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S-Abscisic acid, 2-chloroethylphosphonic acid and indole-3-acetic acid treatments modify grape (*Vitis vinifera* L. 'Cabernet Sauvignon') hormonal balance and wine quality

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Summary

The phenolic composition of red wine strongly determines its quality. Even when the applications of plant growth regulator (PGR) affect grape quality, there is almost no information on the effect of these treatments on the grape's internal hormonal balance and the wine composition and quality. In the present study, changes in the internal hormonal content following the application of (+)-S-abscisic acid (S-ABA), 2-chloroethylphosphonic acid (CEPA) and indole-3-acetic acid (IAA) at veraison were examined to determine their effects on 'Cabernet Sauvignon' grapes and wine composition in a plants in containers experiment and in a commercial vineyard experiment. Applied PGRs had a significant effect on the hormonal balance and phenolic composition of grape skins. The S-ABA-treated grapes showed a significantly higher skin internal free abscisic acid concentration in the plants in container experiment and the CEPA-treated grapes showed a reduction in skin internal IAA concentration in the commercial vineyard experiment. Winemaking was performed in the commercial vineyard experiment. Wine's chemical composition was affected by these treatments and an up-to 63 % increase in malvidin-3-glucoside concentration and an up-to 70 % increase in total tannin concentration were found in wines made from the CEPA-treated grapes. The alcohol content was 10.3 % higher (from 12.6 to 13.9 % v v^{-1}) in wines made from the CEPA-treated grapes. No significant differences in the wine sensory attributes (aroma and mouth-feel) between the control and the PGR-treated wines were identified by a sensory panel.

K e y w o r d s : plant growth regulators, anthocyanins, flavonoids, sensory analysis, alcohol.

A b b r e v i a t i o n s : ABA, abscisic acid; ANOVA, analysis of variance; ANR, anthocyanidin reductase; CEPA, 2-chloroethylphosphonic acid; CVE, commercial vineyard experiment; DAV, days after veraison; DMAC, 4-(dimethyl-amino)cinnamaldehyde; IAA, indole-3-acetic acid; LAR, leucoanthocyanidin reductase; NAA, 1-naphthaleneacetic acid; PAL, phenylalanine ammonia-lyase; PCE, plants in containers experiment; PGR, plant growth regulators; TSS, total solid soluble; S-ABA, (+)-S-abscisic acid; TPI, tannin polymerization index; UFGT, UDP-glucose:flavonoid 3-O-glucosyltransferase.

Introduction

Grape berry ripening occurs under hormonal control (Chervin *et al.* 2004, Symons *et al.* 2006, Davies and Böttcher 2009) and therefore a close relation between the phenolic compound accumulation and the hormonal status has been envisaged (Lacampagne *et al.* 2009, Böttcher *et al.* 2010) as a way to improve grape and wine quality (Kennedy *et al.* 2006).

The treatment of grapes with 2-chloroethylphosphonic acid (CEPA), an ethylene-releasing compound, at véraison increases the internal ethylene content, the expression of the UFGT gene and the level of anthocyanins (El-Kereamy *et al.* 2003, Tira-Umphon *et al.* 2007). Exogenous ABA application increases the internal ABA levels, accelerates the beginning of ripening and increases phenylalanine ammonia-lyase (PAL) activity and UFGT expression, improving the anthocyanin concentration and decreasing the leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR) activity, thereby reducing the tannin content (Gagne *et al.* 2006, Deytieux-Belleau *et al.* 2007, Peppi *et al.* 2008, Lacampagne *et al.* 2009). Finally, the treatment of berries with 1-naphthaleneacetic acid (NAA), an auxinic PGR, causes a delay in the beginning of berry ripening (Böttcher *et al.* 2010) and in the commonly observed ABA peak at veraison, suggesting a possible co-involvement of ABA and auxins in controlling the ripening process (Davies *et al.* 1997, Deytieux-Belleau *et al.* 2007). However, research in this field shows that the hormonal control of grape berry development remains controversial (Davies and Böttcher 2009).

The objective of this study was to determine the effects of the PGRs S-ABA, CEPA and indole-3-acetic acid (IAA) at veraison on the internal hormonal profile of grape skins and on the accumulation of flavonoid compounds in 'Cabernet Sauvignon' grapes and wine.

