Effect of environmentally friendly vineyard protection strategies on yeast ecology during fermentation

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Introduction

Currently, an increasing concern from governments and consumers about environmental sustainability of wine production provides new challenges for innovation in wine industry (Gurerrero and Contos-Villar, 2015). Accordingly, the application of more-environmentally friendly vineyard treatments against fungal diseases (powdery and downy mildew) could have a cascading impact on yeast ecology of wine production (Rantsiou et al., 2020).

This study evaluated the effect of a wide range of environmentally friendly products (Table 1) applied in the vineyard on grape yeast ecology at harvest, as well as during spontaneous and inoculated fermentations in winery and laboratory scale conditions. Yeast ecology was investigated using culture-dependent (plate counts) and -independent (Next Generation Sequencing) methods. Main oenological parameters and volatile compounds were monitored during spontaneous and inoculated fermentations. Spearman's correlation was used to assess associations between ASVs changes and chemical composition observed over fermentation.

Fungicide treatments

Table 1. Description of the fungicide treatments used, including active ingredients and dosages.

#	Category	Active ingredients	Dose (g/ha)
T01	Untreated contol	Untreated control	-
T02	Alternative chemical treatment	Acibenzolar-S-methyl	100
		Sulphur + Copper hydroxide	3200+600
T03	Alternative chemical treatment	Fosetyl-Al	3200
		Sulphur + Copper hydroxide	3200+600
T04	Alternative chemical treatment	Potassium phosphonate	3020
		Sulphur + Copper hydroxide	3200+600
T05	Alternative chemical treatment	Laminarin + Metiram	90 + 1400
		Laminarin + Copper hydroxide	90 + 600
T06	Alternative chemical treatment	Chito-oligosaccharides and oligogalacturonides + Metiram	31.25+1400
		Chito-oligosaccharides and oligogalacturonides + Copper hydroxide	31.25+600
T07	Alternative chemical treatment	Potassium bicarbonate+Metiram	4250+1400
		Potassium bicarbonate + Copper hydroxide	4250+600
T08	Conventional control chemical treatment	Sulphur + Metiram	3200+1400
		Sulphur + Copper hydroxide	3200+600
T09	Alternative chemical treatment	Calcium oxide	884
T10	Alternative chemical treatment	Calcium oxide + Metiram	884 + 1400
		Calcium oxide + Copper hydroxide	884 + 1400
T11	Conventional chemical treatment	Sulphur	3200
T12	Conventional chemical treatment	Metiram	1400
		Copper hydroxide	600

Conclusion

Yeast ecology in fermenting musts may be correlated to specific antifungal products and inoculation protocol employed, suggesting a link between principal active compounds, yeast biodiversity and wine chemical composition. These relationships could help to further control wine quality and can be used to guarantee quality in the pursuit of a sustainable competitive advantage in the market.

References

Guerrero, R.F., & Cantos-Villar, E. (2015). Demonstrating the efficiency of sulphur dioxide replacements in wine: A parameter review. Trends in Food Science & Technology, 42(1), 27-43.

Rantsiou, K. et al. (2020). Impact of chemical and alternative fungicides applied to grapevine cv Nebbiolo on microbial ecology and chemical-physical grape characteristics at harvest. Frontiers Plant Science, 11, 700.

Results

- Significantly lower yeast populations were found for grape berries treated with T04 (Potassium phosphonate + Sulphur + Copper hydroxide) when compared to the controls (untreated grapes (T01) and grapes treated with Sulphur + Metiram + Copper hydroxide (T08), commonly used as conventional treatment in viticulture).

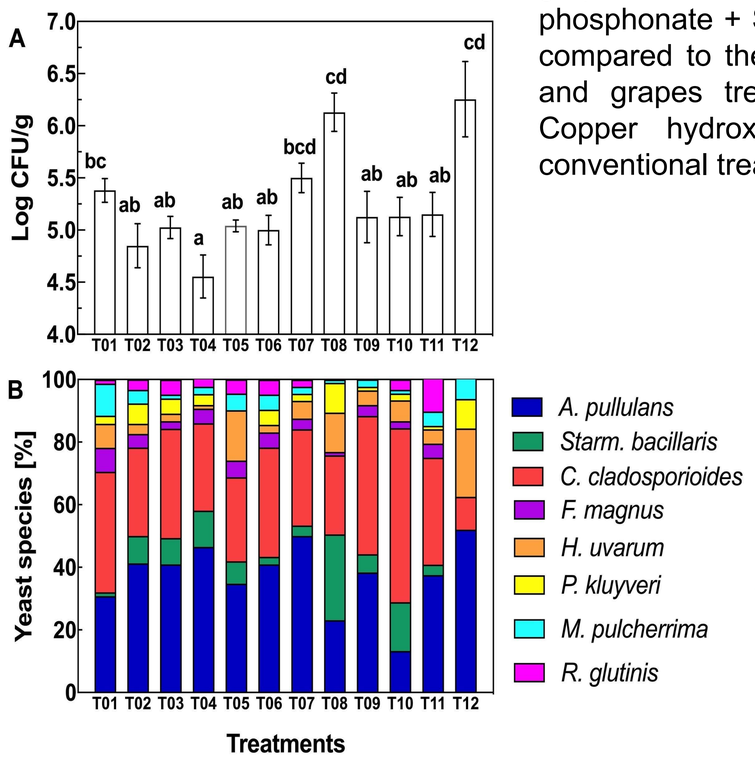

Fig. 1 Total yeast population (Panel A) and yeast species biodiversity (Panel B), registered on Nebbiolo grapes after harvest, as determined by plate counts on WLN medium. Data are expressed as mean ± standard deviation of four biological replicates. Different letters in each column, mean significant differences according to Tukey-HSD test ($P < 0.001$).

