina; [2]Catena Institute of Wine, Bodega Catena Zapata, J. Cobos s/n, Agrelo, Mendoza, Argentina; and [3]Instituto de Fisiología Vegetal (INFIVE), Facultad de Ciencias Agrarias y Forestales, CONICET-Universidad Nacional de La Plata, Diag. 113 Esq. 61, 1900, La Plata, Buenos Aires, Argentina.

*Corresponding autor (fberli@fca.uncu.edu.ar)

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Abstract: It has been proposed that ultraviolet-B (UV-B) radiation activates grapevines antioxidant defense system and abscisic acid (ABA) acts downstream in the signaling pathway. Effects of solar UV-B perceived by high altitude vineyards and ABA sprays on berry quality indicators and fruit yield were studied on *Vitis vinifera* L. cv. Malbec at 5 developmental stages during three consecutive 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 H_2O (–ABA) from 27 days before veraison. Berry skin phenols (anthocyanins and total polyphenols) were increased by +UV-B and +ABA, markedly in concentration (UV-B x ABA significant interaction). The increases in antioxidant capacity, measured as oxygen antioxidant capacity (ORAC) and phenols in the berries exposed

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to +UV-B/+ABA combined treatment were higher compared with –UV-B/–ABA, for the same increase in sugar. Also, +UV-B and +ABA interact to lessen the number of berries, possibly due to higher ethylene emissions, and additively reduce clusters weight, without affecting sugar concentration (smaller berries) at harvest. Antioxidant compounds (protective for plants) are triggered in +UV-B/+ABA at the expenses of sugar accumulation, berry retention and growth (fruit yield). UV-B and ABA effects on berry sugar accumulation and growth depend on the stage of development. UV-B perceived by high altitude vineyards and ABA applications interact to increase red grape berry quality indicators, markedly in concentration (important from a winemaking standpoint), while per berry basis their effects are additives.

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

Introduction

Sunlight is one environmental factor, which drives primary productivity via photosynthesis, but also supplies informational cues vital to plant development. Solar ultraviolet-B (UV-B) radiation (280-315 nm), the most energetic fraction of sunlight that reaches the biosphere, increases as altitude augments since UV-B passes through a thinner atmosphere (less filtering by fewer atmospheric gases) to reach the ground. High altitude vineyards in Mendoza, Argentina *ca.* 1500 m above sea level (asl) receive relatively elevated UV-B levels, with irradiances up to 0.40 W m^{-2} at noon in summertime (Berli et al. 2010). UV-B causes direct and indirect photobiological effects on higher plants, some related to the evoked damage and others as an induced acclimation (Pontin et al. 2010). Those UV-B effects are influenced by other environmental parameters such as photosynthetically active radiation (PAR), thus realistic balances between UV-B and PAR should be used in the experiments (Caldwell et al. 2003).

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Many physiological and biochemical acclimation processes, some of which are common for different stressful conditions, are regulated by the phytohormone abscisic acid (ABA; Seki et al. 2002), therefore, it is feasible to assume that ABA may regulate plant responses to UV-B. Few studies tried to address the relations between UV-B and ABA in plants (Duan et al. 2008), but a promotive effect of UV-B on ABA biosynthesis has been 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 (Berli et al. 2011), where ABA fluctuated during fruit ontogeny, rising at the onset of ripening (veraison) and declining subsequently at harvest (Berli et al. 2011). The latter, in addition to the fact that applications of ABA hastened grape berry ripening (Berli et al. 2011; Jeong et al. 2004), suggest that ABA plays a fundamental role in regulating fruit maturation. Changes in the phytohormone ethylene production around veraison have also been described in non-climacteric grape berries (Tesniere, Pradal et al. 2004). Ethylene regulates many aspects of plant growth and development, including senescence and abscission, playing an important role in the response to many stresses including UV-B (He et al. 2011).

Phenols are secondary metabolites that, besides their biological functions (e.g. attractants for pollinators and seed dispersers, defense compounds against herbivores, pathogens and stressful environmental conditions; Croteau et al. 2000), play a significant role in winemaking, as they contribute to wine quality, determining color, structure, mouthfeel and antioxidant potential (Cheynier 2005). Grape berries phenolic composition (i.e. phenolic acids, stilbenes, anthocyanins, flavonols and flavanols) depends on cultivars (genetic factors), ontogeny (berry development and maturation), environmental conditions and management practices (Downey et al. 2006). In general, their biosynthesis is stimulated by some degree of stress.

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77 Despite the importance of phenols for winemaking there have been relatively few studies on the
78 specific response to UV-B in grape berries (Keller and Torres-Martinez 2004). We previously
79 found that phenols augment in grape berries in response to high solar UV-B, with further
80 increases when combined with applications of ABA (additive effects; Berli et al. 2011). In those
81 experiments, the focus was on phenolic profiles and the results were only analyzed per berry
82 basis, remaining to test associations with antioxidant capacity, and effects on fruit yield. The
83 present work reports independent and interactive effects of solar UV-B perceived at high altitude
84 vineyards and ABA sprays on a field-grown red grapevine cultivar during three consecutive
85 growing seasons, at different developmental stages. Berry antioxidant capacities, anthocyanins
86 and total polyphenols (from a winemaking standpoint, i.e. in concentration), sugar
87 accumulations, and growth were evaluated as fruit quality indicators, while berries number and
88 cluster weight at harvest were used to assess effects on fruit yield.

