To link to this article : http://dx.doi.org/10.1016/j.postharvbio.2009.06.005

Reduction of gray mold development in table grapes by preharvest sprays with ethanol and calcium chloride.

Christian Chervin[1], Daniel Lavigne[2], Pascale Westercamp[3]

[1]UMR 990, INRA, INPT, ENSAT, Université de Toulouse, BP 32607, 31326 Castanet, France
[2]CEFEL, 49 Chemin des Rives, 82000 Montauban, France
[3]CTIFL, CEFEL, 49 Chemin des Rives, 82000 Montauban, France
Corresponding author: chervin@ensat.fr

Abstract:

Preharvest applications of a 16% ethanol (EtOH) solution, containing 1 % of calcium chloride ($CaCl_2$), reduced gray mold development in 'Chasselas' table grapes picked at a late harvest date, the losses due to rotten clusters dropped from 15% in controls to 5% in grapes treated with EtOH+$CaCl_2$. Then over a 6-week cold storage, the losses due to gray mold rots were reduced by 50% when storing EtOH+$CaCl_2$ treated clusters, compared to untreated controls. Preliminary experiments had shown that a 2% EtOH solution was already inducing significant drop of gray mold growth. A range of concentrations up to 50% ethanol had been tested in preliminary trials without observing damages to the vines and clusters. The treatments did not induce significant changes to the fruit quality assessed by sensory analyses on healthy berries.

Introduction

Gray mold rots developing in grapes over the postharvest period are causing severe losses. Solutions to limit pre-harvest treatments with synthetic fungicides are of particular interest to the grape industry as chemical residues are limiting access to many markets, and there is a diminishing number of antifungal compounds that are still registered (Nigro et al., 2006). Many solutions have been studied; one that attracted our attention is the preharvest application of 50% ethanol that proved to be efficient in reducing the rots over the postharvest period (Karabulut et al., 2003). However the quantity of solution sprayed was very high, one liter per five vines, and the preharvest delay very short, 24 hours. In another study, some of these authors further developed the use of ethanol by reducing it (10 to 20%) and combining it with chitosan (Romanazzi et al., 2007), but these treatments were performed postharvest, as were the treatments reported by Lurie et al. (2006) regarding ethanol effects in reducing rots in table grapes. Additionally, Nigro et al. (2006) showed that a series 1% calcium chloride ($CaCl_2$) pre-harvest sprays was efficient to control postharvest rot development in grapes, cv. Italia.

In the present work, we report the effects of a preliminary set of results about preharvest treatments, using a combination of ethanol and calcium chloride, in limiting the gray mold development on a thin skin cultivar, 'Chasselas', that is very sensitive to such rots. Moreover, the challenge was to harvest late and try to store up to Christmas to match a market demand.

Material and Methods

The grapes (cv. 'Chasselas') were grown in Moissac, South West of France. The 12 year old vines are grafted onto SO4, the trellising is in lyre (3.5 x 1.2 m), and the vineyard is drip irrigated. There were 3 replicated blocks of 10 vines each. The vines were treated with two fungicide treatments (mid-June and mid-July), then alternative treatments with EtOH-$CaCl_2$-water solutions were initiated.

During preliminary trials, we tested four percentages of ethanol (EtOH at 2, 8%, 16% and 50 %), and the spraying schedule was: one spray at the beginning of veraison (beginning of August), one spray during the second half of August, another spray mid-September and a last spray at the beginning of October. Each spray was performed with air-pulsed sprayer onto the fruit zone at 150 $L.ha^{-1}$, according to commercial practices. The controls were not sprayed. The 50% EtOH dose was tested to check the potential damages to foliage and potential off-taste by sensory analysis. Combination 16% EtOH and 1% $CaCl_2$ was sprayed according the schedule and conditions described previously from beginning of August. Harvest was performed at the middle of October and for each block, 8 kg of grapes and were placed in a wood tray, which was wrapped in a polyethylene bag with one SO_2 pad (1 g sodium metabisulfite per kg of grapes) and cold stored at 0 to 1°C for 6 weeks throughout. At the end of the cold storage period, the bags were removed and the grapes were left at shed temperature (approximately 15°C) for 24h.

