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# Abscisic acid and ethephon treatments applied to 'Verdejo' white grapes affect the quality of wine in different ways

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**ABSTRACT**: Exogenous ethephon and abscisic acid (ABA) treatments are used worldwide to improve the coloring of red table grapes, but there is little information about their impact on the quality of white grapes and wines. The aim of this work was to evaluate the effects of exogenous ethephon and ABA applications at veraison on the composition of 'Verdejo' white grapes, and on the quality of their wines. To attain this objective, a field trial was carried out in a 'Verdejo'/110 Richter vineyard located in north-central Spain. Two levels of ethephon (0 and 1500 mg $L^{-1}$) and ABA (0 and 800 mg $L^{-1}$) were sprayed on clusters at veraison, in a factorial design for three consecutive seasons. Ethephon and ABA had additive effects, decreasing titratable acidity and increasing pH of the must, which could be exploited by accelerating the grape ripening process in cold-climate zones. Nevertheless, each growth regulator affected the composition and sensory analysis of the wines differently. Ethephon treatments produced wines with lower concentration of acids and better flavor quality than those made from untreated plants, while wines with ABA applications tended to have a higher ethanol concentration and poorer aroma quality than controls. The wines submitted to treatments with ethephon obtained the best overall evaluation in the sensory analysis throughout the trial.

**Keywords**: *Vitis vinifera* L., ABA, growth regulators, ripening, tasting

## Introduction

Growth regulators are chemicals, analogous to plant hormones in cellular activity, which are derived from both natural and artificial sources. Exogenous applications of growth regulators can act in a direct way on hormonal balances that regulate the physiological processes of plants, and this has widespread significance in viticulture. For example, a number of these products can promote the processes of synthesis and accumulation of substances during grape ripening, which help to reach maturity faster and improve the quality of the must (Cantin et al., 2007; Roberto et al., 2012; Szyjewicz et al., 1984). This is very significant under conditions of excessive vigor in the vineyard, especially in cool regions, where heat units for maturing fruit are frequently insufficient.

Application of ethylene releasing compounds, such as ethephon (2-chloroethyl) phosphonic acid), at veraison increases the concentration of polyphenol and anthocyanin, and color in fruits, as it was found in both red table (Amiri et al., 2010; Fitzgerald and Patterson, 1994; Human and Bindon, 2008; Roberto et al., 2013) and wine grape cultivars (Delgado et al., 2004; Gallegos et al., 2006; Shulman et al., 1985). However, contradictory results have been noted in the effects of ethephon on the total solid content, pH, potassium and titratable acidity (TA) of must, depending on the cultivar tested, timing, concentration and application method (Szyjewicz et al., 1984).

Endogenous abscisic acid (ABA) peaks in berries at veraison and plays a very important role in fruit ripening (Deytieux et al., 2007) and, at the same time, is a chemical signal in response to environmental stresses (Ferrandino and Lovisolo, 2014). When ABA is freely marketed at a reasonable cost (Zhu et al., 2016), its exogenous applications can be used as a tool to enhance grape quality and control abiotic stress. ABA treatments at veraison have resulted in significant increases in coloring and anthocyanin concentration in table and wine grapes, as does ethephon (Amiri and Parseh, 2011; Delgado et al., 2004; Koyama et al., 2014; Peppi et al., 2007; Zhang and Dami, 2012; Reynolds et al., 2016; Zhu et al., 2016). In most cases, the applications had little effects on the content of soluble solids or titratable acidity.

Most studies have evaluated the effects of ethephon and ABA sprays on the coloring of red table cultivars, lacking insight into their impact on both the composition of white grapes and the final quality of the wines. The aim of this study was to investigate the effects of ethephon and ABA applications at veraison on must composition of 'Verdejo' white grapes, and on the composition and sensory characteristics of their wines.

## Materials and Methods

A field trial was carried out in the 2004, 2005 and 2006 seasons, in a non-irrigated 'Verdejo'/110 Richter vineyard located in the 'Rueda Appellation of Origin' area, in north-central Spain (latitude 41°26'33.8'' N; longitude 4°50'39.7'' W; altitude 722 m). 'Verdejo' is a white grape cultivar, native of Rueda, which produces famous aromatic wines.

