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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

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Abstract In many cultivars of *Vitis vinifera* periods of mild water stress during ripening are thought to increase grape quality for winemaking, even though yields may be negatively affected. Because abscisic acid (ABA) is involved in the signaling of water stress in plants, we examine the effects of the ABA signal being given without the concomitant water stress. ABA at 250 mg l^{-1} was sprayed weekly or biweekly from bud-burst until harvest onto the leaves of vineyard-grown plants of cv. Cabernet Sauvignon. For ABA-treated plants berry yield per bunch and per plant was significantly increased (1.5- to 2.0-fold) across three consecutive harvests (2005 through 2007). Number of berries per bunch and per plant was the primary basis for the significant crop increases, although bunches per plant also tended to increase (1.1- to 1.3-fold) across all three harvests. Other parameters assessed included number of internodes, shoot length, leaf area, leaf water potential at midday, photosynthesis, and stomatal conductance. These parameters showed no significant change with ABA treatment, although shoot length tended to be reduced, as was leaf area relative to control plants. The significantly increased fruit yields were thus accomplished without accompanying increases in leaf photosynthesis and leaf areas. Juice at harvest had equal levels of sugars (Brix) and somewhat higher levels of anthocyanins and total polyphenols relative to control values. The two latter trends continued for the resultant wine across two vintage years. In conclusion, three seasons of experimental trials have demonstrated that ABA application can significantly enhance yield per plant in the field-grown grape (cv. Cabernet Sauvignon) by favoring increased berry set without diminishing the quality of the fruit for winemaking use.

Keywords Abscisic acid · Berry set · Cabernet Sauvignon · Grape · *Vitis vinifera* L. · Yield

Introduction

In *Vitis vinifera* periods of modest (predawn water potential of ca. -0.8 MPa) water deficit during ripening enhance the polyphenol and anthocyanin contents in the berries (Hardie and Considine 1976; Freeman and Kliewer 1983; Matthews and Anderson 1988; Pérez Peña 2000; Ojeda and others 2002). Hence, this is considered a useful vineyard tool for obtaining high-quality grapes for winemaking, especially for red wine (Dry and others 2000a, b; Vallone and others 2004). These compounds have been found to have organoleptic value (Marais and others 1991) and they may also be important to human health for their antioxidant capacity (Burns and others 2000).

Abscisic acid (ABA) is a phytohormone involved in stress responses, mainly water stress (see reviews by Davies and Zhang 1991; Leung and Giraudat 1998). Increased levels of ABA are associated with growth restriction by water stress, which is postulated to be a mechanism by which the plant can escape from the adverse

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condition imposed by water stress (Creelman and others 1990; Dry and others 2000c; Dood and Davies 2005; Christmann and others 2007; Jiang and Hartung 2008). However, ABA is also known to enhance dry matter accumulation in the leaves and shoots of *Ilex paraguariensis* and this is thought to occur as a consequence of tissue alleviation from water stress (Sansberro and others 2004). Apart from such hydrostatic effects, sink strength may also be enhanced, as has been shown by increased carbohydrate allocation in cereals (Yang and Zhang 2006; Travaglia and others 2007) and other species (see review of Brenner 1987 and references therein). In addition, it has been demonstrated that ABA is involved in the increase of anthocyanins in grape skins (Ban and others 2003; Jeong and others 2004), and applied ABA can improve skin color in table grapes (Peppi and Fidelibus 2008).

Keeping in mind that ABA is involved in the signaling chain of water stress in plants (Christmann and others 2007; Jiang and Hartung 2008), we believe that there is an emerging hypothesis (unpublished) that ABA application to the grape plant may allow growers to meet practical objectives with regard to fruit quality, and do so without the problems associated with placing the plants under water stress. To our knowledge, however, there are no reports of the effect of ABA on grape yield in field experiments. This article reports on the significantly increased berry yield in response to foliar application of ABA across three consecutive years of field experiments with cv. Cabernet Sauvignon, an important cultivar of grape for red wine production.

Material and Methods

Our study was carried out during three consecutive seasons (2004–2005, 2005–2006, and 2006–2007) using grapevines of cv. Cabernet Sauvignon, 10–12 years old, under field conditions at the Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Chacras de Coria, Mendoza, Argentina, located at 68°54′ W and 33°13′ S and 920 m above sea level. The experimental design consisted of completely randomized blocks, with the plants distributed in three rows spaced 1.5 × 2.2 m apart. Grapes were cropped on a bilateral cordon and plants were grown with no soil water restriction. Plants were covered with a plastic net to avoid hail damage and were otherwise managed in the same manner as other vineyards in the Mendoza region.