89 ## Materials and Methods

90 ### Plant material and experimental design

91 The experiment was carried out during three growing seasons, 2009, 2010 and 2011, in a
92 commercial high altitude vineyard (Viñedo Adrianna, Catena Zapata, 1450 m asl, 69º15′37″ W
93 and 33º23′51″ S), Gualtallary, Mendoza, Argentina. The grapevines were a selected clone of
94 *Vitis vinifera* L. cv. Malbec, planted in 1997 on their own roots, trained on a vertical trellis
95 system, arranged in north-south oriented rows spaced 2 m apart, with 1.20 m between plants on
96 the row, and were maintained with no soil water restriction during the whole experiment by
97 using a drip irrigation system. The vines were cane pruned and shoot-thinned to 12 shoots per
98 vine when these shoots reached 10 cm long, and at flowering two clusters per shoot were left.

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A randomized complete block design with a 2 x 2 factorial arrangement of treatments (UV-B and ABA) and five blocks were used (n=5). The experimental unit consisted of 4 plants selected on the basis of their homogeneity from 6 consecutive plants in the row. Two shoot per experimental unit were selected, marked and used to determine the weight of clusters and the number of berries per cluster at harvest (fruit yield), while the rest of the 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 regimens were set for the entire grapevine canopy from 15 days before flowering, stage 21 according to Coombe (1995), mid-November, until harvest at 131 DAF, in early April. A minus UV-B treatment (–UV-B) was given by filtering solar UV-B with a polyester cover that absorb 78% of UV-B and transmit 88% of PAR from sunlight. A full UV-B treatment (+UV-B) was set by covering the canopy with a low-density polyethylene that transmit most of the radiation from sunlight (90% of UV-B and 87% of PAR) to minimize environmental differences between –UV-B and +UV-B. Plastics were set 2.5 m above ground level (*ca.* 30 cm above the grapevines), covered the east and west facing sides of the canopy at an angle of 45° with respect to the soil, and were protected with anti-hail nets (**Figure 1 A** shows a schematic representation of an experimental unit). Transmittance spectral characteristics were previously reported (Berli et al. 2008; Berli et al. 2011). A LI-250 light meter with a LI-190SA quantum sensor (Li-Cor Inc., Lincoln, NE, USA) and a PMA2200 radiometer with a PMA2102 UV-B detector (Solar Light Company Inc., Glenside, PA, USA) were used to measure PAR and UV-B, respectively. **Figure 1 B** shows the solar radiation (UV-B and PAR) received on a typical sunny summer day above

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the canopy at the experimental site on December, January and February (values are means of 2009, 2010 and 2011).

Two ABA treatments were performed using weekly sprays to the aerial part of plants (i.e. including leaves and berries) starting 27 days before veraison, stage 33 according to Coombe (1995), in late January, until harvest. A plus ABA treatment (+ABA) was initiated using a 1 mM aqueous solution of ±-S-*cis*,*trans*-abscisic acid (90% purity; Kelinon Agrochemical Co., Beijing, China), containing 0.1% v/v of Triton X-100 and a minimum amount of ethanol, according to previous works with grapevine (Quiroga et al. 2009). A solution containing H_2O with the concentration of emulsificant and ethanol 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 (maximum differences between dates for the three growing seasons were ± 5 days). Fifty berries per experimental unit were randomly collected in nylon bags (5 berries from each cluster, two top, two middle, and one bottom berry, with berries taken from 10 clusters), kept in dry ice to prevent enzyme degradation and dehydration and taken to the laboratory where berry fresh weight (FW) was determined before storage at -20º C. Then, berries were defrosted at room temperature (25±2º C) and peeled by hand. Relative concentration of sugar (ºBrix) and sugar on a per berry basis (absolute amount) were determined in pulps according to Berli et al. (2011).

Berry skins were extracted with 50 mL of an aqueous ethanolic solution (12% ethanol, 6 g L^{-1} tartaric acid and pH 3.2) at 70º C for 3 h in darkness. Then, the liquid fraction was separated by decanting, maintained 24 h at 4º C and centrifuged 10 min at 10 000x*g* to eliminate tartrates and other sediments. Finally, supernatants were collected and stored at -20º C. Anthocyanins and

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145 total polyphenols index (TPI) were determined as described in Berli et al. (2008), and calculated
146 per berry and per berry FW basis.
147 Oxygen radical absorbance capacity (ORAC) was determined based on Prior et al. (2003) with
148 modifications, as follows. Berry skin extraction solutions were diluted 1:750 v/v in 75 mM
149 potassium phosphate buffer (pH 7.0). Aliquots (50 µL) of diluted samples and Trolox standards
150 were added to a 96-well black plate. Then, 100 µL of fluorescein (20 nM solution) were added,
151 and the mixture was incubated at 37º C for 7 min before the addition of 50 µL of the peroxyl
152 radical generator AAPH [2,2´-azobis(2-amidinopropane) dihydrochloride (Sigma-Aldrich Inc.,
153 St. Louis, MO, USA), 140 mM solution]. Fluorescence was monitored using 485 nm excitation
154 and 538 nm emissions at 1 min intervals for 90 min on a microplate fluorometer (Fluoroskan
155 Ascent FL, Thermo Fisher Scientific Inc., Wilmington, DE, USA). The area under the curve of
156 the fluorescence decay during 90 min was calculated and the ORAC was expressed as mmol of
157 Trolox equivalents (TE) per berry skin and per 100 g berries FW.
158 **Weight of clusters and number of berries (fruit yield)**
159 At harvest (131 DAF), clusters from the two selected shoot per experimental unit, i.e. not used
160 for berry sampling, were collected in nylon bags and weighed. Then, the number of berries per
161 cluster was counted.
162 **Berry ethylene emission**
163 In 2011 growing season, at 52, 72, 96, 112, and 131 DAF, one cluster per experimental unit, i.e.
164 not used for berry sampling, was introduced into a nylon bag (1 L volume). The bags were tied in
165 the peduncle, and ethylene was allowed to accumulate over a two h period (from 10:00 a.m. to
166 noon). Afterwards, the bags were punctured with the needle of a syringe and 10 mL of the inner
167 gas were extracted, the clusters were cut, the syringes were sealed with parafilm, and both

