FT-ICR-MS metabolomics approach to uncover grapevine resistance to downy mildew

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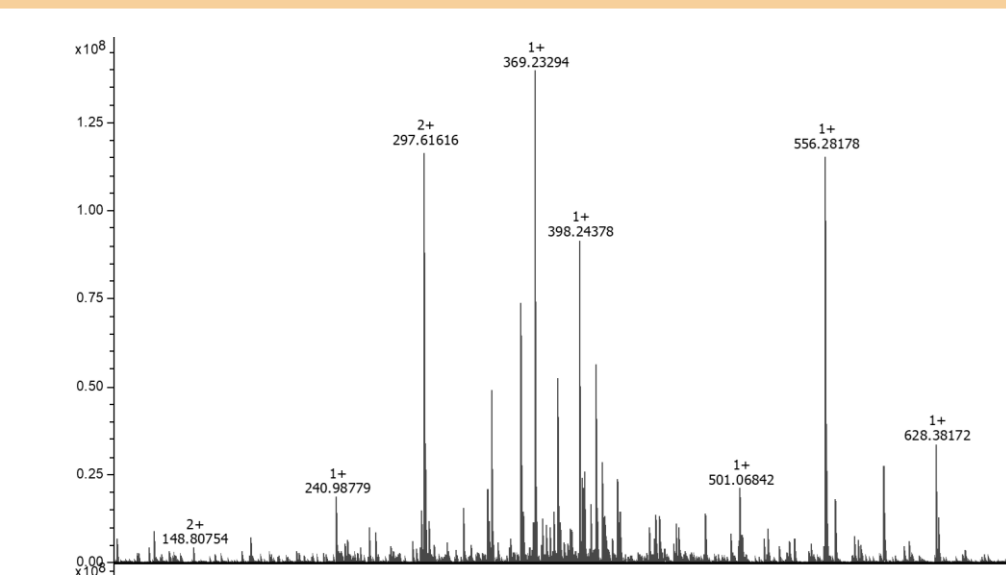# mrmaia@fc.ul.pt

State of the art

Grapevine (*Vitis vinifera* L.) is one of the most important and cultivated fruit plant in the world, mainly due to the wine industry. However, *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 [1].

To understand the innate susceptibility/resistance mechanism of *Vitis*, a metabolic characterization of grapevine leaves is of upmost importance, since plants contain a unique metabolome that change upon pathogen infections [2] 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. To achieve that, we resort to Fourier-Transform Ion Cyclotron-Resonance Mass Spectrometer (FT-ICR-MS), which provides ultra-high-mass accuracy and the highest mass resolution [3]. Moreover, using direct infusion metabolites are analyzed in a high-throughput way, providing a rapid analysis of complex metabolite samples, and eliminating the time-consuming separations [4].
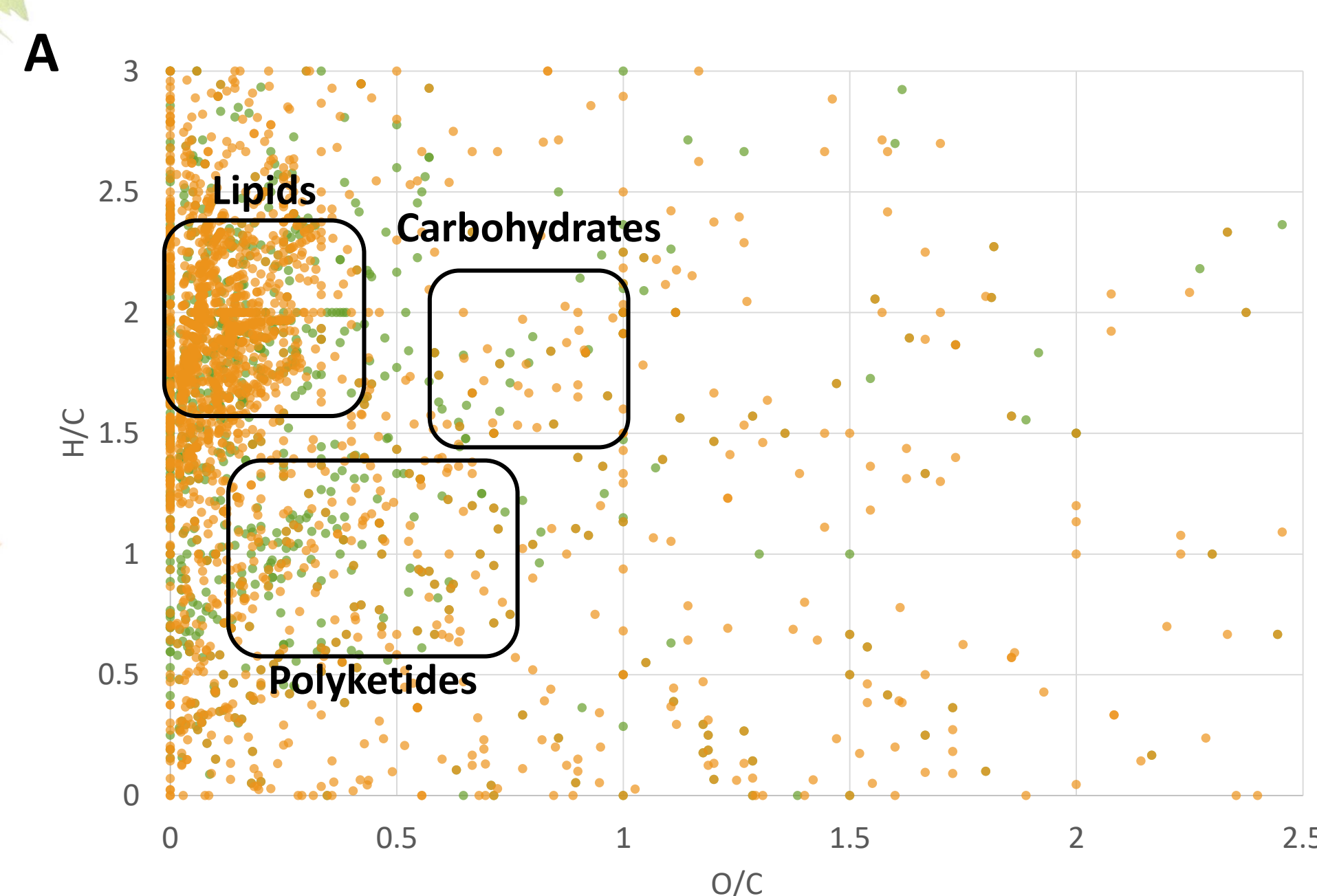
V. Vinifera leaves