Material and Methods

P l a n t m a t e r i a l a n d s a m p l e c o l l e c t i o n : The grape samples were collected from a commercial vineyard experiment (CVE) and a plants-in-70-L containers experiment (PCE), located at the Colchagua Valley (34.18ºS 71.16ºW) and the Maipo Valley (33.30ºS 70.37ºW) of Chile, respectively, during 2009. The plants

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of *Vitis vinifera* L. 'Cabernet Sauvignon' were conducted as a traditional north/south vertical trellis with spur pruning. All the plants had rooted on their own and were between 12 and 15 years old and the CVE planting density was 2,667 vines per hectare.

The CVE layout consisted of three completely randomized 16-plant replicates and the PCE layout consisted of three completely randomized 12-bunch replicate blocks. Thirty random 5-berry bunch fragment samples were collected from the CVE layout at three phenological stages (Table). In the PCE layout, 60 random berry samples per replicate were collected at five phenological stages (Table).

Experimental treatments: In these experiments S-ABA, the naturally-occurring enantiomer is used instead of synthetic ABA, which is a racemic mixture of (+)-S-ABA and (-)-R-ABA, molecules that can be different in inducing gene expression and physiological responses (Zaharia *et al*., 2005). The PGR concentrations were based on manufacturer-recommended doses, 400 $mg \cdot L^{-1}$ for the S-ABA (VBC-30051, Valent BioSciences Corporation, USA) and 480 $mg \cdot L^{-1}$ for the CEPA (Ethrel, Bayer CropScience AG, Germany). For the IAA (Sigma-Aldrich, USA), an equimolar concentration to S-ABA was applied, corresponding to 265 $mg \cdot L^{-1}$. The treatment involved applying the PGR solution containing 0.1 % Tween80 as a wetting agent on the treated grapes or water containing the same Tween80 concentration on the control grapes. The application was done three days after veraison (DAV, with veraison defined as one per-cent color change in the grapes), corresponding to a 40 % color change. In the CVE, all the clusters of the entire plants were treated using an agricultural hand sprayer at a water rate of 1,000 $L \cdot ha^{-1}$. In the PCE, all the grapes were treated by complete cluster immersion for one minute in the respective PGR solution. All treatments were performed at sunset to minimize photodestruction of ABA.

Skin phenolic extractions: Immediately after sampling, the fresh berries were weighed and the berry skins were hand separated. The skins were rinsed with distilled-deionized water, frozen and then lyophilized, before final grinding. One gram of ground lyophilized skins was extracted (Kennedy and Jones, 2001) in 10 mL of a 2:1 acetone/water solution for one hour in a shaker at room temperature and then centrifuged at 4,000 rpm for six min. After centrifugation, the supernatant extract was concentrated under reduced pressure at 35 °C in a rotary evaporator to remove acetone and then dissolved in a 100 mL model wine solution (12 % v v^{-1} ethanol, pH 3.6 and 0.033M tartaric acid). To minimize oxidation, the solutions were sparged with nitrogen gas. From the homogenized berry pulp, we determined the total solid soluble (TSS) by direct reading in a digital refractometer (Pocket PAL-1, Atago, Japan), the pH using a pH meter (Orion 5-Star, Thermo Scientific, Singapore) and the titratable acidity using a pH meter and 0.1N NaOH.

Winemaking: Small-scale winemaking was performed in the CVE for all the biological replicates. The wine was made through a traditional red wine fermentation protocol. The grapes were picked on the commercial harvest date, April 6, 2009 (corresponding to 79 DAV). Twenty-five kilograms of grapes were harvested from each of the 12 replicates of the control and the PGR-treated plants. Fermentation was carried out in plastic 25-L containers in a controlled temperature room with Lalvin EC-1118 selected yeast. Complete spontaneous malolactic fermentation was performed and an adjustment to 30 $mg \cdot L^{-1}$ free sulfur dioxide was made in all the wines prior to bottling.

