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Grapevine resistance to *Plasmopara viticola*: the search for metabolic biomarkers

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Introduction

Vitis vinifera L. cultivars are highly susceptible to downy mildew, caused by *Plasmopara viticola* (Berk. et Curt.) Berl.et de Toni, which affects all the green parts of the vine, causing yield reduction and significant production losses. Thus, if not controlled, it presents serious negative effects in several countries' economy. To cope with this threat, Breeding mildew resistant cultivars is the most effective and environmentally friendly approach against mildew. Resistance to *P. viticola* (RPV) in crossing lines achieved by crossing suitable parent lines or cultivars and the subsequent selection in the offspring to identify desired combinations of traits in particular resistance and quality. However, this process is laborious, takes years to accomplish and in some countries even though there are resistant cultivars, some *P. viticola* pathovars already evolved and surpassed these resistance. Since plants contain a unique metabolome that change upon pathogen infections[1] and could allow us to identify specific compounds associated to either resistance or susceptibility traits.

Our aim is to use an untargeted metabolomics based approach to understand the innate resistance mechanism of cultivars. Once resistance associated metabolites are identified, these can be used as metabolic markers, thus allowing for faster screening on breeding programs. We performed *P. viticola* controlled infections in a new bred cultivar: 2011-64-0002 (created at Julius Kühn Institute - Institute for Grapevine Breeding, Geilweilerhof); and evaluated the lipid profile and characterize the metabolome after 12, 24, 48, 72, 96 hours post-infection and 8 days post-infection.

Materials and Methods

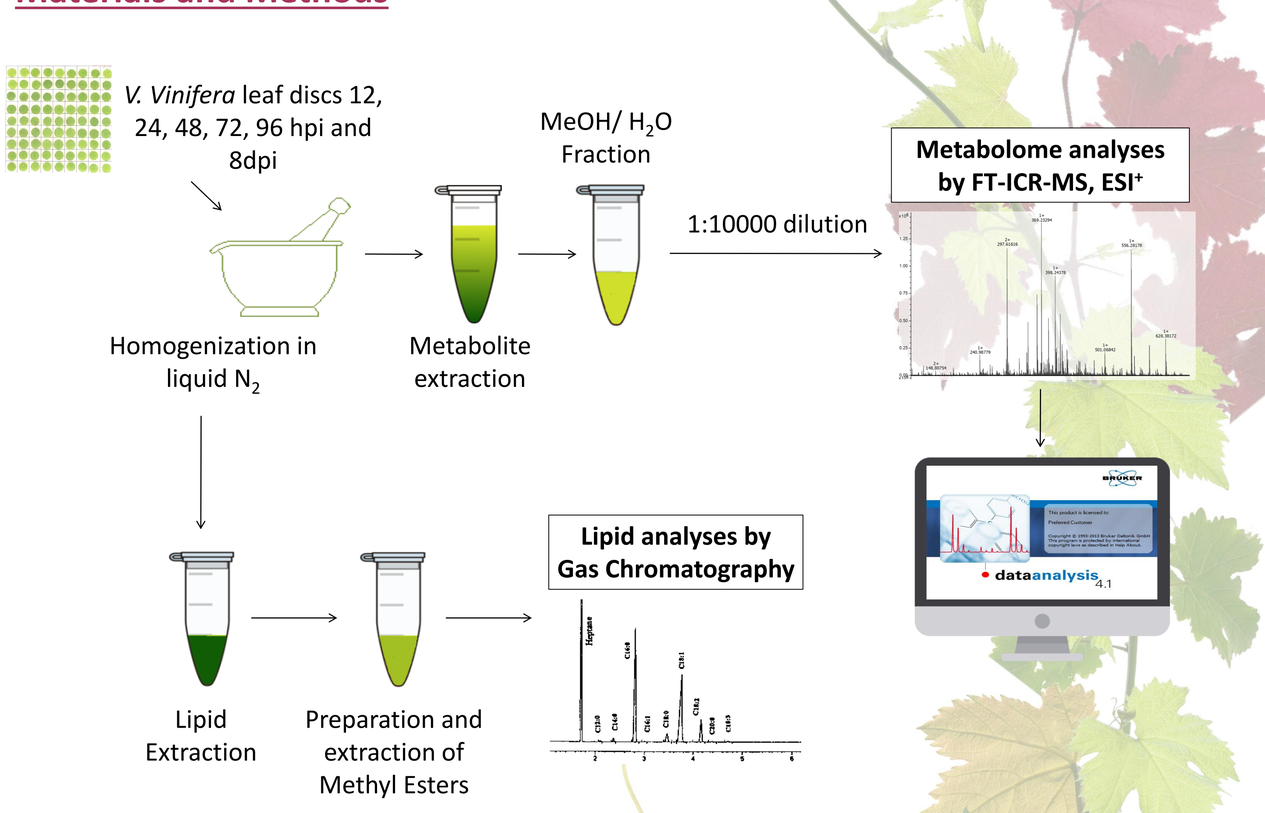

Fig. 1 – Experimental procedure for grapevine leaves metabolite extraction (adapted from Maia et al., 2016[2]) and analysis through FT-ICR-MS and lipid extraction and analysis by gas chromatography.