Metabolic Analysis

Extraction of grapevine leaf metabolome according to [5]

Metabolic comparison to identify resistance associated metabolic biomarkers

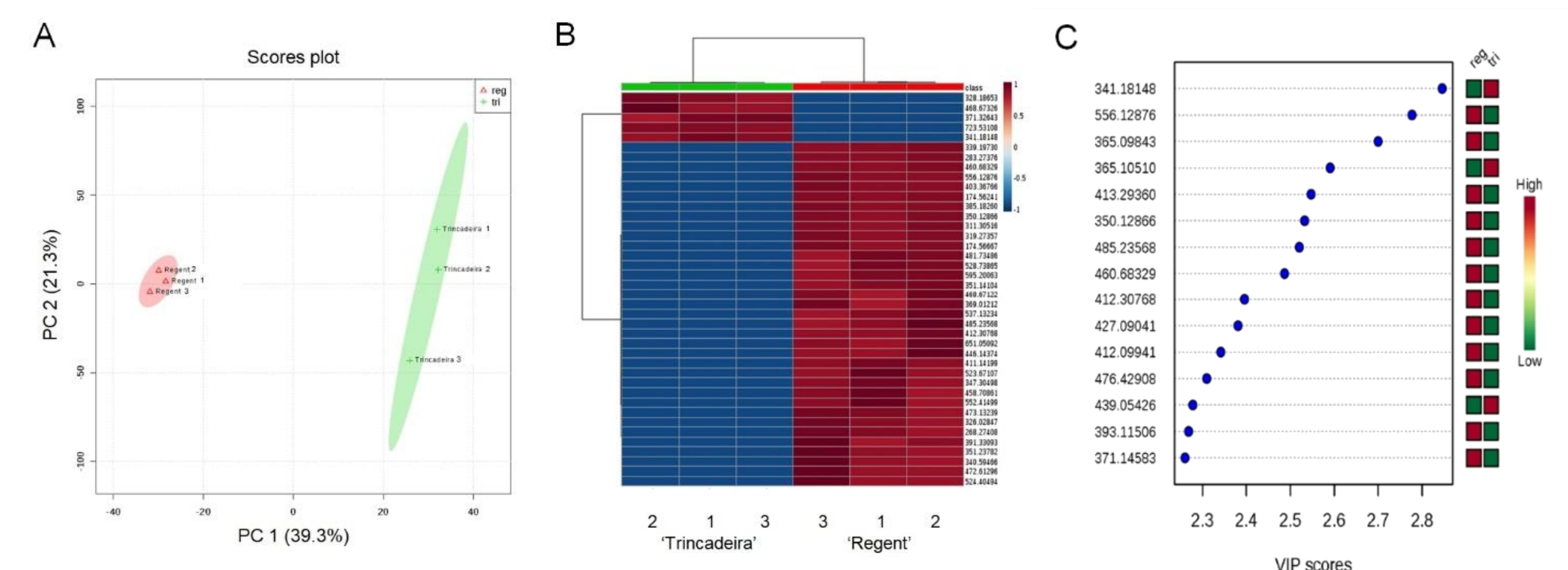### Non-infected Resistant vs Susceptible Analysis