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168 samples were ice cooled and transported to the laboratory. The number of berries per cluster was
169 counted and berries were weighed. Ethylene in the gas samples was determined as described in
170 Beltrano et al. (1994).

171 **Statistical analysis**

172 Repeated measurements multifactorial ANOVA was used to evaluate effects of UV-B, ABA,
173 developmental stages, growing seasons and their interactions under the randomized complete
174 block design ($P \leq 0.05$). A mixed model approach with random effect(s) to account for serial
175 correlation among measures from the same plot at each development stage and season was
176 implemented using SAS Proc Mixed (SAS Institute, 1999). Principal component analysis (PCA)
177 with biplot graphics and standardizing (centering and variance-scaling) data were performed
178 (InfoStat version 2009 software; Grupo InfoStat, Córdoba, Argentina). Linear regression models
179 were calculated for berry's sugar vs. ORAC, vs. anthocyanins and vs. TPI (in +UV-B/+ABA and
180 –UV-B/–ABA treatments), and t-tests were used to compare the slopes of regression lines
181 (InfoStat version 2009 software; Grupo InfoStat, Córdoba, Argentina).

182 ## Results

183 The results are expressed per berry FW basis (concentration) that is dependent of berry size, and
184 per berry basis (absolute amount). The former is important from a winemaking standpoint, and
185 the latter allows to understand physiological effects (biosynthesis and/or accumulation).

186 **Berry skins antioxidant capacity and polyphenols content**

187 **Table 1** shows the effects of UV-B and ABA in berry skin antioxidant capacity (assessed as
188 ORAC), anthocyanins and TPI. Also, the effects of different developmental stages, growing
189 seasons and interactions are included. ORAC per berry basis augmented 75.5% from 52 to 112

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DAF and then remained almost constant, while in concentration (per berry FW basis) was higher at 52 DAF and then decreased. ORAC was increased additively by +UV-B and +ABA (UV-B x ABA interaction effects were not statistically significant). Anthocyanins augmented markedly from 52 to 131 DAF, and were also increased by +UV-B and +ABA (additively per berry basis, but UV-B x ABA interact significantly in concentration amounts, increasing by +ABA markedly in +UV-B). TPI augmented from 72 to 131 DAF, and was affected by +UV-B and +ABA, increasing additively per berry basis. TPI in concentration amounts was enhanced by +ABA distinctly in +UV-B (UV-B x ABA interact significantly). The +UV-B/+ABA combined treatment was higher than –UV-B/–ABA in absolute values (13.0% in ORAC, 12.4% in anthocyanins and 4.0% in TPI) and markedly in concentration amounts (36.3% in ORAC, 33.6% in anthocyanins and 17.9% in TPI). No significant interactions of UV-B and ABA with developmental stages were obtained, and most variables were significantly higher in the 2009 growing season (UV-B x ABA x YEAR interaction effects were not statistically significant). The PCA indicates that considering all the berry´s developmental stages, ORAC, anthocyanins and TPI were associated with +UV-B/+ABA treatment (**Figure 2 A**). In turn, considering only determinations at harvest, the mentioned variables were associated with +ABA treatments (+UV-B/+ABA when they are expressed in concentration, and –UV-B/+ABA when they are in absolute amounts; **Figure 2 B**).

Berry growth, sugar accumulation, ethylene emission and yield

Figure 3 A indicates that berry FW augmented 97.3% from 52 to 112 DAF and then remained almost constant till harvest (sigmoidal growth pattern). Berry growth was reduced additively by +UV-B and +ABA, markedly near harvest (UV-B x DAF significant interaction). Sugar in berry's pulp increased continuously until harvest, and per berry basis it was reduced by +UV-B

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and +ABA, especially near harvest (UV-B x DAF and ABA x DAF significant interactions; **Figure 3 B**). Meanwhile, sugar concentration (ºBrix) was increased by +UV-B and +ABA evidently at veraison (*ca.* 72 DAF; UV-B x DAF and ABA x DAF interactions; **Figure 3 C**). 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 x 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 evidence that at harvest, cluster FW, berries per cluster and berry FW were associated with –ABA treatments (+UV-B/–ABA and –UV-B/–ABA; **Figure 2 B**).

Growing seasons did not affected berries number per cluster, but significant differences in berries growth, sugar accumulation and cluster FW were obtained being reduced in 2011 (UV-B x ABA x YEAR interaction effects were not statistically significant; **Table 2**).

Linear regressions for ORAC, polyphenols and sugars

Figure 5 presents linear regression models for berry's 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´s 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).