Fifteen days after bud-burst, the grapevines' leaves were submitted to a high-pressure foliar spray with distilled water (control) or (S)-*cis, trans*-ABA (90% purity, China Kelinon Co., Beijing, China) applied in a 250-mg l^{-1} solution to drip off (for example, approximately 200 ml [50 mg] of aqueous ABA solution per plant). This ABA dose was chosen after preliminary experiments were performed with field- and pot-grown vines, and according to results obtained from previous experiments using other species (Sansberro and others 2004; Travaglia and others 2007). Both solutions included Triton X-100 at 0.1% v/v as a surfactant. A minimum amount of 95% ethanol was used to initially dissolve the ABA (the same volume of ethanol was also included in the water control solution). Sprays were repeated once every week until harvest. All sprays were performed at dusk (sunset) to minimize ABA photodestruction.

During the 2004–2005 season ABA was tested at 250 mg l^{-1} against distilled water treatments, that is, a simple "control versus ABA" comparison. However, in the 2005–2006 season the treatment was performed on (1) the same plants of the season before (ABA 2nd), and (2) on a new set of vines with no previous applications (ABA 1st). As for the first year's trial, sprays were applied at approximately weekly intervals. In 2005–2006 there was also an additional treatment in which only one application of ABA was applied on a single date, 15 days after bud-burst (ABA early). For the 2006–2007 season the weekly spraying was applied to plants having two (ABA 3rd), one (ABA 2nd), or zero (ABA 1st) years of previous ABA applications. There was, however, no additional once-only spray of ABA for the 2006–2007 season. For each of the three seasons each treatment was replicated six times, and the experimental unit for one replicate consisted of two plants. Experimental results (tissue harvest/measurements) were taken from the east face of the plants (rows were oriented north-south). Nondestructive measurements were performed on one plant, whereas the other plant was used for destructive analysis. Final berry counts/weights were taken from both plants (2 plants × 6 replicates).

The number of internodes and total shoot (cane) length were measured just before harvest for seven shoots per experimental unit. Leaf area (LA) was also assessed just before harvest by using a portable area meter (LICOR 3000, LI-COR Biosciences, Lincoln, NE). Leaf water potential (Ψw_{leaf}) was measured with a Scholander chamber and photosynthetic rate (P) and stomatal conductance (g_s) were measured with an infrared gas analyzer (IRGA, LICOR 6200). The measurements were made at midday, from approximately 1 month after bud-burst (at a frequency of once every month) until harvest. Three leaves constituted the basic experimental unit. From veraison on, three typical berries per bunch from each experimental unit were sampled for anthocyanin content and polyphenol index. These were spectrophotometrically assessed according to Riou and Asselin (1996), and grape Brix degree (°B) was measured with a portable refractometer. Individual grape berry weights and volumes were measured at harvest using a random sample of ten berries per experimental unit.

Grapes were harvested at a ripeness of approximately 24°B in all three seasons and in all treatments. There were no differences in "time to harvest" for ABA-treated versus control plants. In seasons 2004–2005 and 2005–2006 wines were produced according to standard practices for small-scale red wine production to compare the anthocyanin content and polyphenol index with measurements made earlier on juice from individual berries and by using the same methods.

An analysis of variance (ANOVA) was utilized, with treatment and blocks as main factors, using a

