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#### **Changes in Grape Maturity Induced by Spraying Ethanol**

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#### Abstract

Three different ethanol solutions were sprayed onto Cabernet Sauvignon (*Vitis vinifera* L.) clusters during the ripening period: 2.5, 5 and 10% by volume in water. Controls were sprayed with water alone. Three different times of spraying were also tested: 8, 10 and 13 weeks post-flowering. One of the observed changes was a lower titratable acidity in grape samples at harvest, when the clusters were sprayed with ethanol at 10 weeks, in comparison with controls. The wines made with grapes treated with ethanol after mid-veraison, had higher ODs at 520 nm than did the controls. This may due to a combined effect of red pigment levels and acidity. In addition, following malolactic fermentation, the acidity levels of wines made with ethanol-treated grapes were slightly higher than those made with the control grapes. Spraying ethanol at 13 weeks post-flowering increased the berry weight by 10% at harvest without decreasing the °Brix value. The corresponding wines had similar degrees of alcohol. This observation was made for the first time in 2001.

#### **INTRODUCTION**

The application of exogenous ethanol has been reported to inhibit or promote tomato fruit ripening in a dose-dependent manner, correlated to ethylene evolution (Beaulieu and Salveit, 1997). Another report showed that ethanol increased the anthocyanin content of cranberries when used in combination with ethephon (Farag et al., 1992). In a recent study (Chervin et al., 2001), we observed that spraying an aqueous solution of ethanol onto grapes at veraison enhanced internal ethylene production, the absorbance at 520 nm of acidified skin extracts, and related wine color. However, the experimental plan for ethanol dose and spray timing was not optimal. The purpose of the current study was to improve on the design of the experiment. In addition, as the application of 5% ethanol solutions have been tested over the last three years, effects of ethanol sprays on wines made for each of the years are also reported here.

#### **MATERIALS AND METHODS**

The experimental plot was composed of Cabernet Sauvignon vines grafted onto Richter 110 grown in a non-irrigated vineyard (Toulouse, France). Blocks (experimental units) of five vines were sprayed with an aqueous solution of ethanol at four different concentrations: 0, 2.5, 5 and 10%, and at a rate equivalent to 200 L/ha when sprayed only on clusters. The spraying was performed with a hand-held unit at three different dates: 8, 10 and 13 weeks post-flowering, with 10 weeks post-flowering corresponding to midveraison (50 % of the berries had turned red on 50 % of bunches). Three complete replications were set up in three different rows. Fruit was harvested on 4 October, approximately 17 weeks post-flowering. Wines were made with batches of 12 to 15 kg of fruit. A week before harvest, grapes from vines that were treated on the second and third application dates were riper than those treated on the first date (data not shown), and were combined because the five vines did not yield sufficient fruit for wine making. Each of the replicates was kept separate. Wine making was performed according to Sipiora and Gutierrez Granda (1998). Briefly, the maceration of the skins and alcoholic fermentation ran for two weeks in 30 L tanks following inoculation with EC 1118 yeast strain (Martin Vialatte, Epernay, France). Free-run juices and pressings were combined. Malo-lactic fermentations were performed with the UVA Ferm Beta bacterium strain (Lallemand, Toulouse, France). Grape and wine analyses were performed according to Iland et al. (2000). Titratable acidity was assayed with a pH end-point of 7. Wine pH was not adjusted to a similar value prior to spectrophotometric readings.

#### **RESULTS AND DISCUSSION**

One of the major effects of ethanol sprays observed was on titratable acidity of the grape juice (Fig. 1). The results are presented as a percentage of the control values which were variable. It is apparent that spraying ethanol at mid-veraison (10 weeks post bloom) had the greatest effect in decreasing the acidity, regardless of the dose of ethanol applied. According to previous findings, the ethanol mainly decreases the malic acid concentration of grape juice while the tartaric acid increases slightly but not significantly (Chervin et al., 2002). Such a slight increase in tartaric acid may have been linked to the higher acidity observed in wines following the completion of malolactic fermentation (Fig. 2). Detailed analyses of the wine acids have not yet been performed.

