

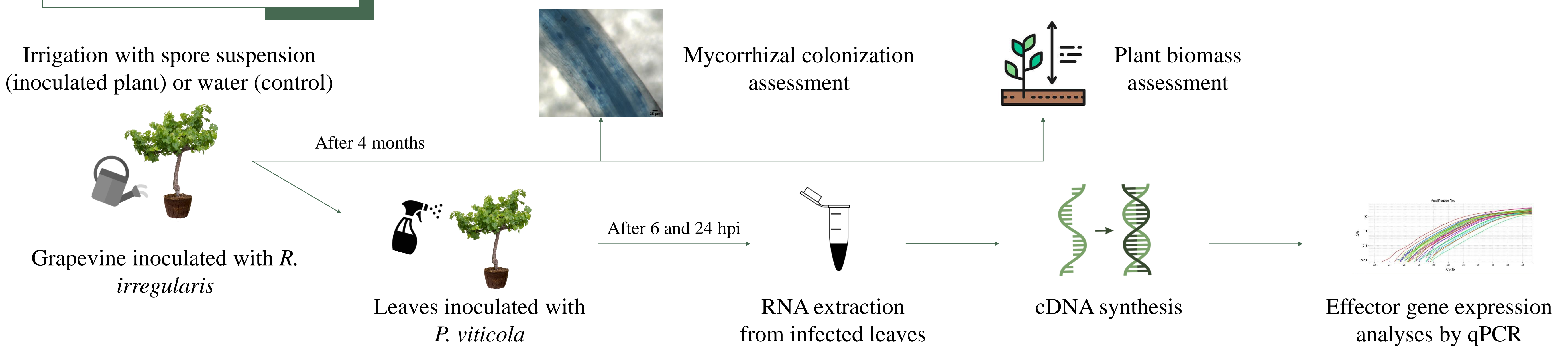
Effect of mycorrhizae on the expression of pathogen effectors during the infection of grapevine with *Plasmopara viticola*

Ana Cruz-Silva^a, Andreia Figueiredo and Mónica Sebastiana

BioISI - Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal | ^a amcsilva@fc.ul.pt

Grapevine (*Vitis vinifera* L.), widely used for berry and wine production, is highly susceptible to the pathogenic oomycete *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni, the etiological agent of grapevine downy mildew disease [1]. In the grapevine-*P. viticola* interaction, it is known that *P. viticola* secretes effectors, many of them target the plant cell nucleus [2–4] and act to suppress plant immunity responses [2–7]. The method commonly used to prevent and control *P. viticola* infection relies on multiple applications of chemical fungicides [1]. However, with European Union goals to lower the usage of such chemicals in viticulture there is a need to develop new and more sustainable strategies. The use of beneficial microorganisms with biocontrol capabilities, such as the arbuscular mycorrhizal fungi (AMF), has been pointed out as a viable alternative [8,9]. With this study, we intended to investigate the effect of AMF colonization on the expression of *P. viticola* effectors during infection of grapevine. Grapevine plants were inoculated with the AMF *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler 2010, and, after mycorrhizae development, plants were infected with *P. viticola*.

Experimental assay



Results and Discussion

The typical AMF structures were clearly observed in the inoculated roots (Fig. 1). The colonization rate of inoculated plants (10%) was significantly higher when compared to the non-inoculated plants (2%). Mycorrhizal inoculation significantly increased the biomass of the grapevine plants (Fig. 2).

