Interreg POCTEFA PALVIP

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Environmental Metabolic Footprinting (EMF) approach to study the quality and the environmental impact of biocontrol products on Mediterranean crops

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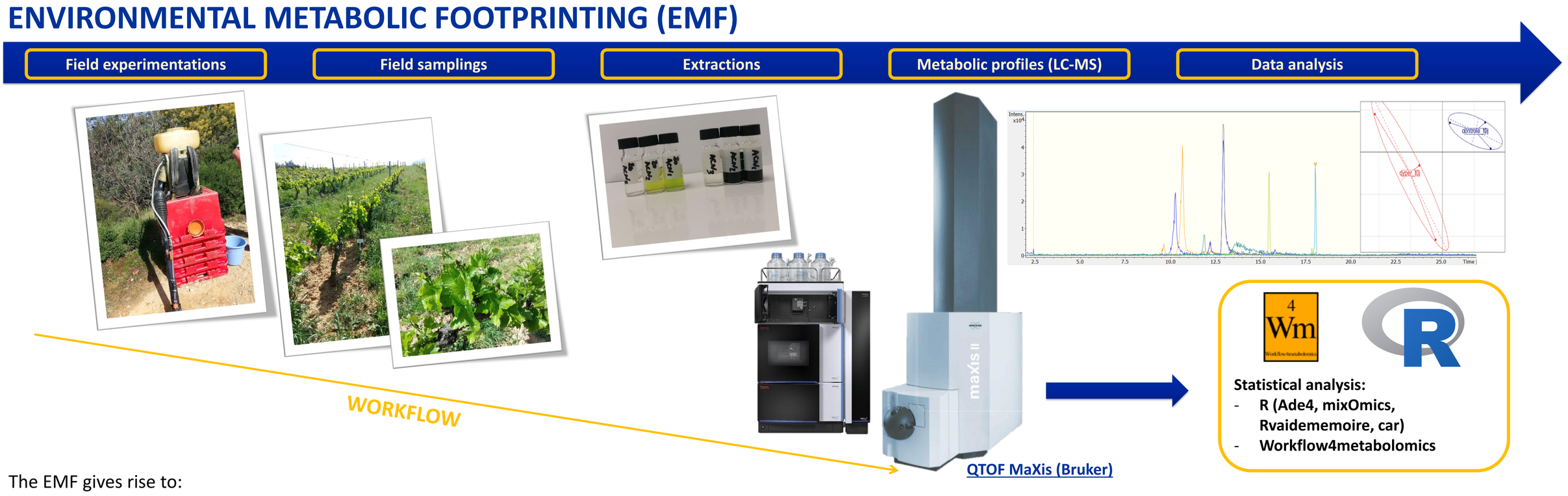
INTRODUCTION

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 Cataluña 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 (University of Perpignan Via Domitia, Universitat de Girona, Futureco Bioscience). Here, a study performed at the UPVD (University of Perpignan Via Domitia), which contributes to the part of the project regarding the evaluation of the environmental impact of these biocontrol products', is presented. For that, we are using an innovative approach based on metabolomics (LC-MS), the Environmental Metabolic Footprinting (EMF).

ENVIRONMENTAL METABOLIC FOOTPRINTING (EMF)

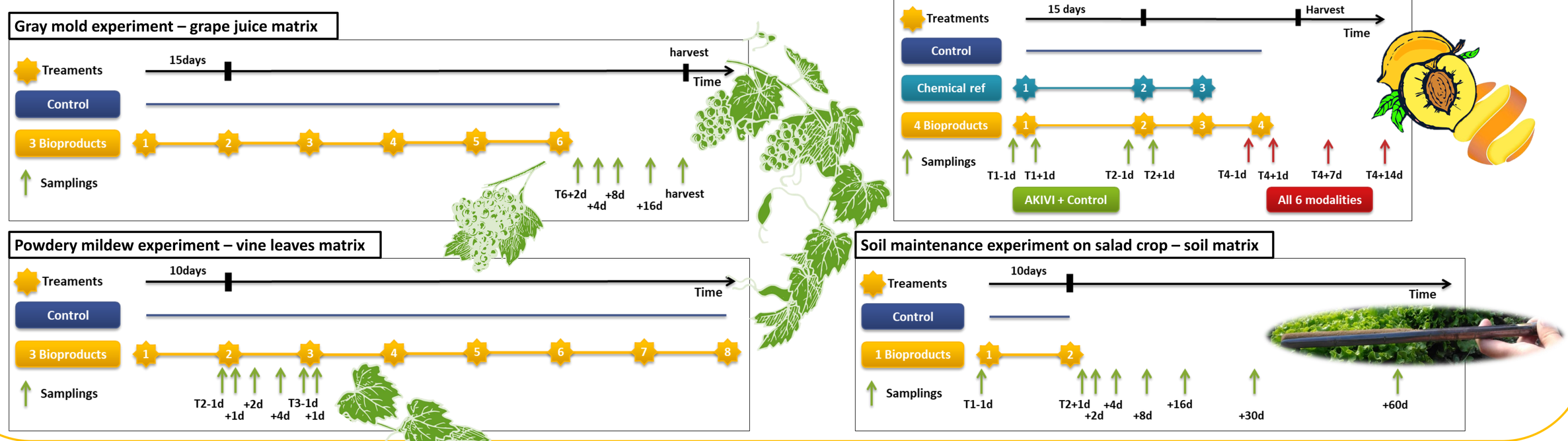


The EMF gives rise to:

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

(2) In this project, **the preharvest interval (PHI)** corresponds to the time delay needed (in days) to have no residue difference between the treated sample and the control.

FIELD EXPERIMENTATIONS AND FIELD SAMPLINGS



EXTRACTIONS

For each matrix an optimization is conducted regarding the extraction protocol.
Up to now, only the powdery mildew experiment is concerned.
The optimization was conducted (below) and the protocol was set up (on the right).

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

2 extraction solvents tested:
- Acetonitrile (*85% recovery for the activity marker*)
- Methanol (*50% recovery for the activity marker*)

→ **Acetonitrile extracts the residues better**

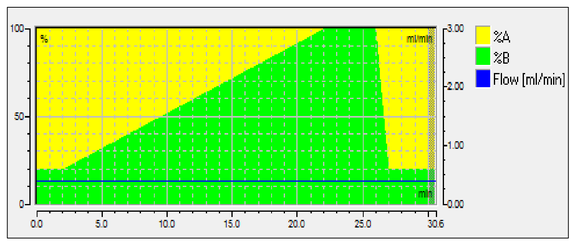
5 leaves
Freeze-drying
Grinding
40mL acetonitrile
Ultrasound
20mL extract recovery
Evaporation to dryness
Dissolution in 1,5mL methanol
Dilution by 15

Extraction steps for powdery mildew experiment

METABOLIC PROFILES (LC-MS)

Extracts are analyzed on an UHPLC-HRMS device Vanquish (Thermofisher) - QTOF Maxis (Bruker) using the parameters:

- Polar C18 column (Luna® Omega 1.6µm Polar C18 100 Å, 100 ×2.1 mm (Phenomenex))
- Methanol elution
- 30min classic gradient:



- Mass analysis mode: ESI +

CONCLUSION AND PERSPECTIVES

Field experiments are done for the 2018 season. Samples are collected and stored waiting for optimization of the extraction and of the analysis.
Regarding the powdery mildew experiment with vine leaves matrix, the optimization was made that 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. The metabolic profiles are currently in acquisition.