The vineyard was planted in 1998 at 3.0 × 1.5 m (2222 vines $ha^{-1}$). Vines were pruned according to the double Guyot system, leaving 20 buds in each, and were trained in a trellis system, adhering to standard practice in the area. The soil was sandy and deep, with low contents of organic matter (11 g kg$^{-1}$) and basic pH (7.8). The values of Olsen phosphorus and exchangeable potassium were 40 and 237 mg kg$^{-1}$, respectively. Rainfall was 357 mm in 2004, 281 mm in 2005, and 342 mm in 2006. Mean air temperature during the ripening period was 18.8 °C, 19.2 °C and 19 °C in the three growing seasons, respectively. There were no late frosts, pest attacks or diseases in any year.

Four treatments were compared in the trial, resulting from combination in a factorial design of two levels of ethephon (0 and 1500 mg L$^{-1}$) and two levels of ABA (0 and 800 mg L$^{-1}$) on aqueous solutions, sprayed on clusters when 70-75 % of the grapes were colored. These doses and the timing were chosen on the basis of previous studies conducted on 'Tempranillo' red grapes in the same region (Delgado et al., 2004; Gallegos et al., 2006). The present experiment was carried out in a randomized complete block with four replications, and four vines in each base plot. Each base plot was isolated from the rest by one vine to prevent overspray from contacting neighboring plants. Treatments were applied to runoff with a 16 L hand sprayer. No adjuvants were added.

In all growing seasons, yield and vigor (pruning weight) of the vineyard were recorded. At harvest, 100 berries were randomly collected and weighed in each experimental treatment. In the must obtained from these samples, total soluble solids content (°Brix), TA, pH, potassium concentration and color intensity were determined according to the official methods established by the European Commission (1990). The color intensity was expressed in terms of absorbance at 420 nm, measured using a UV/VIS spectrophotometer.

A standard wine-making process was used to produce the wine from 10 kg of harvest of each experimental treatment. Hand-picked clusters were destemmed, and then pressed using a pneumatic press (maximum pressure = 0.2 MPa). To all the musts obtained, potassium metabisulfite was added to set free sulfur at 30 mg L$^{-1}$. Approximately, 4 L of must for each experimental treatment was kept for 24 hours at 5 °C, and then racked for vinification. Alcoholic fermentation was induced by the *Saccharomyces cereviseae* yeast. The must, in glass jars, were kept in a refrigerated cell at 17 °C to improve yeast growth. Alcoholic fermentation showed a regular trend, and was considered finished when reducing sugar concentration was lower than 4 g L$^{-1}$. Free sulfur was set at 30 mg L$^{-1}$ with potassium metabisulfite, and wines were clarified and decanted at 4 °C for 48 hours. Next, wines were stored for 30 days at 1 °C for tartaric stabilization in fully filled jars with a volume of 2 L, without 'air space' to avoid oxidation and pollution. Before bottling, setting free once again sulfur at 30 mg L$^{-1}$, the wine composition was analyzed. The alcoholic grade was assessed using a FT-IR spectrophotometer. The contents of glycerol, tartaric, malic and citric acid were determined with a multiparametric autoanalyser.

Finally, a sensory evaluation of wines was performed each campaign by a panel of 25 tasters drawn from final year students of Enology at the University of Valladolid (Spain). Similar to Aleixandre-Tudó et al. (2015), the tasters proceeded to a quantitative assessment, using a scale of 0 to 10 (from lowest to highest intensity) for the following characteristics: color, aroma quality, aroma intensity, bitterness, flavor quality, flavor intensity, and global assessment. Wines were presented in the form of 50-mL samples in clear glasses. All tasting was done on randomized coded samples. Each taster evaluated a maximum of eight wines per session.

Data were subjected to factorial analysis of variance (ANOVA) using SAS statistical software (Statistical Analysis System, version 9.2). Mean values of treated and untreated plants were compared using Student´s t-tests. The relationships between wine composition parameters and organoleptic test scores were studied using Pearson correlation coefficients.