It is well known that acidity has an effect on the resulting color of wines (Jackson, 2000), as it increases the absorbance of anthocyanins at 520 nm. In our study, we found that the absorbance of wines at 520 nm was enhanced by all ethanol doses (Fig. 3). However, the comparison of Figures 2 and 3 proves that acidity is not the only factor involved in increasing the absorbance at 520 nm. Indeed, the wines made with grapes treated with 2.5 % ethanol had lower acidity and higher absorbance readings at 520 nm than did the wines made with the control grapes. Other studies have shown that ethanol increases the concentration of anthocyanin and related synthesis pathways (El Kereamy et al., 2002). Following the addition of HCl (1%) to the wine samples, differences in color were still significant between the wines made with ethanol-treated grapes and those made with the control grapes (Fig. 4). Thus, the ethanol appears to have an effect on both acidity and pigments, the combination of which may cause additive effects on wine color. In future studies, it may be interesting to analyze the major acids present in the wine samples. To date, we have not tested the effects of ethanol-induced changes in the must or on the yeast fermentation.

In a recent experiment performed in Australia (Reynella, South Australia), we found that ethanol sprays had a negative effect on the red pigments in grapes once they had reached 21 °Brix. (unpublished data). A similar trend was previously observed in France where the untreated controls had the same pigment content of the grapes treated with ethanol (Chervin et al., 2001). This appears to be associated with grapevines that are grown in viticultural regions or vineyards that are exposed to warm, sunny weather. The vineyards at Reynella and Toulouse fit into this category as the mean January and July temperatures, respectively, are normally above 19°C.

It is also possible that spraying ethanol too late (four weeks before harvest in Australia) had a smaller impact on anthocyanin biosynthesis than spraying at veraison. However, we did not find any differences in the level of red pigments in the grape skins (methanol extraction) at harvest in all batches from the 2001 experiment in France (data not shown), even though the wine showed differences in color (Figs. 3 and 4).

Unfortunately, variation was introduced into the experiment in 2001 because each replicate of the grape samples was analyzed by a separate student. However, it is worth noting that the analyses of wine color were conducted over three consecutive years (Fig. 5).

When the grapes were sprayed at the later date in France (13 weeks after full bloom and four weeks before harvest) as in Australia, a slight but significant increase in berry weight was observed (Fig. 6), without a change in °Brix. The level of alcohol in the wines was not different (Fig. 7). This trend was also observed for berry weight in the experiment conducted in Australia (unpublished data). However, further studies are required in this area.

Finally, it is worth mentioning that all sprays applied in France using a back-pack unit. Therefore, the ethanol dose delivered per hectare may have been different from that which would be delivered by a commercial sprayer.

#### ACKNOWLEDGEMENTS

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### Figures

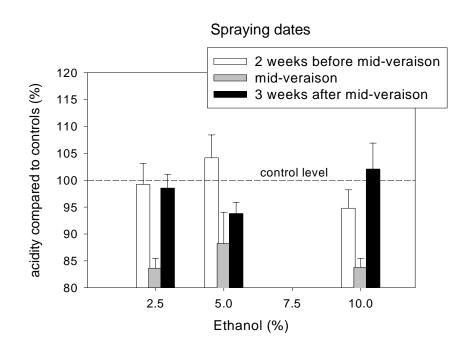


Fig. 1. Effect of spraying ethanol at different dates on grape juice acidity at harvest as a percentage of the control mean acidity, n = 3, error bars represent SE.

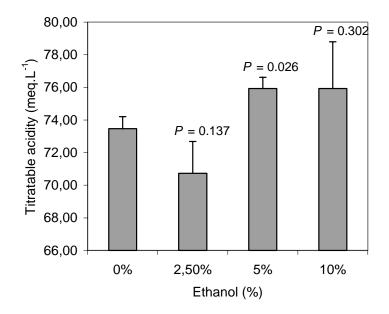


Fig. 2. Effect of spraying ethanol after mid-veraison on the wine acidity following malolactic fermentation, n = 3, error bars represent SE, and *P* the probability that the control mean (0%) is equal to treatment means.