Results

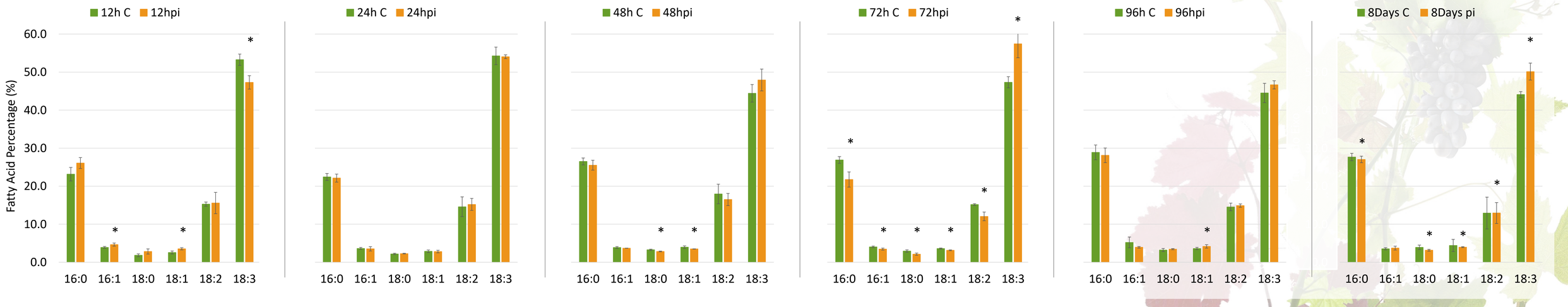

Fig. 2 – Lipid profiling of GC measurements. Total percentage of Palmitic acid (C16:0), Palmitoleic acid (C16:1), Stearic acid (C18:0), Oleic acid (C18:1), Linoleic acid (C18:2) and Free linolenic acid (C18:3). Values correspond to the average of the relative percentage of each fatty acid of inoculated and control samples at each time point post inoculation ± standard error. Mann-Whitney Test was performed to test for significant differences between inoculated and control samples. (* indicates p<0,05).

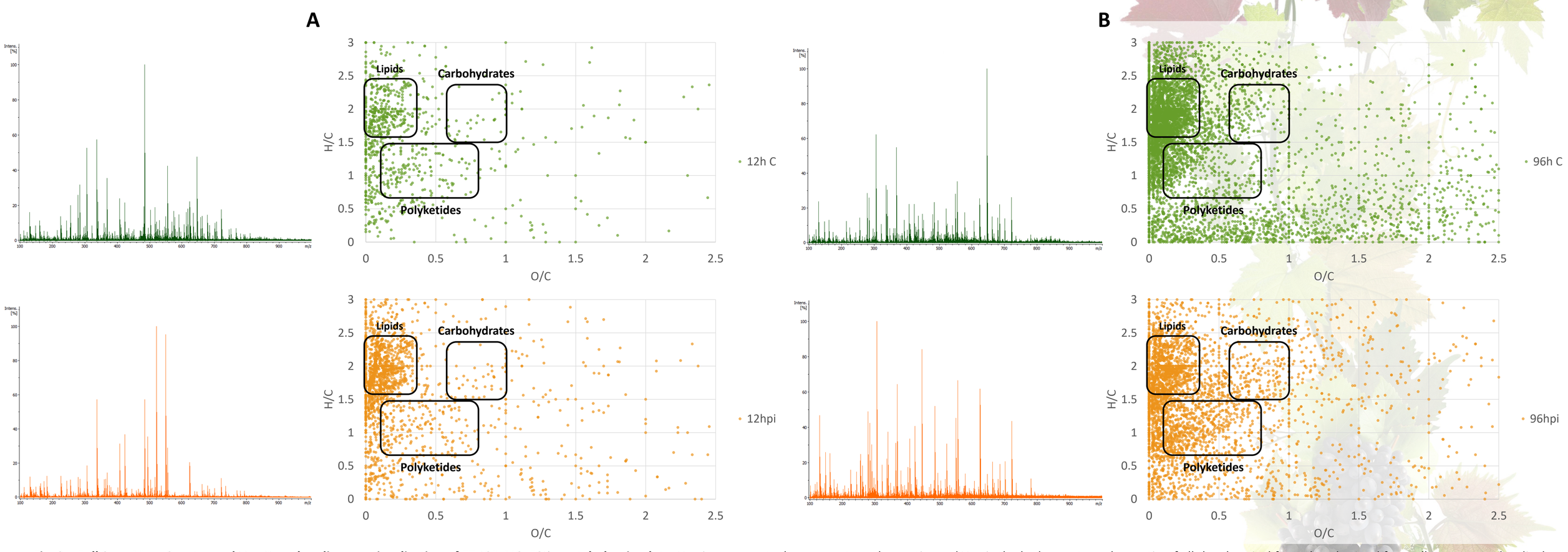

Fig. 3 – Full Scan Mass Spectra and Van Krevelen diagram visualization of FT-ICR-MS, ESI+, metabolomics data. X-axis represents the oxygen to carbon ratio, and Y-axis the hydrogen to carbon ratio of all the chemical formulas obtained from all spectrum. Plot displays the areas of highest point density for the 3 most important major classes of metabolites: lipids, polyketides, carbohydrates. **(A) 12 hours control and post-inoculation with P. *viticola* samples; (B) 96 hours control and post-inoculation with P. *viticola* samples.**

Conclusions

Significant differences in the metabolic profile between controls and inoculated samples were observed;
Major metabolic changes occur after inoculation with *P. viticola*. At late hours of infection different metabolic classes are more represented;
Lipids, polyketides and carbohydrates are the most altered classes;
Molecular biology and statistical work is now being conducted in order to identify specific compounds associated with resistance.

Acknowledgments

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