Influence of organic plant treatment on the terroir of microorganisms.

Influence des traitements organiques des plantes sur les microorganismes du terroir.

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Summary

Several factors like vineyard site, climate, grape variety, ripeness, physical health of the grapes and pest management influence the populations of indigenous yeasts on grapes and later on in spontaneous fermentations. During spontaneous fermentations, so called “wild yeasts” could significantly influence the wine aroma. Some authors certify more complexity and an increase of wine quality to these fermentations. A widespread opinion is that spontaneous fermentation can help to emphasize the characteristics of a specific geographical area or even of one vineyard site.
This was checked in a three years experimental period testing different pest management strategies to replace or reduce copper and sulphur and comparing integrated, organic and biodynamic strategies.
Alternatives to copper or sulphur treatments could however have an impact on the aroma profiles, as they alter the composition of natural yeast populations in the vineyard or lead to changes in yeast metabolism. This was tested with several alternative strategies compared to organic-standard and integrated variants. Effects on spontaneous flora, fermentation course and aroma profiles were analysed.
Yeast populations on grapes and at different stages of grape and must processing were isolated and determined using RFLP analysis of the ITS-region.
Hanseniaspora uvarum and *Metschnikowia pulcherrima* were the dominating species on the grapes in all variants. There was no correlation between the population dynamics of yeast during the processing and fermentation and the different pest management strategies.
In this survey the processing and the ecosystem winery seem to have a more important influence on yeast diversity than the microflora composition on grapes.

Keywords: yeast – spontaneous fermentation – organic pest management – RFLP – sensory analysis

Introduction

The term „terroir“ is mainly used to describe the interaction of soil and vine and in some cases also defined by viticultural and enological human interference. It’s well known for a long time that several factors like vineyard site, climate, grape variety, ripeness, physical health of the grapes, and pest management influence the populations of indigenous yeasts on grapes and later on in spontaneous fermentations.
During spontaneous fermentations, so called “wild yeasts” could significantly influence the wine aroma in a positive and also in a negative way. Some authors certify more complexity and an increase of wine quality to these fermentations. A widespread opinion is that spontaneous fermentation can help to emphasize the characteristics of a specific geographical area or even of one vineyard site. (Maro et al., 2007)
The question is if there is any terroir of yeast in a vineyard and how it is influenced by different pest management strategies.
This was checked in a three years experimental period testing different pest management strategies to replace or reduce copper and sulphur and comparing integrated, organic and biodynamic strategies. The potential influence on the composition of natural yeast populations in the vineyard leading to

changes in yeast metabolism and on aroma profiles was tested with several alternative strategies compared to organic-standard and integrated variants.
Additionally a comparison between conventional, organic and biodynamic pest control was included. Effects on spontaneous flora, fermentation course and aroma profiles were analysed and yeast populations on grapes and at different stages of grape and must processing were isolated and determined using RFLP analysis of the ITS-region.

Material and methods

Vineyard and grapes

The grape material for the examinations came from two different field trials of the Geisenheim Research Center in Geisenheim. Experiments as to replacement and reduction of copper and sulphur in the ecological pest management have been carried out in vineyard M2, which is cultivated with the variety Riesling (clone 293gm, stock 5c, planted 1982). The variants: integrated, eco-standard, phosphite-1 and potassium hydrogen phosphate were examined. The variants are randomly distributed all over the vineyard in four replications. Following variants were tested:
M2/2: Integrated conventional pest management: Sulphur and the fungicide Folpet; M2/4: Organic viticulture: Sulphur, Copper (Copperhydroxid) up to 3 kg/ha and year, Myco-Sin Vin (plant resistence improver); M2/12: Organic viticulture with Copper reduction: Fructogard (containing Potassiumphosphonat) up to BBCH 68, reduced Copper (Copperhydroxid), Sulphur
M2/14: Sulphur-substitution: Potassiumbicarbonate ($KHCO_3$), Copper (Copperhydroxid).
Also integrated, bioorganic and biodynamic pest management were examined and compared in vineyard M4, which is cultivated with the variety Riesling (clone 198-30, stock Börner SO_4, planted 1991). The restructuring of the vineyard according to the suitable pest management is being carried out since three years. Examined Variants: A: Integrated conventional pest management; B: Organic viticulture; C: Biodynamic viticulture
The difference between the three cultivation-forms was based on spray-strategies for plant-protection and soil cultivations, including fertilization. Further on the differentiation in organic- and biodynamic viticulture resulted in the additional use of the biodynamic preparations. The stable manure used for the biodynamic rows of vines was prepared with special compost-preparations. The preparation 500 was applied three times on the soil during spring, whereas the preparation 501 was applied directly on the foliage at the moment before flower, start of ripeness and the physiological ripeness.
The soil of the two vineyards is defined to be sandy and clayey with partially gritty underground.

