

Deciphering grapevine trunk early molecular responses to *P. minimum* and *P. chlamydospora* in the presence of a commercial biocontrol agent (*Trichoderma atroviride*, Vintec[®])

Authors: Ana ROMEO-OLIVAN^{1,*}, Justine CHERVIN^{2,3,4*}, Virginie PUECH-PAGES^{2,3,4}, Sylvie FOURNIER^{2,3,4}, Guillaume MARTI^{2,3,4}, Olivier RODRIGUES², Thierry LAGRAVERE¹, Bernard DUMAS², Alban JACQUES¹.

¹ Unité de Recherche Physiologie, Pathologie, et Génétique Végétales (PPGV), INP PURPAN, Université de Toulouse, Toulouse, France. ² Laboratoire de Recherche en Sciences Végétales, Université de Toulouse, CNRS, UPS, Toulouse INP, Toulouse, France. ³ Metatoul-AgromiX platform, MetaboHUB, National Infrastructure for Metabolomics and Fluxomics, LRSV, Université de Toulouse, CNRS, UPS, Toulouse, INP, France. ⁴ MetaboHUB-MetaToul, National Infrastructure of Metabolomics and Fluxomics, Toulouse, France. * These authors contributed equally to this work. Corresponding author: <u>ana.romeo-olivan@purpan.fr</u>

Context and purpose of the study – Esca, one of the main grapevine trunk diseases, is a complex and poorly understood disease. *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*, two of the main pathogens associated to this disease, are thought to be responsible for the first trunk infections. Little is known concerning grapevine trunk defenses during pathogen infection. Therefore, the aim of the study was to characterize the host responses in the grapevine wood to either pathogen alone. In parallel, we also evaluated how these responses were modified by the presence of a commercial biocontrol agent (*Trichoderma atroviride*, Vintec[®]).

Material and methods – One year-old canes of Cabernet-Sauvignon clone 15 were divided into two-dormant nodes cuttings, then planted in individual pots and grown in controlled conditions (culture tents, photoperiod 12h/12h, 25°C, 45 % humidity). Cuttings were infected with P. minimum and/or P. chlamydospora, in the absence or presence of Vintec[®]. Wood samples were collected at 48 hours post infection (hpi) for global transcriptomics analysis (RNA sequencing) and at 3 weeks post infection (wpi) for metabolomics analyses.

Results - Transcriptomic analysis identified specific sets of differentially expressed genes associated with each pathogen. Functional analysis of these genes revealed differences mainly in "Signaling", "Hormonal signaling" and "Biotic stress response". In addition, we identified clusters of genes differently regulated in the presence of Vintec[®] during the infection. Phenylpropanoid metabolism and stilbene biosynthesis-related genes were significantly represented among the genes differently expressed in the presence of Vintec[®]. Metabolomic analysis highlighted a group of flavonoids and stilbenoids that were overproduced in inoculated plants, compared to non-inoculated plants. Further, metabolomic analysis identified specific metabolites associated with each pathogen. The presence of Vintec[®] resulted in changes in the production of several metabolites. Five relevant 'biomarkers' were chosen for *in vitro* evaluation of their antifungal activity on *P. chlamydospora*. The results suggest that these compounds may play a role in limiting the *in planta* development of the pathogens. Altogether, our results show (i) that the trunk may differently 'perceive' and thus respond to P. minimum and *P. chlamydospora* and (ii) that the Vintec[®] can modify these responses, in a positive way for the plant.

Keywords: Esca, grapevine trunk diseases, plant defense, P. chlamydospora, P. minimum, biocontrol.