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Discussion

A positive effect of the perception of high altitude vineyards UV-B levels and the weekly sprays of ABA (starting *ca.* one month before veraison), was obtained on berry skins antioxidant capacity, anthocyanins and total polyphenols accumulation. Additive effects of UV-B and ABA on berry skin phenols were previously published, but the results were only evaluated in a per berry basis, important to understand the effects on biosynthesis and/or accumulation (Berli et al. 2011). The present work reports that UV-B and ABA effects are more noticeable at expressing the results in terms of concentration. Also, significant UV-B x ABA interaction were observed for anthocyanins and total polyphenols concentrations, meaning that ABA applications were more effective in full UV-B treatment. Their effects on concentration showed to be important since +UV-B and +ABA also reduce additively berry size at harvest. The concentrations of phenols are mandatory from a winemaking standpoint, since wine´s metabolites depends on their concentration in the berries. It is also important that UV-B and ABA effects were consistent during the three years, and most of the variables related with defense were significantly higher in 2009, when higher total solar radiation and air temperatures (mean, maximum and minimum) occur, data previously reported in Berli et al. (2013).

It has been demonstrated that 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 against oxidative stress (Machlin and Bendich 1987). In grapevines, it has been found that UV-B increases berry skin flavonols (Gregan et al. 2012) and that ABA rise flavonols and antioxidant capacity in berry skins (Sandhu et al. 2011) and wines (Xi et al. 2013).

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257 Meanwhile, UV-B combined with ABA additively increases phenols, especially those with
258 higher antioxidant capacity (dihydroxylated anthocyanidins as cyanidin and flavonols like
259 quercetin and kaempferol; Berli et al. 2011).
260 At the beginning of berry maturation, when no anthocyanins are accumulated in the skins yet, the
261 non-anthocyanin phenols seem to be responsible of the antioxidant capacity. At this early
262 developmental stage (50 DAF), flavonols, flavanols, dihydroflavonols, and hydroxycinnamic
263 acids in absolute amounts represent *ca.* 41%, 36%, 13% and 10% of the total phenols,
264 respectively; and the main compound is quercetin-3-glucoside (Berli et al. 2011). In the present
265 experimental conditions, solar UV-B reaches maximum levels in December-January, and then
266 decrease towards harvest in early April. Elicitation of flavonoids by UV-B can be highly
267 dependent on the developmental stage, i.e. despite the fact that grapevine berries are exposed to
268 seasonally higher UV-B levels throughout months prior to veraison, anthocyanins only appears
269 after veraison (Gregan et al. 2012).
270 Effects of +UV-B and +ABA at veraison in promoting sugar accumulation in fruits are different
271 than those at harvest, where the accumulation of sugar per berry decreases and sugar
272 concentration is not affected (+UV-B/+ABA also reduces berry size significantly near harvest).
273 These results are confirmatory of previously reported data in Berli et al. (2011). Stimulation of
274 invertases, hexose transporters and enzymes that soften cell wall were reported for ABA
275 (Koyama et al. 2010), with increases in hexoses (glucose and fructose) accumulation in berries
276 up to veraison (Moreno et al. 2011).
277 Meanwhile, the lower accumulation of sugars and the lower berry growth after veraison in +UV-
278 B/+ABA treatment may be due to decreases in the cell wall elasticity of the berries (Gambetta et
279 al. 2010), and/or degradation of vacuolar invertases that regulate hexose accumulation in berries

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(Giribaldi et al. 2010). Berries on +UV-B/+ABA-treated plants may approach this limit earlier due to an ABA-induced advancement of ripening. The increments promoted by UV-B and ABA of berry skin phenols may be influencing auxins levels since they act as inhibitors of auxins transport (Jacobs and Rubery 1988) so affecting the extensibility of cell walls and therefore the berry growth (Lüthen et al. 1990). Additionally, these can be related with a diminished carbon fixation in +UV-B, caused by reductions in grapevine leaf area and gas exchange (Berli et al. 2013). As well, they may be a response attributable to the cost of forming secondary metabolites to provide protection to plants (Berli et al. 2010), mainly phenolics (Berli et al. 2008; Berli et al. 2011) and terpenes (Gil et al. 2013; Gil et al. 2012).

Reductions of cluster FW at harvest by +ABA markedly in +UV-B are consequence of diminution in berry size, but also reductions of berry number. As clusters number was regulated at the beginning of the experiment, cluster FW is a measure of fruit yield. Hilt and Bessis (2003) correlate high levels of ethylene with grape fruitlets abscission and suggest interactions between ABA and ethylene in regulating fruit abscission. Additionally, Zhang and Zhang (2009) found that grape clusters treated with ABA increased the activity of hydrolases, particularly cellulose and polygalacturonase in pedicels abscission zones, and accelerated the berry falling. The +ABA effect on berries number (through berry retention) depends on berry´s phenological stage (Quiroga et al. 2009).

Conclusion

Solar UV-B at high altitude vineyards and ABA sprays interact to increase red grape berry quality indicators, markedly in concentration (important from a winemaking standpoint), while per berry basis their effects are additives. The quality for winemaking of grape berries integrates

various aspects, but for red wines, especially those to be aged, it has a high correlation with accumulation of phenols and antioxidant capacity (nutraceutical value).Antioxidant compounds which have protective effects for plants, are triggered in +UV-B/+ABA at the expenses of sugar accumulation, berry retention (possibly due to higher ethylene emissions) and growth (fruit yield). UV-B and ABA effects on berry sugar accumulation and growth depend on the stage of development.

