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Metabolomics approach to determine biocontrol products' resilience time and preharvest intervals

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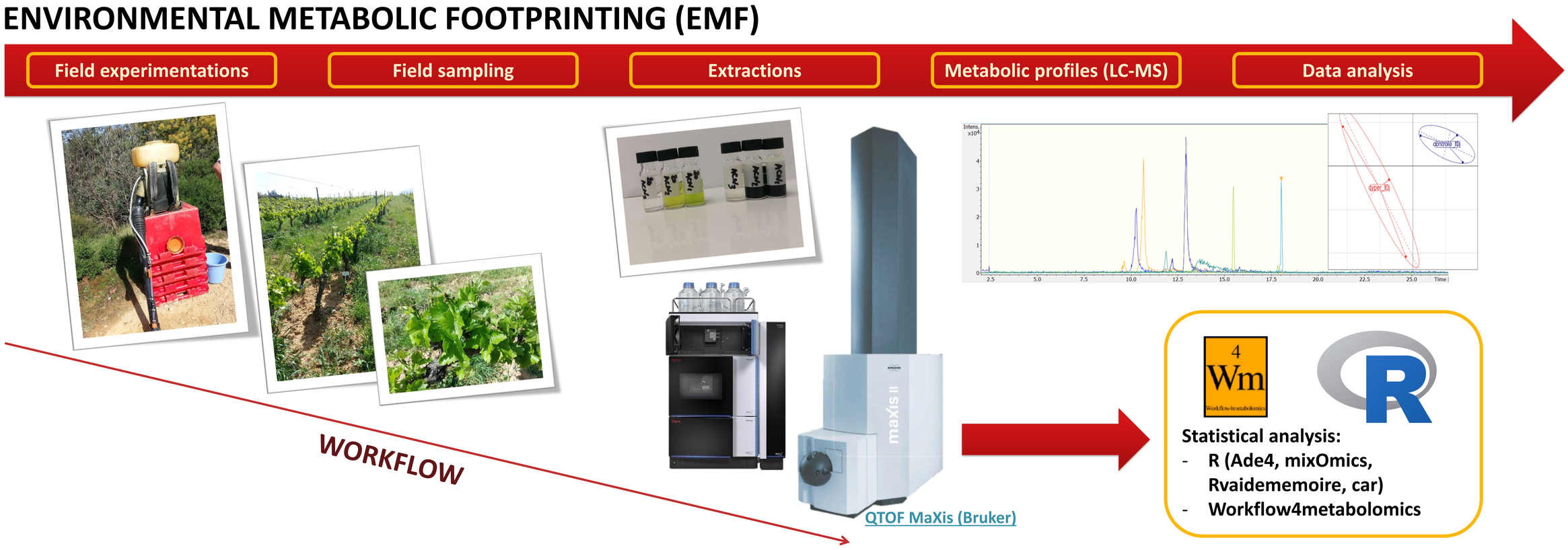
INTRODUCTION

To meet both farmers and consumers' expectations as well as public decisions in the EU (Directive 128/2009), the use of conventional pesticides must be reduced in favor of the use of plant protection products from natural sources, the biopesticides. Although the use of biopesticides is increasing significantly (+15% per year), data are needed in terms of their efficacy and eco-toxicological properties. Based in Catalonia and Roussillon, the EU funded PALVIP project (local Mediterranean crops' alternative protection) associates universities and technical structures in order to evaluate new biocontrol products developed by the local SMEs. To reach that goal, the biopesticides selected in the project will be studied according to their efficiency through field experimentations (Chambre d'Agriculture 66, INCAVI), their effect on plants (Universitat de Girona, Universitat Autonoma de Barcelona) and their environmental impact (Université de Perpignan Via Domitia, Universitat de Girona, Futureco Bioscience). In a first stage, the UPVD (University of Perpignan Via Domitia) will contribute to the part of the project regarding the evaluation of the environmental impact of these biocontrol products'. For that, we will use an innovative approach based on metabolomics (LC-MS), the Environmental Metabolic Footprinting (EMF).