- No significant differences were observed among the alternative and conventional treatments, compared to the controls, in terms of alpha diversity index or microbial composition as a function of treatment

- Fermentation data suggested that complete alcoholic fermentation was correlated to the application of antifungal treatment in the vineyard, as well as the inoculation protocol used. Spontaneous fermentations conducted in the laboratory using laminarin treated grapes showed a higher relative abundance of *Saccharomyces cerevisiae* than other treatments. The resultant wine had floral and fruity aroma without an increase in levels of acetic acid.

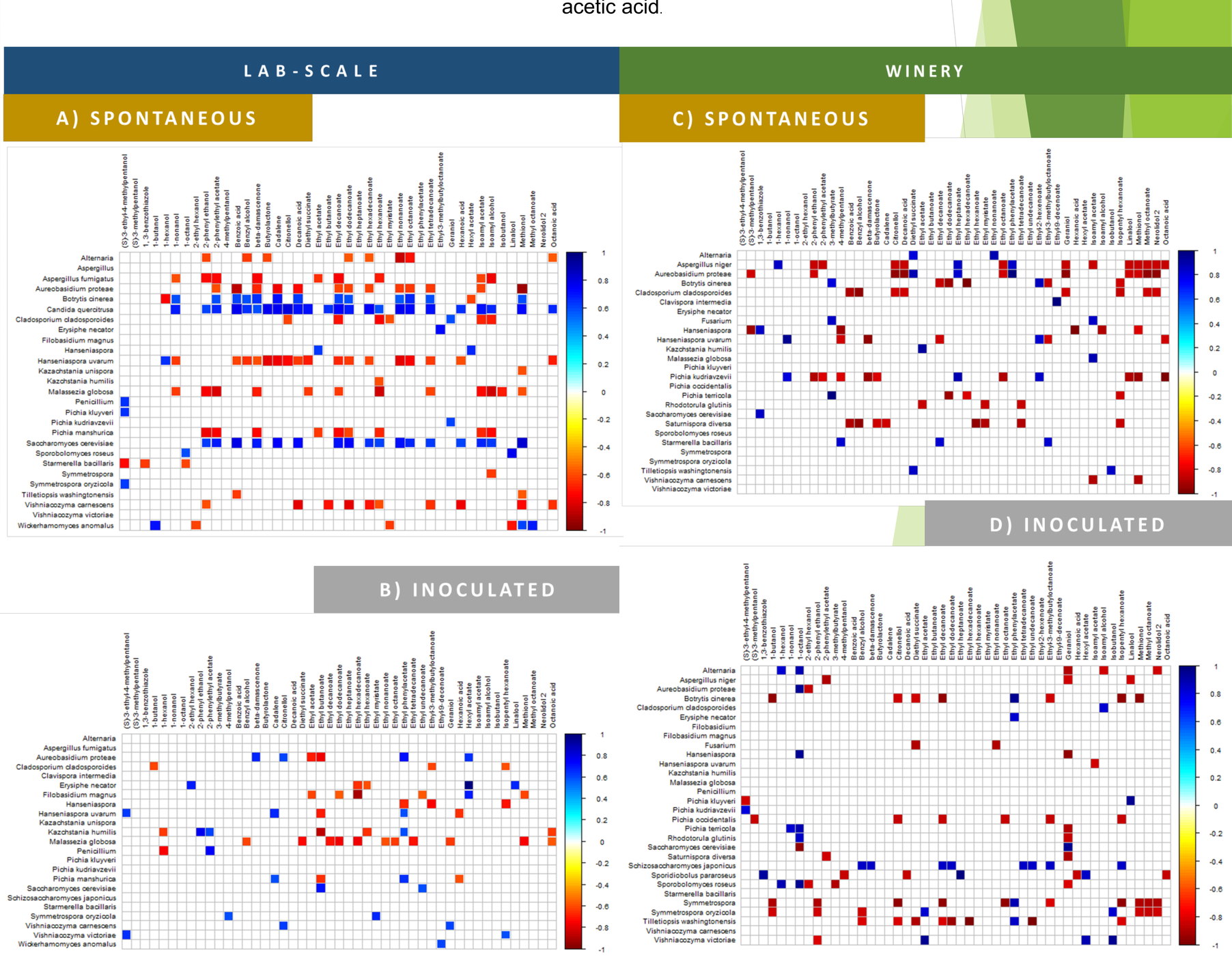

Fig. 2 Correlation plot showing Spearman's correlation between the fungal ASVs and volatile compounds observed with an incidence above > 1% in at least 2 samples during spontaneous and inoculated winery and laboratory fermentation. Only significant associations between the relative frequency of fungal ASVs and the concentration of metabolites are shown ($P < 0.049$). The intensity of the colors represents the degree of correlation between the fungal ASVs and volatile organic compounds, as measured by Spearman's correlation, where the blue color represents a positive degree of correlation and red a negative correlation between the metabolites and OTUs.