3rd European Conference on Xylella fastidiosa and XF-ACTORS final meeting

Phenometabolomics of olive plants infected with *Xylella fstidiosa* using Nuclear Magnetic Resonance, high Resolution Mass Spectrometry, Hyperspectral Reflectance, and Integrative Chemometrics Analysis

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INTRODUCTION

- Comparison of metabolic profiles and hyperspectral reflectances of Xf-infected olive plants can be exploited to identify infection markers and related wavelengths and set up early disease detection strategies through Remote Sensing.

MATERIALS & METHODS

Experimental design

- **Plants**: 100 young olive trees (3-4 years)
- **Variety**: Cellina di Nardò
- **Inoculum**: *X. fastidiosa* (ST53 strain)

  Phaeoacremonium group:
  - *P. rubrigenum* (N20 strain)
  - *P. aleophilum* (B1a strain)

  Pseudophaeomoniella group:
  - *P. oleae* (Fv84 strain)
  - *P. oleicola* (M24 strain)
  - *P. oleicola* (M51 strain)
- **Sample**: Number of mature leaves statistically significant (10-15)
- **Measurements**: spectral signatures, NMR and HRMS spectra, molecular analysis on the same leaves
- **Period**: December 2017 (2 years after inoculation)
- **Controlled climatic conditions**: 24 °C ± 2 °C; Relative Humidity over 80 %

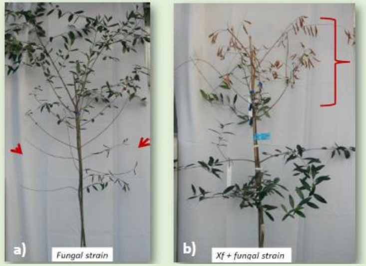
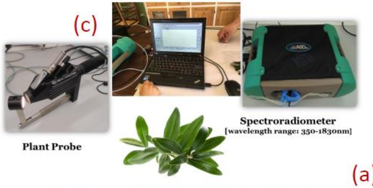


Figure 1: Symptoms in olive plants a) inoculated with fungi and b) inoculated with fungi and *Xf.*



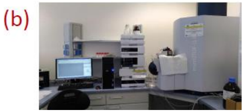


Figure 2: Nuclear Magnetic Resonance (NMR) (a), Mass Spectrometric (MS) (b) and Hyperspectral Reflectance (HR) (c) techniques used in this study

- Trees undergo phenotypic and metabolic changes during the infection period, as demonstrated by a recent non-targeted metabolomic study under thermo-conditioned greenhouse of Xf-infected olive plants co-inoculated with xylem fungi (Jlilat *et al.*, 2021). All acquired spectra were analyzed using statistical chemometric techniques.

RESULTS

- Statistical characterization of primary (NMR) and secondary (MS) metabolites associated with Xf infection, in combination or not with fungal isolates, indicated that Xf-infected samples differed from those infected only with fungi and from the control (Jlilat et al., 2021).

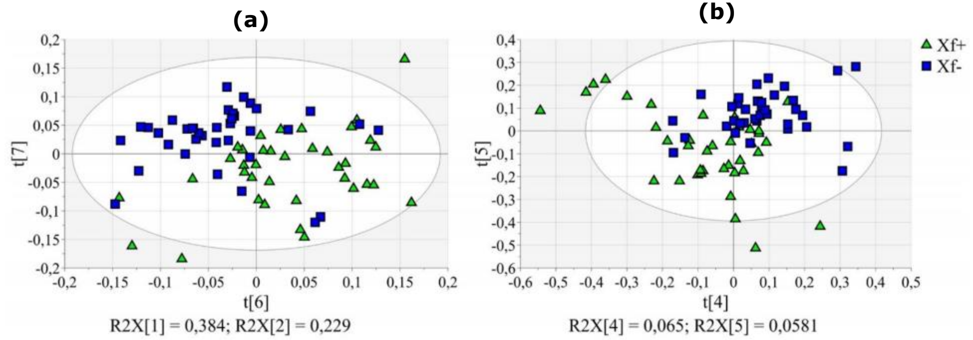


Figure 3: Principal Component Analysis (PCA) applied to NMR and MS data obtained from olive leaf analysis: (a) PCA applied to NMR data; (b) PCA applied to MS data.