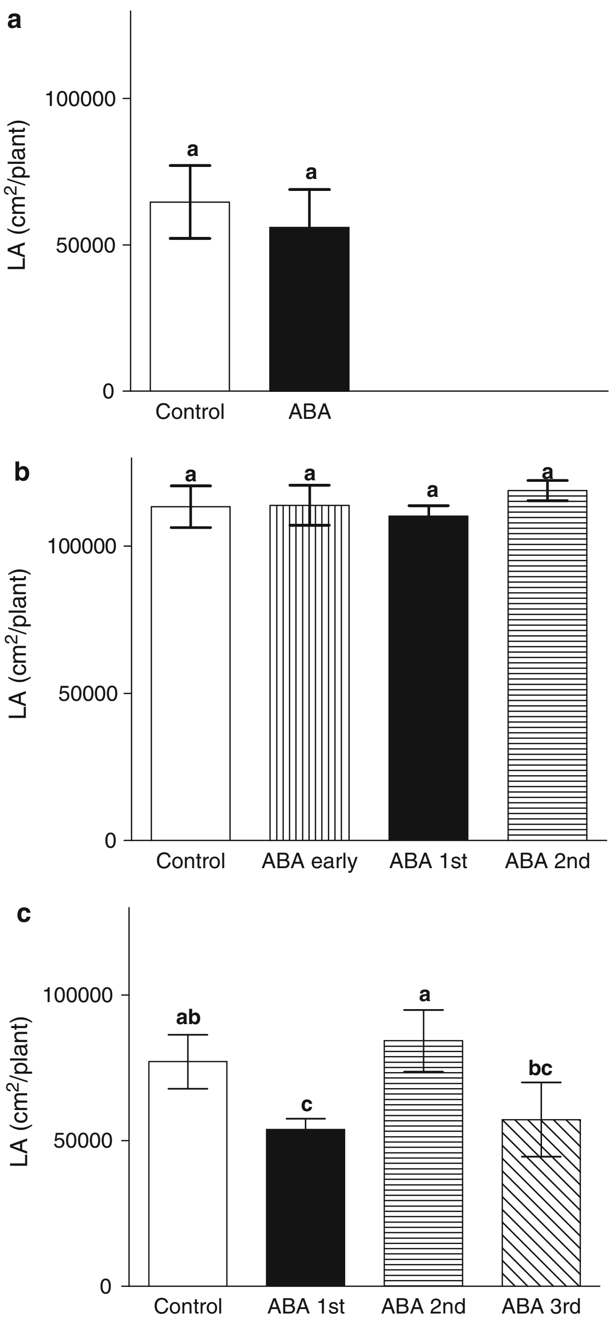

Fig. 1 Influence of foliar-applied ABA on leaf area (LA, cm^2/plant) of grape plants assessed just before harvest for the seasons 2004–2005 (**a**), 2005–2006 (**b**), and 2006–2007 (**c**). Different letters indicate significant differences at $p \leq 0.05$ (LSD test)

