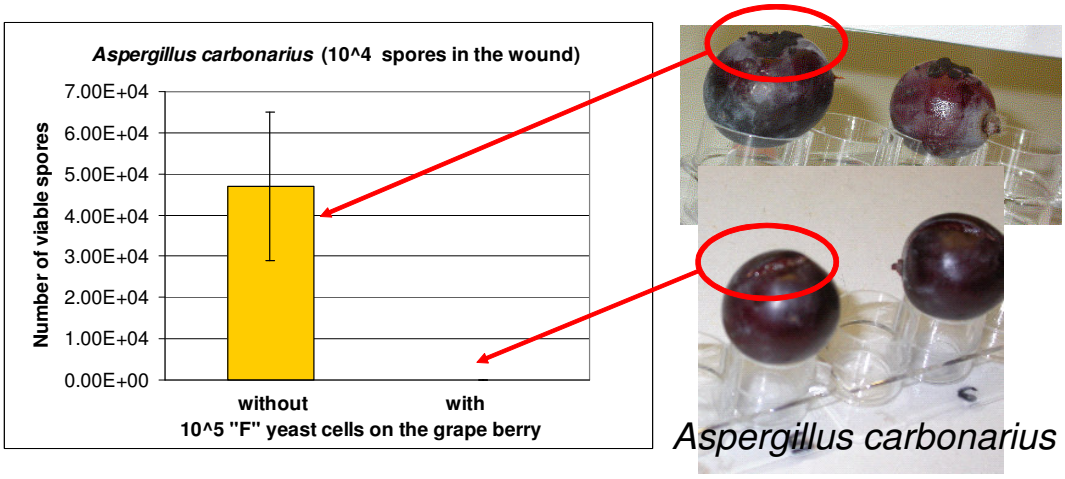
EVALUATION OF YEAST SPRAYING AS A TOOL FOR REDUCING FUNGUS DISEASES ON GRAPEVINES

Jean-Michel SALMON
INRA Montpellier, France

Extract from Research Results in Code of Best Practice for Organic Winemaking, produced under the EU FP6 STREP project ORWINE

Introduction

The main objective of this task was the potential reduction of microbial diseases on damaged grape berries by spraying *S. cerevisiae* yeasts on the grapes by creating competition amongst micro-organisms on their surfaces. Surface competitions were successfully performed to control post-harvest diseases (moulds) of fruits or vegetables by pre-harvest applications of yeasts. Natural saprophytic yeasts were generally used for this purpose. Such natural yeasts (mainly *Cryptococcus* and *Rhodotorula* ssp.) are known to colonize plant surfaces or wounds for long periods under dry conditions utilizing available nutrients for rapid multiplication and to be minimally affected by pesticides. Limitation of the use of such yeasts relies on the fact that their mass production on an industrial scale is very difficult, or even impossible. However it is not known whether anyone has tried to test standard industrial *S. cerevisiae* strains, which are easily available in large amounts, for their ability to control fungus development. The choice of oenological *S. cerevisiae* strains was dictated by the fact that most of these available strains were originally isolated from grapes or wines, and therefore seemed more adapted to the specific substrate as represented by damaged grape berries.



Effect of simultaneous yeast inoculation on *A. carbonarius* infected damaged berries

Fig. 1: Effect of simultaneous yeast inoculation on *Aspergillus carbonarius* infected damaged berries.

Results and Conclusion

The first trial was on the effect of *S. cerevisiae* on the development of undesirable bacteria or fungi at the surface of deliberately damaged grape berries.

The effectiveness of yeast spraying by different commercial *Saccharomyces cerevisiae* strains was evaluated on two different species of fungus diseases: *Botrytis cinerea* (invasive disease fungus) and *Aspergillus carbonarius* (undesirable fungus responsible for ochratoxin A (OTA) production), as well as on an invasive bacterial species (*Gluconobacter oxydans*).

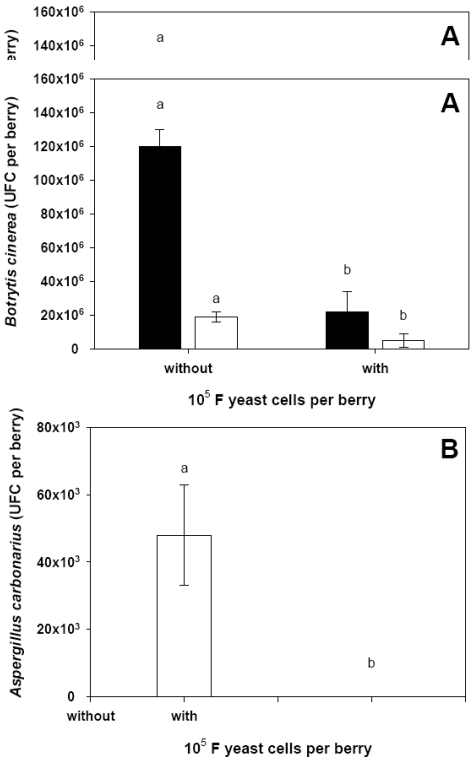


Fig. 2: Effect of the inoculation of 10^5 *S. cerevisiae* F cells on the surface of wounded grape berries previously and then inoculated with (A) 10^6 *B. cinerea* M04/51 (black boxes) and M04/63 (white boxes) spores or (B) 10^4 *A. carbonarius* spores. Assessment of Fungi was carried out after 48 h incubation at 28°C (mean and standard deviation of two replicates of three grape berries for each situation). The same letters indicate homogeneous groups at the 95% confidence level, as tested by Tukey statistical test.

