



Gembloux Agro-Bio Tech

University of Liege

ML Fauconnier
Karlsruhe
17/01/2019

Faculty Gembloux Agro-Bio Tech

Four masters in Bio-engineering

- Environmental sciences
- Forest and natural spaces
- Agronomy
- Chemistry and bio-industries

→ 1000 students (from 40 countries)

Research center TERRA

11 axis
7 technical platforms
320 Ph D students



Laboratory of Chemistry of Natural Molecules

Extraction, purification and characterization of plant
secondary metabolites for applications in agronomy or food
industries

Laboratory of Chemistry of Natural Molecules

Prof. ML Fauconnier

3 post-docs
20 Ph D students
4 technicians

Laboratory of Chemistry of Natural Molecules

Essential oils

Other VOCs

Oxylipins

Other secondary metabolites

1. Essential oils

Oilprotect Project

EOs for Protection of cereals against insects
Tierry Kenne and Saskia Sergeant
Coll. UCL- private companies

Tree Injection Project

EOs for Protection of trees against insects
Pierre-Yves Werrie Ph D student
Coll. UCL- private companies

EOs production in Senegal

Two senegale Ph D students
Founded by WBI

EOs slow release for biopesticide develop.

Use of new polymers for encapsulation
Chloé Maes Ph D student
Coll. Reims University

Ecoextraction of Apiaceae seed EOs

Iness Bettaieb post-doc
Coll. Center of Biotechnology of Borj Cedria (Tunisia)

HERBIOIL project

Eos as herbicides study of the action mechanisms
LBMI + CGO + CA

EOHUB project

Erasmus + Knowledge alliance
Coll. different countries

Collaboration with different universities in Morocco, Tunisia, Algeria, ...

2. Other VOCs

VOCs as markers for mycotoxin contamination (Agrosensor project)

Develop. of molecular imprinted polymer sensors
Laurie Josselin PhD student- Guo Xiaodong PhD student CAAS
Coll. Umons-Materia Nova- U Gent- private companies

VOCS as marker of abiotic plant stress (Solindic project)

Bastien Durenne Ph D student
Coll. CRAW

Vocaction project

VOCs as antifungal agents in plant protection
Caroline Declercq post-doc
Founded by FNRS leader: phytopathology lab

VOCs and food process

- Marinated pork Han Dong PhD student CAAS
- Peanut oil LT Hu Hui PhD student CAAS
- Fruits/vegetables Wu Xinye PhD student CAAS

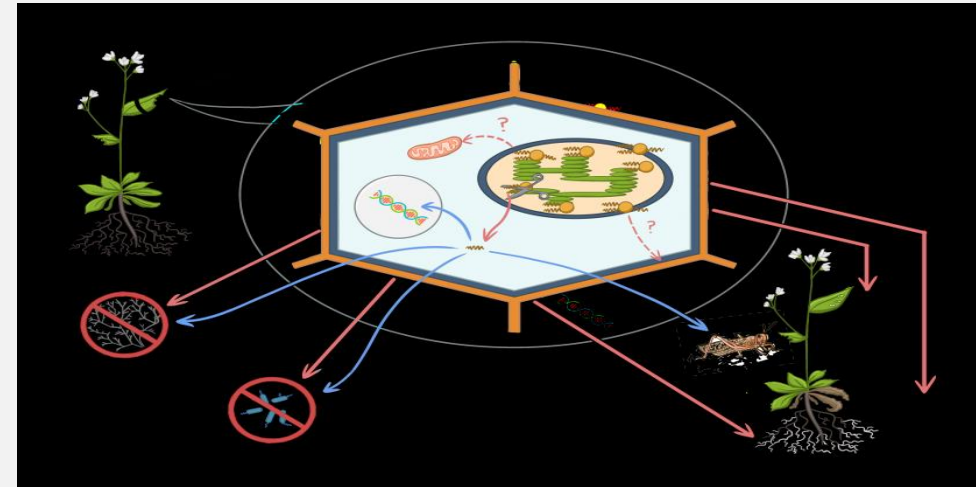
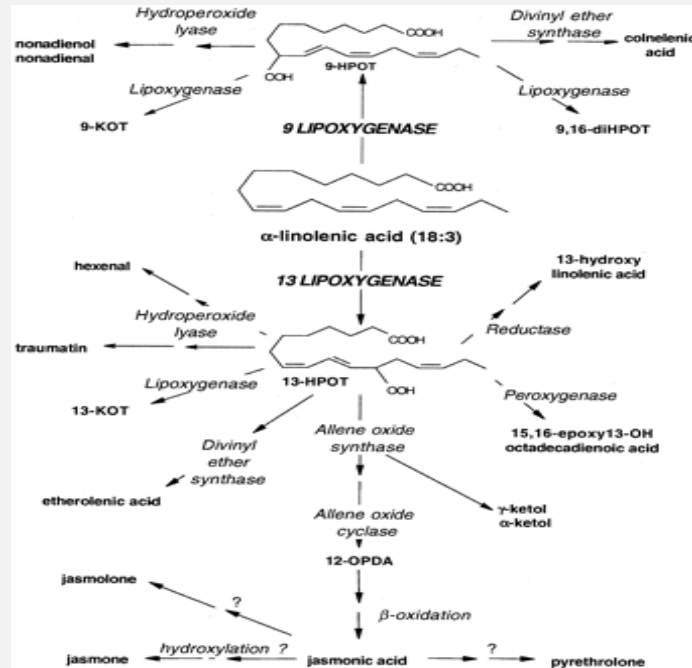
Flavor profiling , aroma formulation collaboration with different companies (Firmenich, Orval, ...)

VOCS in traditional fermented products

VOCs in biological communications

Plant-plant, Plant-insect , plant-fungi
Different coll. inside and outside ULg

3. Oxylipins



Fatty acid hydroperoxides new elicitor/biopesticide

Structure –function relationship

Estelle Deboever PhD student

Co-promotion LBMI

Esterified oxylipins : roles in plants (arabidopsis project)

- Total synthesis (41 steps !)

Firmin Obonou Akong post-doc

Coll. UNamur

- Extraction, dev. Analytical methods, localization, structure-function relationship

Manon Genva PhD student

Co-promotion LBMI

4. Other plant secondary metabolites

Secondary metabolites from african plants for cosmetic/medicinal applications

- Matthew Saive : valorization of Mahoran flora Ph D student
- Fatimata Nea : valorization of endemic plants from Ivory Cost, PhD student
- Evelyne Tanoh : valorization of endemic plants from Ivory Cost, PhD student
- Liu Jiang : valorization of secondary metabolites from sweet potatoes, Ph D student CAAS

Fabaceae root exudates to improve soil pollution bioremediation

Marie Davin PhD student
Co-promotion with soil dep


Allelopathy: structure-function relationship

Simon Lebeque PhD Student
Leader LBMI


Analytical platform



Volatolomics

- SAFE (Solvent assisted flavor extraction)
 - Supercritical CO2 extraction
 - Pilot distillation
 - 3 GC-FID
 - 3 GC-MS including the following injection modes
 - Liquid injection
 - SPME
 - Static Headspace
 - Dynamic headspace
 - MVM (multivolatiles method)
 - DHS-TDU
 - Twister
- 

Non volatile

- 4 HPLC (DAD, RI, ELSD, fluo)
 - 1 prep HPLC (DAD, RI, ELSD, fluo)
 - 1 LC-MS
 - 1 IR, 1 UV-vis
- 

LBMI

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<http://www.gembloux.ulg.ac.be/biophysique-moleculaire-aux-interfaces/>

The team

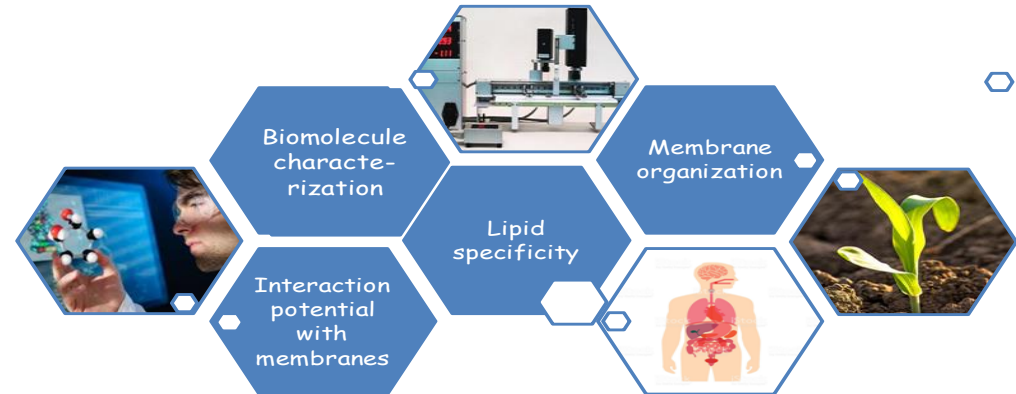
- 2 permanents (FNRS)
- Non permanent researchers:
 - 1 post-doc
 - 7 PhD students
- Technicians:
 - 0.25 FTE lab technician
 - 0.1 FTE secretary

