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# Metabolomics approach to study the environmental impact and residues of biocontrol products (BP)

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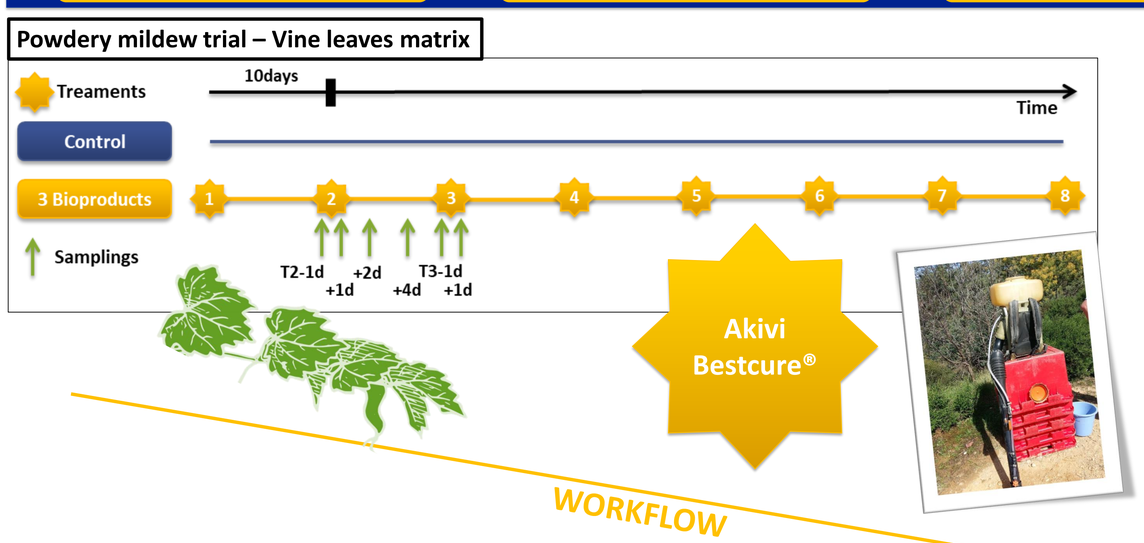
## INTRODUCTION

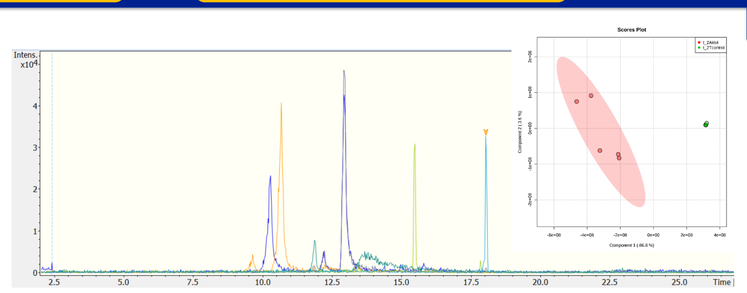
Biopesticides or Biocontrol Products (BP) represents an interesting alternative to ~~the~~ conventional pesticides. However, there is a need of technical efficacy studies and ecotoxicological profile references. The PALVIP project (local Mediterranean crops' alternative protection) aims to fill that gap while evaluating new BP developed by small local businesses partners. To reach that goal, the BP selected in the project are studied according to their efficiency through field experimentations, their effect on plants and their environmental impact.

In a first stage the CRIOBE / University of Perpignan Via Domitia will contribute to the part of the project regarding the evaluation of BPs' environmental impact. To date, the half-life, t1/2, was often used to study the fate of pesticides in environmental matrices. However, this value doesn't give any information regarding the formation of by-products and the effect on biodiversity. Consequently, an innovative approach based on metabolomics (LC-MS), the Environmental Metabolic Footprinting (EMF), was recently developed in the lab.

