

# Exploiting Metabolomics to Unravel Priming of Defence in Oak

seedlings against Powdery Mildew
Rosa Sanchez-Lucas, Jack Bosanquet, Victoria Pastor, Estrella Luna
1: BIFOR, University of Birmingham, Birmingham B15 2TT, United Kingdom; 2: Metabolic Integration and Cell Signalling Group, University Jaume I, Castellon 12071, Spain



Introduction

## Plants are continually exposed to multiple stresses and have highly

- sophisticated strategies to fight against these threats. • Priming of defence is a sensitisation of defence mechanisms for a faster and stronger activation upon subsequent attack.
- Some chemicals are known to trigger priming but there have been a lack of studies in oak seedlings.
- Oaks predominate deciduous European forest and are endangered by climate change and pathogens.
- Powdery mildew (PM) is a bottleneck to natural regeneration in UK woodlands (seedling mortality, reduced photosynthetic capacity). reduction,

#### Aims

- To determine whether oak seedlings can express chemically-induced priming resistance against PM.
- To unravel priming mechanism by LC-MS/MS QTOF Untargeted Metabolomic Analysis.

#### Materials and Methods Elicitor treatments on 3 month old seedlings Sodium salicylate + 5mM SA 0.002% EtOH Spraying Water + 0.002% EtOH (0.05% Silwet) Jasmonate + 0.002%

PM inoculum applied by spraying 1.5x10<sup>6</sup> Erysiphe

Untargeted metabolomic approach

MS/MS QTOF.

Leaves collected at 0, 1 and 2 dpi for LC-

Spectra-data analysis

Inoculation after 1 week

*alphitoides* spores/mL + 0.05% Silwet

XCMS R script----Statistic Analysis (METABOANALYST) ----Identification (MarVis-Suite)

### Results

Priming phenotypes at early times

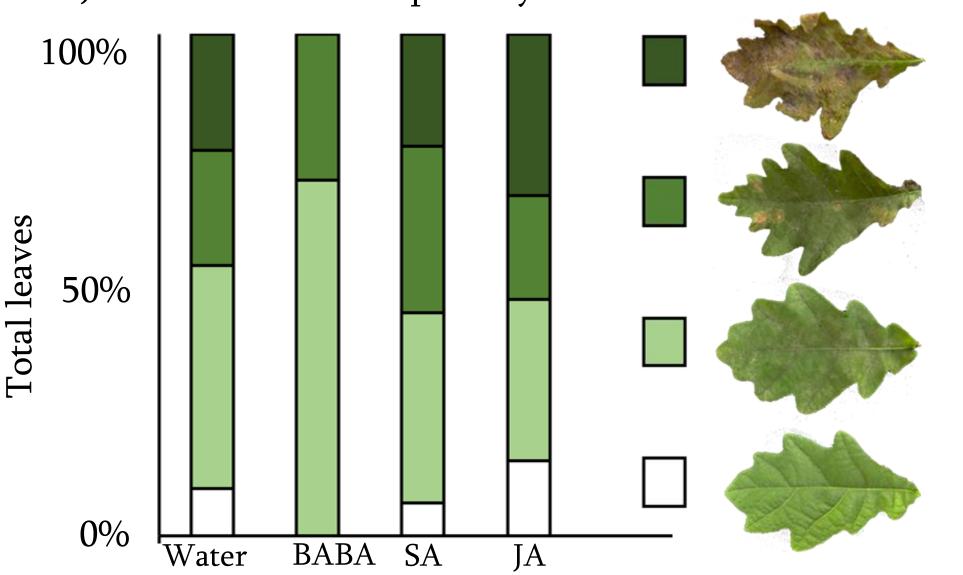
Resistance by SA and BABA was based on priming of SA-

Soil Drench

(Watering)

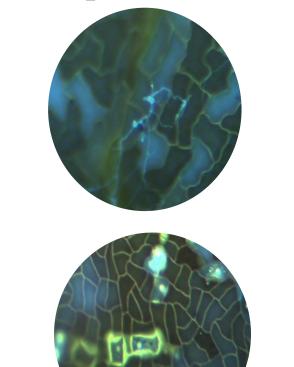
Disease resistance phenotypes

Treatments with SA and BABA result in enhanced resistance to PM and JA in enhanced susceptibility.



dependent gene expression and callose deposition, respectively.