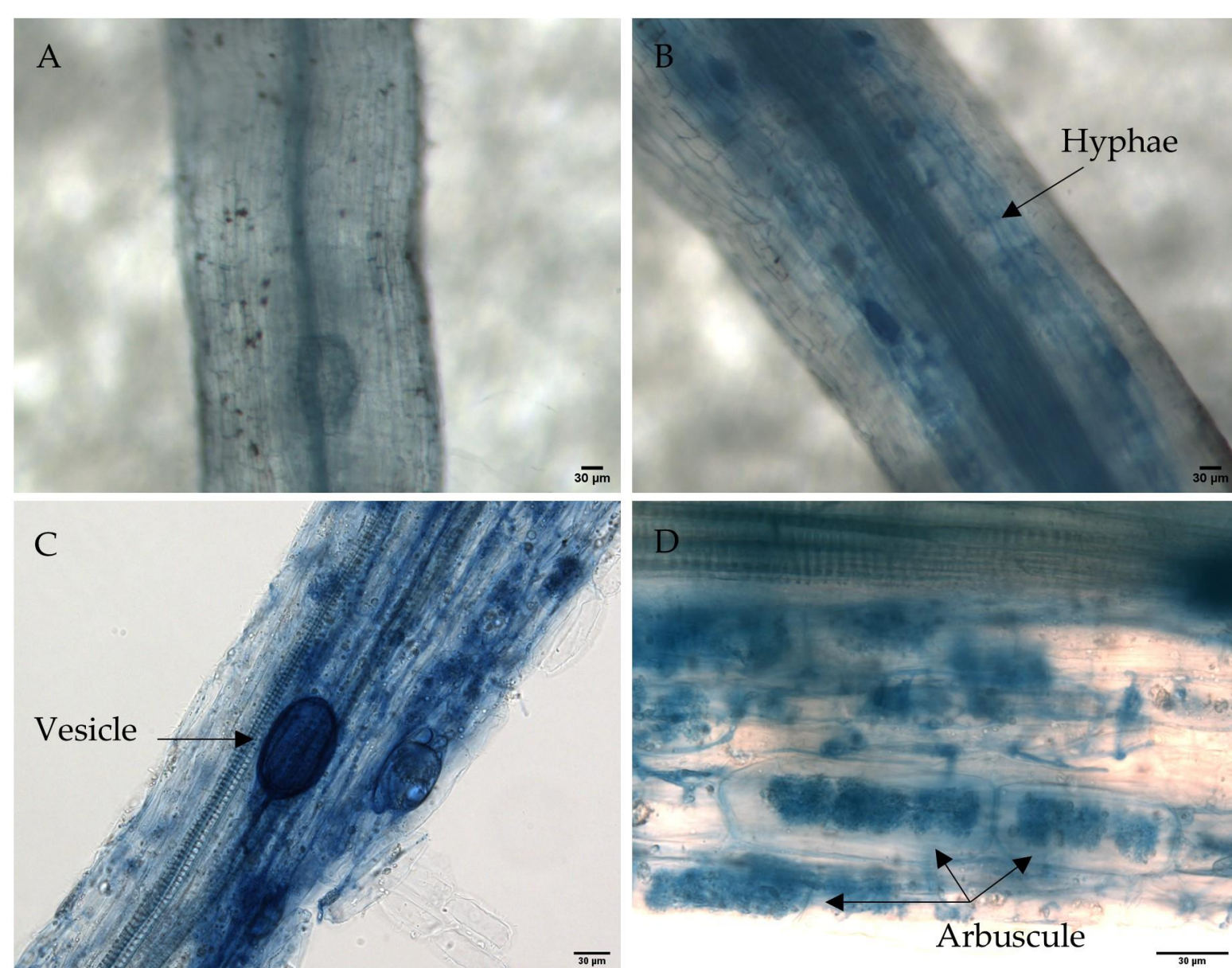


Fig. 1. Mycorrhizal structures observed in grapevine roots, after *R. irregularis* inoculation (Trypan blue staining). (A) Non-colonized root segment. (B–D) Colonized root with AMF structures.