## ENVIRONMENTAL METABOLIC FOOTPRINTING (EMF)

Field experiment → Field sampling → Extractions → Metabolic profiles (LC-MS) → Data analysis - Results




Qexactive™ (Thermo Fisher Scientific)

**Statistical analysis:**
- **Workflow4metabolomics**
- **MétaboAnalyst**
- **R (Ade4, mixOmics, Rvaidememoire, car)**

The EMF gives rise to:

**(1)** a new integrative proxy, **the resilience time** that corresponds to the time needed for the compound dissipation and its effects on the matrix.

**(2) the preharvest interval (PHI)** that corresponds to the time needed to have no residue difference between the treated samples and controls.

## ANALYSES (LC-MS)

Extracts are analysed on the UHPLC-HRMS Vanquish – Qexactive™ instrument (Thermo Fisher Scientific) according to the following parameters:

### Liquid chromatography (LC)

- Polar C18 column (Luna® Omega 1.6µm Polar C18 100 Å, 100 ×2.1 mm (Phenomenex)),

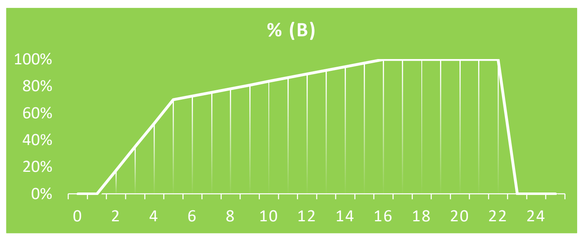
$t_{column}$ = 30°C

- **Elution:**

(A) 65% eau/ 35% methanol

(B) 100% methanol

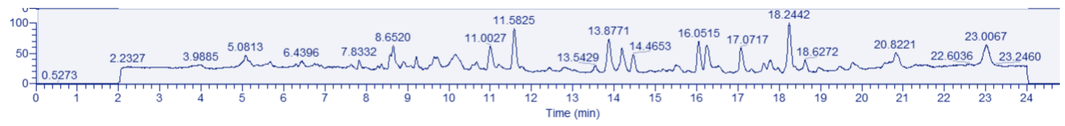
- 0.35 mL/min flow
- 25min optimized gradient