Figure 1 - Metabolome difference and discrimination between 'Trincadeira' (Susceptible) and 'Regent' (Tolerant). (A) PCA scores with sample labels and 95% confidence regions shown. (B) Sample hierarchical clustering and heatmap using the top 50 most significant (t-test p-values) MS peaks. (C) Top discriminative peaks in a PLS-DA classification model, shown by decreasing scores of Variable Importance in Projection (VIP) over the first component.
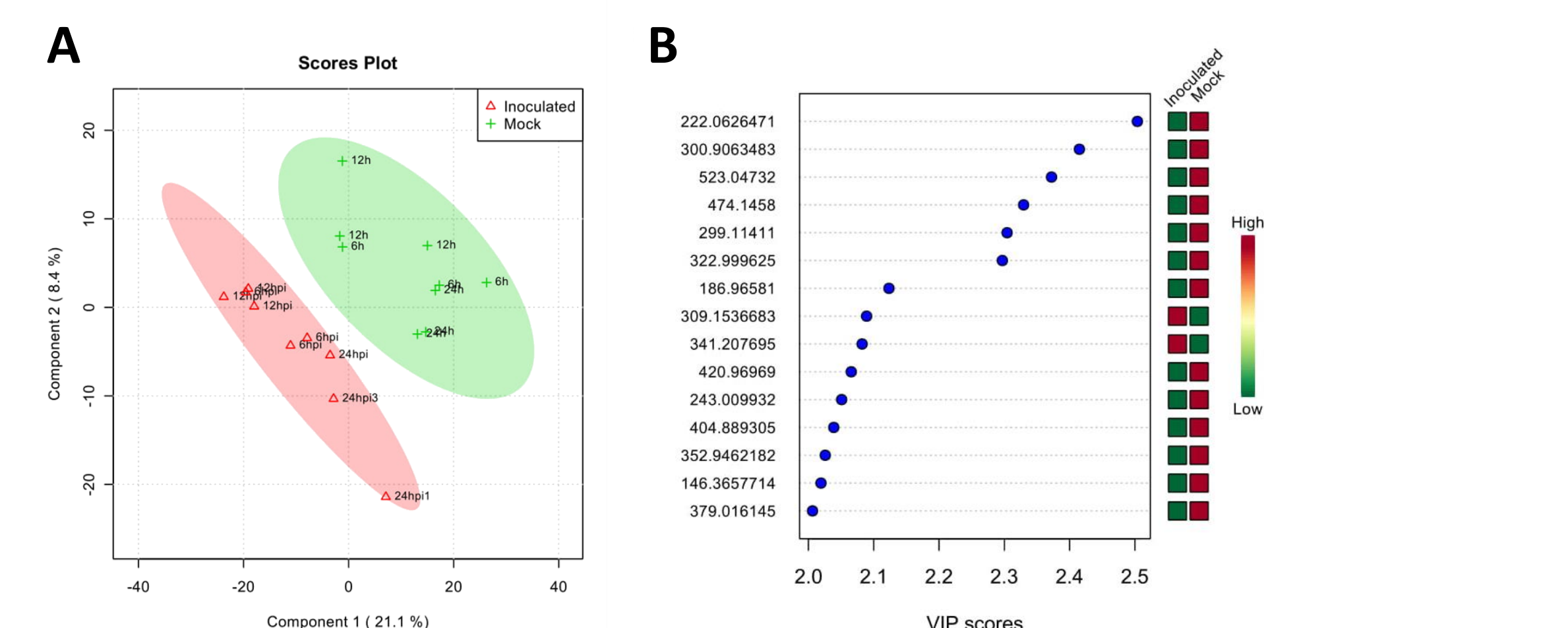
Infected Susceptible Cultivar Analysis