Callose deposition at 2 dpi



PR1 gene expression at 1 dpi

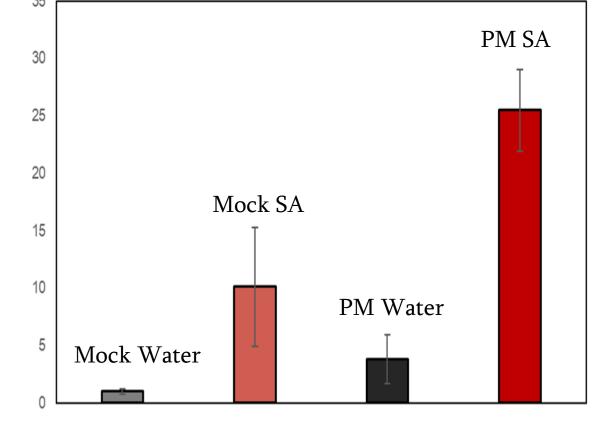
Pot volume/10 of

5mM BABA

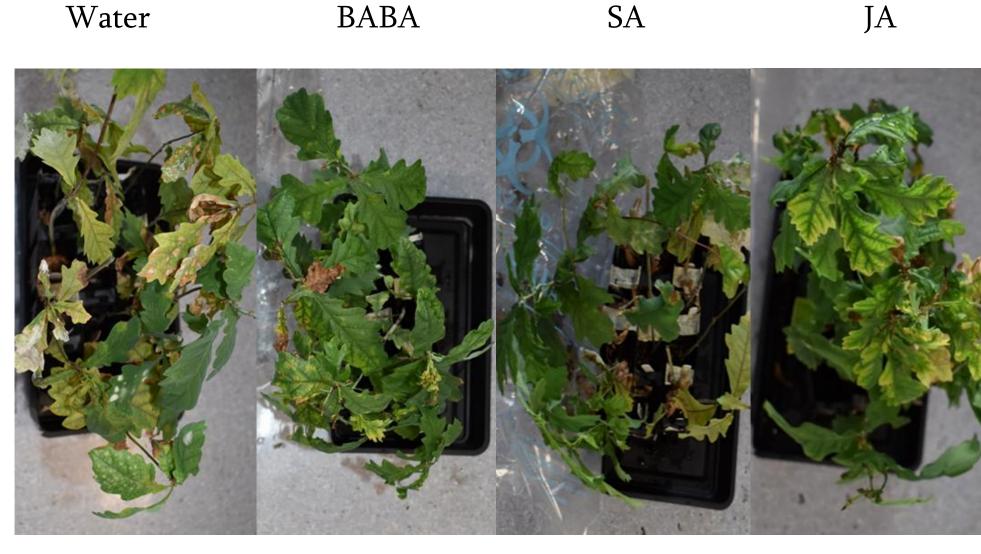
Pot volume/10 of

**BABA** (10x)

water

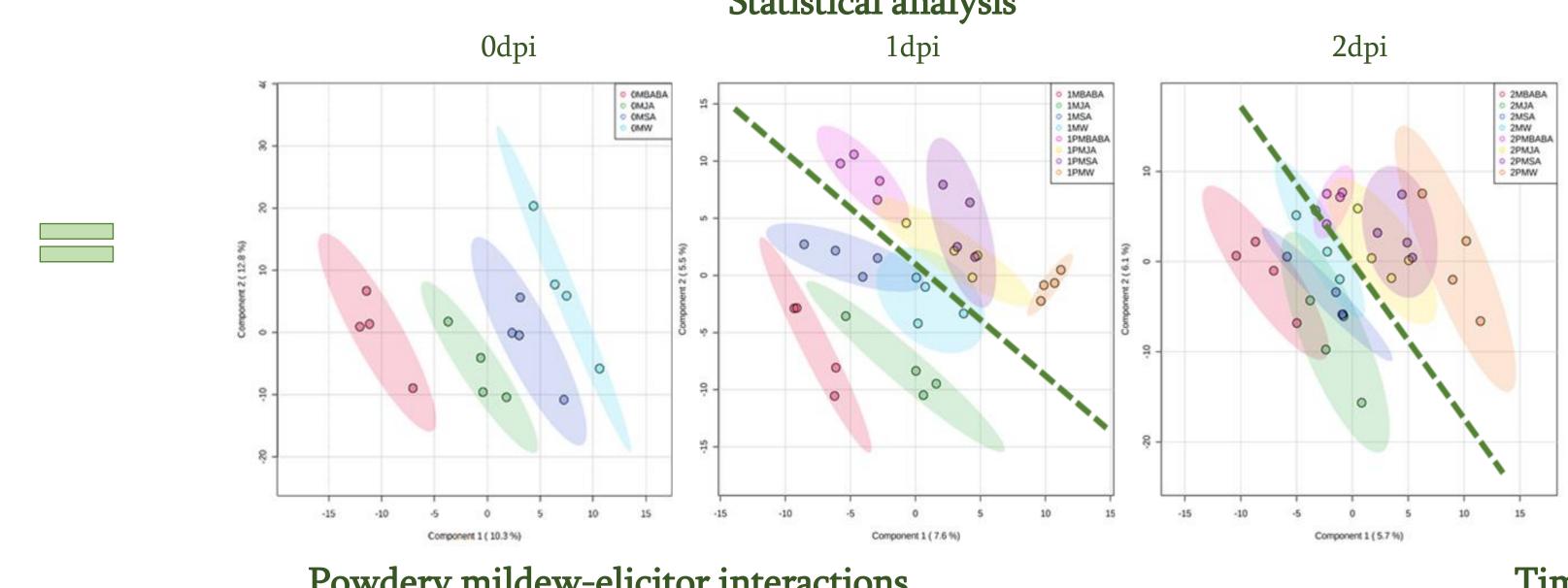


Long-lasting resistance phenotypes

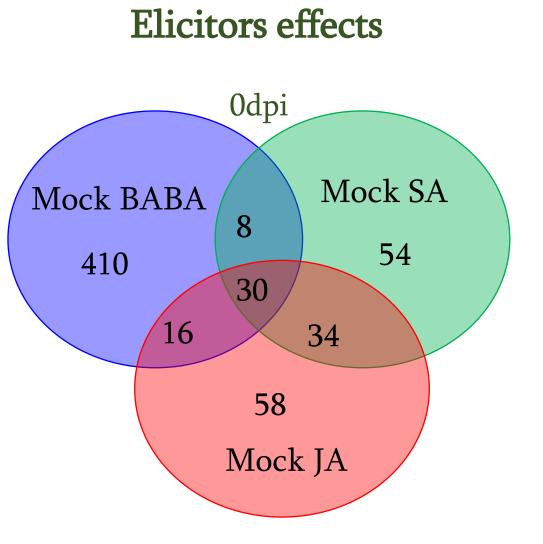


Statistically different m/z

LC-MS/MS QToF untargeted metabolomics Statistical analysis



658 ESI-POS 514 ESI-NEG 377 1dpi 378



ESI-POS

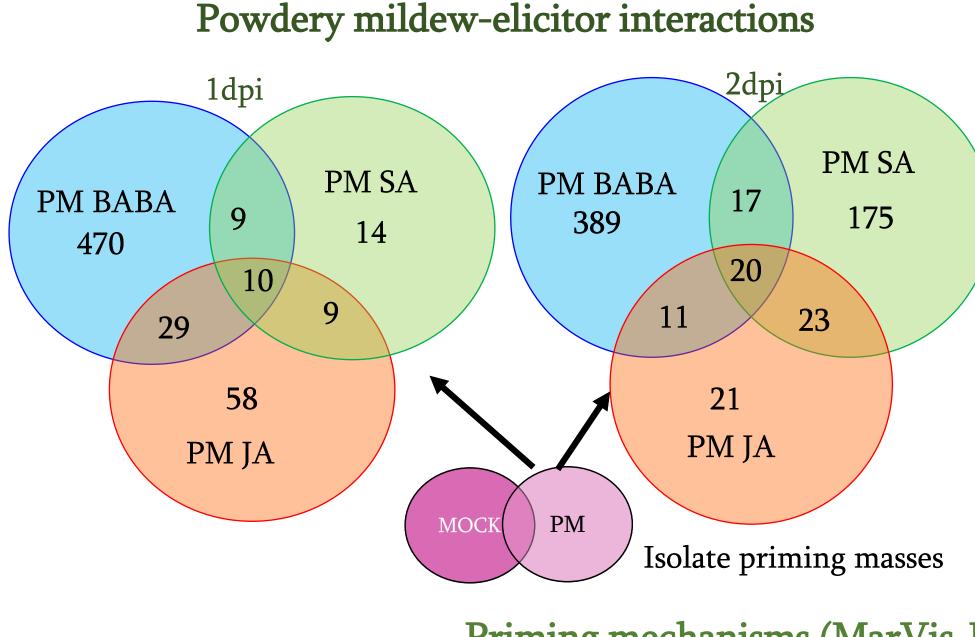
17865 putative

masses

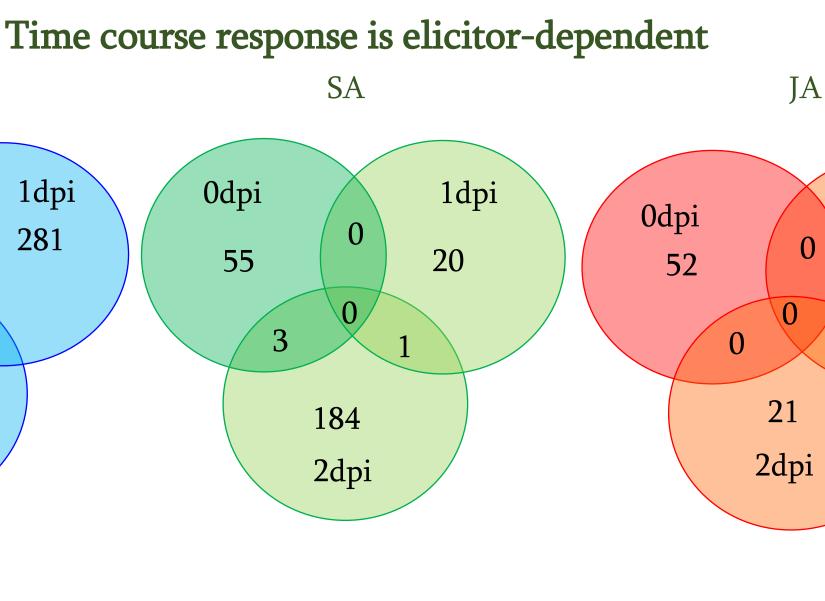
ESI-NEG

9408 putative

masses



BABA 0dpi 1dpi 279 74 105 183 2dpi



Priming mechanisms (MarVis-Pathway) Primed masses for BABA treatment