## Results and Discussion

The ANOVA showed that almost all variables measured were strongly affected by season (Table 1), which is probably due to interannual meteorological conditions. Berry weight, vigor of the vineyard and yield (average values from 5.5 to 7.0 kg vine$^{-1}$ in the three seasons) were not affected by experimental treatments. Most reports suggest that neither ethephon (Lurie et al., 2010; Shulman, et al., 1985) nor ABA applications at the beginning of ripening (Cantin et al., 2007; Roberto et al., 2012) impacted either yield or berry size.

The ANOVA showed that ethephon and ABA had an additive effect on TA and pH of the must (Table 1). Nevertheless, wine composition parameters were differently affected by each growth regulator: ethephon impacted TA, pH, malic and tartaric acid concentrations, and affected the flavor quality of the wines, whereas ABA modified alcoholic grade, aroma quality and global assessment in the organoleptic analysis. No interaction effects ($p > 0.05$) were detected between the two plant growth regulators in any of the variables studied.

**Must composition**

Table 2 demonstrates that ethephon spraying had little influence on total soluble solids content, but it produced a significant increase over the untreated plants in 2006. Although it is known that ethylene promotes the transport of sucrose into berries during ripening (Chervin et al., 2005), most of the studies in the literature show that treatments with ethephon either increased must soluble solids concentration or did not affect them at all (Amiri and Parseh et al., 2011; Szyjewicz et al., 1984). On the other hand, ethephon sprayings decreased the TA of the must in 2005 and 2006 seasons (Table 2), which could be mainly due to

**Table 1** – F-values of factorial analysis of variance of yield, vigor, must and wine quality data obtained with ethephon (ET) and abscisic acid (ABA) treatments applied at veraison in the 2004, 2005 and 2006 seasons.

| Parameters | Source of variation | | | | |
|---|---|---|---|---|---|
| | Model | Season | ET | ABA | ET*ABA |
| Yield and vigor | | | | | |
| Yield | 9.73** | 34.56** | 2.82 | 0.12 | 0.34 |
| 100 berry weight | 83.29** | 328.28** | 4.43 | 0.67 | 0.77 |
| Pruning weight | 15.18** | 53.52** | 0.08 | 0.11 | 0.96 |
| Must composition | | | | | |
| ºBrix | 10.35** | 35.16** | 2.52 | 0.64 | 1.57 |
| pH | 8.54** | 26.78** | 3.51* | 6.72** | 1.79 |
| Titratable acidity | 27.75** | 79.77** | 28.17** | 17.68** | 3.27 |
| Potassium content | 34.07** | 131.97 | 2.11 | 2.41 | 1.36 |
| Absorbance 420 nm | 123.76** | 484.37** | 0.70 | 1.26 | 0.82 |
| Wine composition | | | | | |
| Alcoholic grade | 6.14** | 17.25** | 0.37 | 4.11* | 1.28 |
| pH | 4.67** | 1.42 | 25.49** | 0.39 | 1.09 |
| Titratable acidity | 5.00** | 4.46* | 19.61** | 2.13 | 0.45 |
| Potassium content | 11.10** | 29.32** | 22.72** | 1.10 | 2.45 |
| Tartaric acid | 13.62** | 42.09** | 19.46** | 1.33 | 0.75 |
| Malic acid | 31.16** | 107.76** | 13.70** | 2.13 | 0.60 |
| Citric acid | 33.01** | 126.58** | 1.49 | 0.05 | 0.76 |
| Glycerol | 4.88** | 8.22** | 2.58 | 2.74 | 0.98 |
| Wine sensory analysis | | | | | |
| Color intensity | 5.391** | 36.48** | 0.386 | 0.326 | 5.77 |
| Aroma quality | 6.814 ** | 48.94 ** | 0.290 | 3.201* | 0.11 |
| Aroma intensity | 0.349 | 1.86* | 0.106 | 0.008 | 0.29 |
| Bitterness | 0.588 | 2.16* | 0.093 | 0.020 | 0.49 |
| Flavor quality | 4.488** | 32.88** | 0.284* | 0.622 | 0.57 |
| Flavor intensity | 1.168** | 6.79** | 0.305 | 0.012 | 0.26 |
| Overall assessment | 5.330** | 39.83** | 0.022 | 1.381* | 0.12 |

*Significant $p < 0.05$; **Significant $p < 0.01$.