ABA and IAA analysis: Internal "free" ABA was determined following the method described by Antolin *et al.* (2003) and internal IAA was determined according to the method of Abbas *et al.* (2000). For the extraction of both hormones, 500 mg of the ground lyophilized skins of 100 berries were extracted with 100 mL methanol 80 % v v^{-1}, containing BHT (2.6-di-*tert*-butylphenol) as an antioxidant, with continuous stirring at 4 °C overnight. After filtration, the extract was concentrated under reduced pressure at 35 °C to remove methanol and adjusted to a pH of 3.0 (± 0.05). The residue was dissolved in ultrapure water and mixed with polyvinyl poly-pyrrolidone (5 %, w v^{-1}) at 4°C for 20 min. After filtration and the adjustment of pH to 2.5 (± 0.05), the extract was subjected to three con-

Table

Grapevine growth stage, sampling date, days after veraison and total solid soluble of the grape samples

	Sample	Stage[a)]	Date	DAV	TSS (°Brix)
Container plants	1	35: Berries begin to color and enlarge	19/01	5	12.7
	2	35: Berries begin to color and enlarge	28/01	14	16.2
	3	36: Berries with intermediate sugar values	06/02	23	20.1
	4	37: Berries not quite rape	25/02	42	21.5
	5	38: Berries harvest-ripe	27/03	72	23.9
Commercial vineyard	1	35: Berries begin to color and enlarge	29/01	12	16.7
	2	37: Berries not quite ripe	05/03	47	22.6
	3	38: Berries harvest-ripe	30/03	72	23.9

[a)] Grapevine growth stages as defined by the Modified E-L system (Coombe 1995).

secutive liquid-liquid extractions with diethyl ether (v v^{-1}). The organic phases, containing free ABA and IAA, were pooled and dried under reduced pressure. The residue was dissolved in one mL diethyl ether and stored at -80 °C until analysis. Prior to HPLC analysis, the extract was dried under nitrogen, dissolved in 300 µL of methanol and filtered through a 0.45 µm membrane.

The extract was automatically injected and processed by an HPLC (Thermo Fisher Scientific, Illkirch, France) equipped with a reverse-phase column (4.6x250 mm Hypersyl® BDS C18, 5 µm). A gradient solvent system was used with methanol as solvent A and 0.1 M H_3PO_4 in ultrapure water as solvent B. The elution program had the following proportions of solvent A: 0-7 min, 60 %; 7-7.5 min, 60-65 %; 7.5-11 min, 65 %; 11-11.5 min, 65-60 %; 11.5-20 min, 60 %. The flow rate was one mL per minute and the column was kept at room temperature. The detection was performed in combination with UV spectrophotometry for ABA (λ: 254 nm) and with fluorescence spectrometry for IAA at 280 and 360 nm excitation and emission wavelengths, respectively. The data acquisition and processing were performed using the Chromquest 4.2 software (Thermo Fisher Scientific, Illkirch, France).

The ABA and IAA calibration curves were established using commercial ±-*cis*,*trans*-ABA (Sigma-Aldrich, Saint Quentin Fallavier, France) and IAA (Sigma-Aldrich, Saint Quentin Fallavier, France).

Phenolic compounds analysis: The phenolic composition of the grapes and wine was determined using a UV/Vis spectrophotometer model Spectronic Genesys 2 (Milton Roy, Rochester, NY). The total anthocyanins were determined at 520 nm according to the method described by Puissant and Leon (1967), the total phenols were determined by DO280 and the total tannin was determined by precipitation with methyl cellulose (Sarneckis *et al*. 2006). The tannin determination by the 4-(dimethyl-amino)cinnamaldehyde (DMAC) assay, in accordance with the method described by Vivas *et al.* (1994), was performed to secure a tannin polymerization index (TPI), calculated as the quotient between the total tannin and the DMAC index. All the analyses were performed in duplicate.

Anthocyanins analysis by HPLC: The chromatographic system for the HPLC-DAD analysis of the anthocyanins consisted of a LaChrom Elite® HPLC system with a 1,024 photodiode-array detector (Hitachi LaChrom Elite, Japan). Separation was performed using a Purospher® STAR (Merck, Germany) reverse-phase C_{18} column (250 mm X 4,6 mm i.d., 5µm) at 20ºC. The detection was carried out at 520 nm. The elution gradient consisted of two solvents: Solvent A was water/formic acid 90:10 v v^{-1} and solvent B was acetonitrile, following the methodology described by Fanzone *et al.* (2010). After filtering through a 0.45 µm pore size membrane, a 150 µL aliquot of grape skin extract was injected. Delphinidine-3-glucoside, peonidine-3-glucoside and malvidin-3-glucoside (Extrasynthese, Lyon, France), were used as standards and the other anthocyanins were identified by comparison of the standard retention times.