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Table 1 Oxygen radical absorbance capacity (ORAC), anthocyanin and total polyphenols index (TPI) of skin berries for +UV-B and –UV-B in combination with +ABA and –ABA, at 52, 72, 96, 112 and 131 DAF, in 2009, 2010 and 2011 growing seasons.

	ORAC (mmol TE per berry skin)		**ORAC** (mmol TE per g berries FW)		**Anthocyanin** (μg per berry)		**Anthocyanin** (mg per 100 g berries FW)		**TPI** (TPI per berry)		**TPI** (TPI per 100 g berries FW)	
UV-B												
+UV-B	564.45	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 x 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[a]												
$P_{(UV\text{-}B)}$	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	
$P_{(UV\text{-}B\ x\ ABA)}$	0.1162		0.9010		0.0907		0.0259		0.2490		0.0099	
$P_{(UV\text{-}B\ x\ DAF)}$	0.3544		0.6834		0.3270		0.0774		0.4786		0.5674	
$P_{(ABA\ x\ DAF)}$	0.6235		0.7981		0.6059		0.0676		0.8270		0.7803	
$P_{(UV\text{-}B\ x\ ABA\ x\ YEAR)}$	0.4474		0.4855		0.7199		0.3168		0.1580		0.0583	

Values are means for each factor and different letters indicate significant differences (LSD Fisher, $P \leq 0.05$).
[a] $P_{(UV\text{-}B)}$, $P_{(ABA)}$, $P_{(DAF)}$ and $P_{(YEAR)}$: effects of UV-B, ABA, developmental stage and growing season; $P_{(UV\text{-}B\ x\ ABA)}$, $P_{(UV\text{-}B\ x\ DAF)}$, $P_{(ABA\ x\ DAF)}$ and $P_{(UV\text{-}B\ x\ ABA\ x\ YEAR)}$: interaction effects of factors.

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Table 2 Cluster fresh weight (FW), number of berries per cluster and berry FW at harvest (131 DAF) for +UV-B and –UV-B in combination with +ABA and –ABA, in 2009, 2010 and 2011 growing seasons.

	Cluster FW (g per cluster)		**Berries per cluster**		**Berry FW** (g per berry)	
UV-B						
+UV-B	83.41	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	1.58	a
2011	73.87	b	66	a	1.15	b
UV-B x 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[a]						
$P_{(UV\text{-}B)}$	0.2377		0.6837		0.0010	
$P_{(ABA)}$	0.0568		0.1593		0.0373	
$P_{(YEAR)}$	0.0074		0.4903		0.0001	
$P_{(UV\text{-}B\ x\ ABA)}$	0.0848		0.0417		0.8036	
$P_{(UV\text{-}B\ x\ ABA\ x\ YEAR)}$	0.4673		0.1018		0.7218	

Values are means for each factor and different letters indicate significant differences (LSD Fisher, $P \leq 0.05$).
[a] $P_{(UV\text{-}B)}$, $P_{(ABA)}$ and $P_{(YEAR)}$: effects of UV-B, ABA and growing season; $P_{(UV\text{-}B\ x\ ABA)}$ and $P_{(UV\text{-}B\ x\ ABA\ x\ YEAR)}$: interaction effects of factors.

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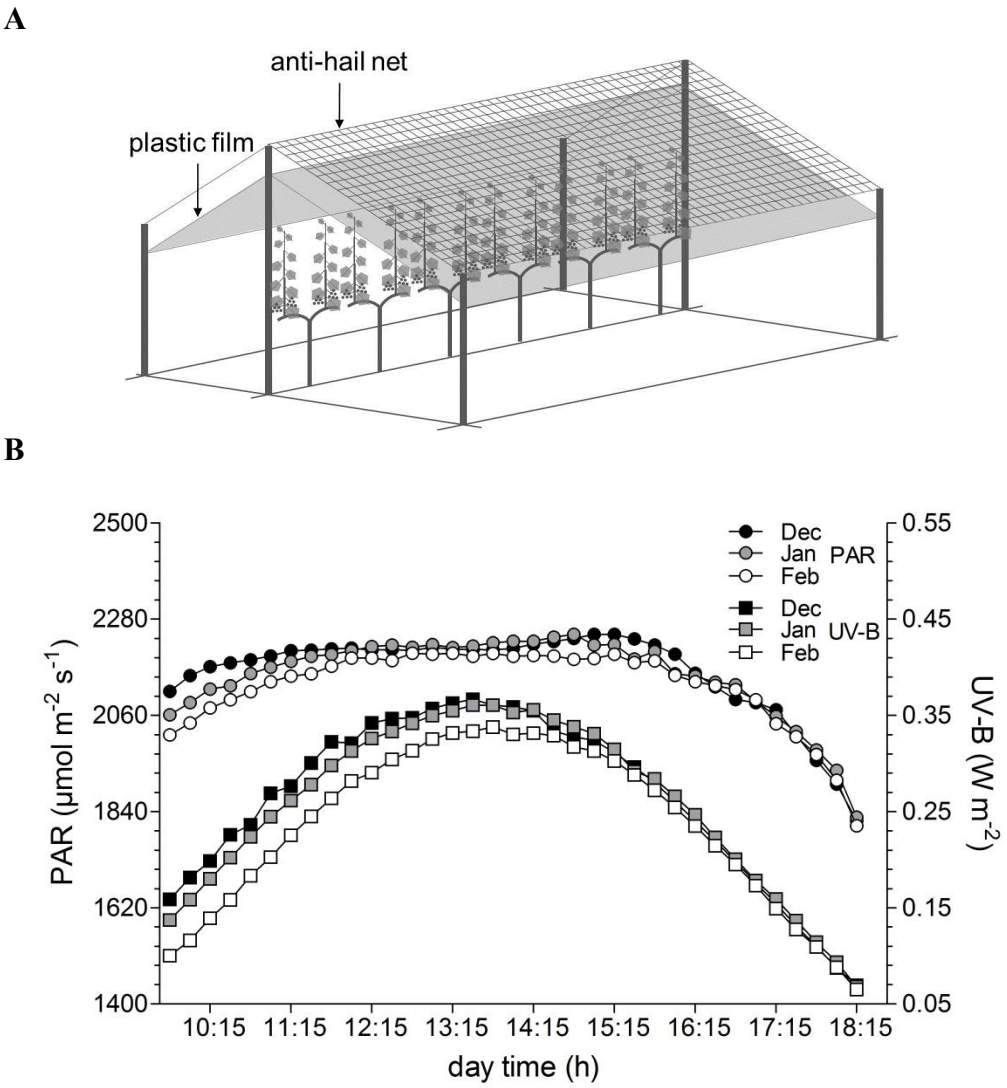

