

INTRODUCTION

Microorganisms can establish different types of interactions with superior organisms: plants host in their internal tissues a set of fungal and bacterial endophytes, which may play a beneficial role on the host fitness under biotic and/or abiotic stress. Therefore, this work is focused on the quantitative and qualitative assessment of the cultivable endophytic bacterial communities present in the leaves of grapevines affected by downy mildew, under organic control management. This work was included in the framework of the project "LIFE GREEN GRAPES - New approaches for protection in a modern sustainable viticulture: from nursery to harvesting", that compared different strategies for *Plasmopara viticola* control.

EXPERIMENTAL SITE AND DESIGN



The experimental trial was conducted in wine production vineyard (Chianti Classico area) of the «Castello di Gabbiano» farm, where 5 different types of management were compared:

- 1) Integrated Pest Management (IPM)
- 2) IPM partly integrated by resistance inducers (*)
- 3) Organic management
- 4) Organic with partial integration of copper by resistance inducers (*)
- 5) Resistance inducers (*) (with a small amount of copper because of high incidence of the disease)

(*) These products are known to have resistance induction activity, registered in the fertilizer biostimulant categories.

Fig.1 Farm and vineyard of sampling (Castello di Gabbiano, Impruneta FI)

MATERIALS AND METHODS

- The sample material was composed by leaves of *V. vinifera* cv. Sangiovese. Isolation of endophytic bacteria was carried out in petri dishes, using TSB (Tryptic Soy Broth) medium.
- Total DNA was extracted by thermal lysis and the 16S rRNA genes were amplified by specific PCR (primers P0 and P6).
- ARDRA (Amplified Ribosomal DNA Restriction Analysis), using restriction enzyme Msp1, permitted to detect and group the isolates with the same electrophoretic profile into haplotypes.
- DNAs of different haplotypes were sequenced and compared with the NCBI bacterial sequences database.

RESULTS AND DISCUSSION

➤ The ARDRA allowed to clusterize the isolates with the same electrophoretic profile into three different haplotypes (Fig. 2) with members of genera *Kocuria* (1, 2) and *Bacillus* (3). The widely dominant haplotype resulted to be the species *Bacillus amyloliquefaciens* (Fig. 3), a gram-positive bacterium that has biocontrol properties.

➤ The *B. amyloliquefaciens* CFU obtained on culture medium from tissues of vines with organic management increased from treatment 3 to 5 (Fig. 4), while copper was gradually substituted with defence inducers (Fig. 5 and 6).

➤ Despite there isn't any significant correlation between inducers and CFU ($p=0,11$), the fact that their trends are similar permit to suppose a potential role in increment of endophytes and in defence of plant.

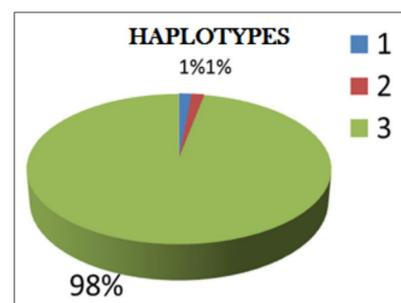


Fig. 2 Percentage of haplotypes



Fig. 3 *Bacillus amyloliquefaciens*

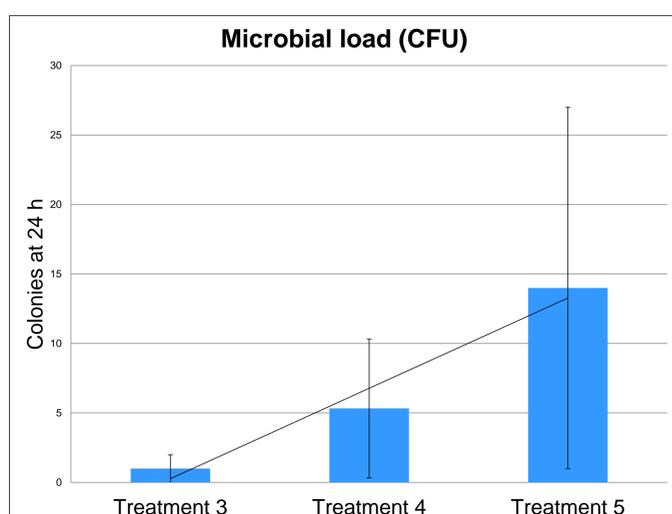


Fig. 4 CFU from treatments after 24 h

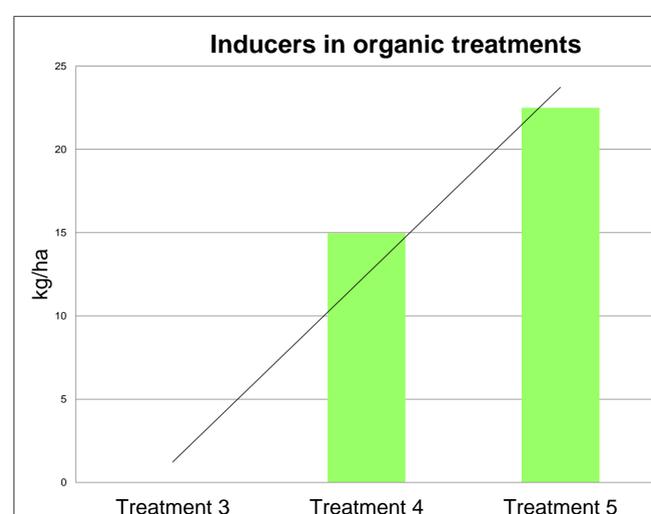


Fig. 5 Amount of defence inducers applied in every treatment

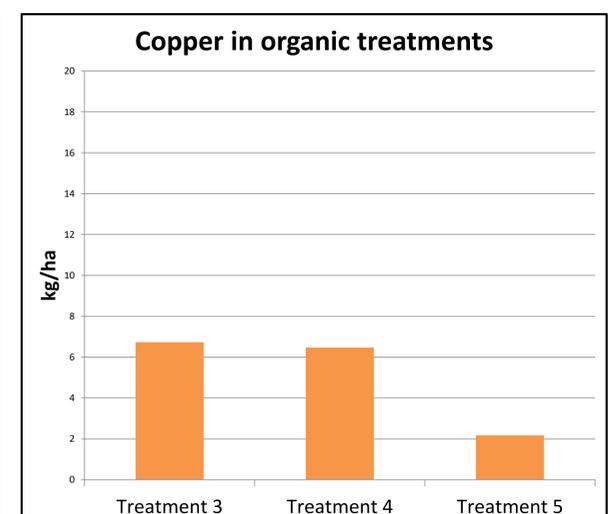


Fig. 6 Amount of copper applied in every treatment

CONCLUSIONS

The increasing presence of *B. amyloliquefaciens* in the samples following treatments with increasing amounts of defence inducers opens questions and research perspectives on the possible relationship between defence inducers, also – but not only – associated with a lower amount of copper, and a positive increase in the beneficial endophytic microflora.