ENVIRONMENTAL METABOLIC FOOTPRINTING (EMF)



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. It has the potential to evaluate all the post-application effects of the biopesticide - (2) the preharvest interval (PHI) that corresponds to the time needed to have no residue difference between the treated sample and the control.

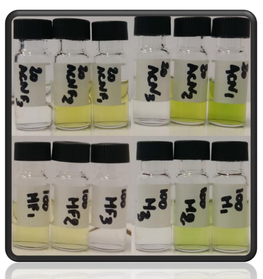
EMF approach will be used to detect bioproducts residues in vine leaves, grape juice, peach peels and salad growing soils. In the case of soils, the resilience time will be evaluated besides the PHI in order to study the impact of the biocontrol products used on the soil matrix (study of the soil meta-metabolome [1],[2]).

OPTIMIZATION OF THE LEAVES EXTRACTION

Extraction steps:
- 8 leaves
- Freeze-drying
- Grinding
- 40mL extraction solvent
- Ultrasound
- 20mL extract recovery
- Evaporation to dryness
- Dissolution in 1,5mL MeOH

Ivy as model leave

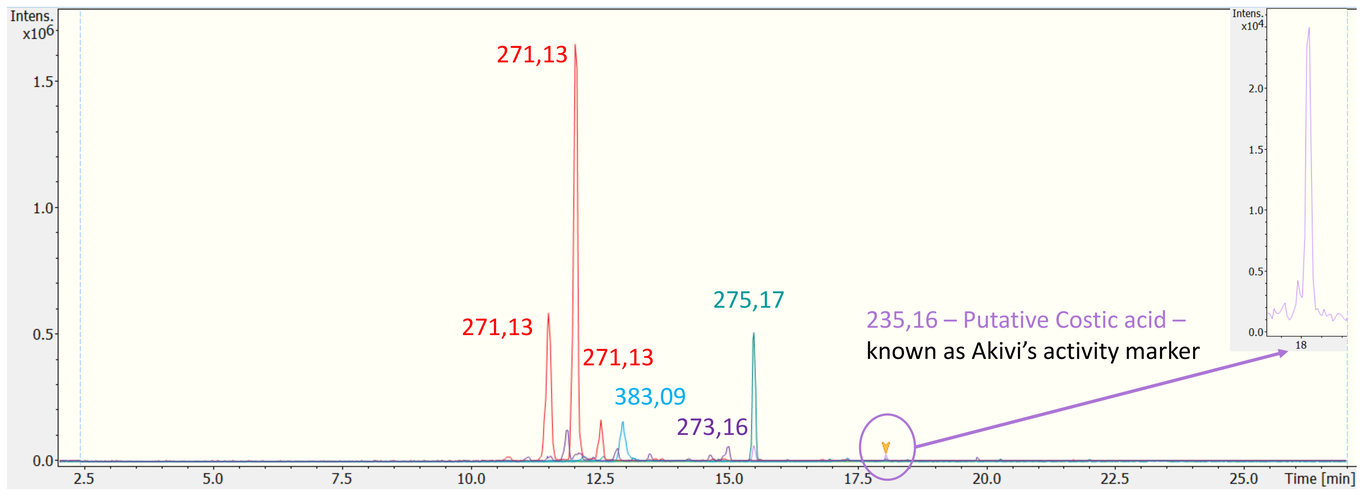
Evaluation of the extraction recovery 2 *vs.* 3:
1: untreated leaves
2: treated leaves with Akivi (AkiNaO)
3: biopesticide (Akivi) alone, it will be the 100% extraction yield



Extraction solvent's tests

4 extraction solvents tested:
- Acetonitrile
- Acetonitrile + formic acid
- Methanol
- Methanol + formic acid