Vinification

Grapes were on the one hand sterilely removed from the vineyard in small quantities immediately before harvest and on the other hand regularly harvested and processed. The pressed must was clarified by sedimentation in a cold storage house for 12 hours at 4 °C. The then racked musts were sulphited with 30 mg/l. Fermentation was effected in glass balloons with volumes between 3.5 and 10 l according to variant and available must quantity. For each variant one balloon was inoculated with the yeast starter culture EC1118 (Lallemand, 20 g/hl) and two approaches were fermented spontaneously. The sterilely from the vineyard drawn grape samples before harvest were pressed manually and 200 ml each were prepared for microvinifications. Fermentation course of the vinifications was observed by measuring the density or by investigating the weight loss.

Sample drawing

For the investigation of the yeast flora, samples were drawn every two days during the first fermentation third from the spontaneous fermentation of the different variants and the microvinification ones. The samples were diluted in a decadal series and plated on malt agar for the isolation of the single yeast colonies. 100 at random selected yeast colonies were isolated for further characterization for each variant and sample drawing date. This was effected using cultivation on lysine and HDM medium.

Identification of species

Selected isolates were identified using Restriction-Fragment-Length-Polymorphism (RFLP) of the ITS-region of the 5.8 S rRNA-gene. For the necessary isolation of the total DNA, the yeasts were cultivated for 24 hours in an incubation shaker in liquid YPD-medium. 1.5 of these cultures were collected after 5 min of centrifugation at 5000 rpm and the DNA was isolated according to the method of Rose et al., 1990. The amplification of the ITS-region was effected in a mastercycler ep (Eppendorf, Hamburg) using the Primer ITS1 and ITS4 (Esteve-Zarzoso et al., 1999). PCR was carried out according to following parameters: 3 min initial denaturation at 95 °C, followed by 35 cycles consisting of 1 min denaturation at 95 °C, 2 min annealing at 52 °C and one extension of 2 min at 72 °C with a final extension of 10 min at 72 °C.
The resulting PCR products were restricted according to manufacturers' instructions with the restriction enzymes CfoI, HaeIII and HinfI (Fermentas, St. Leon). The resulting fragments and the PCR-products were put together with a 100 bp-ladder from Fermentas as standard marker on a 2 % agarose gel, stained with ethidiumbromide and photographed. For species identification, size of the DNA bands of the ITS-products and resulting restriction fragments were determined using 1D-Gel Electrophoresis Software LabImage (Kapelan GmbH, Halle/Saale). The species identification was made through a correlation comparison of the band samples with a data base.

Sensory analysis

The wines were tasted by a panel of 18 trained tasters and evaluated by sensory descriptive analysis. The sensorial characteristics were quantified on a eight-point scale using several descriptors such as fruity, bitter, peach, smell of mercaptane, intensity of aromatic substance (smell) and typical (taste of Riesling).

Results and Discussion

In all cases the spontaneous approaches need a longer time to finish the fermentations as shown in figure 1 by examples of the copper and sulphur replacement experiments of the vintages 2005 and 2007.