Figure 2 - Partial least squares-discriminant analyses (PLS-DA) PC1/PC2 score plots and variable's importance (VIP scores) of secondary metabolite profiles of *Vitis vinifera* cv. Trincadeira at 6, 12 and 24 hpi with *P. viticola*.
(A) PLS-DA score plot of ESI(-) samples; (B) VIP scores of Component 1 in ESI(-). Three biological replicates were performed per analysis.
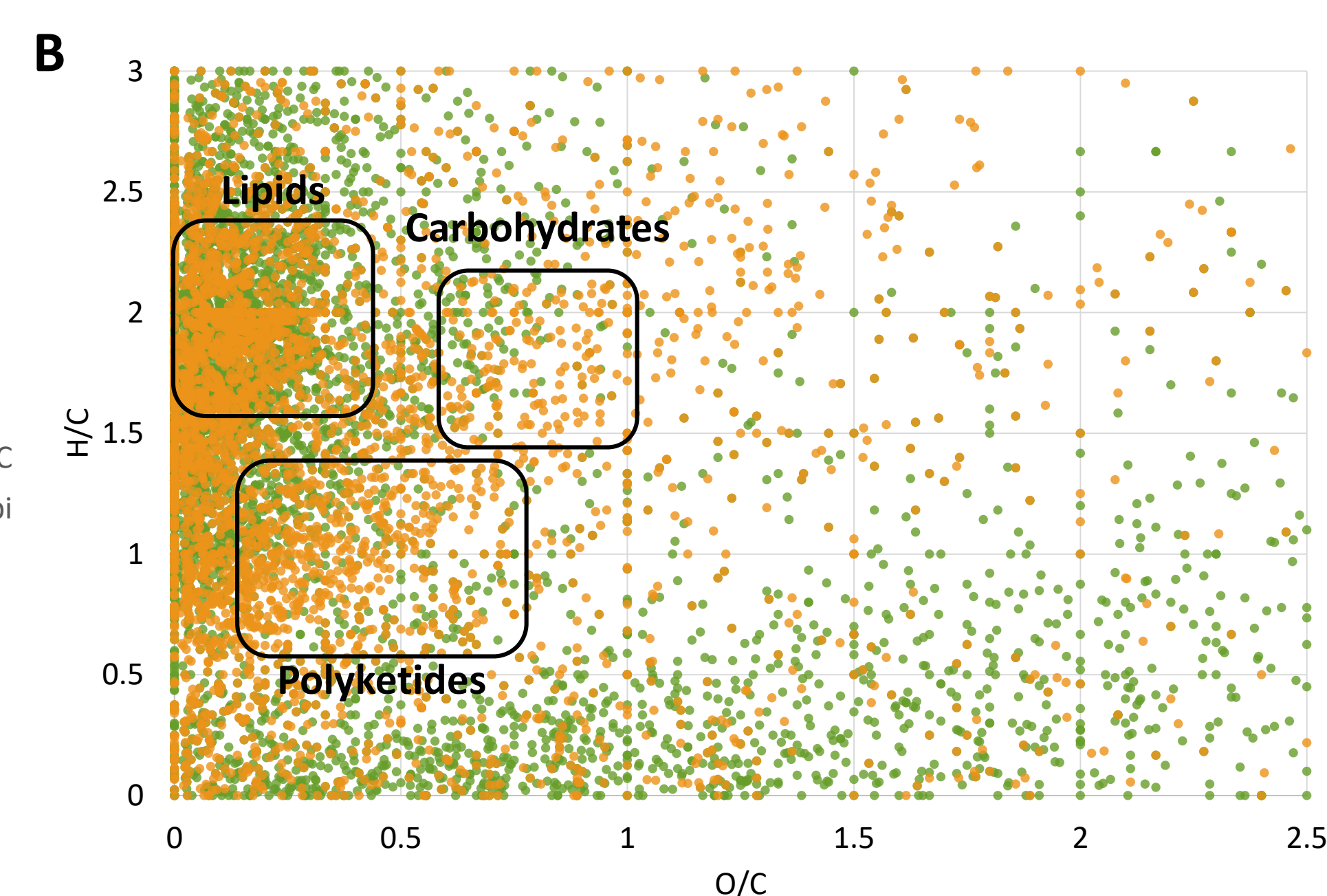
Infected Resistant Cultivar Analysis

Figure 3 – Van Krevelen diagram visualization of FT-ICR-MS, ESI+, metabolomics data. The X-axis represents the oxygen to carbon ratio, and the 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.

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