Main equipment

- Langmuir film balance
- Isothermal titration calorimetry
- IR spectroscopy
- Fluorescence spectroscopy
- Circular dichroism
- Computer platform (Linux station bi-xeon quad core)

Main research topics

- ✓ Study of the **structure-function relationships** of biomolecules
 - Design of new active molecules
- ✓ Molecular understanding and description of **mechanisms** involved in biological phenomena related to interactions with **biological membranes**
 - Perception of biomolecules by cellular membranes
 - Molecular organization within a membrane



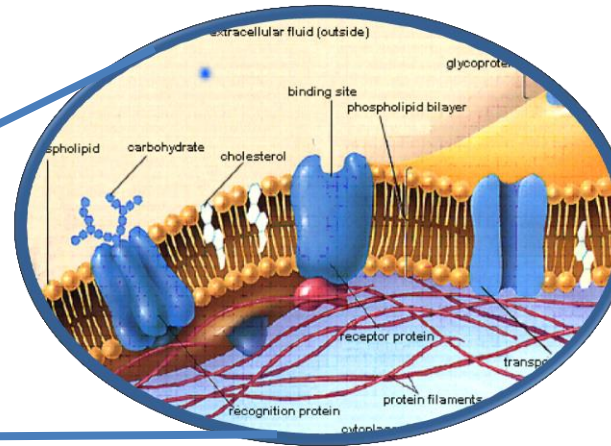
Integrative strategy with complementary *in vitro* and *in silico* biophysical approaches

Human health

Plant protection

Biological membranes

Biological membrane = Bilayer with a complex composition and organization

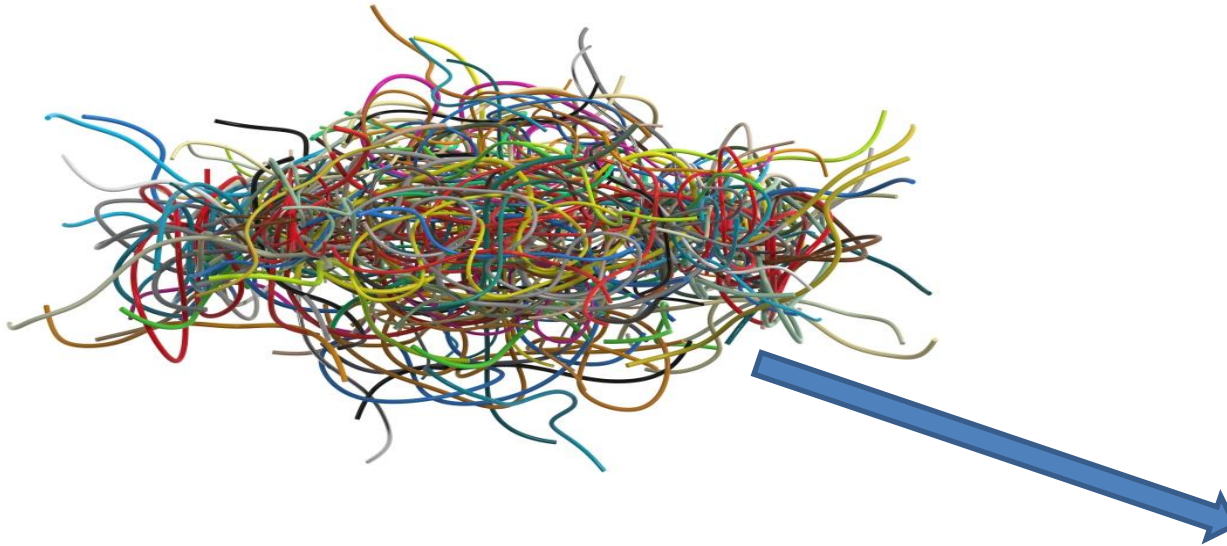


Molecular recognition,
Signal transduction,
Enzymatic catalysis,
Cellular adhesion,
Membrane fusion

First contact point between
molecule and cell

**Biomolecule/membrane interaction:
a key event for the biomolecule
to be active**

Lipid membrane models

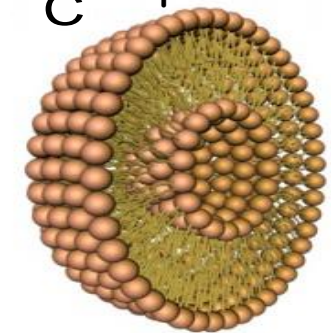


Working on the lipid part = No proteins !

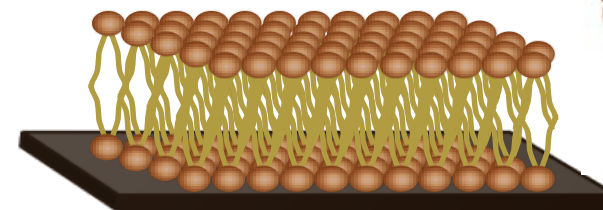
A Lipid monolayer



C Liposome

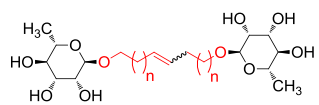


B

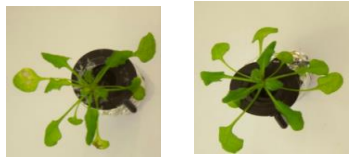


Supported lipid bilayer

Species	Glycerophospholipids	Sphingolipids	Sterols
Plant	PLPC	GPES*	Sitosterol
Fungi	DOPC POPE	Phytosphingosine**	Ergosterol
Bacteria	POPE POPG CL		
Mammalian	POPC	Sphingomyelin	Cholesterol

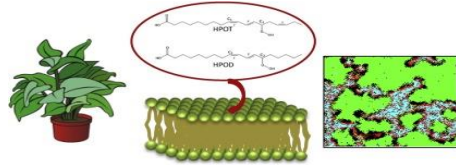


Elicitors



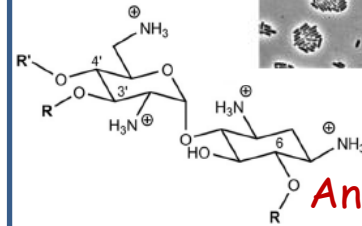
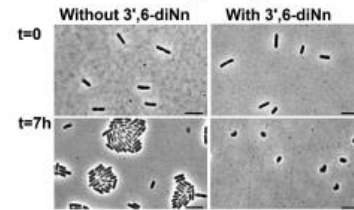
Luzuriaga-Loaiza et al., *Sci. Reports*, 8, 8534 (2018)

Key intermediate oxylipins



Deleu et al., *Colloids .Surf. B*, 175 (2019)

Pseudomonas aeruginosa growth



Neamine derivatives

Antibiotics

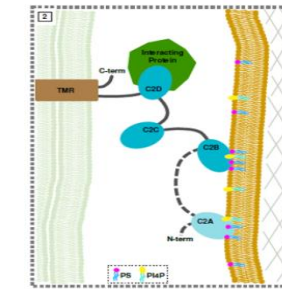
Sautrey et al., *JBC*, 291, 13864-13874, (2016)

Bioremediation



BestBioSurf

C2 domains and MCTPs at plasmodesmata



Plant membrane contact sites

Brault et al. *EMBO J*, submitted



Ghrelin

Drug delivery

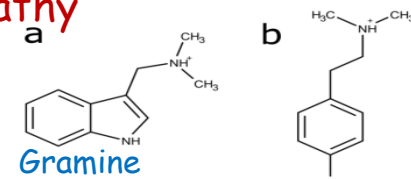
Salade et al., *Int.J.Nanomedecine*, 12, 8531, (2017)

