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Research Note

Ethanol application at veraison decreases acidity in Cabernet Sauvignon grapes

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Summary: Spraying ethanol (5 % v/v in water) onto grape clusters at mid-veraison led to a 30 % drop in the malic acid concentration at harvest. As a consequence, titratable acidity also dropped by 10 %. The concentration of tartaric acid did not change significantly. The mode of action of ethanol on malic acid metabolism is discussed.

K e y w o r d s : *Vitis vinifera*, ripening, malate, tartrate, ethanol, acidity.

Introduction: As shown previously, spraying aqueous solutions of ethanol onto Cabernet Sauvignon clusters at mid-veraison increased the ethylene concentration in berries in comparison to controls sprayed with water (Chervin *et al.* 2001). This is similar to results obtained after spraying ethanol solutions onto tomatoes (Beaulieu and Saltveit 1997). Since ethylene is known to increase plant cell respiration (Abeles *et al.* 1992) which is known to catabolise some of the malate pool in grapes (Ruffner 1982), we studied the influence of ethanol sprays on grape acidity.

Material and Methods: The experiments were conducted over three consecutive years (1999-2001) in a vineyard close to Toulouse, southwestern France. Cabernet Sauvignon vines (*Vitis vinifera* L.) grafted to 110 Richter rootstocks (*Vitis Berlandieri* x *V. rupestris*) were used. Bunches were sprayed with an aqueous solution of ethanol (5 % v/v) using a back-pack spray unit (200 $l \cdot ha^{-1}$). Controls were sprayed with water. This was done when approximately 50 % of the berries had started to change colour, 8 to 9 weeks after full bloom. Observations by Hale *et al*. (1970) suggest that grapes respond to ethylene only after veraison is initiated. At harvest, 8 weeks later, batches of 60 berries were picked from a block of 5 vines. Three blocks were used per treatment. The experimental blocks were set up on different vines each year. For analyses, berries were weighed and then blended for 2 min in a Seb blender, 15 ml of this slurry were then centrifuged for 3 min at 2,000 *g* at room temperature. Brix values were obtained with a hand-held refractometer (Atago, Japan) using 200 µl of clear supernatant. Titratable acidity was assessed using 5 ml of supernatant with 0.1 NaOH (to a pH of 7.0). The concentrations of malic and tartaric acids were assessed by capillary electrophoresis on aliquots of the remaining supernatant as described by Arellano *et al*. (1997).

Results and Discussion: Compared to controls titratable acidity of grape juice at harvest was reduced by 11 % due to ethanol sprayed at veraison (Table). This reduction was significant at the 0.05 % level, even though calculation was based on sets of data from three consecutive seasons with different weather conditions and different sites in the vineyard with individual effects on vigour and ripening (data not shown). Detailed analyses of the two major grape acids showed that the decrease in acidity was due to a significant decrease (33 %) in the malic acid content in ethanol-treated grapes, compared to the controls (Table). Tartaric acid concentration was not significantly altered and there was no significant change in the pH of the must.

The decrease in titratable acidity can be explained by the change in malic acid content. Calculations showed that cation contents were unaffected (Table). This may indicate that there was no change in the rate of phloem loading between ethanol-treated and control plants. Potassium accumulation was shown to be a continuous process in ripening berries (Possner and Kliewer 1985).

T a b l e

Berry juice acidity and calculated cations at harvest, as a function of spraying Cabernet Sauvignon clusters at veraison with an aqueous ethanol solution (5 %); n=9 (over three seasons, 1999 to 2001)

	Malate (meq/l)	Tartrate (meq/l)	Titratable acidity (meq/l)	pH	Calculated cations (meq/l)
Control	33.72 ± 10.28	46.54 ± 10.08	66.94 ± 5.75	3.38 ± 0.09	13.3
Ethanol-treated	22.40 ± 5.85	50.90 ± 9.23	59.48 ± 7.13	3.34 ± 0.15	13.8
t-test (*P* value)	0.016	0.380	0.035	0.530	

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The mean Brix values of the batches were not significantly different (19.2 ± 0.9 for controls and 20.0 ± 1.0 for treated grapes; means ± SE with n = 9 over three seasons; t-test $P = 0.114$). Thus the differences in acidity induced by ethanol cannot be explained by a global increase in maturity. They cannot be explained by a difference in berry weight either (1.30 ± 0.21 g for controls and 1.29 ± 0.19 g for treated grapes; means ± SE with n = 9 as above; t-test $P = 0.904$).

In a recent study, we reported that ethanol treatment enhanced colour of red wines and suggested that ethanol affects anthocyanin accumulation through an increased ethylene production (CHERVIN *et al*. 2001). The decrease in the malic acid content, observed here, agrees herewith, since the malate is a substrate of both respiration and gluconeogenesis. The rates of both of these pathways happen to be increased by ethylene (BEAUDRY *et al*. 1989; ABELES *et al*. 1992). However, ethanol may also act independently of ethylene. It is well known that malate respiration begins simultaneously with sugar accumulation in berries, just before colour changes (RUFFNER 1982; COOMBE 1992). The onset of sugar storage is accompanied by a compartmentation breakdown of the apoplast, phloem unloading strongly increasing as a result of xylem rupture (LANG and DÜRING 1991; COOMBE 1992; DREIER *et al*. 1998). Regarding the effects of ethanol on phloem unloading we got no clear indication about changes in water, sugars and potassium (berry weight, Brix and calculated cations). Malate respiration may result from increased permeability of the vacuolar membrane (TERRIER *et al*. 2001). Ethanol and its oxidation product, acetaldehyde may conceivably increase malate decompartmentation and respiration (TERRIER and ROMIEU 1998).

Whether the treatment could have commercial applications or not will depend on further research on and development of commercial sprayers. Indeed the optimal ethanol concentration will vary according to the spraying equipment.

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