**Table 2** – Main effects of ethephon and abscisic acid (ABA) treatments on must composition in the three seasons studied.

| Treatment | Season | Level | Soluble solids | Titratable acidity | pH | Brix/Titratable acidity |
|---|---|---|---|---|---|---|
| | | mg L$^{-1}$ | ºBrix | g tartaric L$^{-1}$ | | |
| Ethephon | 2004 | 0 | 20.73 a | 5.45 a | 3.60 a | 4.28 b |
| | | 1500 | 20.61 a | 4.87 a | 3.69 a | 4.60 a |
| | 2005 | 0 | 20.04 a | 4.30 a | 3.71 a | 4.90 a |
| | | 1500 | 20.27 a | 3.86 b | 3.74 a | 5.13 a |
| | 2006 | 0 | 21.34 a | 4.82 a | 3.64 a | 4.56 a |
| | | 1500 | 21.58 b | 4.43 b | 3.80 b | 4.64 a |
| ABA | 2004 | 0 | 20.77 a | 5.50 a | 3.60 a | 4.35 b |
| | | 800 | 20.72 a | 4.26 a | 3.71 a | 4.59 a |
| | 2005 | 0 | 20.03 a | 4.94 a | 3.62 a | 4.96 a |
| | | 800 | 20.22 a | 3.88 a | 3.73 a | 5.11 a |
| | 2006 | 0 | 21.77 a | 5.14 a | 3.87 b | 4.38 b |
| | | 800 | 22.24 a | 4.33 b | 4.00 a | 4.93 a |

Within seasons, means with different letters are significantly different ($p$ < 0.05, t-test).

a reduction in malic acid concentration in the grapes (Chervin et al., 2002). Recent studies (Liu et al., 2016) have revealed that the effects of ethephon treatments depend on the degree of light exposure to sunlight, so that the reduction in must TA would be produced only when spraying is performed on directly exposed clusters.

A slight increase in must pH can be associated with ethephon treatments (Table 2), with significant differences in 2006, as reported by others (Delgado and Martín, 2002; Gallegos et al., 2006; Shulman et al., 1985).

The soluble solids content in harvest musts were similar in grapes treated with ABA and untreated (Table 2), consistent with other authors' findings (Amiri and Parseh, 2011; Delgado et al., 2004). Nevertheless, as with ethephon, ABA tended to decrease the TA and raise the pH of the must in all seasons studied, which is consistent with previous results obtained in table grape cultivars (Omran, 2011; Peppi et al., 2007; Reynolds et al., 2016; Yamamoto et al., 2015). However, most studies in the literature have not observed changes in must TA associated with the application of ABA (Cantin et al., 2007; Delgado et al., 2004).

As a consequence, the must from grapes treated with ethephon or ABA (Table 2) tend to reach higher values in the soluble solids / titratable acidity ratio than untreated grapes in all seasons, registering significant differences in 2004 and 2006. The additive effect of both growth regulators on this technological maturity index could be exploited by accelerating fruit ripening in cold-climate grape growing regions.

There were no differences between experimental treatments in terms of color intensity of the must in any season (absorbance at 420 nm from 0.20 to 0.27), which is consistent with the results obtained by Uzquiza et al. (2013), when applying ethephon to 'Verdejo' clusters close to harvest. In the present study, must potassium concentration (from 929 mg L$^{-1}$ to 1362 mg L$^{-1}$) remained unaffected by both ethephon and ABA.

## Wine composition and quality

As a result of its effects on must composition, the application of ethephon tended to increase the ethanol, and decreased malic and tartaric acid concentrations of the wine compared to controls (Table 3). Citric acid concentration also tended to decline in wines made from treated grapes. A higher concentration of potassium, together with the decrease in TA produced by ethephon, caused a rise in the pH of the wine from treated grapes in all study seasons. This contribution to increasing must and wine pH is a negative effect of ethephon, particularly in warm climates where the TA of winegrapes tends to be too low. High pH values increase the relative activity of harmful micro-organisms, lower the color intensity, bind more sulphur dioxide and reduce the free $S0_2$ concentration in the wine, and can reduce the ability of wine to age (Jackson and Lombard, 1993).