The percentage of gray mold rot was measured visually; preliminary experiments showed that no other rots develop in such experimental conditions. The decay at harvest was calculated by adding the weight of clusters with more than 5 % rotten berries to the weight of rotten berries removed from clusters with less than 5% rotten berries. After storage, the percentage of commercial grapes was calculated from the weight of clusters that were not discarded as they had less than 5 % of rotten berries, these latter being removed before commercialisation. The sensory analyses were performed by hedonic test (Poste et al., 1991), using 10 cm scoring lines from 0: “I dislike extremely” to 10: “I like extremely”. Using these scales, the 21 panelists (staff from the experimental station and students) were asked to appreciate the overall visual quality and the overall taste quality of small portions of cluster.

The analyses of variances and calculation of *P* and LSD values were performed with DSAASTAT v.1.0192 (Andrea Onofri, DSAA, Perugia, Italy).

Results and Discussion

In the search for synergistic effects, we tested aqueous sprays with 16% EtOH, that was shown to be the best dose in preliminary trials with the ‘Chasselas’ cultivar, in combination with 1% calcium chloride that was shown to be an efficient pre-harvest treatment limiting fungal development in table grapes (Nigro et al., 2006).

In Figure 1, the efficacy of the combination EtOH and $CaCl_2$ reducing decay due to gray mold rots is clearly observable. In comparison to controls, there was a 10% reduction of rots at harvest (Figure 1), the fruit being of commercial value reached 95% of harvested grapes (only 5% of decay in the EtOH+$CaCl_2$ treatment plots). This result is very encouraging showing it is possible to replace anti-Botrytis sprays that may be phased-out in the near future.

A storage experiment made with these late harvest grapes showed there was a 50% rot reduction after 6 weeks of storage with SO_2 pads (Figure 2). This would enable the grape industry to propose such grapes during the winter festive season in the Northern hemisphere, and this is a target for local growers.

Sensory analyses using hedonic tests were mainly set-up to check if ethanol & $CaCl_2$ were inducing taste differences, and no significant difference was shown, neither in the visual aspects of the healthy berries ($P = 0.085$), nor in the taste ($P = 0.275$). The probability of the visual difference was not far from the critical value of 0.05, as indeed the treated berries tended to be greener than controls (unshown data), but for the sensory analyses, batches of three berries were given to the panellists, thus the difference of visual aspect was less obvious than in a crate.

It is worth noting that these experiments were performed with thin skin grapes, cv. ‘Chasselas’, very sensitive to Botrytis rots. Other cultivars with thicker skin, like Italia, Muscat, Red Globe and Thompson Seedless, that are commercialised worldwide, may have lower levels of rots in controls and may show even better results using EtOH+$CaCl_2$ combinations.

In a recent study (Nigro et al., 2006), $CaCl_2$ preharvest applications were performed twice before harvest with a two-week-interval. Here we chose to adapt our treatments to the industry spraying schedule already in place. Different schedules may be tested in further studies.

Over the preliminary experiments, the grapes did not present variations in titratable acidity or refractometric index, and the use of 50% ethanol solution in water generated neither foliar damage onto the vines and nor significant visual and taste differences assessed by sensory analyses (data not shown).

The fungicidal effect of ethanol may be due temporally to the ethanol effect *per se* (Karabulut et al., 2005) or the effect of its oxidised form: the acetaldehyde, usually a lot more toxic compound (Pesis, 2005). We were surprised by the noticeable limitation of gray mold development by ethanol doses as low as 2% (data not shown). It is very improbable that ethanol would have had a direct effect on fungus growth at this low percentage as Lichter et al. (2003) showed that at least 30% ethanol are necessary to prevent *Botrytis cinerea* spore germination. Thus the 2% ethanol dose is more likely to induce plant defense either through acetaldehyde signalling (Pesis, 2005) or directly through ethanol as a primary signal (Tomsett et al., 2005).