Wine sensory analysis: A nine person sensory analysis panel was formed consisting of students and staff of the Enology Laboratory of the Faculty of Agricultural Sciences, "Pontificia Universidad Católica de Chile". All the panel members were familiarized with the method of sensory analysis and aromatic standards during a 2-week training (four sessions). For the vegetal aroma, 3-isobutyl-2-methoxypyrazine and 3-isopropyl-2-methoxypyrazine standards (Centro de Aromas y Sabores, DICTUC, Chile) were used. For the astringency, tannic intensity and bitterness, the method proposed by Delteil (2000) was used. A structured 9-unit linear scale was used. The samples were individually three-digit coded and presented in random order to the panelists. The assessments were made following general requirements for sensory testing conditions. Each wine sample was tasted three times by each panelist.

Statistical analysis: The results were compared by one-way (for completely randomized experiments) and two-way (for completely randomized block experiments) analysis of variance (ANOVA) and Tukey's HSD multiple comparison procedure, with $P < 0.05$ statistical significance between treatments, using Statgraphics Plus (Statistical Graphics Corp., Princeton, NJ, USA).

Results

Skin internal "Free" ABA and IAA status: The variation in internal "free" ABA content in the grape skins during veraison and ripening is shown in Fig. 1. Soon after the onset of ripening, at the first sampling point (5 DAV, two days post treatment for the CVE and 12 DAV, nine days post treatment for the PCE), when 50-80 % of the berries had changed color, the treatments showed significant differences. The S-ABA-treated grapes

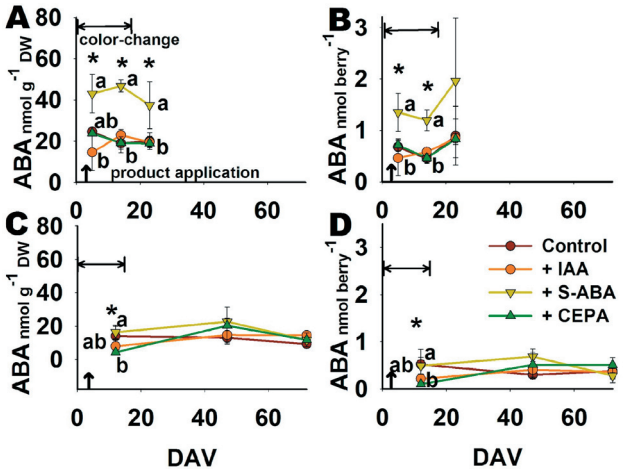

Fig. 1: The skin internal "free" ABA content in nanomoles per gram dry weight (**a**, **c**) and nanomoles per berry (**b**, **d**) in a 'Cabernet Sauvignon' containers (**a**, **b**) and commercial vineyard (**c**, **d**), of treated and control plants. DAV: days after veraison. The data represent the mean of three independent replicates ± standard deviation (error bars). The asterisk and letters indicate, for the same date, a significant difference between treatments ($p < 0.05$; ANOVA and Tukey's HSD multiple comparison procedure).

showed significantly higher levels of internal "free" ABA, as compared with all the other treatments at 5, 15 and 23 DAV in the PCE, expressed either as a concentration or on a per-berry basis. There were no significant differences in internal "free" ABA contents between the control and the S-ABA treated grapes in the CVE, skins of the S-ABA-treated grapes showed higher contents when compared with the CEPA-treated grapes and the skins of the CEPA-treated grapes showed lower contents when compared with the control skins expressed as a per-berry basis. The skins of the IAA-treated grapes showed no significant differences when compared with the control grapes.

The variation in the internal IAA content in the grape skins during veraison and ripening is shown in Fig. 2. In the CVE, the CEPA-treated grapes showed a significant reduction in internal IAA levels as compared with all the other treatments at 12 DAV, expressed either as a concentration or on a per-berry basis. No significant differences between treatments were observed at 47 and 72 DAV in the CVE or at any of the three sampling dates in the PCE.

Berry maturation: No significant berry weight differences between the treatments were found in both experiments and no significant differences in crop weight in the CVE were evidenced between the treatments at harvest time (data not shown). However, the PGR applications altered the pulp maturation parameters (Fig. 3). The CEPA-treated grapes showed a decrease in TSS content (Fig. 3 A and D). This reduction was significant for the first sampling date (12 DAV) in the CVE and for the two final sampling dates (42 and 72 DAV) in the PCE. The CEPA-treated grapes showed a significant increase in pH at 72 DAV (Fig. 3 B) and a significant decrease in total acidity at 47 DAV in the CVE (Fig. 3 C). Similar but not significant tendencies for the pH and total acidity could be observed in the PCE (Fig. 3 E and F).