Table 3 – Main effects of ethephon treatments on wine composition parameters in the three seasons studied.

| Season | Ethephon | Alcoholic grade | Titratable acidity | pH | Potassium | Tartaric acid | Malic acid | Citric acid |
|---|---|---|---|---|---|---|---|---|
| | mg $L^{-1}$ | % | g tartaric $L^{-1}$ | | mg $L^{-1}$ | | g $L^{-1}$ | |
| 2004 | 0 | 11.9 a | 7.31 a | 3.02 b | 631 b | 3.77 a | 3.24 a | 3.56 a |
| | 1500 | 12.1 a | 6.20 b | 3.14 a | 788 a | 3.01 b | 3.06 b | 3.26 b |
| 2005 | 0 | 11.2 a | 6.97 a | 3.07 b | 834 b | 4.91 a | 2.36 a | 2.71 a |
| | 1500 | 11.4 a | 6.58 a | 3.13 a | 919 a | 4.44 b | 2.15 a | 2.67 a |
| 2006 | 0 | 11.7 a | 6.96 a | 3.15 b | 768 b | 4.23 a | 3.21 a | 3.61 a |
| | 1500 | 11.9 a | 6.41 a | 3.32 a | 946 a | 3.48 b | 3.04 b | 3.52 a |

Within seasons, means with different letters are significantly different ($p < 0.05$, t-test).

ABA applications did not modify any wine composition parameter (Table 1) except the alcoholic grade, which increased in treated grapes compared to untreated controls in 2005 (13 % *versus* 11%, $p < 0.05$) and 2006 (12 % *versus* 11 %, $p < 0.05$). Contrary to these results, Luan et al. (2014) have showed that ABA treatments decreased the TA and increased pH of 'Yan 73' and 'Cabernet Sauvignon' red wines, compared with the controls.

Neither ethephon nor ABA influenced wine glycerol concentration (Table 1), recording average values of 5.8 g $L^{-1}$ in 2004, 5.2 g $L^{-1}$ in 2005, and 7.1 g $L^{-1}$ in 2006. Additionally, the color intensity of wines was similar in treated grapes and controls. This parameter reflects the degree of oxidation, which would increase when the pH was higher (Bonorden et al., 1986).

As well as described for wine composition analysis, ABA and ethephon applications affected the organoleptic parameters in a different way (Table 1). Wines from grapes treated with ethephon in 2005 and 2006 received a higher score on flavor quality than controls (Figure 1), whereas grapes treated with ABA produced, in 2004, wines with poorer aroma quality than controls (Figure 2). In line with these effects, the wines made from ethephon treated grapes tended to obtain the highest global value in the sensory analysis throughout the trial in the three seasons studied, while the wines corresponding to ABA application recorded a lower overall score than controls in 2005 (Figure 3).

The correlation coefficients between composition parameters and sensory properties (Table 4) reveal that, in general terms, the tasters gave higher scores in aroma and flavor (quality and intensity) to the wines with a higher alcoholic grade, malic and citric acid concentration, and lower concentration of tartaric acid. The equilibrium between acidity and sweet taste of alcohol is crucial for balanced quality in dry white wines (Peynaud, 1978), especially in those obtained from grapes with great aromatic potential such as 'Verdejo'.

Some of the aroma and flavor substances are confined mainly to the skin, and accumulate during the final stages of fruit ripening (Keller, 2015), so that it is to be expected that more advanced maturity in grapes will be translated into increases in wine quality. Nevertheless, in the present study, this impact has been observed with ethephon but not with ABA treatments. Exogenous ABA and ethephon can enhance the total phenolic content in the grapes, but its effects on individual phenolic compounds could be different. Similarly, ripening of aroma compounds in the skin might be affected differently by both growth regulators, and may also produce wines with different quality levels. Further research on these points is needed.

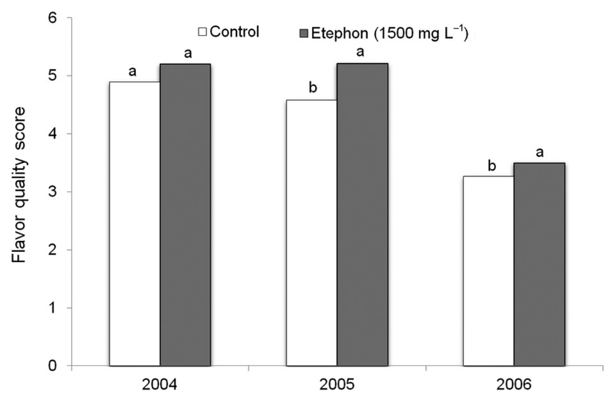


**Figure 1** – Influence of ethephon treatments on the flavor quality score of wines (within a season, values with different letters are significantly different, $p < 0.05$).

