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Potential for ethanol vapours to limit table grape berry shatter and to limit ethylene evolution from clusters.

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Abstract:
We have shown previously that ethanol vapours (given by 2 ml per kg of grapes) can prevent Botrytis development and stem browning, two of the major problems in postharvest quality of table grapes. In the present paper, we will give emphasis to preliminary results about (i) the role of ethanol vapours in the inhibition of berry shatter and (ii) the control of ethylene evolution from grapes bunches by ethanol vapours and the link to the control of Botrytis.

INTRODUCTION

Ethanol is known to influence ripening and senescence (Podd and Staden, 1998), reduce decay (Gabler and Smilanick, 2001) and kill insect contaminants (Dentener et al., 1998). Table grapes are routinely treated with sulfur dioxide (SO_2) to reduce the incidence of postharvest decay during storage and transportation; however SO_2 treatment may cause damage to the grapes and result in sulfite residues which are unacceptable to some consumers. Application of ethanol to table grapes by dipping has been shown to effectively improve storage, mainly by limiting botrytis growth (Lichter et al., 2002; Karabulut et al., 2003). In the search to adapt such a treatment to commercial practices of placing SO_2 pads on the top of the grape crates, we have investigated the efficacy of paper pads soaked in ethanol in order to generate vapours to control rots. We already published the first year results (Chervin et al., 2003).

MATERIAL AND METHODS

Chasselas grapes were picked in a local vineyard (Montauban, France) at the end of September each year and packed in 5 kg wooden crates. The experiment was set-up as follows, including five treatments: control, one SO_2 pad per crate, ethanol 1.25 ml/kg, ethanol 3.75 ml/kg and ethanol 7.5 ml/kg. The crates were stored at 0°C for one or two months. The experimental unit was a 5 kg crate, replicated 3 times for each treatment and storage duration. At the end of each storage period, the bags were removed and crates were left at 8°C for half an hour to limit condensation on the fruit and then transferred to ambient temperature. Quality assessments were performed 3 days later. Botrytis rot incidence was visually assessed by counting the number of affected berries per cluster on all the clusters in each crate, when the average number of rotten berries per cluster exceeded 20, the cluster was considered as "rejected", i.e. not suitable for sale. Indeed, during the packaging process, the manual removal of rotten berries is in use in some areas. Berry shatter was assessed by shaking one cluster randomly chosen from each crate, twice (Ahumada et al., 1996).

Ethanol concentration in the crate headspace was measured with a Dräger pump fitted with specific glass tubes. Internal ethylene was assessed by gas chromatography as described previously (El-Kereamy et al., 2003), with a five minute incubation time under -700 mm Hg partial vacuum in a NaCl saturated solution to limit ethylene solubility. The laccase activity was assessed according to Grassin and Dubourdieu (1989).

RESULTS AND DISCUSSION

We observed over the two year experiment that a dose of 2 ml ethanol / kg of fruit was sufficient to control Botrytis growth, without affecting the fruit sensory quality assessed by consumer panels. These results have been published elsewhere. Overall, a dose of five ml ethanol / kg of fruit gave headspace concentrations of 200 to 400 ppm of ethanol at 0°C.

Would berry shatter be reduced by ethanol vapours?
In a preliminary set of experiment in 2001, we studied the potential anti-shatter role of the ethanol vapours and found that indeed they were reducing berry shatter when applied after the cold storage (Table 1).

This early set of results was confirmed in a another set of experiments (Chervin et al., 2003). This effect on berry shatter may be directly due to effects of ethanol on Botrytis development, and we did not design experiments to differentiate between shatter due to Botrytis and "physiological" shatter. However, Chasselas may not be the best cultivar to illustrate this point as it is not known to present serious shatter problems over the postharvest period.

Would ethanol vapours reduce Botrytis development by reducing ethylene evolution from grapes?
This question came from the knowledge that ethylene is known to be associated to Botrytis development (Qadir et al., 1997) and that ethanol reduces ethylene evolution from treated fruit (Beaulieu and Saltveit, 1997).

We observed that gassing the grape clusters with 1 ppm ethylene as they were taken out of cold storage increased the percentage of rots in comparison to controls, at the end of a three day period at 20°C (Figure 1).