- Interaction with lipid membranes
- Structure-function relationships



Allelopathy

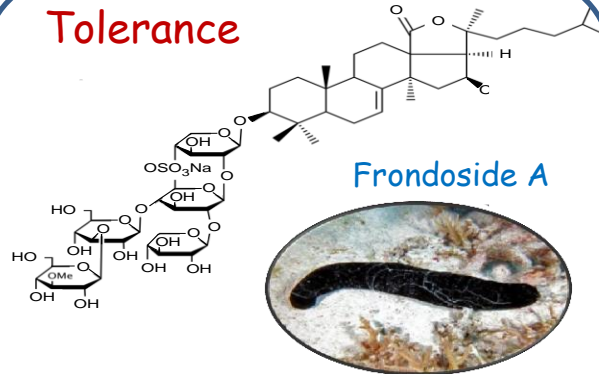
Hordenine



Gramine

Lebecque et al., *Sci. Reports*, 8, 9784 (2018)

Tolerance



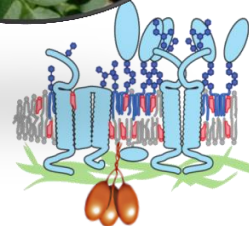
Frondoside A



Claereboudt et al., *Sci. Reports*, 8, 10845 (2018)



Plant protein anchor into PM

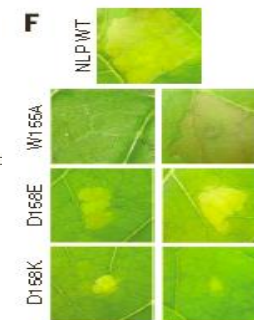
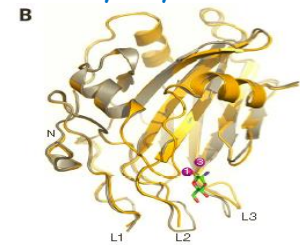


Remorin

Gronnier et al., *eLife*, 6:e26404 (2017)

Plant toxin

Microbial NLP cytolysin



Lenarcic et al., *Science*, 358, 1431, (2017)


Surfactants



Sugar esters



Meline et al., *Enz. Microb. Technol.*, 112,14(2018)



Relationship between fatty acid
hydroperoxides and their biological
activities as potential biopesticides

DEBOEVER Estelle

PhD Student

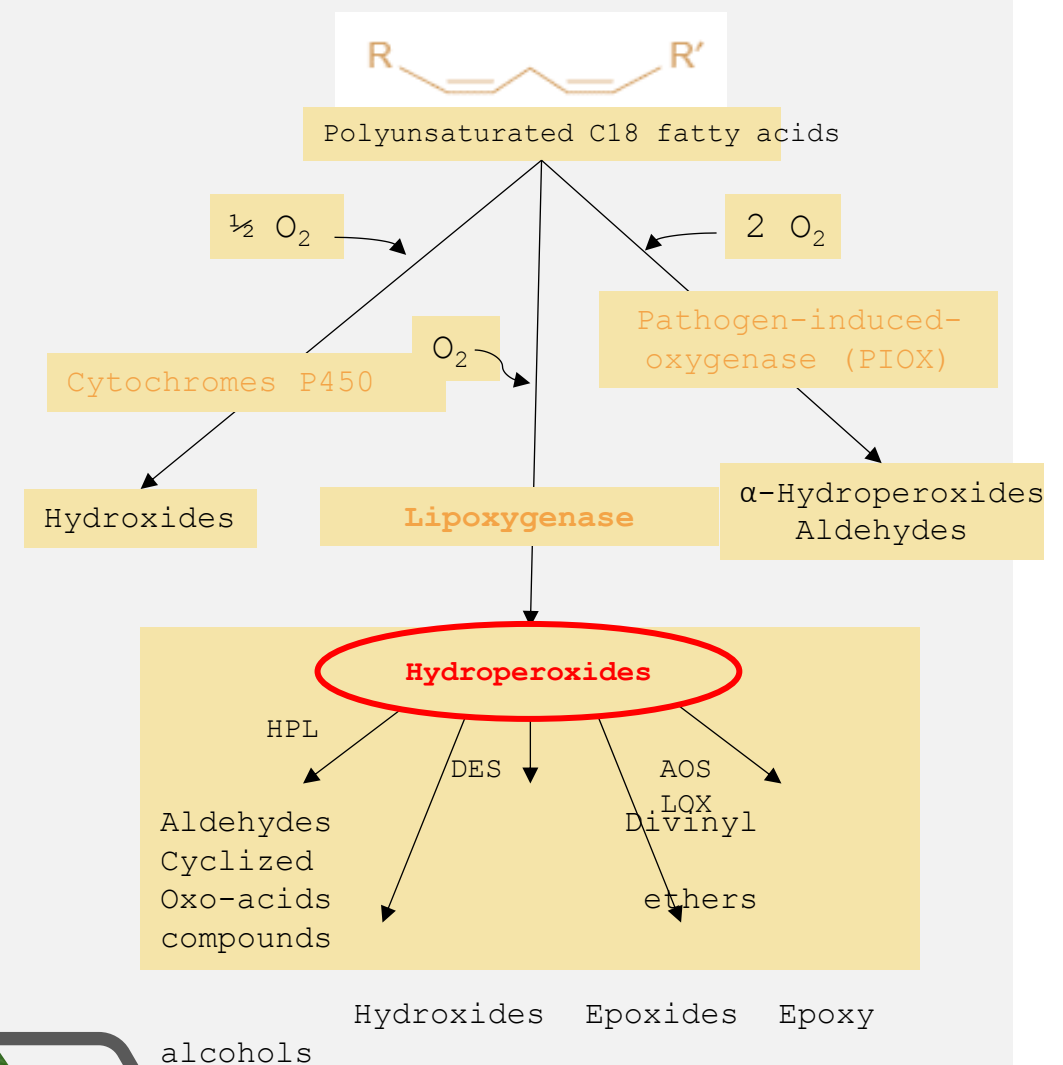
About the context

- (A)biotic stresses \gg plants
- Complex environmental conditions
- Adaptation strategies

The LOX pathway

- Emerging pathway in plant defence
- Oxylipins
 - Free fatty acids \rightarrow HPO \rightarrow ...