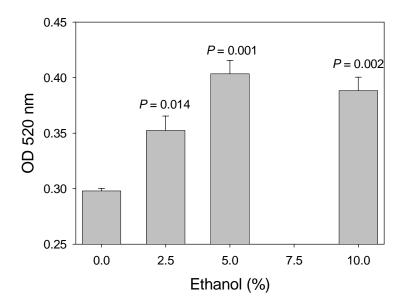


Fig. 3. Effect of spraying ethanol after mid-veraison on the wine OD at 520 nm six months after harvest. Measures were performed in 1 mm quartz cuvette without dilution, n = 3, error bars represent SE, and *P* is the probability that the control mean (0%) is equal to the treatment mean.

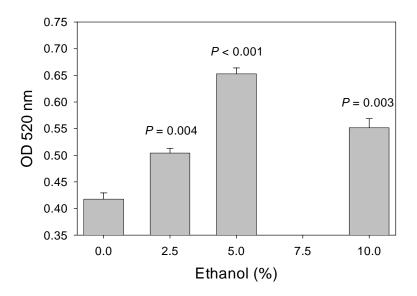


Fig. 4. Effect of spraying ethanol after mid-veraison on the wine OD at 520 nm six months after harvest, after addition of HCl 1% (v/v). Measures were performed in 1 mm quartz cuvette without dilution, n = 3, error bars represent SE, and *P* is the probability that the control mean (0%) is equal to the treatment mean.

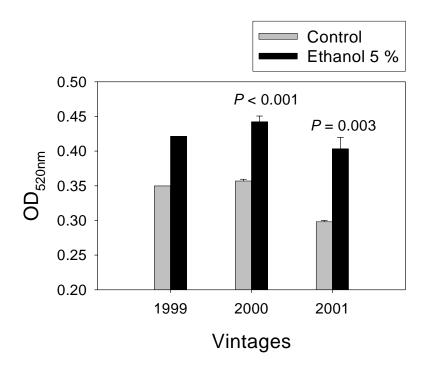


Fig. 5. Effect of spraying ethanol after mid-veraison on the wine OD at 520 nm. Measures were performed in 1 mm quartz cuvette without dilution, n = 3, except for 1999 where n = 1, error bars represent SE, and *P* is the probability that the control mean is equal to the treatment mean.

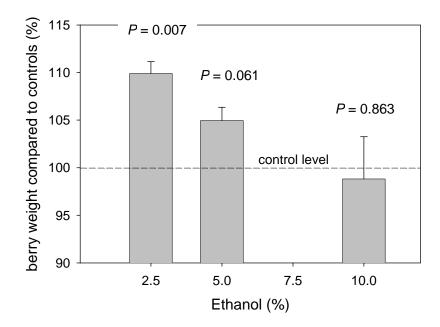


Fig. 6. Effect of spraying ethanol three weeks after mid-veraison on the berry weight as a percentage of the control mean, n = 3, error bars represent SE, and *P* is the probability that the control mean is equal to the treatment mean.

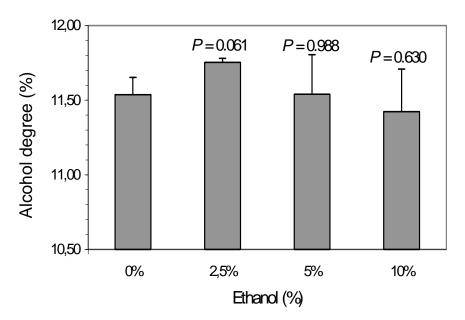


Fig. 7. Effect of spraying ethanol after mid-veraison on the wine alcohol degree, n = 3, error bars represent SE, and *P* is the probability that the control mean (0%) is equal to the treatment mean.