Injection in ESI(+) & in ESI(-)
→ESI(+) produce more intense peaks



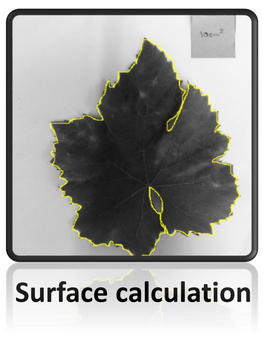
Extraction ion chromatograms of the Akivi characteristic peaks (sample 3)
Extraction recoveries calculation by integrating those peaks (samples 2 *vs.* 3)

	ESI positive				ESI negative			
Rt	18,1min							
m/z	235,16				233,14			
Solvent	ACN	ACNF	MeOH	MeOHF	ACN	ACNF	MeOH	MeOHF
Yield	85%	75%	52%	24%	76%	61%	57%	30%

Yield calculation for Akivi's activity marker compound

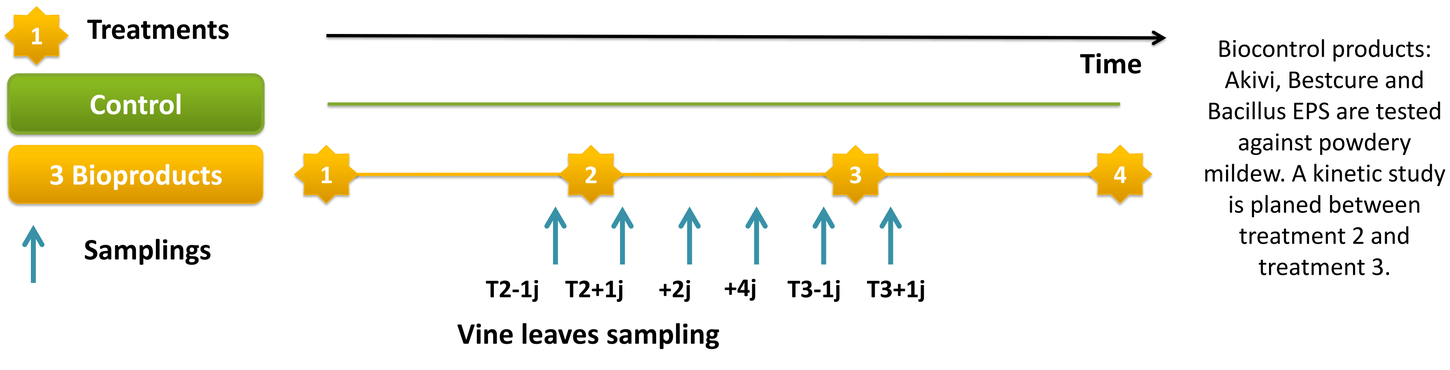
TREATMENT AGAINST POWDERY MILDEW ON VINE LEAVES EXPERIMENT

The field experiment on vine just started and we will soon analyze these field samples with the optimized method. In order to study the kinetic degradation of the product we have to estimate the quantity of residue per leave and to compare several treatments per leave surface.



Surface calculation

Leaves' surface will be calculated using *ImageJ* in order to quantify the amount of residues per leave surface
Here the leave area is 119cm²



Vine leaves sampling

Biocontrol products: Akivi, Bestcure and Bacillus EPS are tested against powdery mildew. A kinetic study is planed between treatment 2 and treatment 3.

CONCLUSION AND PERSPECTIVES

The optimization made on ivy leaves with Akivi allowed us to choose an extraction solvent: acetonitrile, and a mass analysis mode: ESI positive.
The analytical method optimized is able to detect residues of the 3 products tested: Bacillus EPS, Bestcure, Akivi.
We are ready to start vine leaves analysis in order to provide new data on the efficiency and the environmental impact (EMF approach) of the biocontrol products tested in this project.

[1] Patil C *et al* (2016). *Environmental Metabolic Footprinting : A novel application to study the impact of a natural and a synthetic β-triketone herbicide in soil.* Science of the Total Environment 566-567 (552-558).
[2] Salvia M-V *et al* (2017). *Environmental Metabolic Footprinting (EMF) vs. half-life: a new and integrative proxy foe the discrimination between control and pesticides exposed sediments in order to further characterize pesticides' environmental impact.* Environnemental Science and Pollution Research.