HPLC-DAD grape skin anthocyanin composition: The HPLC-DAD grape skin anthocyanin compositions for the PCE at 42 DAV and for the CVE at 47 DAV are shown in figure 4. For the PCE at 42 DAV, of the subtotal anthocyanins, only total malvidin and total delphinidin showed significant differences. When the levels were compared to control grapes, skins from the IAA-treated grapes showed a significant decrease in total malvidin and skins from the S-ABA- and CEPA-treated grapes showed a significant increase in total malvidin, with a greater increase found in skins from the CEPA-treated grapes (Fig. 4 A). When anthocyanins were grouped into di- or trihydroxylated forms, the effects of treatments were only significant for the trihydroxylated anthocyanins (*i.e.*, malvidin, petunidin and delphinidin) and these forms showed the same effect as total anthocyanins, with skins from the CEPA- and S-ABA-treated grapes showing higher concentrations than skins from IAA-treated and control grapes (Fig. 4 B). In the CVE at 47 DAV, significant differences were observed in total malvidin, total petunidin, total peonidin, total cyaniding, subtotal dihydroxylated, subtotal trihydroxylated and total anthocyanins. Only the skins from the CEPA-treated grapes showed higher significant concentrations (Fig. 4 C and D).

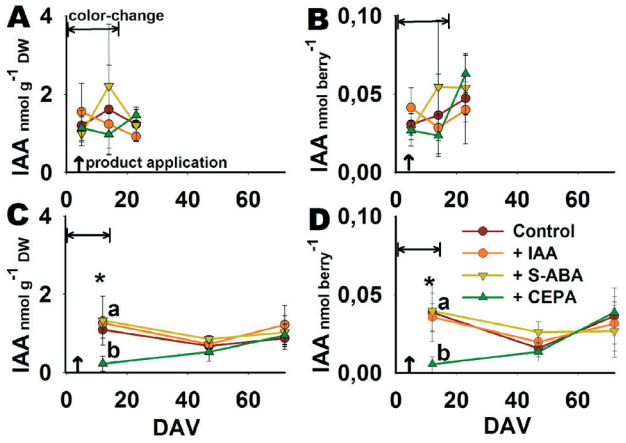

Fig. 2: The skin internal IAA content in nanomoles per gram dry weight (**a**, **c**) and nanomoles per berry (**b**, **d**) in a 'Cabernet Sauvignon' containers (**a**, **b**) and commercial vineyard (**c**, **d**) of treated and control plants. DAV: days after veraison. The data represent the mean of three independent replicates ± standard deviation (error bars). The asterisk and letters indicate a significant difference between the treatments ($p < 0.05$; ANOVA and Tukey's HSD multiple comparison procedure).

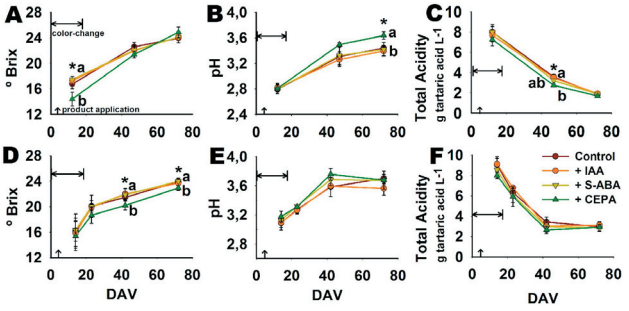

Fig. 3: The Total Soluble Solids (**a**, **d**), pH (**b**, **e**) and titratable acidity (**c**, **f**) of a commercial vineyard (**a**, **b**, **c**) and a container plants experiment (**d**, **e**, **f**) for berry pulp samples of treated and control grapes. DAV: days after veraison. The data represent the mean of three independent replicates ± standard deviation (error bars) and an asterisk indicates a significant difference between the treatments at the same date ($p < 0.05$; ANOVA).

Wine composition: The effect of treatments on wine composition is summarized in Fig. 5. Wine analysis showed that wines from the CEPA-treated grapes were significantly higher than the control in alcohol content, while wines from S-ABA- and IAA-treated grapes did not show significant differences. Wines showed no significant differences in pH, total acidity, residual sugar, volatile acidity, color intensity, color hue and TPI. Wines from CEPA-treated grapes showed significantly higher levels in DO280 index, total anthocyanins and total tannin, while wines from S-ABA treated grapes only showed a significantly higher level of total anthocyanins. Wines from IAA-treated grapes did not show any significant difference when compared to control wines (Fig. 5).