The results of Figure 2 show that ethanol in the headspace over a two month period of cold storage inhibited the ethylene evolution over the first hours of re-warming at the end of the cold storage, this ethanol effect was strong whatever the ethanol rate in the crate at the beginning of storage. However by comparing with the results of the Figure 3, the limited ethylene evolution does not seem to be a cause of limited rejection due to rots, e.g. there was a strong inhibition of ethylene evolution at a 1.25 ml/kg dose compared to control (Figure 2), whereas there was as much rejection in the 1.25 ml/kg samples than in controls (Figure 3).

Moreover a series of treatments with 1-MCP (1-methylcyclopropene, blocker of the ethylene receptors), applied over the first hours at 20°C following the removal of the crates from the cold store, did not limit rot development during the 3-day period at 20°C (data not shown).

The Figure 4 shows that the pattern of laccase activity as a function of the ethanol doses is not well correlated to the ethylene evolution pattern of Figure 2. This reinforces the previous comment about the link between ethylene and fungus development.

CONCLUSION

Overall there is no evidence to indicate whether ethylene is a cause or a consequence of fungus development in these experiments.
Regarding berry shatter, ethanol vapours may have some inhibiting effects; it should be checked over cold storage with a cultivar that is more sensitive to shatter than Chasselas. Whether this shatter inhibition is due to a direct effect of ethanol on shatter or an indirect effect through inhibition of Botrytis development is unclear.

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Table 1: Reduction of Chasselas berry shatter by ethanol vapours. The ethanol treatment was performed after one month cold storage, with 3.75 ml of ethanol / kg of fruit for 3 days at 20°C, followed by one week in air at 20°C before shatter assessment.

Control	402 berries dropped off 94 clusters (76 % of the fallen berries were rotten)
Ethanol	81 berries dropped off 99 clusters (23 % of the fallen berries were rotten)

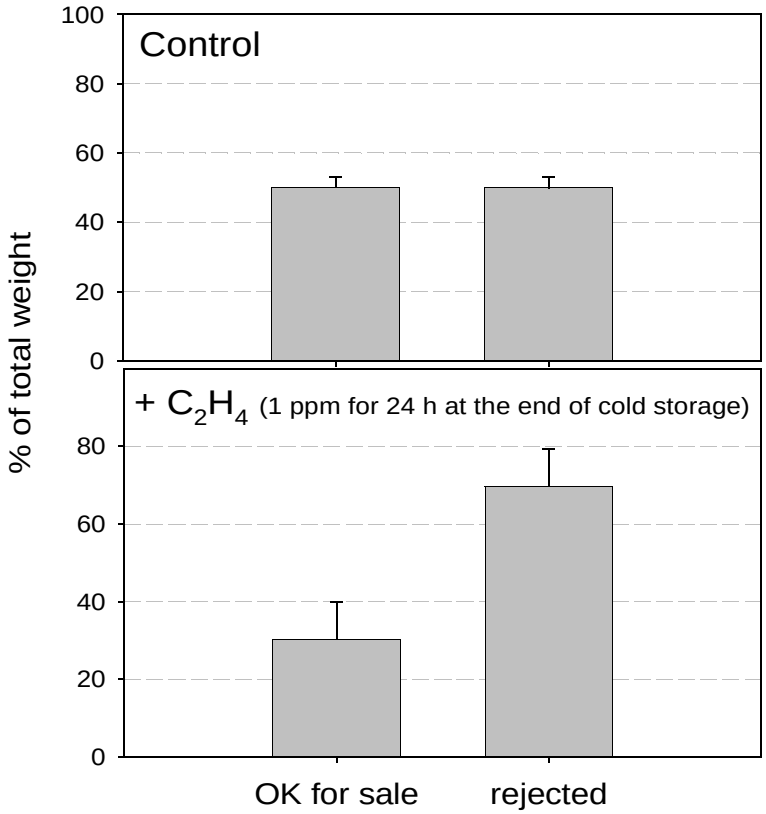

Figure 1: Effect of gassing Chasselas grapes with 1 ppm ethylene on the percentage of rejection due to rots, visual assessment after 3 days at 20°C; gassing was performed when the crates were removed from cold storage after a 2 month period at 0°C and transferred at 20°C; n = 3 replicates of 5 kg each, error bars show SE.