Figure 1 Schematic representation of an experimental unit in the minus UV-B and full UV-B treatments (polyester and low-density polyethylene films, respectively) protected with anti-hail nets (**A**). Solar UV-B and PAR registered on a typical sunny summer day (December, January and February) above the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

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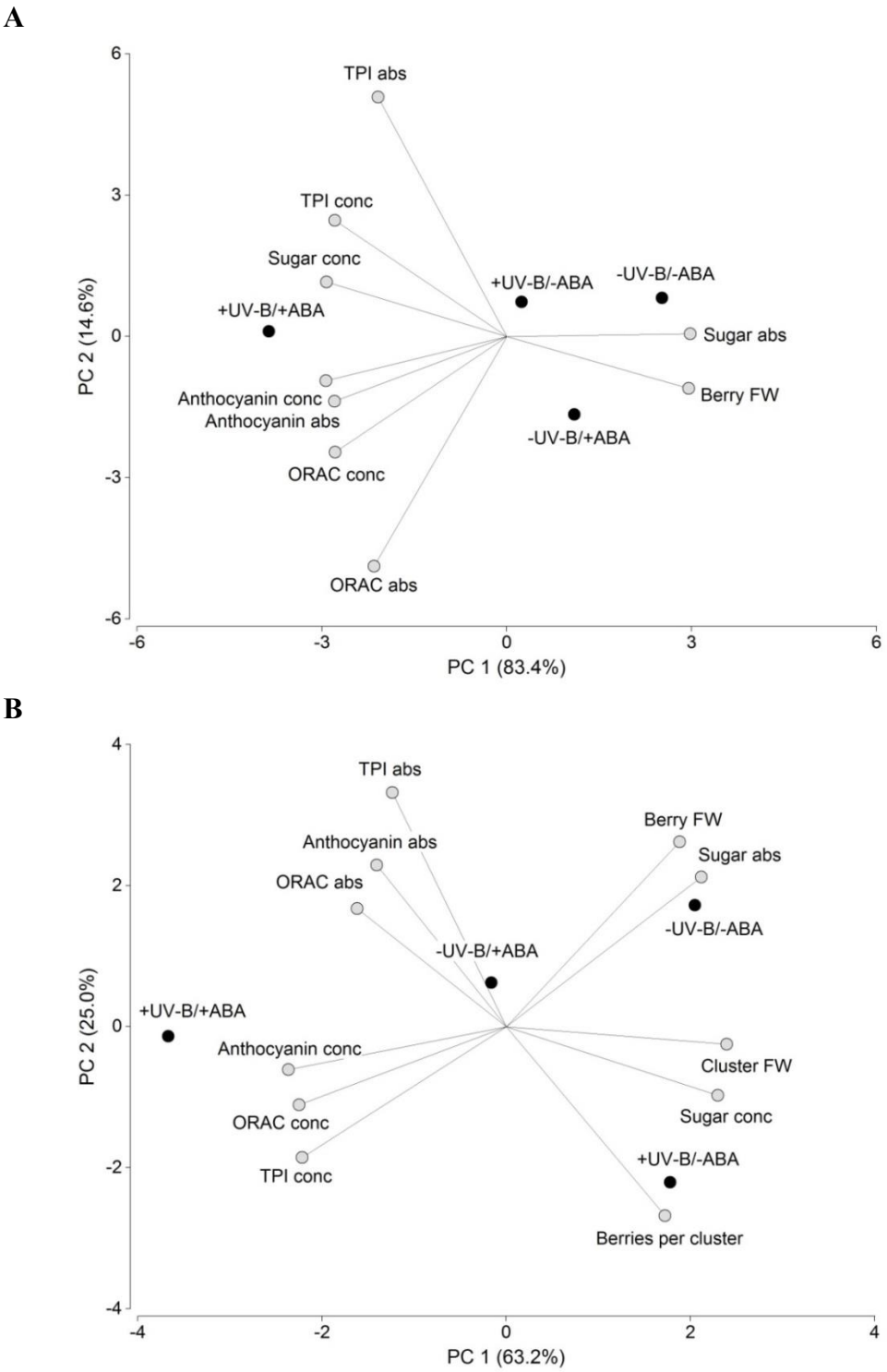

Figure 2 Biplot display of the principal components analysis (PCA) of the variables determined in berries for the treatments +UV-B and –UV-B in combination with +ABA and –ABA, during three seasons considering all the berry´s developmental stages analyzed (**A**) and only at harvest (**B**). The variables are expressed in absolute (abs) and in concentration (conc) amounts.

