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## TOWARDS A METABOLOMIC CHARACTERISATION OF THE GRAPEVINE RESPONSE TO FLAVESCENCE DORÉE INFECTION BY NMR AND LC-MS PROFILING.

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Flavescence dorée (FD) is a quarantine disease of grapevine, involving interactions between the plants, leafhopper vectors (phytophagous insect *Scaphoideus titanus* Ball, *Cicadellidae* family), and Flavescence dorée phytoplasma. This disease is a major threat to vineyard survival in different European grape-growing areas.

Differences in FD susceptibility between grapevine varieties exist, in terms of multiplication of the associated phytoplasma and its spread in the plant. Cabernet Sauvignon (CS) is highly susceptible to FD, whereas Merlot (M) is less susceptible. The objective of this project is to determine which primary and specialised metabolites are altered in grapevines following infection by FD phytoplasma under controlled conditions. Plants of CS and M were infected with FD phytoplasma on a single leaf via the natural insect vector *S. titanus* in a high containment greenhouse. Sampling took place 7-day, or 7-week post-inoculation. The leaves were harvested and immediately frozen in liquid N<sub>2</sub> to quench metabolism. Phytoplasma titers were determined on fresh powder of each leaf. Metabolomic analyses were performed on a selection of the leaf sample extracts by <sup>1</sup>H-NMR, LC-LTQ/Orbitrap-HRMS, and LC-QqQ-MS. NMR spectra were acquired at 500 MHz and processed using NMRProcFlow (nmrprocflow.org). For both LC-MS, metabolites were separated by reverse-phase chromatography with a H<sub>2</sub>O/MeCN gradient. Untargeted experiments were performed on a UHPLC-ESI-LTQ/Orbitrap-MS with an ESI probe operated in positive mode and processed using MS Dial v4.7.8. Targeted experiments were performed on an LC-QqQ-MS system with a MRM method in positive or negative mode and the quantification by using calibration curves built with standards. The phytoplasma titre data, metabolite quantification data or metabolomic signatures of leaf samples were managed with ODAM tool.

Visual observations of the NMR spectra revealed several tendencies: (i) 'insect bite' effects, (ii) 'grapevine variety' effects in infected leaves and (iii) 'infection' effects in M. These effects are reflected in a variation in the content of certain metabolites. Twenty-one primary metabolites including sugars, amino and organic acids were identified by NMR and 15 specialised metabolites (flavonoids, stilbenes and cinnamic acids) by LC-MS. For the annotation of still unknown signals, further NMR and LC-MS acquisitions will be performed. PCA of  $^1\text{H}$ -NMR data on primary metabolites, untargeted LC-HRMS and targeted LC-MS data both on specialized metabolites showed group discrimination according to grapevine variety but also between the healthy and infected states. The spectral variables from untargeted analyses and specialized metabolites from targeted analysis differentiating the varieties or infection levels could be used as biomarkers of response to infection and should contribute to the identification of the pathways disrupted by infection.

These preliminary results are encouraging. The metabolome experiments and the statistical analyses of the metabolomic profiles will continue with the aim of highlighting metabolites and pathways that respond differentially between CS and M varieties in order to identify which ones are associated with better resistance to this disease. These data will allow a better understanding of the grapevine-FDp interactions and will be useful in the context of varietal selection.

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