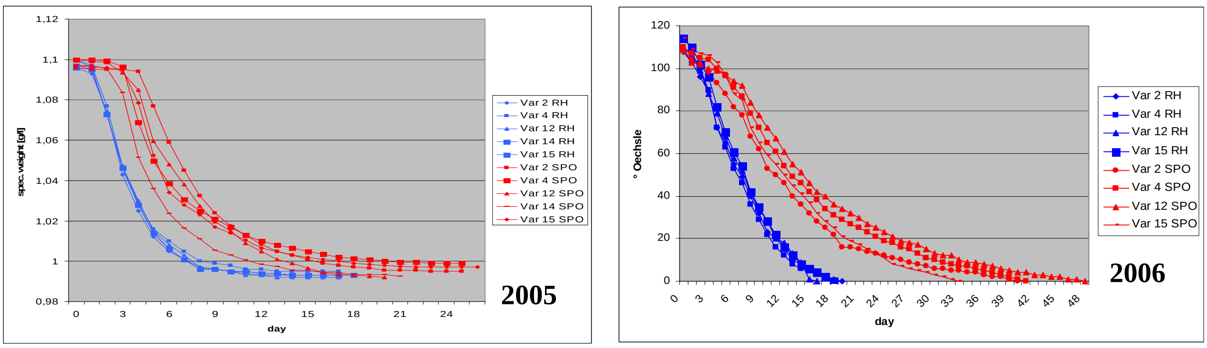

Figure 1 Examples for courses of spontaneous and inoculated fermentations with grapes from different copper and sulphur replacement experiments (M2-2005, M2-2006). EC1118 (Lallemand, 20 g/hl) was used as pure dried yeast. Spontaneous fermentations are marked in red, inoculated fermentations in blue.

Using RFLP analysis for the identification the yeast flora on the grape berries and at different stages of fermentation was investigated.
On the grape berries the two species *Hanseniaspora uvarum* and *Metschnikowia pulcherrima* dominated, whereas a switch to other yeasts like *Pichia* and *Candida* species was detected after fining the must by sedimentation and sulphiting. Peculiar is the predominance of *Candida stellata* during some of the vinifications. But most of the fermentations were finished in a well known manner by *Saccharomyces cerivisiae* (data not shown).