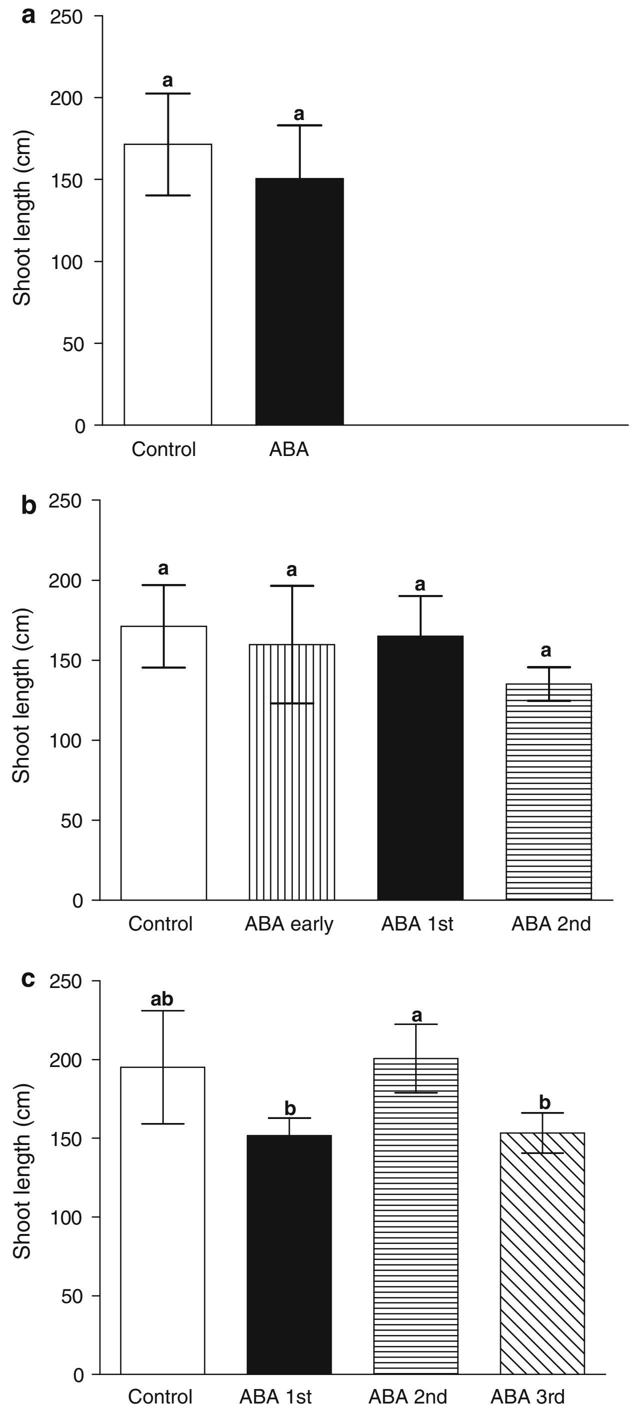

Fig. 2 Influence of foliar-applied ABA on cumulative shoot length (cm) of grape plants assessed just before harvest for the seasons 2004–2005 (**a**), 2005–2006 (**b**), and 2006–2007 (**c**). Different letters indicate significant differences at $p \leq 0.05$ (LSD test)

STATGRAPHICS PLUS 4.0 software program. The Fisher's least-significant-difference (LSD) procedure was used for discriminating among the means of the variables ($p \leq 0.05$).

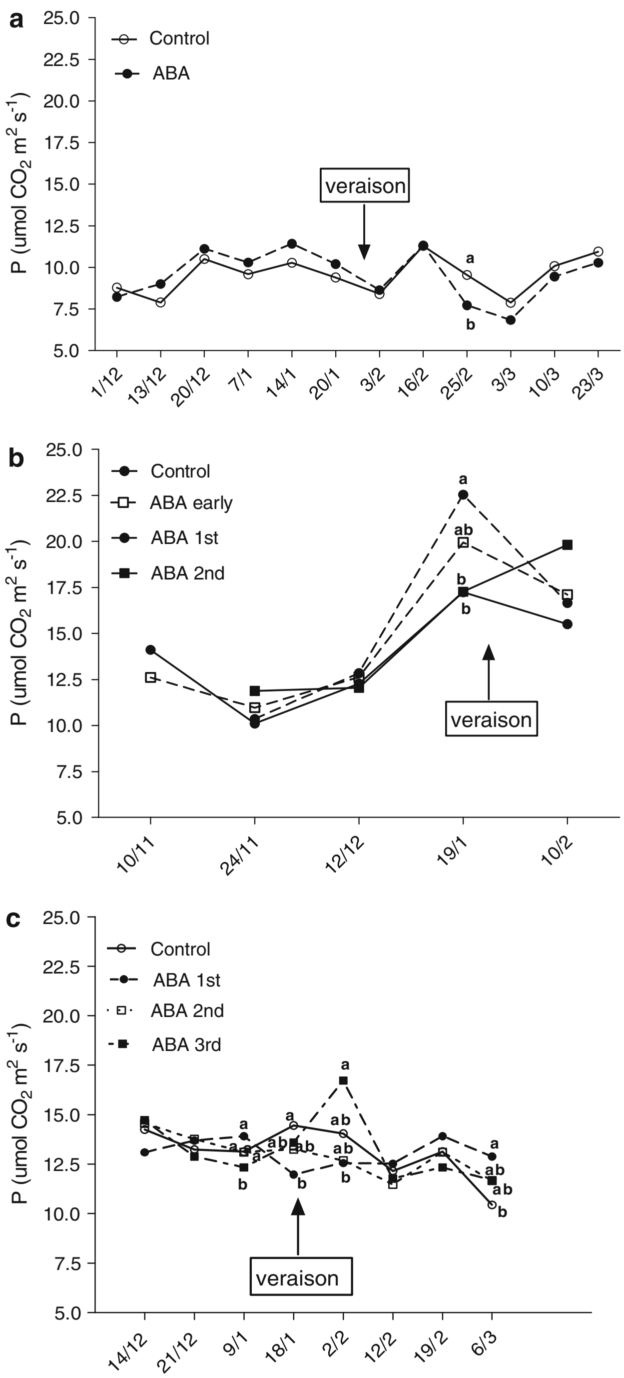

Fig. 3 Influence of foliar-applied ABA on photosynthesis (μmol CO_2 m^{-2} s^{-1}) in grape plants assessed from 15 days after bud-burst until harvest for the seasons 2004–2005 (**a**), 2005–2006 (**b**), and 2006–2007 (**c**). Different letters for the same date indicate significant differences at $p \leq 0.05$ (LSD test)