The HPLC wine anthocyanin composition is shown in figures 4E and 4F. Only wines made from CEPA-treated grapes had significantly higher concentrations of total malvidin, total cyanidin, trihydroxylated and total anthocyanins

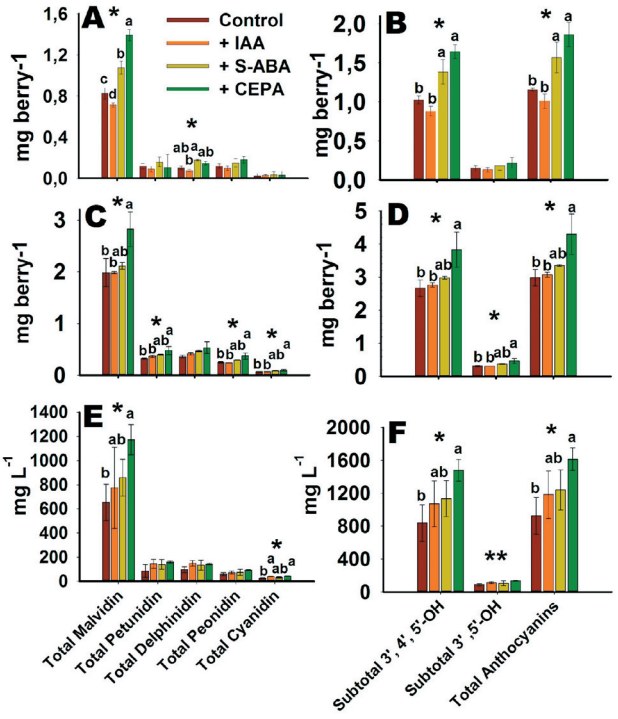

Fig. 4: Grape skin and wine anthocyanin composition, including malvidin, petunidin, delphinidin, peonidin and cyanidin (**a**, **c**, **e**) and trihydroxylated, dihydroxylated and total anthocyanins (**b**, **d**, **f**) of PGR-treated and control grapes from 'Cabernet Sauvignon' container plants at 42 DAV (**a**, **b**), a commercial vineyard at 47 DAV (**c**, **d**) and wine made of commercial vineyard grapes. The data represent the mean of three independent replicates ± standard deviation (error bars) and an asterisk indicates a significant difference between treatments at the same date ($p < 0.05$; ANOVA).

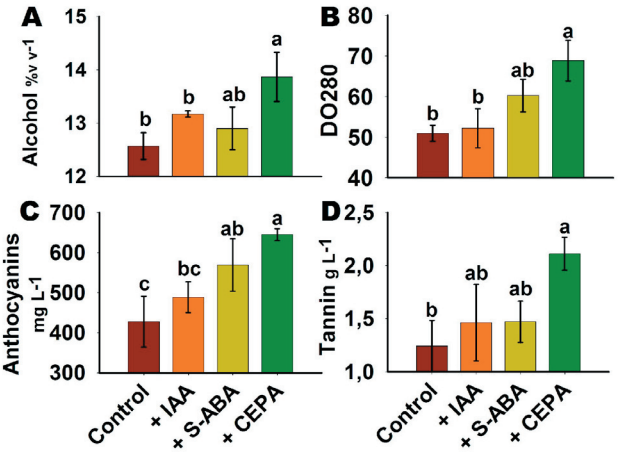

Fig. 5: The composition of wines, including the alcohol content (**a**), DO280 index (**b**), total anthocyanins (**c**) and total tannin (**d**) in wines made from PGR-treated and control grapes from a 'Cabernet Sauvignon' commercial vineyard. The data represent the mean of three independent replicates ± standard deviation (error bars) and letters indicate significant differences between the treatments ($p < 0.05$; Tukey's HSD).