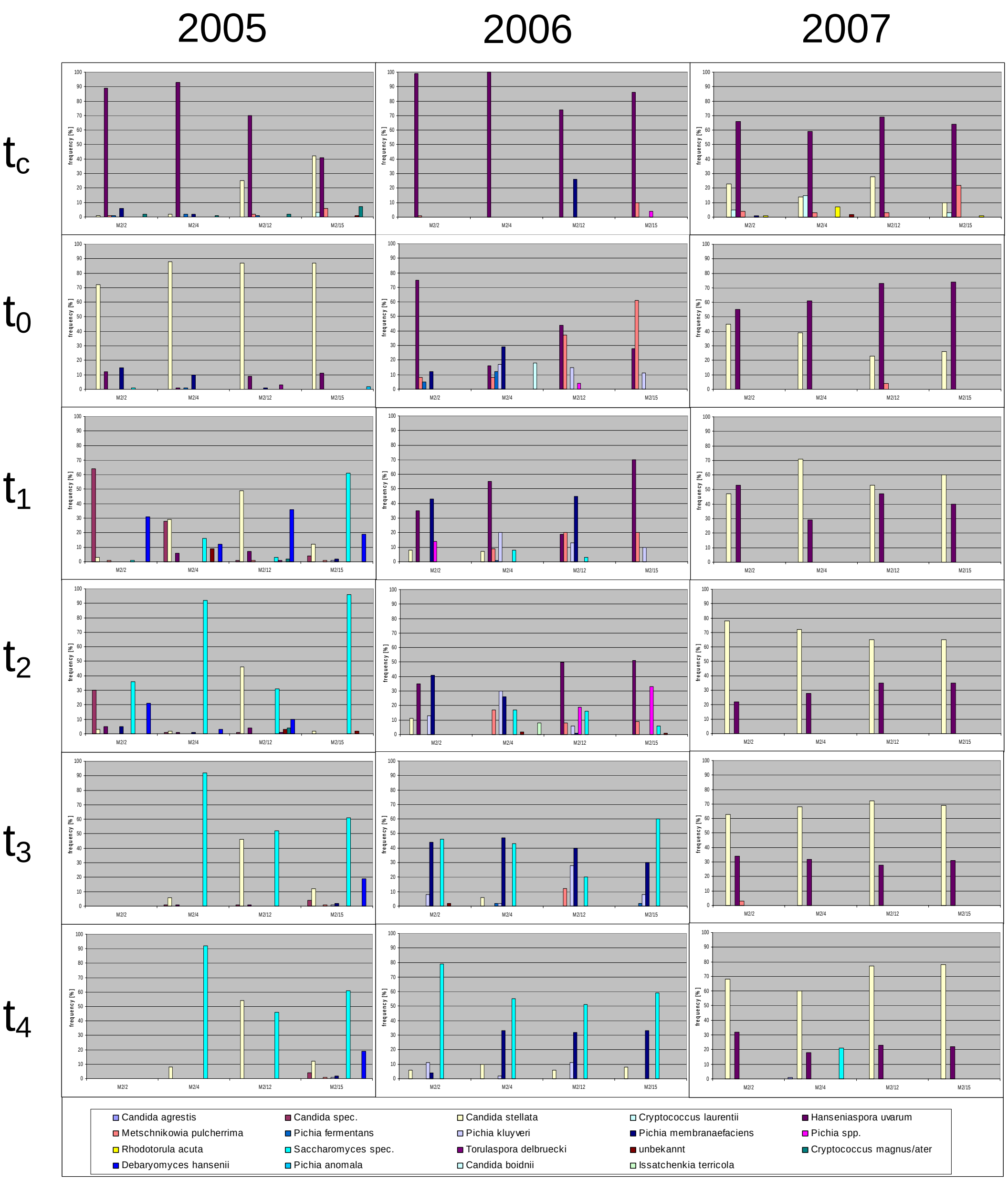

Figure 2 Yeast population dynamics of spontaneous micro vinifications with grapes from different copper and sulphur replacement experiments. The results of three consecutive years are presented. t_c stands for the status directly after pressing the grapes without other treatment, t_0 for the start of fermentation after fining the must by sedimentation and sulphiting. Further samples were taken every two days.

The vinifications of the comparative studies between conventional, organic and biodynamic plant protection management didn't show significant differences between the three variants. In 2007 *Metschnikowia pulcherrima* was the only yeast isolated from the must of the conventional variant after pressing the grapes. But a big difference could be observed for the vintages 2006 and 2007. In 2007 *Candida stellata* predominated during a large period the spontaneous fermentations of all three variants.

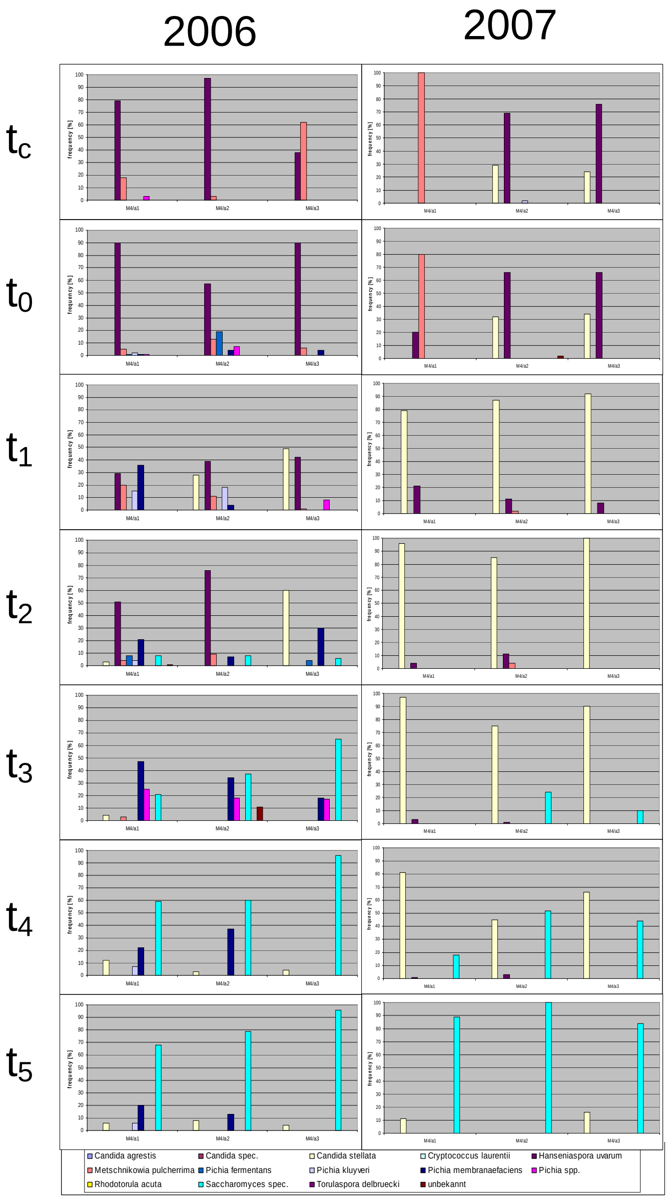

Figure 3 Yeast population dynamics of spontaneous micro vinifications with grapes from comparative experiments between conventional, organic and biodynamic plant protection treatment. The results of two consecutive years are presented. t_c stands for the status directly after pressing the grapes without other treatment, t_0 for the start of fermentation after fining the must by sedimentation and sulphiting. Further samples were taken every two days.