Results

ABA-treated plants showed a tendency to have a reduced leaf area (LA, Fig. 1a–c, significant for ABA 1st) and reduced shoot length (Fig. 2a–c, significant for ABA 3rd). Differences in photosynthesis (P), primarily just after veraison, were occasionally significant, but trends were not consistent (Fig. 3a–c). For stomatal aperture, g_s, as for P, showed differences between treatments that were occasionally significant at the 5–10% levels, but trends

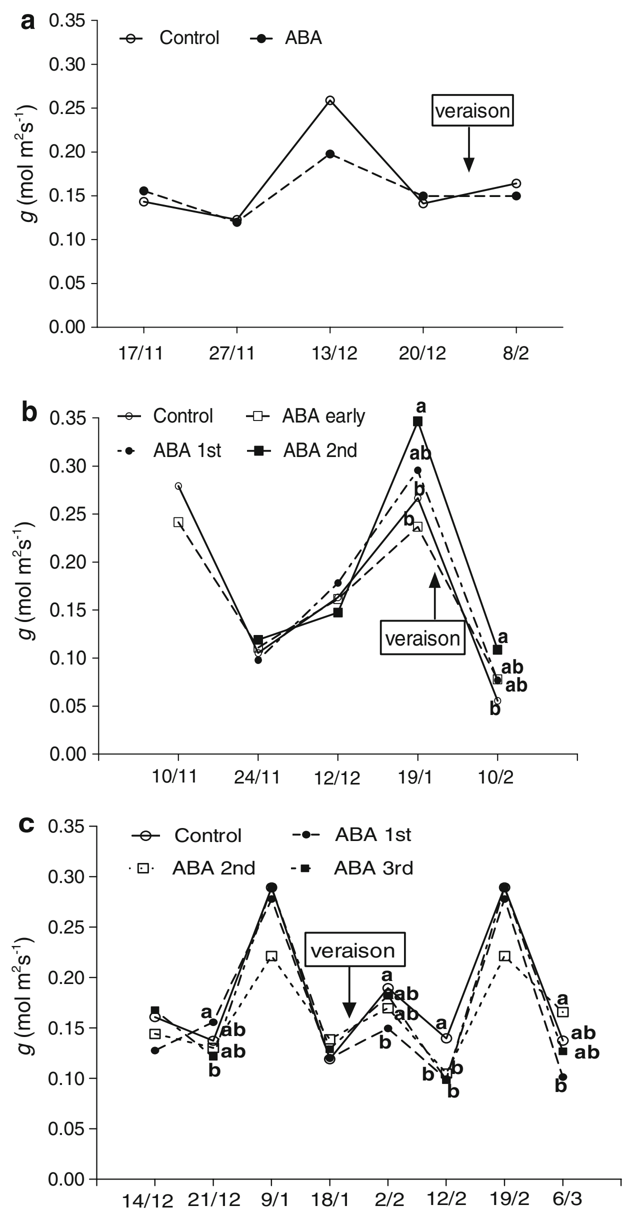

Fig. 4 Influence of foliar-applied ABA on stomatal conductance (g_s, mol m^{-2} s^{-1}) in grape plants assessed from 15 days after bud-burst until harvest for the seasons 2004–2005 (**a**), 2005–2006 (**b**), and 2006–2007 (**c**). Different letters for the same date indicate significant differences at $p \leq 0.05$ (LSD test)

Table 1 Influence of foliar-applied ABA on yield parameters in grapevine field experiments for 2004–2005, 2005–2006, and 2006–2007

Treatment	Kg per plant	Bunches per plant	Average bunch weight (g)	Berries per bunch	Average berry weight (g)	Average berry volume (ml)
2004–2005						
Control	2.376b	41.8a	53.4b	67.3b	0.794a	1.088a
ABA	4.822a	46.2a	82.1a	131.3a	0.622a	1.007a
2005–2006						
Control	2.536c	31.2b	81.5b	86.2b	0.97a	1.16a
ABA early	3.125abc	34.5ab	92.2ab	98.2b	1.04a	1.13a
ABA 1st	4.188a	40.2a	107.7a	137.3a	0.81a	1.13a
ABA 2nd	3.758ab	39.2ab	97.1ab	120.4ab	0.82a	1.09a
2006–2007						
Control	2.737b	36.1a	77.5b	78.05b	0.99a	1.07a
ABA 3rd	4.191a	37.3a	112.2a	114.3a	0.99a	1.00a
ABA 2nd	3.773ab	41.3a	92.2ab	104.1ab	0.88a	1.05a
ABA 1st	3.308ab	36.1a	93.1ab	103.8ab	0.90a	0.94a

For each season different letters in the same column indicate statistical significance at $p \leq 0.05$

were not consistent (Fig. 4a–c). The number of internodes and midday Ψw_{leaf} were not affected by the ABA treatment (data not shown).

