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Evaluation of a oxygenate based plant protection treatment in viticulture against fungal diseases

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Abstract. Over the last decades the use of pesticides in vine protection, e.g. copper is under severe discussion and is becoming a major concern in viticulture. Since the effectiveness of oxygenates against various microorganisms had been proven in the medical field a strategy for oxygenate-based plant protection was developed and evaluated over three vintages. The production of the oxygenate is following the Criegee-mechanism using O_3 and unsaturated natural plant derived fatty acids forming so called ozonides. Therefore the effect of the treatment has been evaluated in a holistic approach, covering the efficiency against fungal diseases, protection of desired beneficial insects, the micro flora, various secondary metabolites of the grapevine and the resulting sensory profile of the wines. The biological effectiveness has been measured by using different in-vivo and in-vitro studies. The influence on desired berry compounds, e.g. anthocyanins, have been determined by classical GC-MS and HPLC methods. Positive effects against downy and powdery mildew could be demonstrated. No negative effects against insects, naturally occurring microorganisms, and desired berry compounds were observed. Even spontaneous fermentation was not inhibited. Quantitative descriptive sensory analysis as well as CATA/RATA showed no negative effect of the treatment.

1 Introduction

The control of fungal diseases is an essential problem in viticulture and alternative strategies are needed to further reduce the use of plant protection products and to provide alternatives to the use of copper-containing plant protection products, especially for organic viticulture. Currently, agriculture and viticulture are facing fundamental challenges (e.g. farm to fork strategy of the European Commission, Green Deal, climate crisis, energy crisis, etc.). The use of agrochemicals is one of the major anthropogenic determinants of the grape microbial community. Nevertheless, resistance development of plant pathogenic organisms is threatening viticulture. Thus, research must be conducted concerning new antimicrobial active compounds in plant pest management. A promising field of development is concerned with ozonized plant oils. The broad effective spectrum of these compounds is based on a rather unspecific mode of action conducted by trioxolanes, peroxides and aldehydes. The efficiency against human pathogenic yeasts and bacteria has been proven in various publications in the past two decades as well as for almost all kind of microorganisms [1,2,3].

2 Materials and methods

2.1 Ozonides

Ozone was generated by passing oxygen from a bottle (99.5%) through an ozone generator COM-AD-08 (Anseros Klaus Nonnenmacher GmbH, Tübingen Germany) with a constant flow rate of 100 L/h. The ozone concentration was measured by an ozone analyzer GM-OEM-6000 from Anseros. The generated ozone was bubbled into 250 mL of oleic acid (Sigma-Aldrich, 65-88%, St. Louis, MO, USA) in a 500 mL glass reactor with a concentration of 130 g/m^3 for four hours. During ozonation, ozone and oleic acid were mixed by vigorous stirring on a high-speed laboratory stirrer from IKA (EUROSTAR 20 high-speed control). The reactor was tempered at 12 °C during the reaction time. At the end of the reaction, the product (ozonized oleic acid, C250/10 V1) was obtained as a highly

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viscous colorless liquid with a peroxide value of 1800. Peroxide values were determined by measuring the amount of iodine via titration with sodium thiosulphate solution (volumetric standard solution, Carl Roth). The ozonized oleic acid was provided by Anseros, Tübingen. Anseros provided a system (Oxygenat System (OXY400)) which enabled the production of a homogeneous spray solution with the desired concentration.

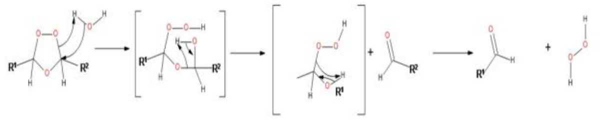

Figure 1. Formation of oxygenate according to the Crigee reaction using ozonized oleic acid and the resulting formation of reactive oxygene species [4].