A general inhibition effect was observed *in vitro* by a set of 17 industrial *S. cerevisiae* strains against *B. cinerea* and *A. carbonarius* mycelia growth, but not against bacterial growth (*Gluconobacter oxydans*). However, only few of them are really very efficient. Thus only the most promising *S. cerevisiae* strain, named F was conserved.

In a second set of experiments, it was demonstrated that the spreading of *S. cerevisiae* F strain at the surface of previously damaged grape berries contaminated with different microbial species was very efficient for reducing fungus mycelium growth after 48 h of incubation (Figure 2). This was not the case for bacterial *G. oxydans* contamination, where no effect is observed. From this first part of the work, it could be roughly concluded that *S. cerevisiae* F spraying by its mass impact could lower grape infection by fungi.

In a third set of experiments it was demonstrated that yeast spraying should be done about 2-5 days after initial infection by the fungi in order to get an optimal antagonistic effect. After this period, the potential of fungi to initiate disease remains, indicating that a competition for nutrients has taken place between protagonists. The effect of yeast spraying on *A. carbonarius* development on the grape berries was particularly significant. From all these experiments it is thought that such yeast spraying before grape harvest could represent for the viticulturist a biological alternative for limiting the occurrence of *A. carbonarius* in the vineyard.

In subsequent field scale experiments performed during 2007 and 2008 vintages, it is shown that yeast spraying with the selected industrial *S. cerevisiae* F strain on an artificially *A. carbonarius* infected vineyard was able to reduce the spread of *A. carbonarius* inside the grape berries, even if the external black mycelia form of *A. carbonarius* is not observed at the grape berry surfaces. From the obtained results, it is possible to deduce that yeast spraying on the surface of intact grape

berries partially reduces *A. carbonarius* penetration into undamaged grapes. Moreover, the reduction of the spread of *A. carbonarius* was accompanied by a significant reduction in the final level of ochratoxin A in the corresponding wines (Figure 3). The chemical and sensory properties of the final wines were also not detrimentally affected by yeast spraying.

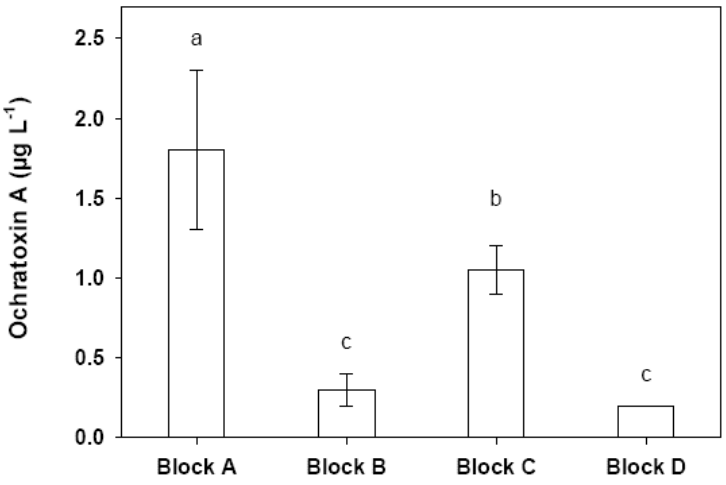


Fig. 3: Ochratoxin A levels (µg L^{-1}) in the finished wines (mean and standard errors of duplicates of 2007 vintage).

Each block contains 2 rows of 38 vine stocks of Mourvèdre variety. Two blocks (A and C) were contaminated by A. carbonarius spores (10^3 spores per bunch), one month before harvest. Two blocks (B and D) were sprayed with commercial S. cerevisiae "F" cells (10^7 cells per bunch), one week before harvest. The 304 vine stocks of the 4 separate blocks were hand-picked, separate fermentations (2 x 1 HL) were performed on the grapes harvested in each block: identical starter yeast inoculation, identical alcoholic fermentation conditions, and wine ageing. The same letters indicate homogeneous groups at the 95% confidence level, as tested by Tukey statistical test.

Fig. 4: Additional infected berry by *Aspergillus carbonarius*; natural infected berries by *Botrytis cinerea*, *Penicillium expansum*, *Trichothecium roseum* and Acetic-acid bacteria's.

ACKNOWLEDGEMENT
The authors gratefully acknowledge from the European Community financial participation under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities, for the Specific Targeted Research Project "ORWINE" SSPE-CT-2006-022769.

DISCLAIMER
The views expressed in this publication are the sole responsibility of the author(s) and do not necessarily reflect the views of the European Commission.
Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of the information contained herein.