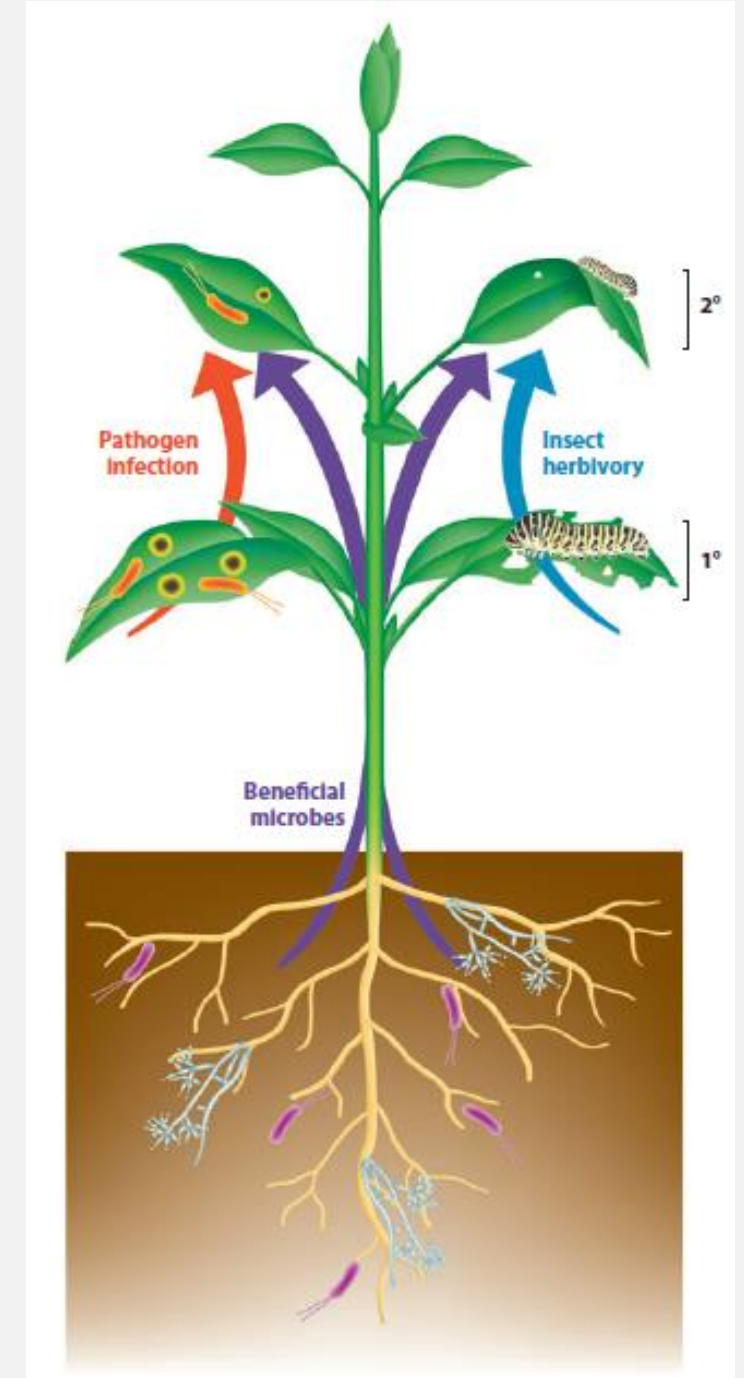
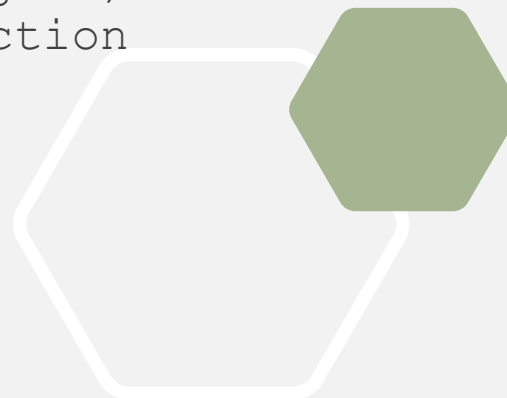
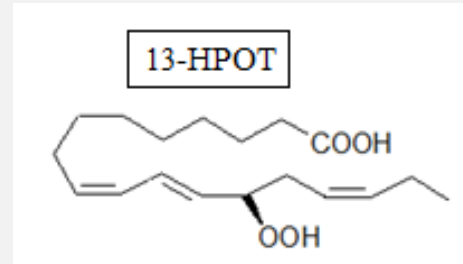
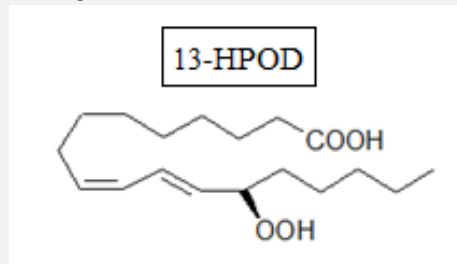
- HPOs in the literature



Objectives

The goal of my PhD project is to answer 3 main scientific questions:

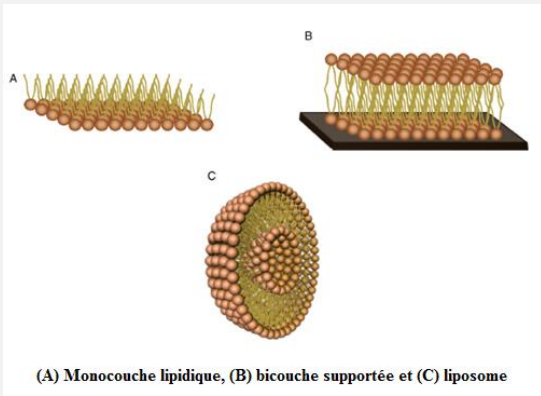
- In planta, are 13-HPO antimicrobial agents ? Are they sufficiently powerful to eliminate totally a pathogen when applied exogenously ?
- When applied exogenously, are 13-HPO acting as elicitors ?
- How interactions between 13-HPO and plasma membranes (plants or pathogens) can explain those mechanisms of action ?



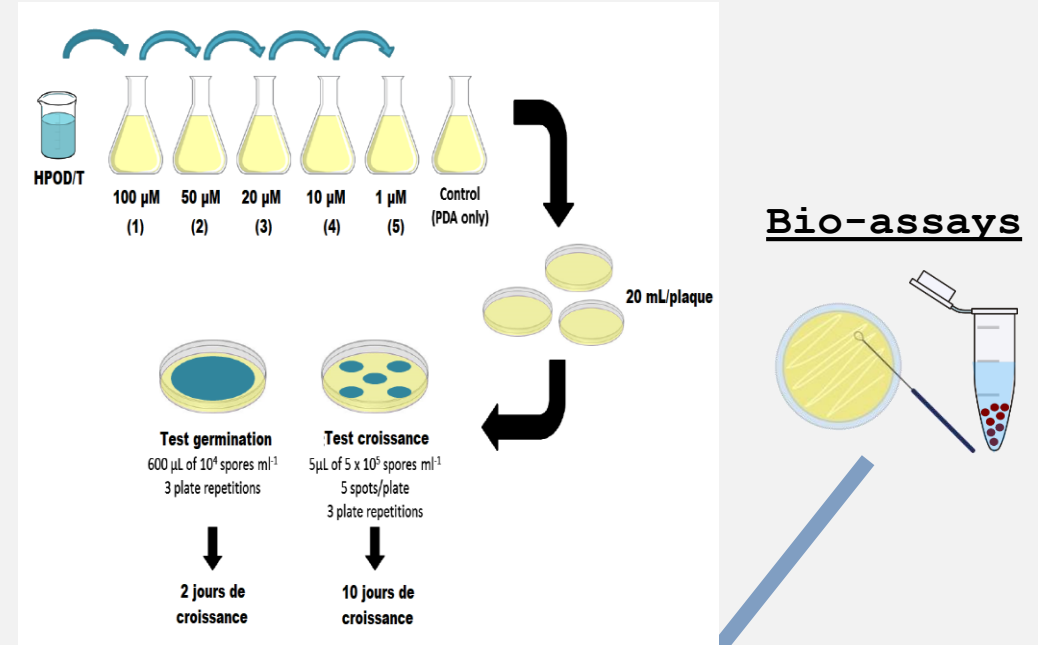
Multidisciplinary strategy

Use of a multidisciplinary but complementary approach

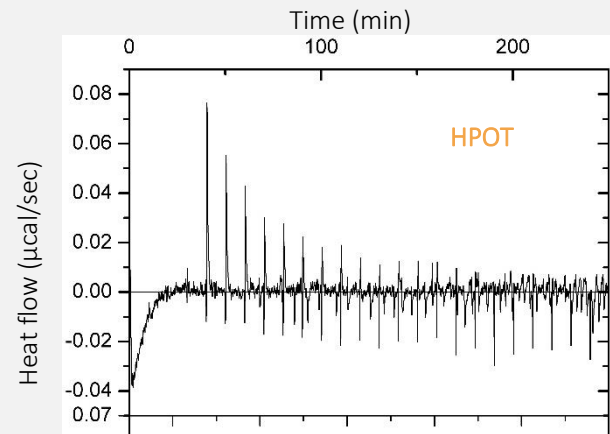
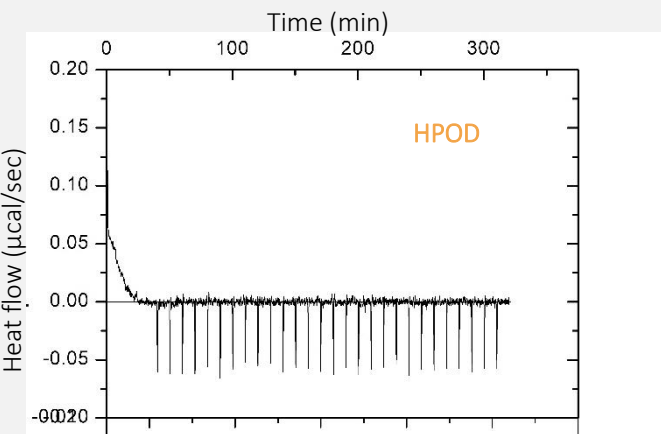
Bioinformatic and Biophysic tools