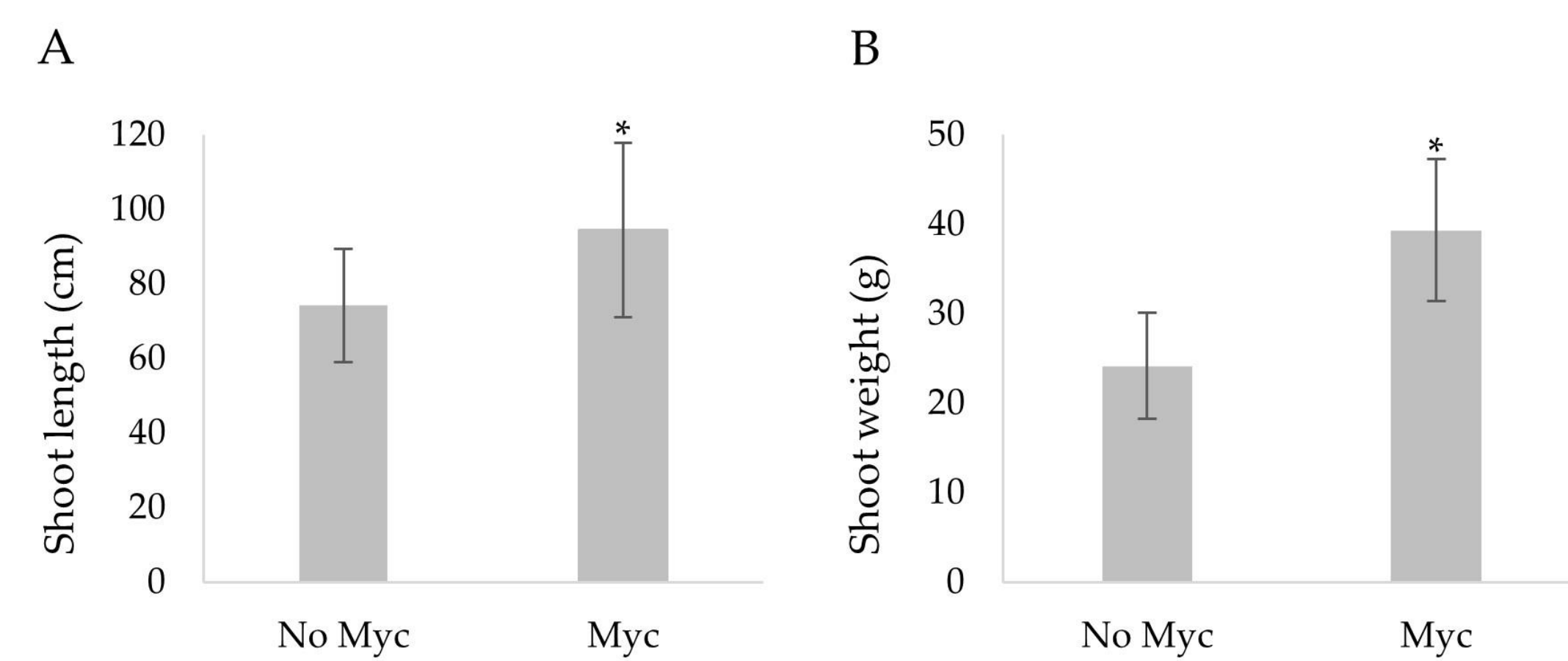


Fig. 2. Effect of mycorrhizal inoculation on grapevine shoot growth. (A) Shoot length of mycorrhizal (Myc) and non-mycorrhizal plants (No Myc). (B) Shoot weight of mycorrhizal (Myc) and non-mycorrhizal plants (No Myc). Statistical significance is represented by (*) (t-test $p < 0.05$).

Effector genes exhibited changes of expression in mycorrhizal plants when compared with non-mycorrhizal plants upon infection with *P. viticola* (Fig. 3). These results show for the first time that pathogen effector gene expression may be altered by plant mycorrhization.

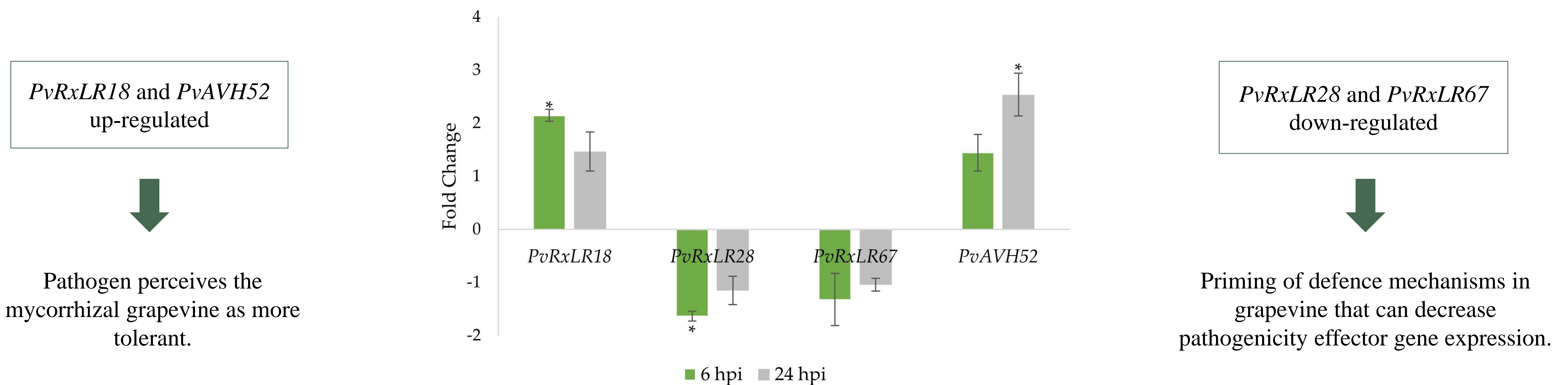


Fig. 3. Effect of mycorrhizal colonization on *P. viticola* RxLR effector gene expression on grapevine leaves, 6 hpi and 24 hpi. Statistical significance from comparing the expression of each effector on mycorrhizal and non-mycorrhizal plants, in each the time point, is represented by (*) ($p < 0.05$).

Conclusions

- The results suggest that pre-mycorrhization of grapevine plants may be of importance to decrease the pathogenicity of *P. viticola* and limit its ability to infect grapevine leaves. These further support the use of mycorrhizal fungi as an alternative for a more sustainable control of the downy mildew disease in viticulture.