Table 1 shows yield parameters assessed across all three seasons. ABA treatment significantly increased yield (kg per plant) for the 2004–2005 season, as did the ABA 1st and ABA 2nd treatments for the 2005–2006 season. For the 2006–2007 season only the ABA 3rd treatment showed significantly increased yields per plant. However, even though the other ABA treatments (ABA sprayed once only in 2005–2006 and ABA 2nd and ABA 1st in 2006–2007) were not statistically significant, the trends were positive and the yield increases ranged from +1.23-fold to +1.38- and +1.21-fold, respectively (Table 1). Thus, there was a clear tendency for ABA to enhance fruit yield at harvest across all treatments and years. The number of berries per bunch was the single yield component that showed significance. Thus increased berry numbers/weight accounted for the improved yield performance of the ABA-treated plants across the three seasons. Berry size, as assessed by volume, showed no differences among treatments, although average berry weight tended to be reduced in five of the six primary treatment comparisons (Table 1). Also, sugar content of the berries, as assessed by Brix (data not shown), was not affected. Although ABA was applied for 2 and 3 years on some treatments, there were no cumulative effects when compared to the 1-year application.

One possible consequence of an enhanced number of berries per bunch might be a lower concentration of anthocyanins and polyphenols, that is, a reduced grape quality for winemaking. Figure 5a–c shows the anthocyanin concentrations for the different treatments across the three seasons. There were no decreases in anthocyanin levels. In fact, for the 2004–2005 season statistically significant increases in anthocyanin content were seen (Fig. 5a). Total polyphenol index values did not differ between treatments in any of the three seasons (Fig. 6a–c). For wine made from grapes collected from two of the seasons (2004–2005 and 2005–2006), no differences were found in either anthocyanin content or total polyphenol index (Fig. 7a, b).

Discussion

Yield increases in fruit of Cabernet Sauvignon were accounted for solely by an increased number of berries per bunch. The simplest explanation is that the applied ABA “somehow” increased berry set or reduced early berry abortion. Because no obvious water stress occurred (data not shown), alleviation of such stress by the applied ABA does not seem to be a reasonable explanation. Although no significant differences in vegetative shoot growth were measured, leaf area did tend to be reduced for almost all ABA treatments. Thus, berry set and subsequent growth (of significantly increased berry numbers on slightly increased numbers of bunches) took place under circumstances where photosynthesis (per unit leaf area) was not significantly influenced by ABA treatment. Even so, the significantly increased numbers of berries on ABA-treated plants accumulated similar amounts (per berry) of photoassimilate (as evidenced by Brix), although weight per berry tended to be lower on ABA-treated plants (all relative to