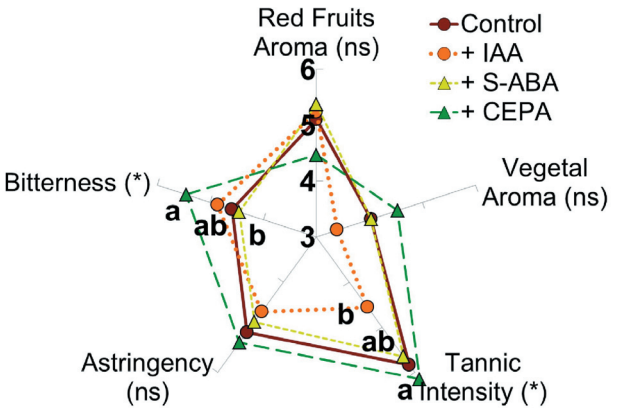

Fig. 6: Wine sensory analysis, including red fruits aroma, vegetal aroma, tannic intensity, astringency and bitterness of wines made from PGR-treated and control grapes from a 'Cabernet Sauvignon' commercial vineyard. The data represent the mean of three independent replicates, an asterisk indicates a significant difference between the treatments ($p < 0.05$; ANOVA).

when compared to control wines. No significant effects of S-ABA in wines could be observed (Fig. 4 E and F).

Wine sensory analysis: The effect of treatments on wine sensory analysis is summarized in Fig. 6. CEPA-treated grapes produced wines with significantly higher bitterness than S-ABA-treated wines and significantly higher tannic intensity than IAA-treated wines. No significant difference between control and PGR-treated wines could be observed. Moreover no significant differences in the red fruit aroma, vegetal aroma and astringency could be found between any treatments.

Discussion

The results shown here demonstrate that grape PGR treatments at veraison have significant effects on the grape ripening process, grape TSS, pH and titratable acidity levels, wine alcohol concentration and accumulation of phenolic compounds in grapes and wine, including DO280 index, total tannin, anthocyanin concentration and composition. No effects on crop weight could be found and some effects on wine aromatic and mouth-feel sensory attributes were found.

The expression of genes related to metabolism of ABA, auxins, ethylene and brassinosteroids have been shown to be significantly modified at the onset of ripening (Symons *et al*. 2006, Pilati *et al*. 2007). In this study, the exogenous application of S-ABA increased the level of internal "free" ABA in the grape skins in the PCE. This effect was shown in previous studies by applying a racemic mixture of synthetic S- and R-ABA (Deytieux-Belleau *et al*. 2007). However, no effect of the S-ABA treatment over internal IAA was found in both experiments. The decrease in internal IAA obtained with the CEPA treatment in the CVE may be explained by an acceleration of the normal peroxidase activity that could be responsible for increasing IAA degradation, thereby rendering the tissue sensitive to ethylene and enhanced ripening (Szyjewicz *et al*. 1984). The results of this study suggest that the role of ethylene as a promoter of ripening could result in a reduction of inhibitors of ripening, such as auxins. Previously, it has been

reported that pre-veraison benzothiazole-2-oxyacetic acid treatment caused increases in grape ethylene concentration (Coombe and Hale 1973, Weaver and Singh 1978). At 47 and 72 DAV in the present study, there were no significant effects on the internal ABA or IAA. This was expected as hormone peaks occurs while the color change is developing, and after that time no additional changes in the hormonal profiles take place (Deytieux-Belleau *et al*. 2007).

In accordance (El-Kereamy *et al*. 2003) or in contrast (Davies *et al*. 1997, Chervin *et al*. 2008) with previous reports, we did not find any significant differences in mean berry weight with CEPA or IAA treatments in either experiment. Our results show that IAA and S-ABA treatments did not affect grape TSS in either experiment, as was the case with wine alcohol content. These results agree with the hypothesis that ABA treatments may be able to hasten the initiation of sugar accumulation when applied early (before veraison) but cannot enhance it once ripening has already commenced (Davies and Böttcher 2009). On the other hand, our results showed that IAA treatment did not affect TSS content in both experiments. Böttcher *et al*. (2010) showed that pre-veraison treatment of 'Shiraz' berries with NAA, another auxinic hormone, significantly delayed ripening as measured by the accumulation of TSS and anthocyanins. These discrepancies may be explained by differences in application time (Giribaldi *et al*. 2010), applied products and varieties studied. A generally controversial subject is the effect of CEPA treatments on TSS content (Szyjewicz *et al*. 1984). In this study, CEPA application significantly decreased TSS content of grapes in some sampling dates in both experiments. This effect was also observed by Delgado *et al*. (2004) with 'Tempranillo'. But as is shown in Fig. 3, CEPA-treated grapes in the CVE increased their TSS levels as ripening advanced. Even when CEPA-treated grapes significantly increased in grape pH and decreased in total acidity in the PCE, no significant differences could be found in these parameters in CVE grapes and wines. CEPA-treated grapes in the PCE evidenced higher pH and lower total acidity values, but simultaneously lower TSS values were found, showing an independent control of acid degradation and TSS accumulation.