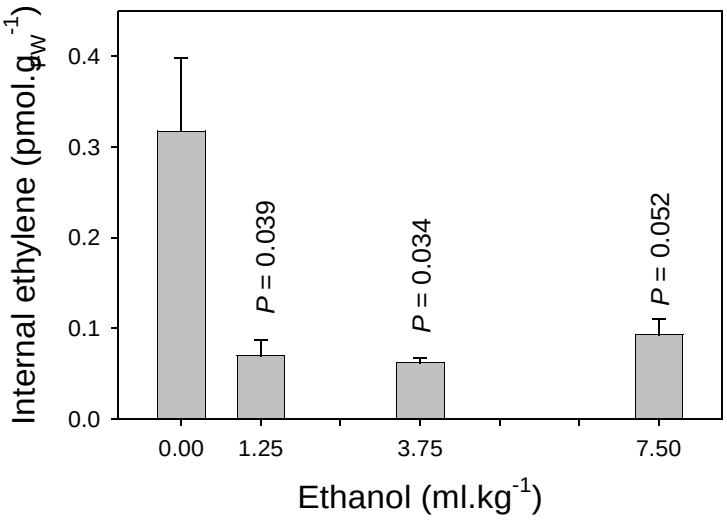

Figure 2: Effect of various ethanol doses on the ethylene evolution from Chasselas grapes, after 2 to 4 hours at 20°C, just after removing from cold store (2 months at 0°C); n = 3 clusters, error bars show SE, *P* is the probability of the mean to be equal to control mean.

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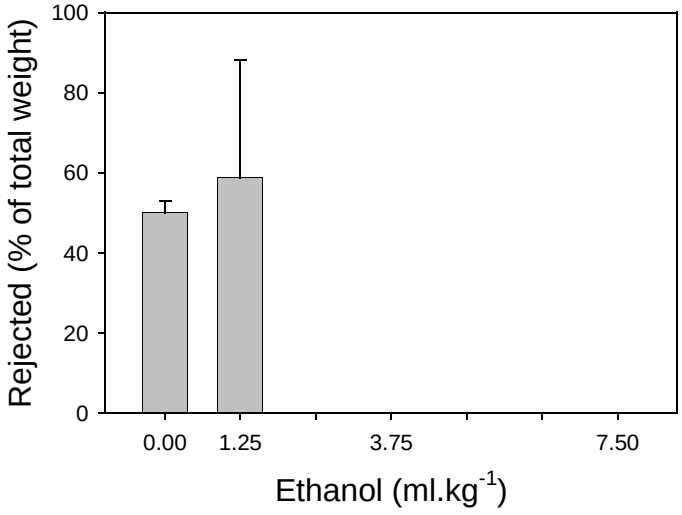

Figure 3: Effect of various ethanol doses on the percentage of rejection due to rots in Chasselas grapes, after a 2 month period at 0°C, plus three days at 20°C; n = 3 replicates of 5 kg each, error bars show SE.

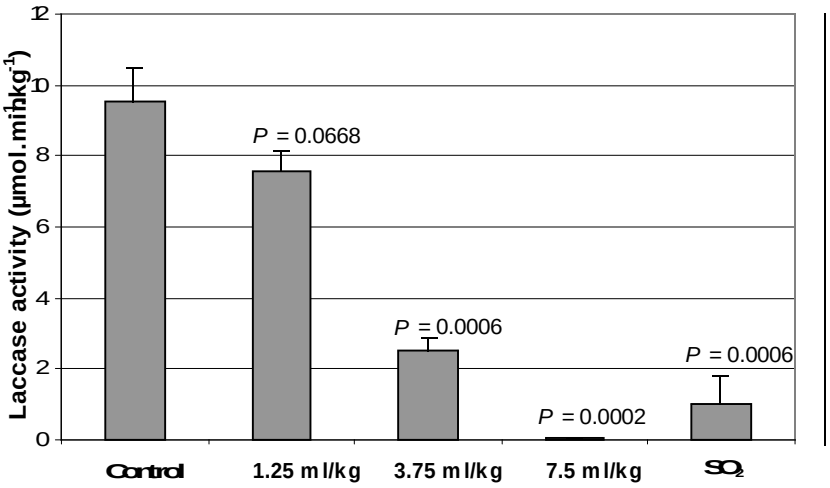

Figure 4: Effect of various ethanol doses on the laccase activity assayed in extracts of Chasselas berry tissues, stored for 2 month at 0°C plus 3 days at 20°C; n = 3, error bars show SE, *P* is the probability of the mean to be equal to control mean.