109 putative masses primed 37 tentative identifications Major represented pathways: alkaloids/flavonols

ESI-NEG

upper figure: prototypes lower figure: cluster size

460 putative masses primed 51 tentative identifications Major represented pathways: Lignans Aromatic compounds ESI-POS

Primed masses for SA treatment upper figure: prototypes | lower figure: cluster size 32 putative masses primed

180 putative masses primed

21 tentative identifications 2 identification max per pathway

13 tentative identifications 2 identification max per pathway

**ESI-POS** 

1dpi

ESI-NEG

#### Conclusions

- Trees can be primed successfully by chemicals, showing different defence responses with SA, JA and BABA treatments.
- BABA treatment causes greater metabolome changes.
- SA and BABA mediate different response mechanisms.
- BABA triggers earlier metabolome changes than SA.
- Preliminary enrichment analyses are inconclusive (low percentage of identified masses)
- > BABA priming is mostly dependent on alkaloids biosynthesis, whereas SA failed to identify any specific pathways.

#### Future perspectives

The next steps in the project are:

- 1) To identify the metabolites responsible for priming by BABA and SA and propose putative biomarkers.
- 2) To test these elicitors against other stresses including:
  - Acute Oak Decline (bacteriosis disease)
  - Drought
  - Climate change resilience Increased CO2 levels
- 3) To link metabolome and transcriptome changes.