2.2 In field application and in vitro assays

The effectiveness against *Plasmopara viticola* and *Erysiphe necator* was verified over three vintages in parcels of vineyards *Vitis vinifera* L. cv. (Portugieser, Pinot Noir, Pinot Blanc and Müller-Thurgau) in Neustadt (Weinstraße), Rhineland-Palatinate, Germany. Within the vineyard, parcels chosen in a randomized block design samples received weekly treatments with 0.8% [v/v] ozonized oleic acid (OT), a conventional treatment (CT) or no treatment (NT) from developmental stage BBCH 13 to BBCH 83.

Two acetic acid bacteria (AAB), four lactic acid bacteria (LAB) and nine saccharomyces and non-saccharomyces yeasts were investigated with in vitro assays.

2.3 Chemical and sensory analysis

Spectrophotometry (color intensity & tone), HPLC-DAD (characterization of phenolic composition such as total anthocyanins and flavan-3-ols), GC-MS after enzymatic treatment (aromatic precursors), HA-Assay (Polymeric Pigments), as well as descriptive sensory analysis (CATA/RATA) were used to analyze the differences between the treatments.

3 Results and discussion

An amplicon metagenomic approach was used to determine the effect of repeated treatments with ozonized oleic acid on the microbial community of grapevine. Differences in community composition of treated vineyards were compared to non-treated and conventionally treated samples regarding the prokaryotic and eukaryotic microblome. Every treatment showed effects both on occurrence and abundance of microorganisms and the community assembly while compared to non-treated vineyards. Conventional treatment revealed a slight drop in microorganism diversity compared to non-treated and ozonized oleic acid treatment [5].

The results in terms of minimum inhibitory concentration of ozonized oleic acid Brettanomyces *bruxellensis*, Saccharomyces *cerevisiae*, Pediococcus *sp*. and Acetobacter *aceti* revealed the highest sensitivities against ozonized oleic acid (LIQUENSO® Oxygenat). Culture growth of these organisms was significantly reduced at an ozonide concentration of 0.25% (v/v), which corresponded to a quarter of the concentration used in the vineyard.

The living cell counts per berry for yeast and bacteria after treatments (Pinot Blanc) between the control (no treatment), conventional treatment (conv), conventional combined with the Oxygenat (conv + ox) and the plain Oxygenat (ox) did not show any significant impact between the modalities (Fig. 2). As a result spontaneous fermentation was successful in all fermentations (data not shown).

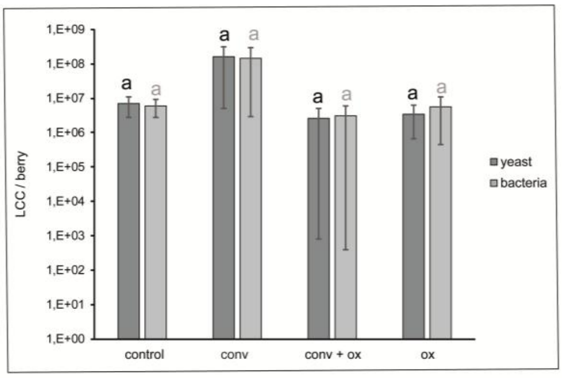

Figure 2. Living cell counts (LCC) of yeast and bacteria per berry after different plant protection treatments var. Pinot Blanc (*n* = reps. x 100 berries).

The efficiency against fungal attack, visually determined by disease incidence and disease severity, is shown in Fig. 3.

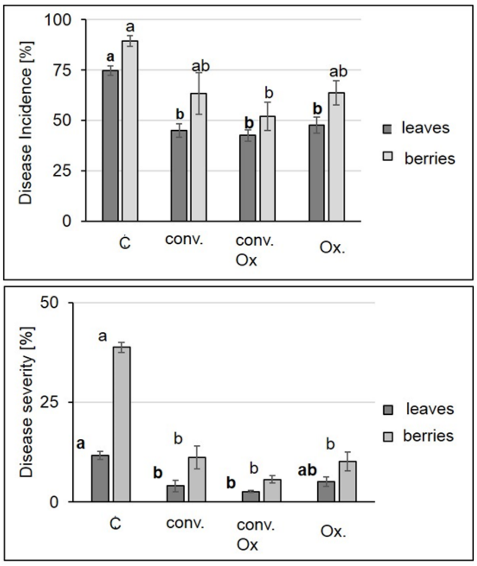

Figure 3. Disease incidence (above) and disease severity (below) for *Plasmopara viticola* after the treatments on Pinot Noir (*n*=3).