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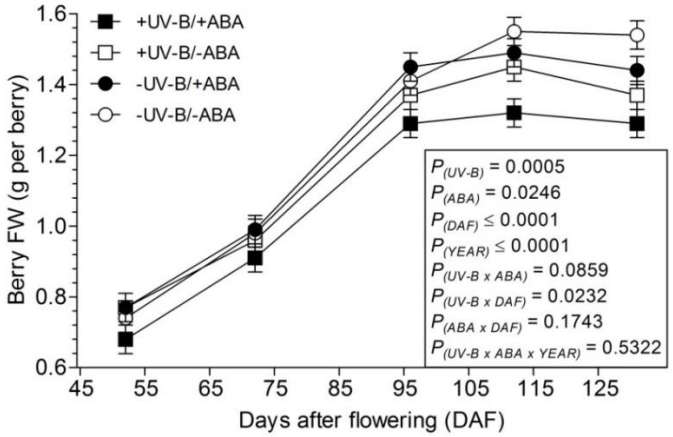
A



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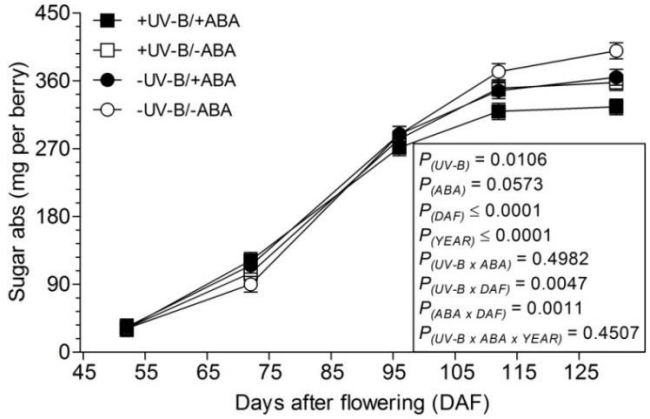

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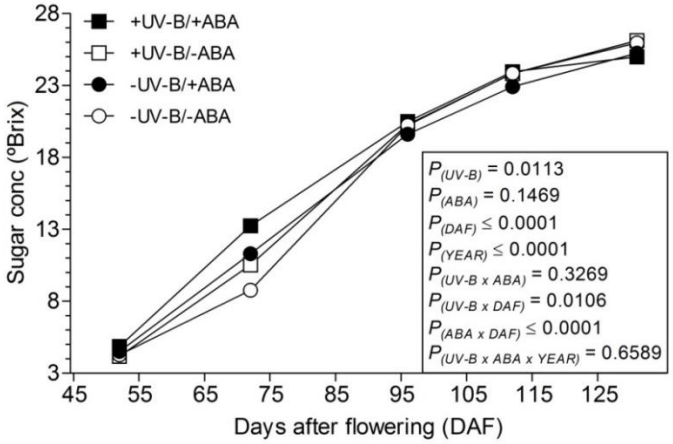

Figure 3 Berry fresh weight (FW; g per berry; **A**), sugar in absolute (abs; mg per berry; **B**) and in concentration (conc; ºBrix; **C**) amounts for +UV-B and –UV-B in combination with +ABA and –ABA at 52, 72, 96, 112 and 131 DAF. Values are means ± SE for 2009, 2010 and 2011 growing seasons. $P_{(UV-B)}$, $P_{(ABA)}$, $P_{(DAF)}$ and $P_{(YEAR)}$: effects of UV-B, ABA, developmental stage and growing season; $P_{(UV-B \times ABA)}$, $P_{(UV-B \times DAF)}$, $P_{(ABA \times DAF)}$ and $P_{(UV-B \times ABA \times YEAR)}$: interaction effects of factors.

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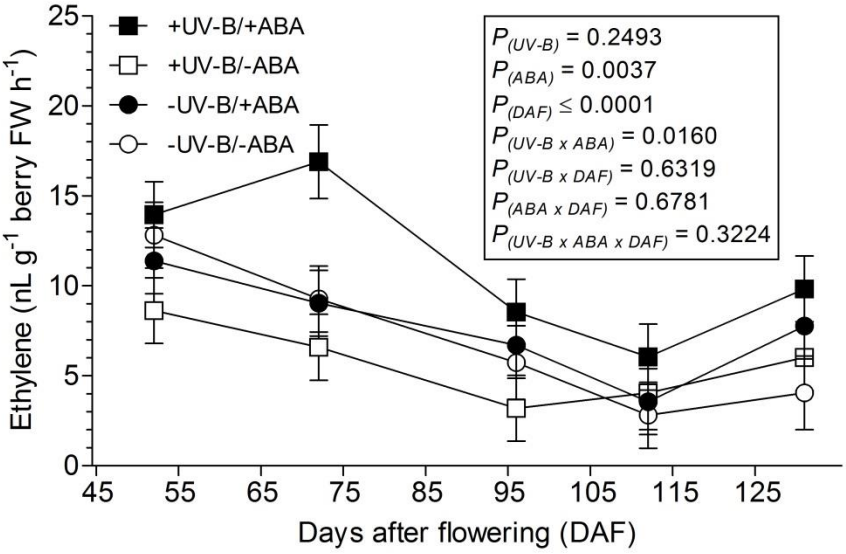

