

# Differential phenolic production of *Vitis vinifera* cv. Alvarinho leaves affected with Esca disease

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## Introduction

Esca is a destructive disease that affects *Vitis vinifera* around the world leading to important losses in wine production. Information on the response of *Vitis vinifera* plants to this disease is still scarce.

To study the defence mechanisms of *Vitis* plants to Esca, we analysed leaves from both infected and non-affected boughs of *Vitis vinifera* cv. Alvarinho, of the Vinho Verde region (North of Portugal). Phenolics were analysed by HPLC-DAD-MS and by multivariate statistical analysis.

## Experimental

### Sample collection:

Several leaves with and without visible symptoms were collected from infected boughs (respectively diseased (d) and apparently healthy (aph) leaves); leaves were also collected from asymptomatic boughs (healthy leaves (h)).

### Phenolic Analysis:

HPLC-DAD analysis of methanolic extracts from leaves: reverse phase column with gradient elution. Moreover samples were subjected to LC-MS analysis; Agilent 1100 LC/MSD Trap; ESI, negative ion mode; scan range 200-1500 amu.

### Multivariate Statistical Analysis:

Peak areas were corrected by the amount of biomass extracted of the corresponding sample. Peaks were aligned one by one based on relative retention time and UV spectra. Data was analysed by principal components analysis (PCA) with SIMCA-P software (Umetrics, Umea, Sweden).

## Results

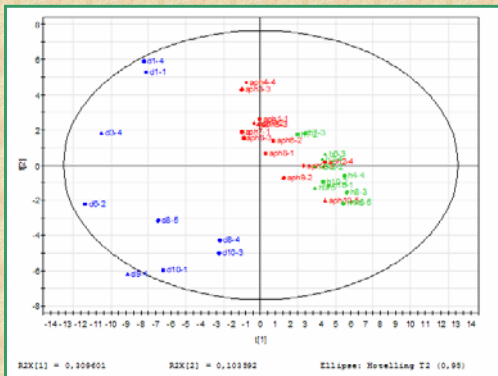


Figure 1 – Scores plot of PCA analysis of HPLC data, showing separation of diseased (d), apparently healthy (aph) and healthy (h) leaves based on the compounds contributing to components 1 and 2 extracted in PCA analysis. The ellipse represents the Hotelling T2 with 95% confidence.

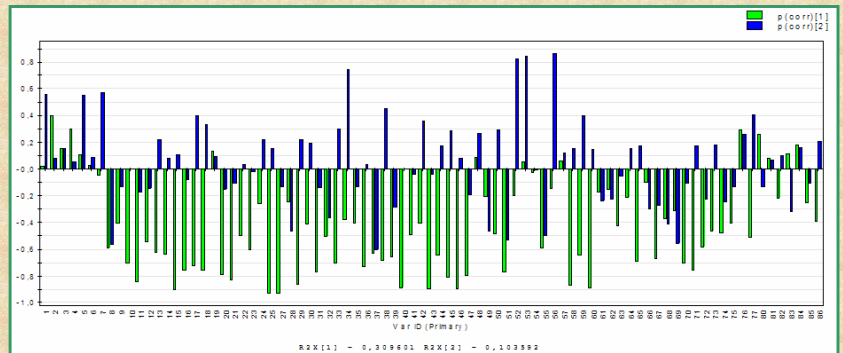


Figure 3 – Plot showing the correlation of each compound detected by HPLC (named 1 to 86) and components 1 (green) and 2 (blue) extracted by PCA analysis.

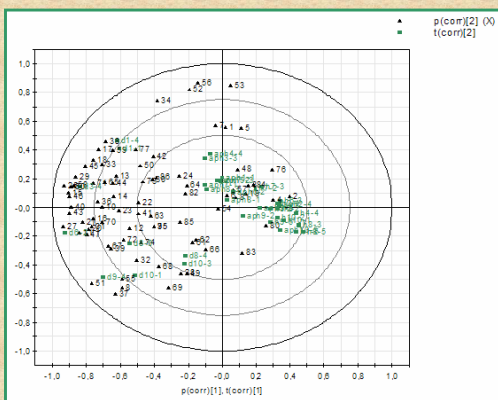


Figure 2 – Loadings and scores bi-plot of PCA analysis of HPLC data, showing the association of d, aph and h leaves with the compounds (named 1 to 86). The circle represents correlation.

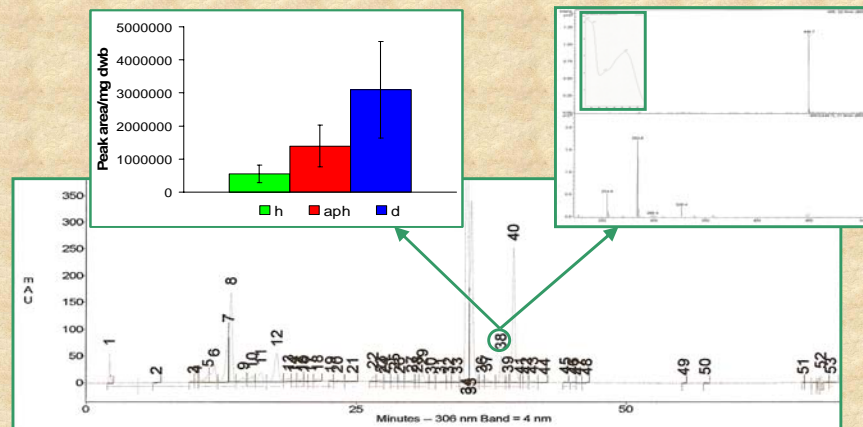


Figure 4 – Chromatogram representative of d leaves; mass spectra and UV profile of peak 38 (upper right) identified as Kaempferol-3-glucoside; amount of Kaempferol-3-glucoside in h, aph and d leaves (upper left).

## Conclusions

- Differences in phenolic profile between d, aph and h leaves were revealed by multivariate statistical analysis (Figures 1, 2 and 3).
- The PCA analysis showed a clear separation between d, aph and h leaves, with the aph samples clustered in a median position (Figure 1).
- This indicates a change in phenolic production, induced by the presence of pathogens, even before appearance of disease symptoms.
- Several compounds were correlated with d leaves, e.g. Kaempferol-3-glucoside was highly correlated ( $p < 0,01$ ) (Figure 4).