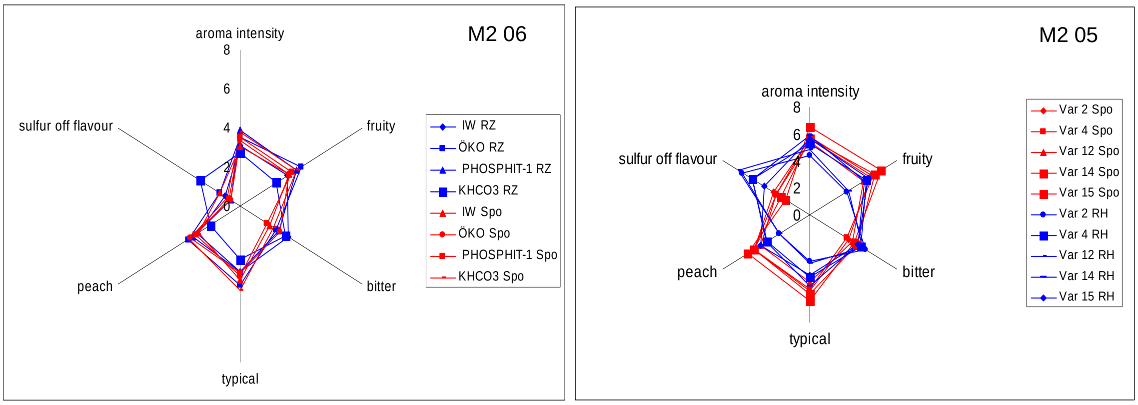

Figure 4 Descriptive sensorial analysis of wines produced from grapes of different copper and sulphur replacement experiments (M2-2005). Results of wines produced by spontaneous fermentations are indicated in red, results of wines produced with EC1118 (Lallemand, 20 g/hl) are marked in blue.

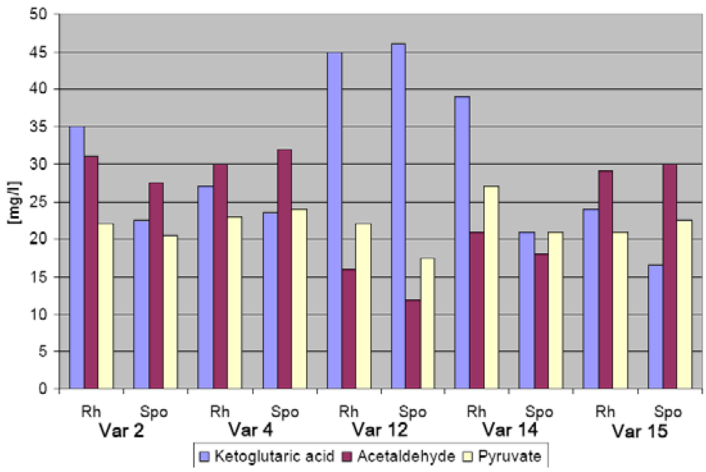

Figure 5 Analysis of sulphur binding compounds in wines produced from grapes of different copper and sulphur replacement experiments (M2-2005).

Conclusion

The yeast population dynamics during fermentation could not be linked to the different spray–strategies. No correlation between the population dynamics of yeast during the processing and fermentation and the different pest management strategies could be observed.

In this survey, the processing and the ecosystem winery seem to have a more important influence on yeast diversity than the microflora composition on grapes. In this case, the winemaking process, level of sulphiting and the winery ecosystem seem to have a more important influence on the yeast diversity. But more investigations are necessary and further research is in progress.

References

DI MARO E., ERCOLINO D. and COPPOLA S. (2007) Yeast dynamics during spontaneous wine fermentation of Catalanesca grape. *Int J Food Microbiol* **117**, 201-210

ESTEVE-ZARZOSO B., BELLOCH C., URUBURU F. and QUEROL A. (1999) Identification of yeast by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. *Int J Syst Bact* **49**, 329-337

ROSE M.D., WINSTON F. and HIETER P. (1990) Methods in Yeast Genetics: A Laboratory Course Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York