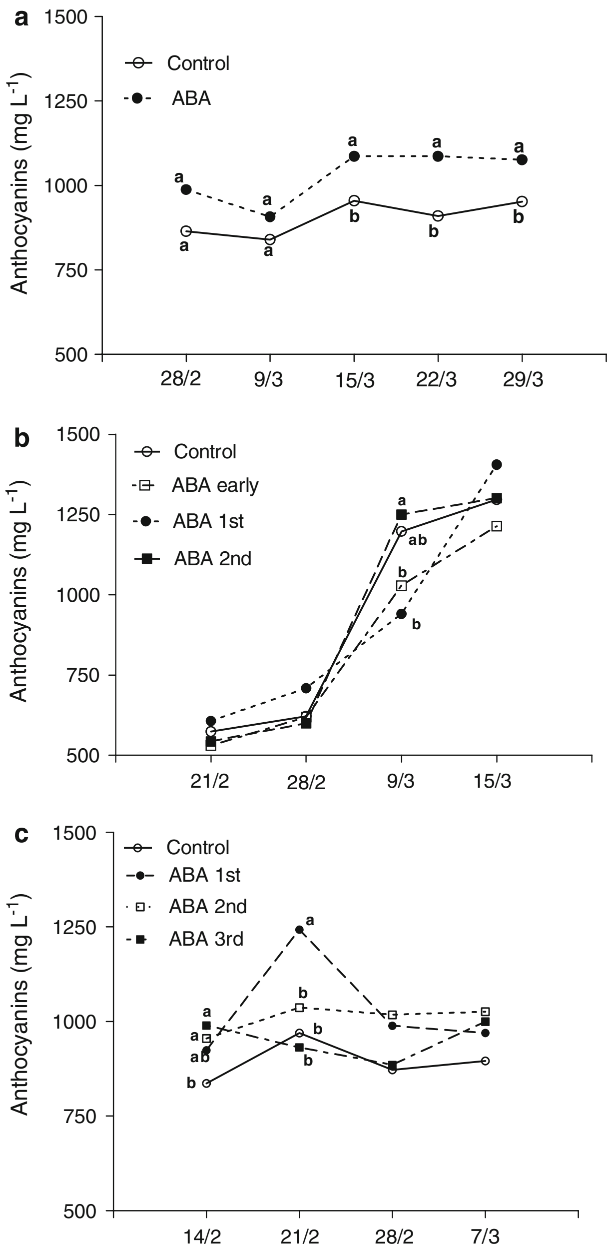

Fig. 5 Influence of foliar-applied ABA on anthocyanin content in grapes from veraison until harvest for the seasons 2004–2005 (**a**), 2005–2006 (**b**), and 2006–2007 (**c**). Different letters for the same date indicate significant differences at $p \leq 0.05$ (LSD test)

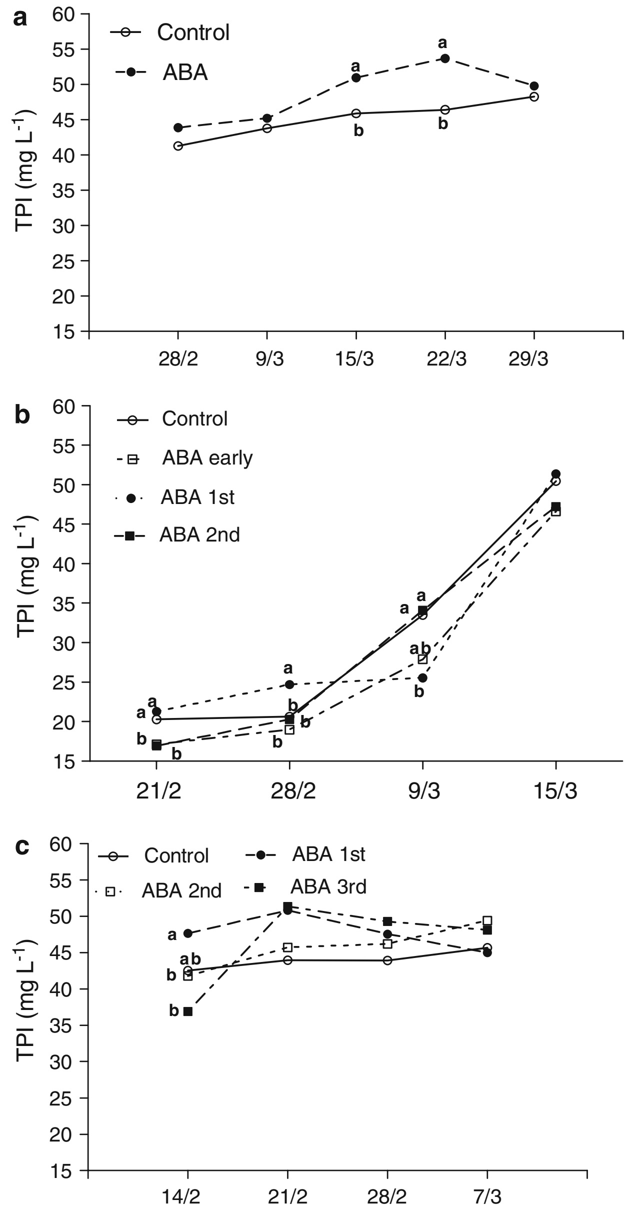

Fig. 6 Influence of foliar-applied ABA on total polyphenol index (TPI) in grapes from veraison until harvest for the seasons 2004–2005 (**a**), 2005–2006 (**b**), and 2006–2007 (**c**). Different letters for the same date indicate significant differences at $p \leq 0.05$ (LSD test)