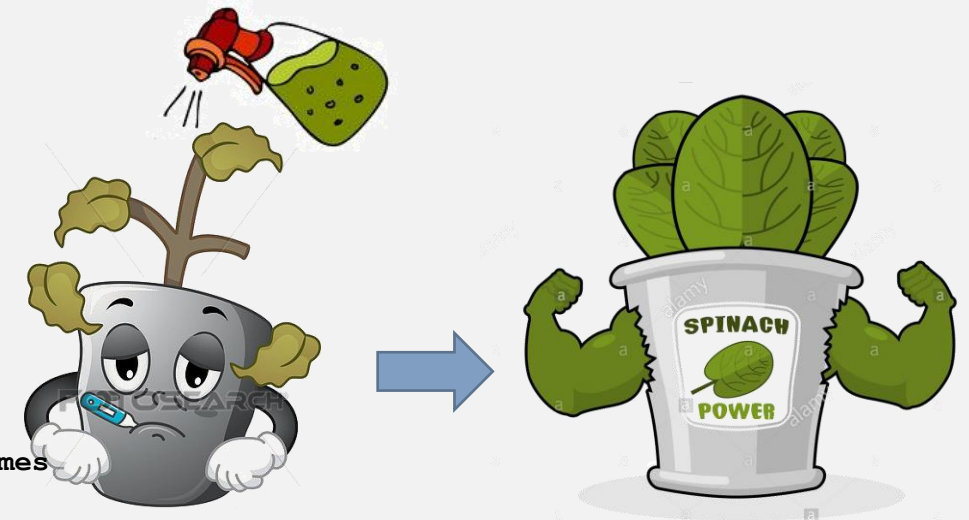
- Langmuir trough
- Isothermal titration calorimetry
- Neutron reflectometry
- SAXS/WAXS
- Cryo-TEM
- Etc.



Complementarity

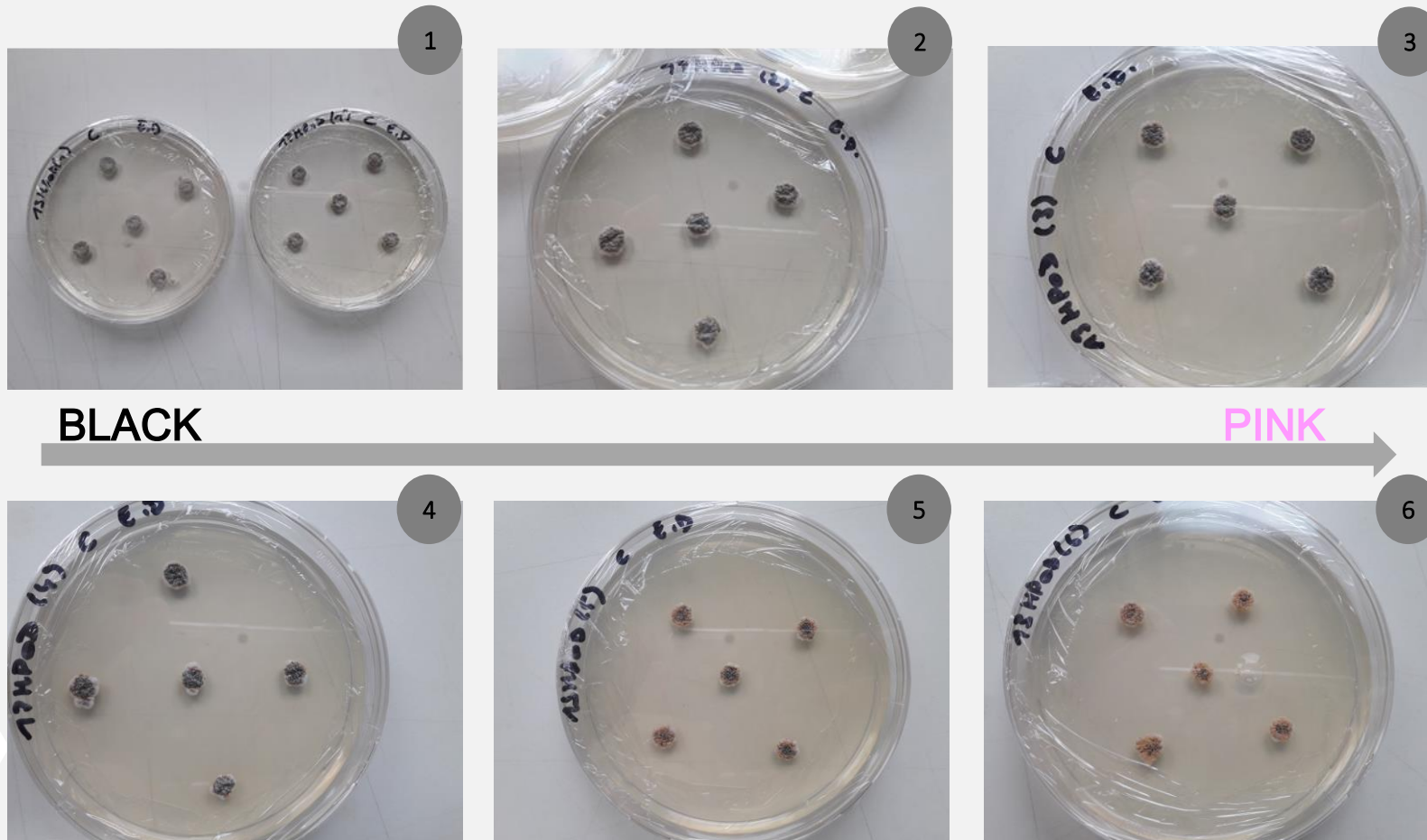


An example of raw data obtained for the partitioning of HPO in POPE/POPG/CL liposomes



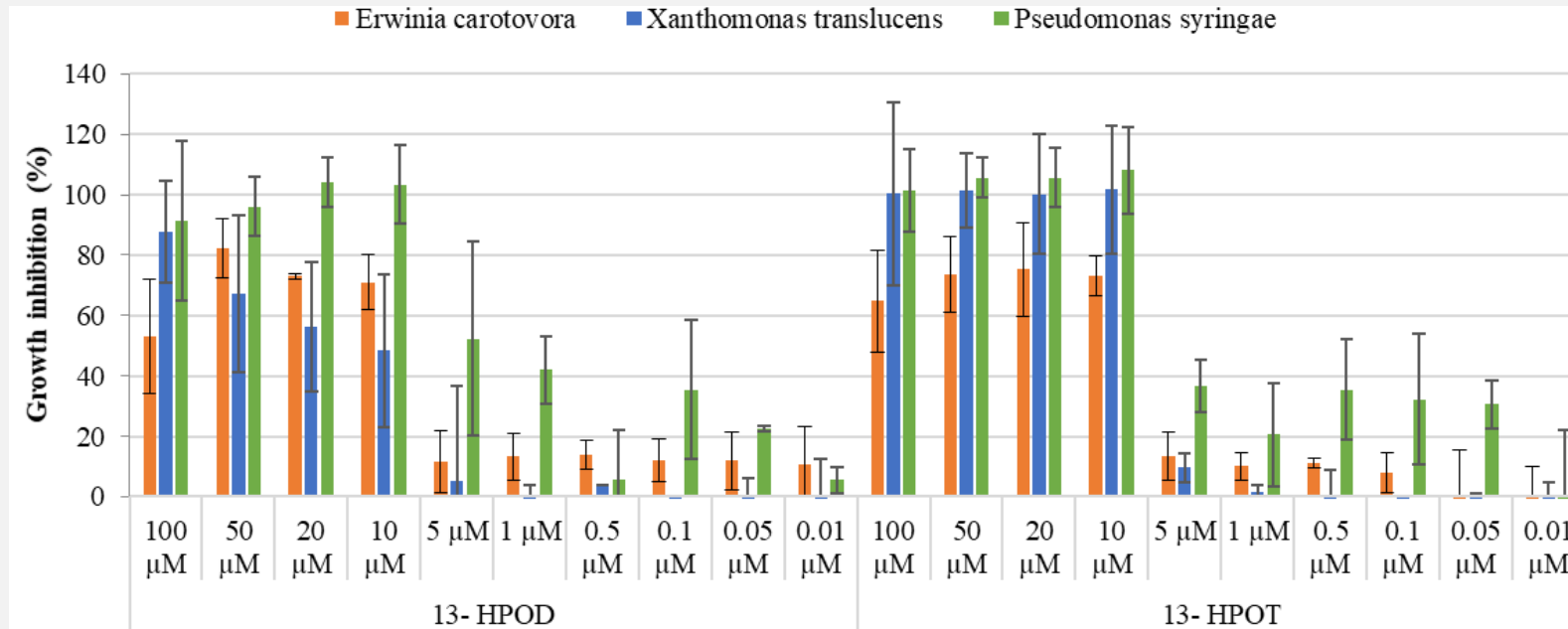
Comparison

In planta, are 13-HPO antimicrobial agents ?