Figure 4 Berry ethylene emission (nL g^{-1} berry FW h^{-1}) for +UV-B and –UV-B in combination with +ABA and –ABA at 52, 72, 96, 112 and 131 DAF. Values are means ± SE for 2011 growing seasons. $P_{(UV-B)}$, $P_{(ABA)}$ and $P_{(DAF)}$: effects of UV-B, ABA and developmental stage; $P_{(UV-B \times ABA)}$, $P_{(UV-B \times DAF)}$, $P_{(ABA \times DAF)}$ and $P_{(UV-B \times ABA \times DAF)}$: interaction effects of factors.

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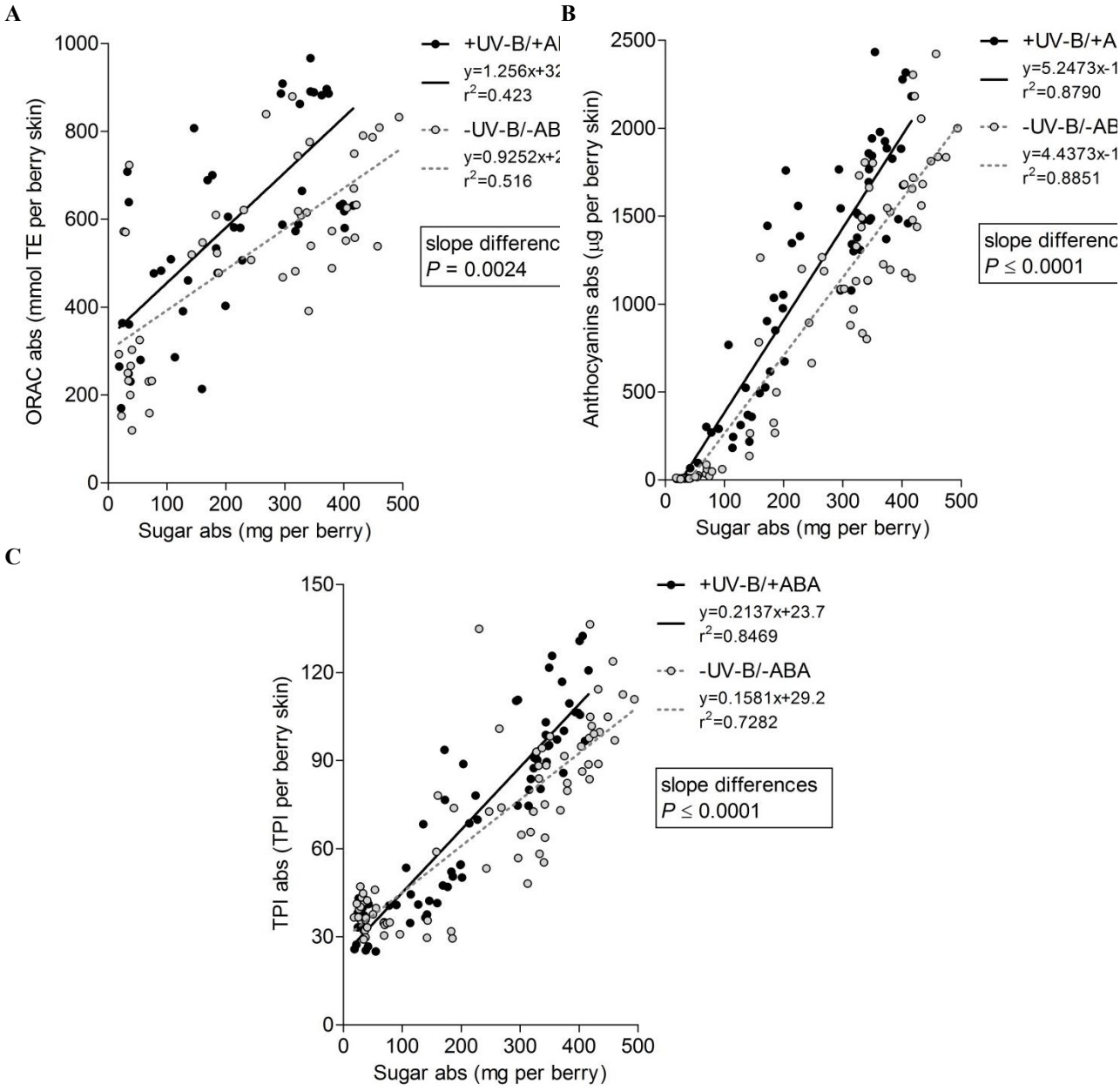

Figure 5 Linear regression between berry sugar and oxygen radical absorbance capacity (ORAC; **A**), berry sugar vs. anthocyanins (**B**) and berry sugar vs. total polyphenols index (TPI; **C**), for +UV-B/+ABA and –UV-B/–ABA combined treatments, considering all the growing seasons and berry´s developmental stages. Variables are in absolute amounts (abs; sugar: mg per berry, ORAC: mmol TE per berry skin, anthocyanins: µg per berry skin and TPI: TPI per berry).