control plants). Hence, leaves of ABA-treated plants were able to meet the increased photoassimilate demands of a significantly increased crop load, despite a somewhat reduced leaf area. There is thus a strong indication that the applied ABA has not only increased berry set and/or prevented premature berry abortion, but has also enhanced the sink strength of berries on ABA-treated plants (Brenner 1987 and references therein). Although it would be reasonable to expect lower polyphenol and/or anthocyanin content with an increased crop load, this did not happen, that is, yield increases per plant were obtained without reducing the grape quality for winemaking. One explanation for these results is that ABA increased the photosynthetic efficiency, for example, by augmenting pigments protective of the photosynthetic apparatus (carotene, see Brenner 1987 and references included therein; Travaglia and others 2007). However no consistent or significant improvement of photosynthesis per unit leaf area was found for ABA-treated plants.

In wheat (Travaglia and others 2007) and rice (Yang and Zhang 2006), ABA was shown to increase photoassimilate

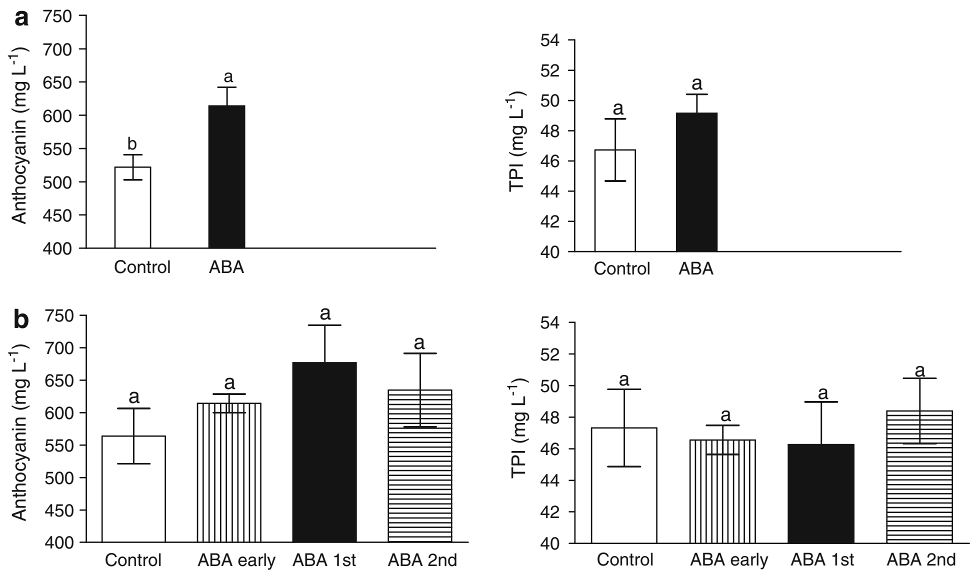

Fig. 7 Influence of foliar-applied ABA on anthocyanin content (on the left) and total polyphenol index (TPI, on the right) in wines made from the grapes collected for the seasons 2004–2005 (**a**) and 2005–2006 (**b**). Different letters indicate significant differences at $p \leq 0.05$ (LSD test)

allocation to the developing grains. Our results with grape seem consistent with such a hypothesis because more berries (of similar weight) were produced, both per bunch and per plant, in ABA-treated plants relative to control plants. Residues of plant trimming did not show any differences in leaf biomass among treatments (data not shown), although young shoots may not be the main sink for reserves. Finally, there is also a possibility of reduced photoassimilate being allocated to roots and/or trunk wood in ABA-treated plants, but these were not assessed in our experiments.

The enhancement of anthocyanin biosynthesis by ABA, which was applied directly to grape berries, had been shown by Jeong and others (2004). Thus, the ability of these ABA-treated grape plants to maintain (or even increase) anthocyanin concentrations in an overall increased crop load may be explained by the direct effects of ABA on anthocyanin biosynthesis.

In conclusion, across three seasons of experiments, we have shown that ABA enhanced grape yield in cv. Cabernet Sauvignon grown under standard field conditions in the grape-growing region of Mendoza. The most obvious reason for this increased yield is an ABA-induced berry set (or retention), followed by an ABA-promoted allocation of photoassimilate to these significantly increased berry numbers. It should be noted that early work by Mullins and Osborne (1970) with Cabernet Sauvignon plants grown in pots demonstrated that ABA applied to leaves enhanced growth of inflorescences. This is perhaps another very relevant demonstration of ABA's ability to "enhance" sink strength in reproductive organs.

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