Comparison

In planta, are 13-HPO antimicrobial agents ?

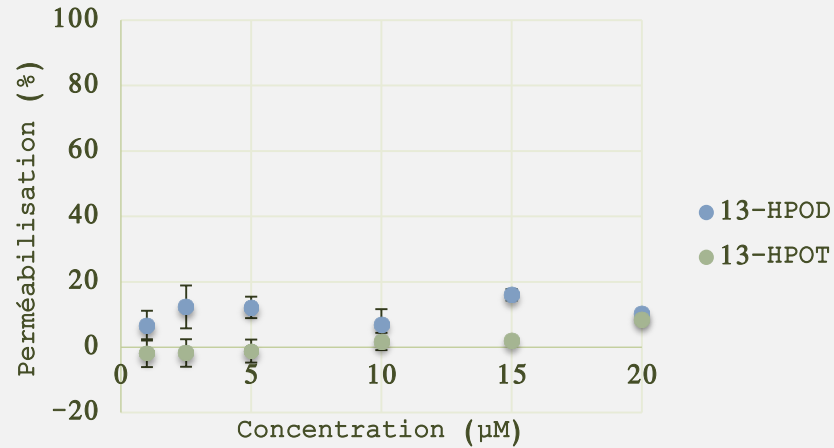


Comparison

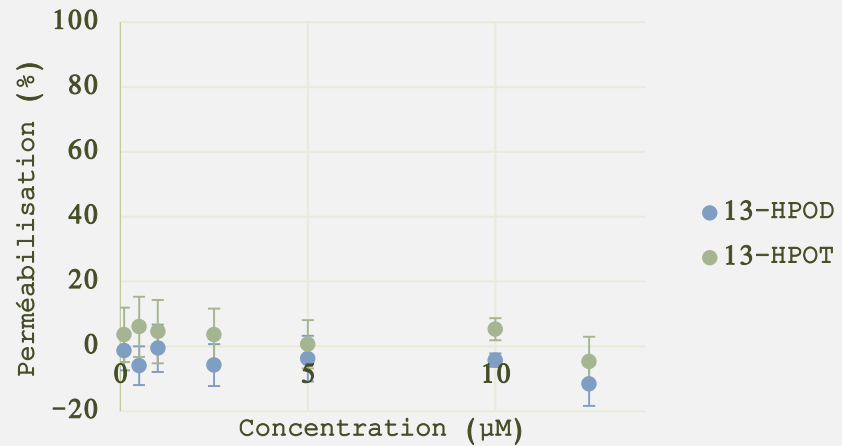
How interactions between 13-HPO and plasma membranes (plants or pathogens) can explain those mechanisms of action ?



Permeabilisation of plant PM
PLPC/Sito/GluCer



Permeabilisation of bacterial PM
POPE/POPG/CL

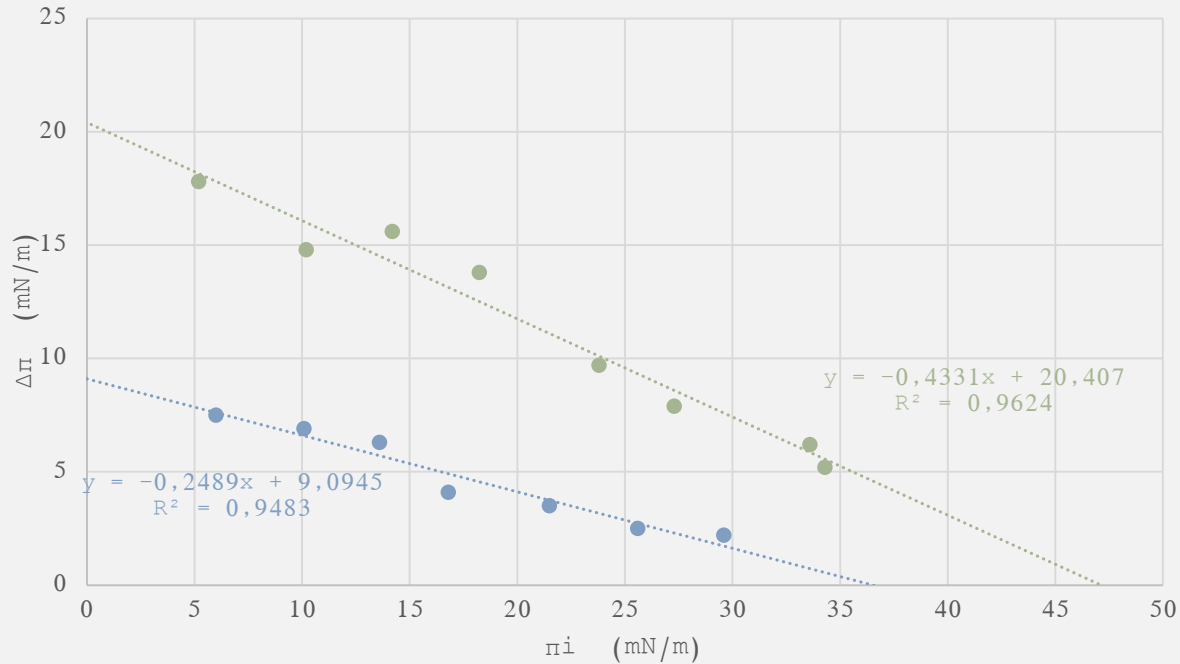


Comparison

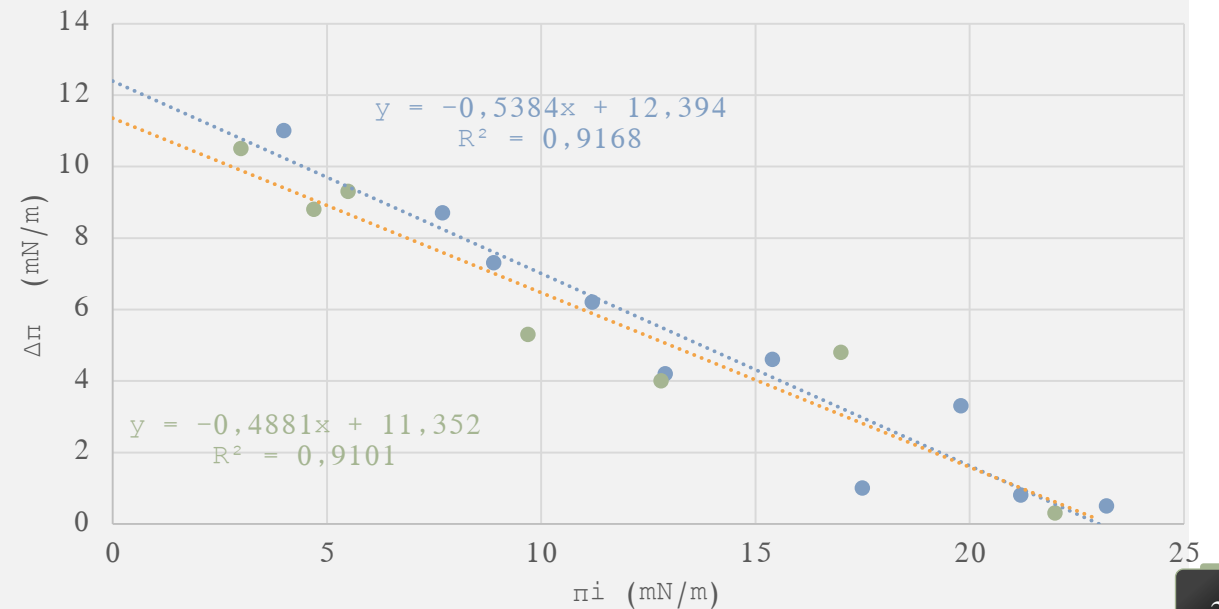
How interactions between 13-HPO and plasma membranes (plants or pathogens) can explain those mechanisms of action ?