### Mass spectrometry (MS)

- Mass analysis with ESI+ mode

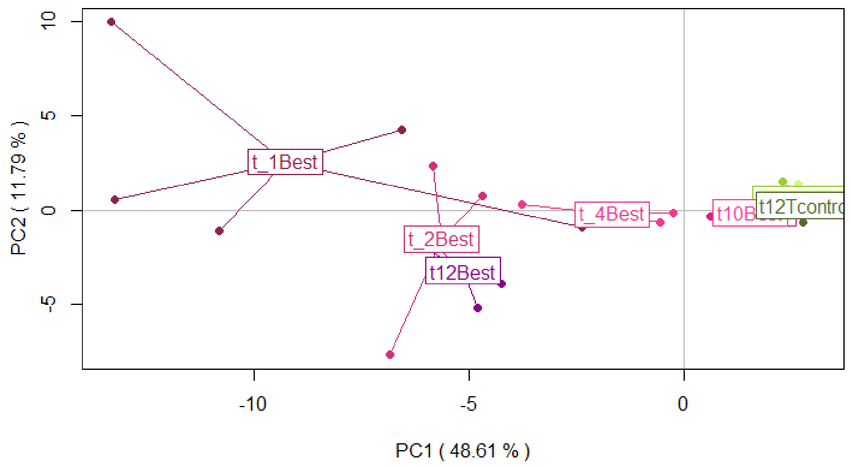
### Metabolic profile



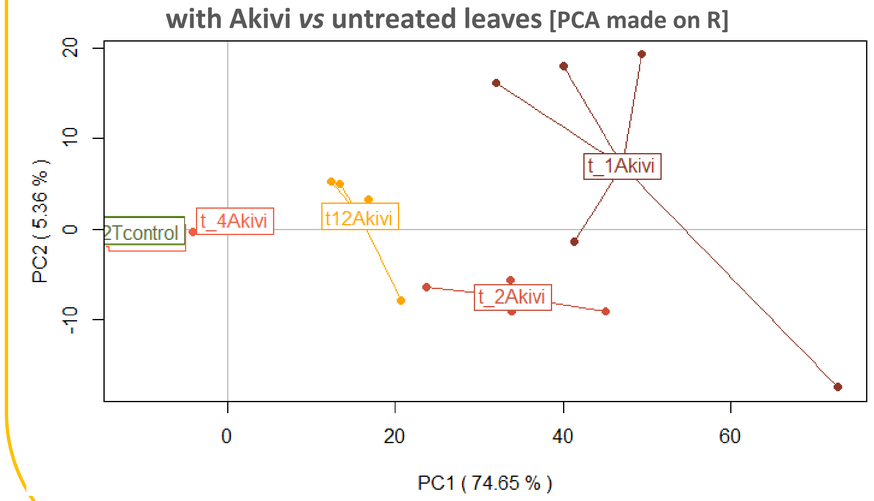

## DATA ANALYSIS - RESULTS

Xenometabolome study: BP residues and by-products

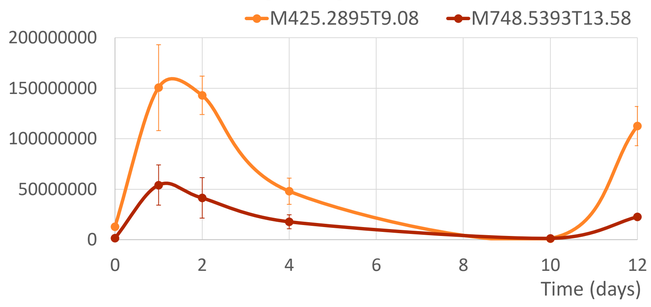
**Xenometabolome evolution of vine leaves treated with Bestcure® *vs* untreated leaves [PCA made on R]**



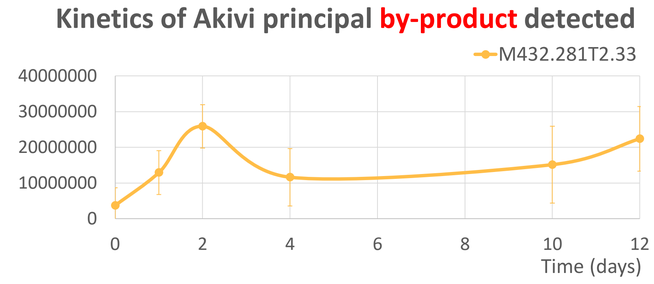

**Xenometabolome evolution of vine leaves treated with Akivi *vs* untreated leaves [PCA made on R]**



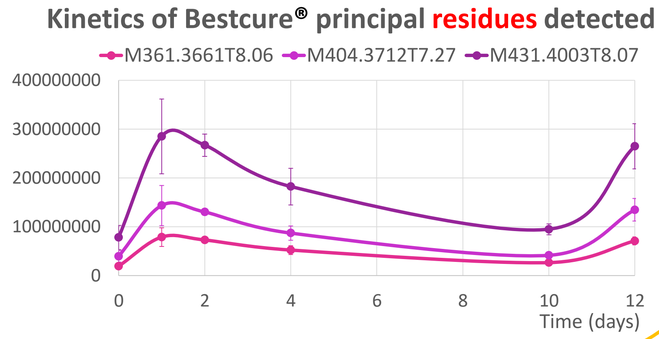

**Kinetics of Akivi principal residues detected**




**Kinetics of Akivi principal by-product detected**




**Kinetics of Bestcure® principal residues detected**




## CONCLUSION

The degradation of 2 fungicides BPs on vine leaves was monitored. The extraction steps were optimized and the extracts were analyzed using a UHPLC-HRMS instrument. For now, only the xenometabolome was studied and the preliminary results showed a degradation kinetics for the 2 fungicides BP with a PHI between 4 and 10 days. The effect of the leaves metabolites (endometabolome) is currently analyzed.

The same study will be performed for the 2019 field experiment (the project lasts 3 years (from 01/2018 to 12/2020) and sampling are done each year). Also, other matrices will be studied in the project: peach treated against brown rot, grape treated against grey mold and soil treated against weed development.