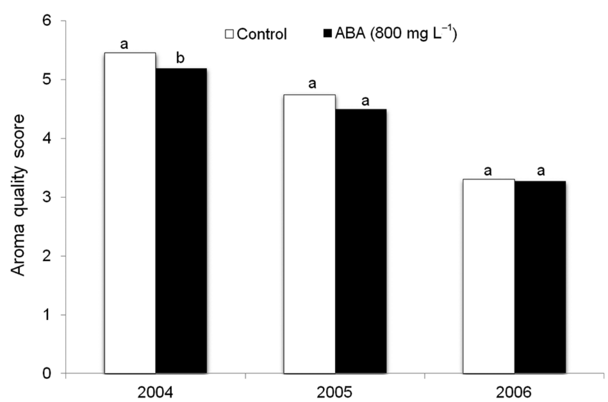


**Figure 2** – Influence of ABA treatments on the aroma quality score of wines (within a season; values with different letters are significantly different, $p < 0.05$).

**Table 4** – Pearson correlation coefficients between wine composition parameters (rows) and organoleptic test scores (columns) in the whole trial in the 2004, 2005 and 2006 seasons (n = 48).

| Parameters | Color intensity | Aroma quality | Aroma intensity | Bitterness | Flavor quality | Flavor intensity | Overall assessment |
|---|---|---|---|---|---|---|---|
| Alcoholic grade | 0.46** | 0.29** | 0.20* | 0.16 | 0.31** | 0.29** | 0.29** |
| Total acidity | 0.02 | 0.20* | 0.10 | 0.01 | 0.12 | 0.08 | 0.13 |
| pH | 0.04 | -0.08 | 0.01 | -0.02 | -0.02 | 0.01 | -0.04 |
| Tartaric acid | -0.54** | -0.35** | -0.09 | -0.17 | -0.36** | -0.23** | -0.33** |
| Malic acid | 0.50** | 0.50** | 0.17* | 0.17* | 0.47** | 0.28** | 0.43** |
| Citric acid | 0.60** | 0.53** | 0.19* | 0.22* | 0.50** | 0.36** | 0.47** |
| Glycerol | 0.32** | 0.12 | 0.18* | 0.08 | 0.16 | 0.22* | 0.13 |
| Potassium | -0.34** | -0.27** | -0.13 | -0.12 | -0.23** | -0.13 | -0.20* |

*Significant $p < 0.05$; **Significant $p < 0.01$.

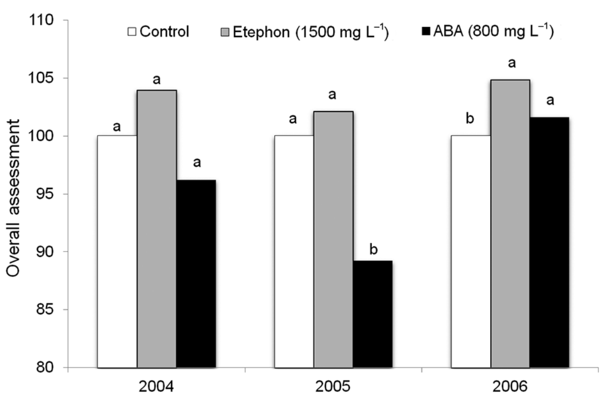


**Figure 3** – Overall assessment score in the sensory analysis of the wines referred to the controls (average value = 100) in the three seasons studied. Within a season, values with different letters are significantly different ($p < 0.05$).

## Conclusion

Both ethephon and ABA applications allow for increases in the sugar / acidity ratio of the must of Verdejo' grapes, which could be exploited to accelerate grape ripening in cold-climate zones. However, the impact of each growth regulator on the composition, aroma and flavor of the wines is very different. The wines derived from treatments with ethephon, with lower concentrations in organic acids than controls, presented the best overall evaluation in the sensory analysis throughout the trial.

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