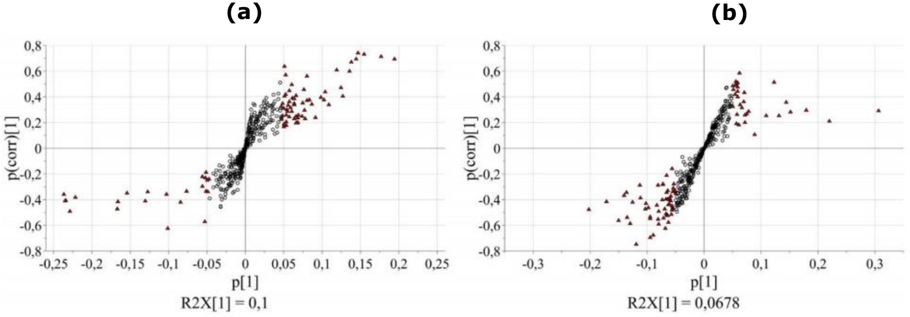


Figure 4: Identification of metabolites with the highest potential as biomarkers (red triangles) by applying Partial Least Square-Discriminant Analysis (OPLS-DA) to NMR (a) and MS (b) data.

RESULTS

- Higher amounts of **malic acid**, **formic acid**, **mannitol**, and **sucrose** were found in Xf-infected plants than in non infected plants.
- In contrast, infected plants revealed lower amounts of **oleuropein** and **glucose**.

Signal	Metabolite	δ (ppm)	MMW	Correlated MS bucket $[M-H]^-$
1	Oleuropein	1.57, 2.49, 2.65, 5.76, 6.03, 7.51	540.184	539.182
2	Oleuropein aglycone	2.85 – 2.89, 8.91, 9.33	378.132	377.118
3	Malic acid	2.54, 2.75, 4.45	134.022	133.013
4	Mannitol	3.66 – 3.90	182.079	181.073
5	Glucose	3.25, 3.38 – 3.56, 3.65 – 3.90, 4.65 (anomeric-H β-glucose), 5.24 (anomeric-H α-glucose)	180.063	179.057
6	Sucrose	5.40, 4.21, 4.06	342.116	341.111
7	Aromatic compounds	6.80 – 7.00	-	-

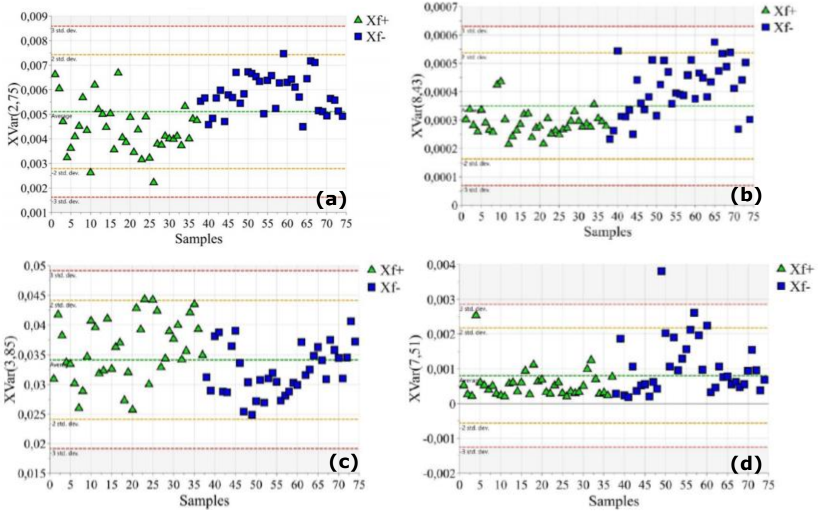


Figure 4. Assignment of relevant metabolites in the 1H NMR spectrum of *X. fastidiosa* infected leaves and statistic of the buckets attributable to malic acid (a), to formic acid(b), to mannitol(c) and to oleuropein (d). Xf+: samples of Xf-inocultaed plants; Xf-: samples of uninoculated plants.

RESULTS

- Covariance matrices between NMR, MS, and HR methods were used to link HR spectral features with NMR and MS diagnostic signals.
- Several wavelength ranges *positively* and *negatively* related with the assigned metabolites were associated:

Visible: [480-520 nm]; [625-645 nm] e [670-705 nm]

Infrared: [950-1000 nm]; [1165-1190 nm] e [1420-1485 nm].