Conclusion

Finding alternatives to synthetic fungicides, which are under scrutiny nowadays, is of critical importance to many growers. Here we report significant effects of pre-harvest sprays with ethanol and calcium chloride solutions that limit gray mold decay in late harvested 'Chasselas' grapes, and moreover after a cold storage period of six weeks the growers would have sufficient quantities of healthy grapes to access markets over the winter festive season. Further trials with other cultivars should be worth a try, and longer shelf life after cold storage should also be simulated, as actual results are useful for immediate consumption.

Acknowledgements to Jean-Claude Pech, Alain Latché and Mondher Bouzayen, UMR 990, Génomique et Biotechnologie des Fruits, for their support, and for the partial funding by the Midi-Pyrénées regional council. Thanks to two anymous reviewers for their time and comments.

References

Karabulut, O.A., Smilanick, J.L., Gabler F.M., Mansour M., Droby S., 2003. Near-harvest applications of Metschnikowia fructicola, ethanol, and sodium bicarbonate to control postharvest diseases of grape in central California. Plant Dis., 87, 1384-1389.

Karabulut, O.A., Romanazzi G., Smilanick, J.L, Lichter A., 2005. Postharvest ethanol and potassium sorbate treatments of table grapes to control gray mold. Postharvest Biol. Technol., 37, 129-134.

Lichter, A., Zhou, H.W,. Vaknin, M., Dvir, O., Zutchi, Y., Kaplunov, T., Lurie, S., 2003. Survival and responses of *Botrytis cinerea* after exposure to ethanol and heat. J. Phytopath., 151, 553-563.

Lurie, S., Pesis, E., Gadiyeva, O., Feygenberg, O., Ben-Arie, R., Kaplunov, T., Zutahy, Y., Lichter, A., 2006. Modified ethanol atmosphere to control decay of table grapes during storage. Postharvest Biol. Technol., 42, 222-227.

Nigro, F., Schena, L., Ligorio, A., Pentimone,I., Ippolito, A., Salerno, M.G., 2006. Control of table grape storage rots by pre-harvest applications of salts. Postharvest Biol. Technol., 42, 142-149.

Pesis, E., 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. Postharvest Biol. Technol., 37, 1-19.

Poste, L.M., Mackie, D.A., Butler, G. and Larmond E., 1991. Hedonic scaling test. Laboratory Methods for Sensory Analysis of Food, ed. Agriculture Canada, publication 1864/E, pp. 64-67.

Romanazzi, G., Karabulut O.A., Smilanick J.L., 2007. Combination of chitosan and ethanol to control postharvest gray mold of table grapes. Postharvest Biol. Technol., 45, 134-140.

Tomsett, B., Tregova, A., Garoosi, A. and Caddick, M., 2004. Ethanol-inducible gene expression: first step towards a new green revolution? Trends Plant Sci., 9, 159-161.