The application of oxygenate treatment could show the same effects against *Plasmopara viticola* like the treatment with conventional plant protection. Only the control (not treated) shows significantly increased disease severity. Against *Erysiphe necator* a significantly positive effect could only be observed while doubling the concentration of the oxygenate in the spaying solution.

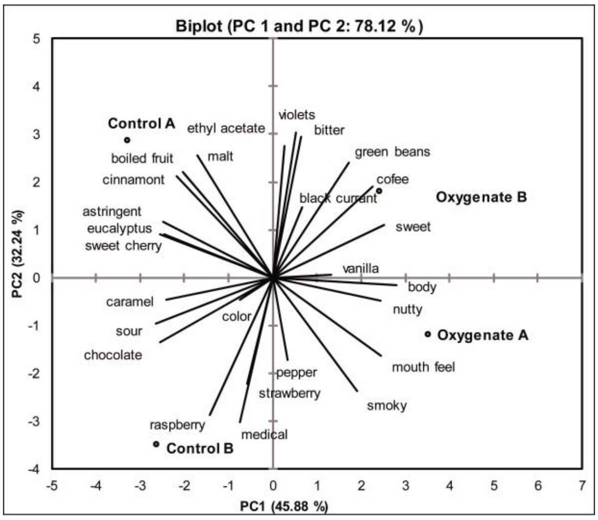

Figure 4. PCA of sensory data (RATA) for Pinot Noir in 2020.

Regarding the impact of the treatments in terms of sensory profile for the finished wines using RATA (Fig. 4) and other descriptive methods, no negative effect, independent of the variety used, could be observed. Nevertheless Oxygenate treatments could be described with nutty, coffee, sweet and body as well as mouth feel. The Control repetitions are more separated by PC2 than the Oxygenate treatments and described by either sour, raspberry or boiled fruit and ethyl acetate. Even if there is no negative alteration of the sensory profile after oxygenate treatment no conclusion regarding ageing potential can be given.

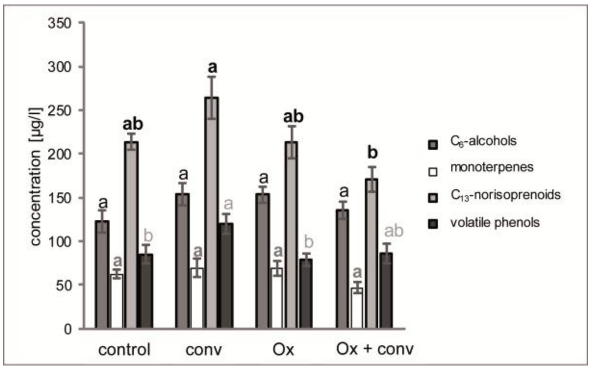

Figure 5. Concentration of aromatic precursors after treatment of Pinot Blanc berries at harvest.

As shown in Fig. 5 there is almost no significant difference in aromatic precursors at harvest. Regarding volatile phenols there is no evidence that the Oxygenate treatment might induce stress related formation of those in the plant. Highest concentrations of C_{13}-norisoprenoids can be found for the conventional plant protection treatments. The might be an interaction between the combination of conventional and Oxygenate application as the concentration of C_{13}-norisoprenoids is reduced compared to the single use of Oxygenate or conventional treatment.

4 Conclusion

The plant protection by using Oxygenates is very forward looking to reduce classical plant protection products such as copper and to comply with the new Green Deal regulations. Thus will help producers to reduce classical fungicide application by maintaining grape quality. The initial skepticism that Oxygenate treatment might have a negative effect on fermentation, berry composition and wine sensory could not be shown. As valuable insects are also not affected by Oxygenates a fundamental and natural alternative in combination with classical plant protection products is proposed.

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