13-HPO in PLPC/Sito/GluCer monolayer

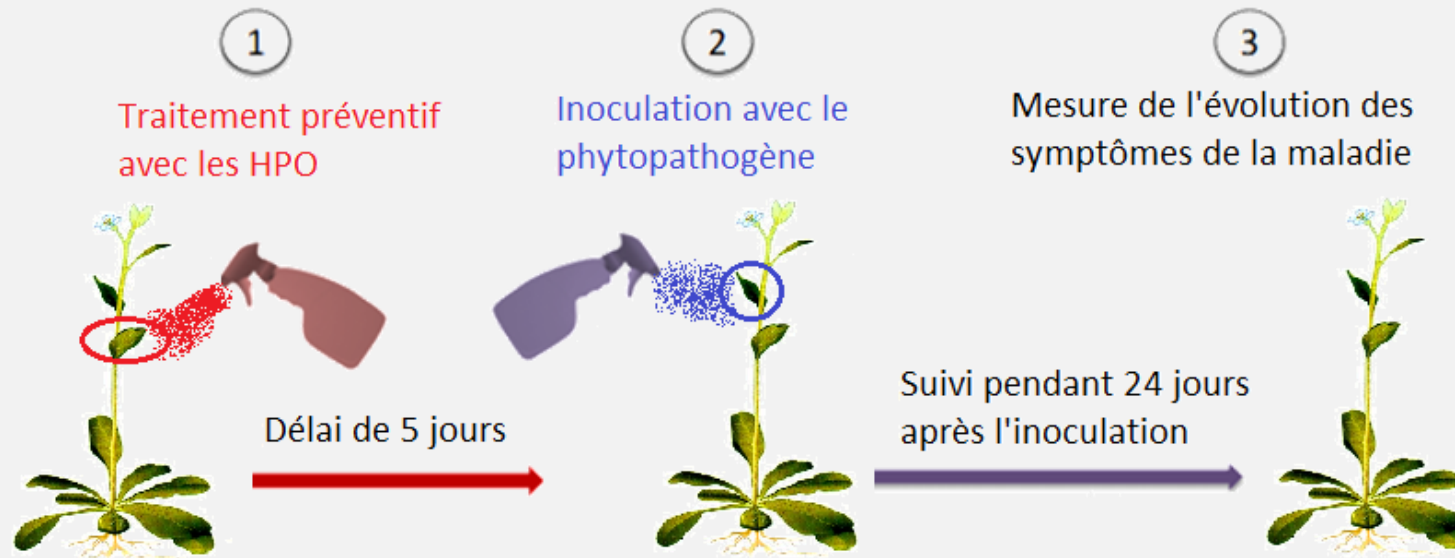


13-HPO in POPE/POPG/CL monolayer



What next ?

- Preliminary results show that:
 - HPO have a significant *in vitro* antimicrobial activity
 - Difference between HPOD/HPOT forms: HPOT >>> HPOD
 - Interactions with biomimetic plasma membrane: HPOT >>> HPOD
 - Correlation between antimicrobial activity and interactions with plasma membrane
- Need to do the *in planta* tests :
 - In planta, are 13-HPO antimicrobial agents ?
 - When applied exogenously, are 13-HPO acting as elicitors ?





Thank You
for your
attention



Estelle DEBOEVER



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.be

ESTERIFIED OXYLIPINS
STRUCTURE - FUNCTION
RELATIONSHIPS

GENVA MANON

Context

- **Plant oxylipins**
 - Free or esterified → galactolipids, phospholipids, ...
- **Arabidopsides**

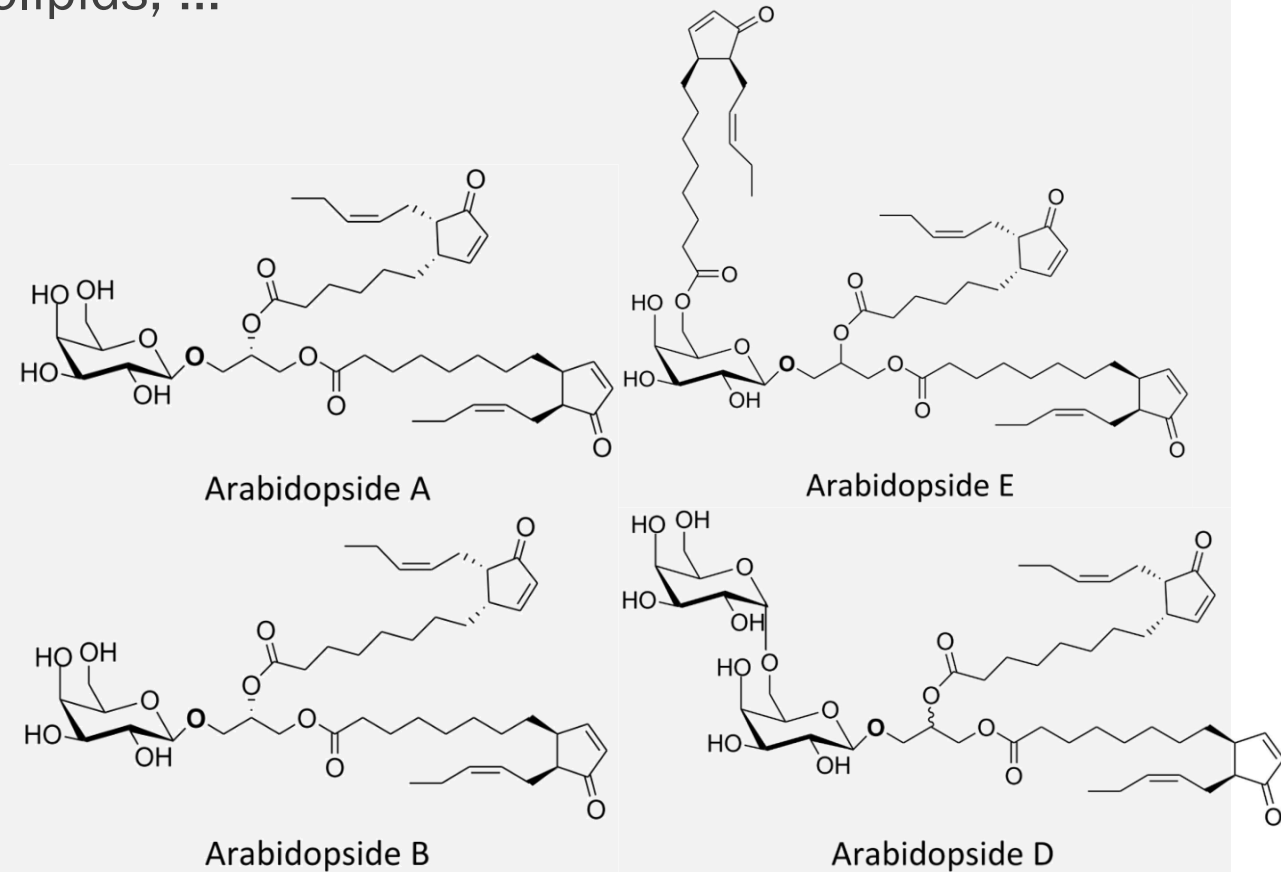
Stress →

Wounding, cold,
pathogen, ...