The results show a greater accumulation of anthocyanins in skins from CEPA-treated grapes in both experiments. This issue is also reported in other studies (Delgado *et al*. 2004, El-Kereamy *et al*. 2003, Szyjewicz *et al*. 1984). The skins of S-ABA-treated grapes did not show this effect, contrary to the results obtained by Peppi *et al*. (2006) in 'Flame Seedless' table grapes, where ABA applied at veraison was superior to CEPA applied at any of the times tested. These results contribute to the hypothesis that the ethylene signal is likely a regulator of grape UFGT expression in grapes and this stimulation has shown to be independent from the MYBA1 transcription factor (Tira-Umphon *et al*. 2007). This evidence suggests that S-ABA and CEPA treatments could be acting by different mechanisms but in the same direction and there may be a positive interaction between ABA and ethylene in the expression of the UFGT gene (Chervin *et al*. 2009). The effects of S-ABA and CEPA were particularly significant for the trihydroxylated anthocyanins in the PCE, therefore S-ABA and CEPA treatments may have more effect on the expression/activity of the flavonoid-3'5'-hydroxylases than on the flavonoid-3'-hydroxylases (Castellarin *et al*. 2006). This could have an effect on the color of grapes and wine as dihydroxylated anthocyanins produce predominantly orange hues while trihydroxylated ones confer red-purple hues (Heredia *et al*. 1998). Our results did not show any significant difference in wine hues, but other reports have shown that ABA treatments alter the color value and decreased the berry hue (Delgado *et al*. 2004).

IAA can have a negative effect on maturation by delaying veraison and diminishing coloration by reduction of anthocyanin concentration in some non-climacteric fruits such as strawberries and grapevines (Given *et al*. 1988, Ban *et al*. 2003, Deytieux-Belleau *et al*., 2007, Böttcher *et al*. 2010). In accordance with these studies, applied IAA in this study showed a negative effect on some grape skin anthocyanin glycoside content in the PCE.

Information about the effects of PGR applications on wine composition and sensory characteristics is poor. We observed that CEPA treatment caused significant increments in wine alcohol, anthocyanins, DO280 index and tannins, while S-ABA treatment caused only a significant increase in anthocyanins and minor non-significant increments in the other wine parameters (Fig. 5). These results confirm the superior effect of CEPA over S-ABA in the conditions of the present study for improving the accumulation of phenolic compounds in wine. The results show a greater accumulation of anthocyanins in wines from the CEPA- and S-ABA-treated grapes. This effect was higher with the CEPA treatment than with the S-ABA treatment, in accordance with what Venburg *et al.* (2009) show.

Wines made of the CEPA-treated grapes had higher levels of bitterness and tannic intensity. These effects could be related to the higher concentrations of tannin in these wines and could be influenced by the higher alcohol content that they present, which is known to enhance bitterness (Vidal *et al*. 2002). However, no significant differences between the control and PGR treatments were found in wines by sensory analysis, results which agree with those obtained by Böttcher *et al.* (2010), who did not find significant differences in sensory properties between small-scale wine lots made from control and NAA-treated fruit. No significant differences in TPI were found in grapes or wine, therefore no relation with the differences in sensory attributes could be explained with this tannin quality index.

Even when CEPA treated grapes showed lower TSS content in all sampling dates in the PCE and in the two first sampling dates in the CVE, the last sampling date in the CVE showed a slight increase in TSS and wines made of CEPA treated grapes showed higher alcohol content. This could be explained by the effect of CEPA on plant-berry vascular fluxes (Mailhac and Chervin 2006) and the consequences on sugar transport and berry weight. It seems that due to this higher loss of berry weight between 47 and 72 DAV in CEPA treated grapes (19 % in CEPA treated

grapes and between 4 to 9 % in the other treatments), grape TSS at 72 DAV and wine alcohol concentrations became higher.

Our results show an important effect of the ethylene-releasing compound CEPA, which is greater than that of S-ABA, showing the relevance of ethylene levels at veraison on the grape ripening process and grape and wine quality. Even if changes in the ethylene gas levels during grape berry ripening are small compared to those of climacteric fruits, the physiological response to ethylene may be modulated more by changes in the sensitivity of the perception of this hormone than by its levels (Davies and Böttcher 2009).

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