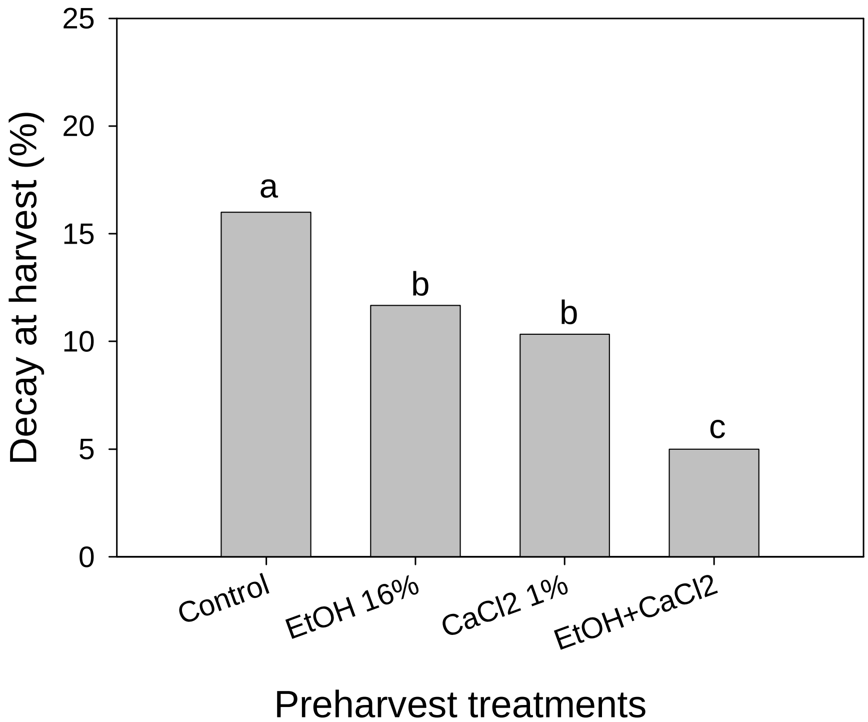

Figure 1: Decay of ‘Chasselas’ grapes due to gray mold at harvest. In the combination, the ethanol and $CaCl_2$ were used at similar concentrations to single treatments; n = 3, mean comparison performed by ANOVA and Tukey HSD test, different letters ‘a’, ‘b’ and ‘c’ show means that are significantly different at the 0.05 level. “EtOH” and “$CaCl_2$” stand for ethanol and calcium chloride sprayed in aqueous solutions at various times before harvest, see Materials and Methods.

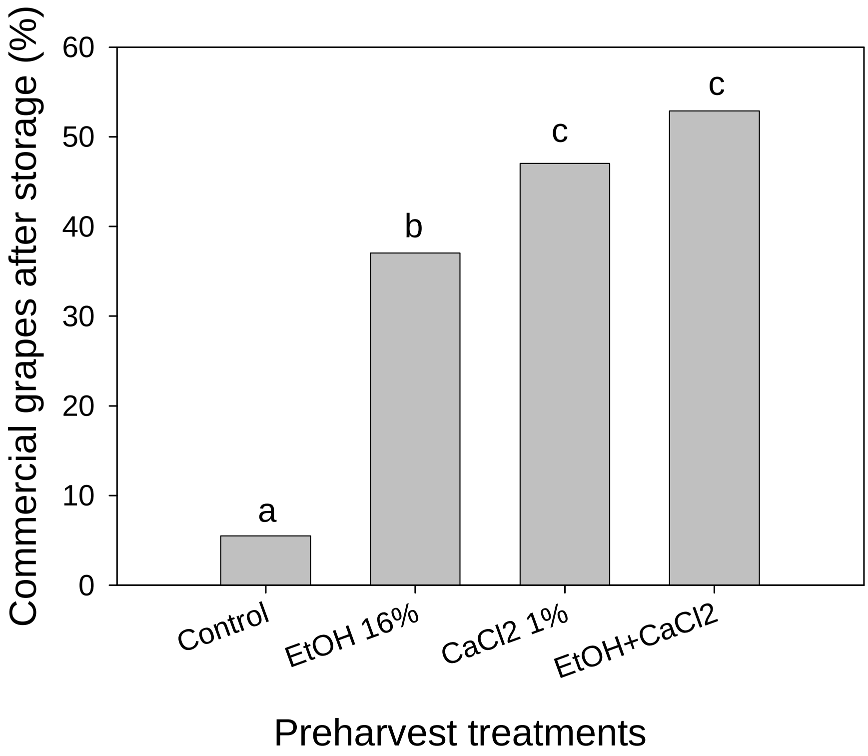

Figure 2: Percentage of commercial ‘Chasselas’ grapes after 6 weeks of cold storage,. In the combination, the ethanol and $CaCl_2$ were used at similar concentrations to single treatments; n = 3, mean comparison performed by ANOVA and Tukey HSD test, different letters ‘a’, ‘b’ and ‘c’ show means that are significantly different at the 0.05 level. “EtOH” and “$CaCl_2$” stand for ethanol and calcium chloride sprayed in aqueous solutions at various times before harvest, see Materials and Methods.