Arabidopsis thaliana (L.)
Heynh.
& other plants

← **Arabidopsides**
and POL
production



Context

- **Arabidopsides**

- **Localization**

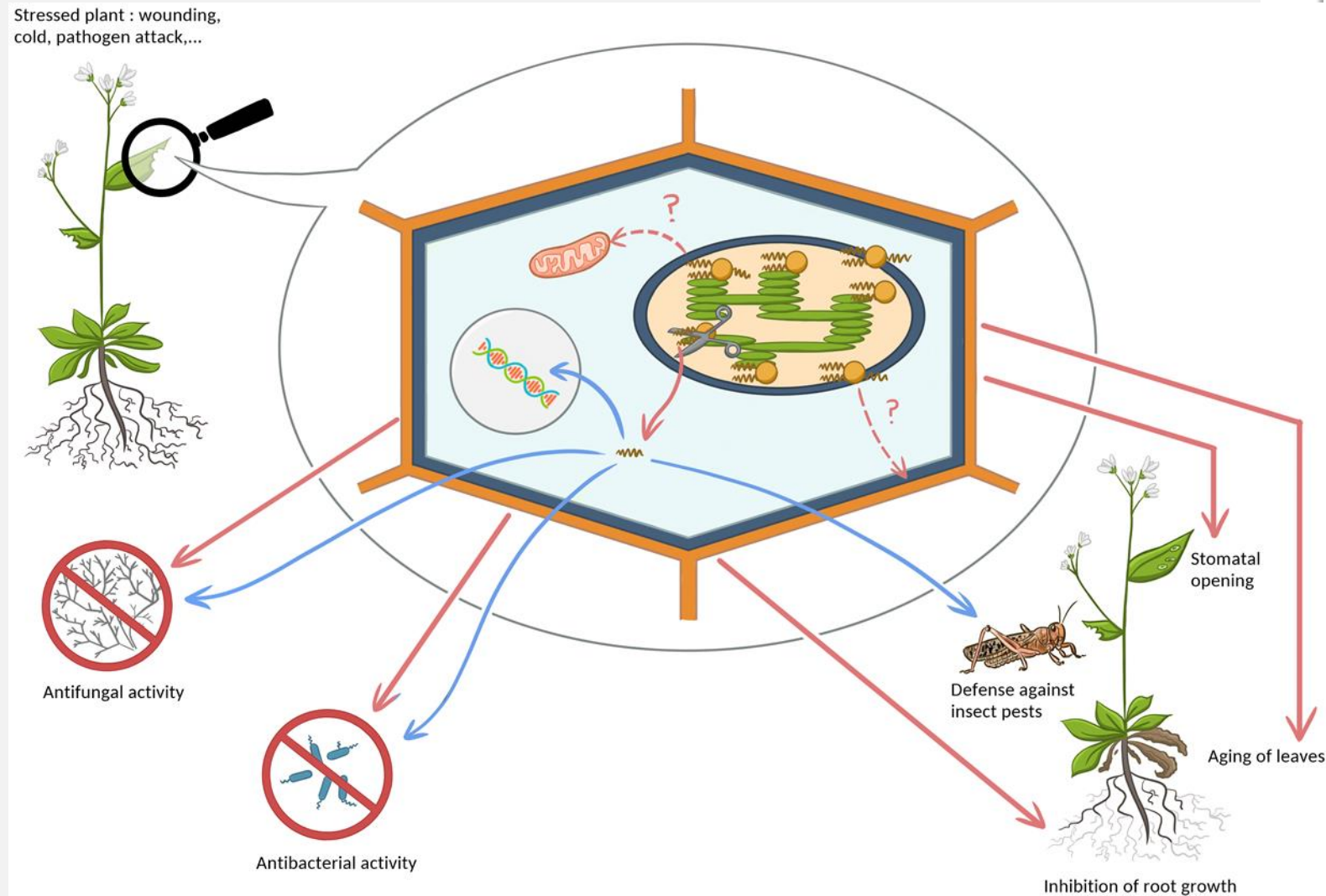
- Chloroplast membranes?

- **Synthesis**

- Following MGDG & DGDG oxidation

- **Precise functions: unknown**

- Roles in
 - Plant defence processes
 - Developmental processes
- Direct or indirect functions



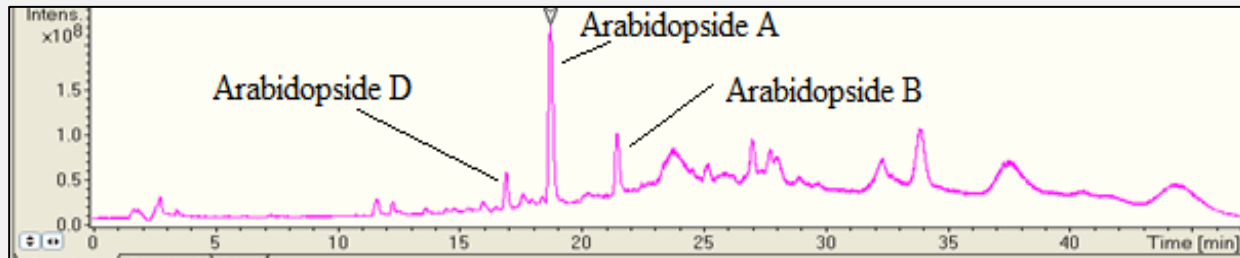
Genva, M., Obounou Akong, F., Andersson, M.X. et al. Phytochem Rev (2018).

Thesis overview

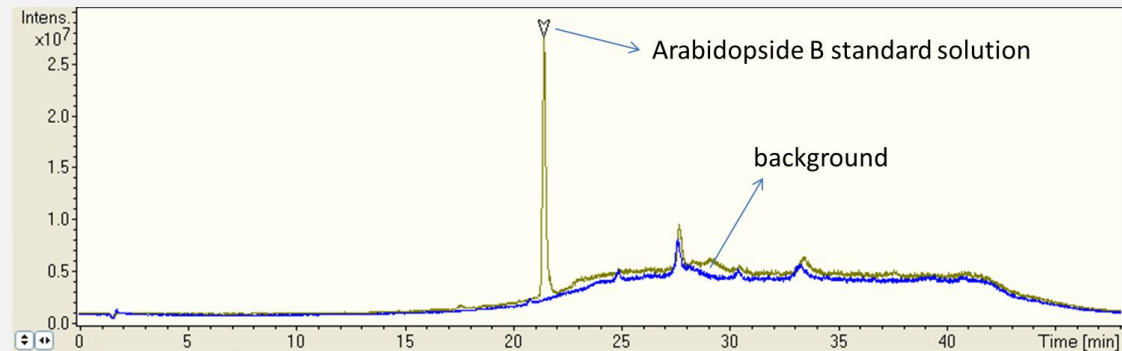
Part 1

- Arabidopsides extraction and purification
- Arabidopsides characterization
- Development and validation of an HPLC-MS method to detect and quantify arabidopsides

- Obtention of pure arabidopsides A, B, D & E
 - Lipid extraction
 - (Semi-) preparative HPLC purification
- HPLC-MS separation and detection of arabidopsides



- Utilization of purified standards for arabidopsides quantification by HPLC-MS



- Method validation almost finished
- Will be used for arabidopside determination in other *Brassicaceae* plants

Thesis overview

Part 2

How does arabidopside modify structure and functions of plant membranes ?

- **Detection and quantification of arabidopsides in plant membranes**

- **Preliminary assays: arabidopsides detection among chloroplast membranes**
 - Detection of arabidopsides in chloroplast external membranes and thylakoïds
 - Will be repeated this year using Rpm1/col-0 plants to induce arabidopside production

Thesis overview

Part 2

How does arabidopside modify structure and functions of plant membranes ?

- **Detection and quantification of arabidopsides in plant membranes**
- **Study of arabidopside interactions with those membranes *in-vitro* & *In-silico***

- **Preliminary assays: arabidopsides detection among chloroplast membranes**
 - ***In-vitro* and *in-silico* assays**
 - Interfacial properties of arabidopsides are different from non oxidized galactolipids
 - Arabidopsides A, B & D can interact favourably with chloroplast lipid
 - Arabidopsides A and B are able to permeabilize chloroplast membranes
- arabidopside presence in chloroplast membranes under stress may modify chloroplast structure and functions

Thesis overview

Part 2

How does arabidopside modify structure and functions of plant membranes ?

- Detection and quantification of arabidopsides in plant membranes
- Study of arabidopside interactions with those membranes *in-vitro* & *In-silico*
- Study of arabidopsides effect on chloroplast functions

- Preliminary assays: arabidopsides detection among chloroplast membranes
- *In-vitro* and *in-silico* assays
- Arabidopside effects on chloroplast function: planned for this year
 - Flow cytometry analysis of arabidopsides-containing chloroplasts shape (Rpm1/col-0 , col 0)
 - Photosynthesis ability

Thesis overview

Part 3

Are arabidopsides used as a pool of free oxylipins or does arabidopsides have direct functions?

- **Biological assays: study of esterified oxylipins formation and remobilization under stress conditions**

- **Planned for next years**

Infection 1
Avirulent *Pseudomonas syringae*

Infection 2
Virulent *Pseudomonas syringae*



Free and esterified oxylipins quantification after each infection and at different times

Correlation between oxylipin production & disease symptoms

Col-0 & LOX2 mutant

Are arabidopsides produced following the first infection used to generate free oxylipins during the second infection?

Postdoctoral fellowship

- Arabidopside total synthesis (41 steps)

The background of the slide is a close-up photograph of several white flowers with yellow centers, set against a dark green background. The flowers are in various stages of bloom, with some in sharp focus and others blurred.

**THANK YOU FOR YOUR
ATTENTION**

Funding ?

FNRS : short term grants, post doc grant

ULg : co-fund post doc grants

Interreg North-West : 3 partners, companies ... (March-April 2019)

Belgium, Germany, Switzerland, SME of Gembloux Agro-Bio Tech, ...

ERAnet SusCrop

<https://www.suscrop.eu/>

ERAnet CoreOrganic

<https://www.coreorganic.org/>