



ALCOVE ITEM 2020-2024

Coordinator A.M. FARNET DA SILVA



New agricultural practices harmless for human health and agrosystems, based on a circular and local economy

Chemometrics
Infrared spectroscopy

N. DUPUY

Biotechnology
Solid-state
fermentation

P. CHRISTEN

Plant physiology
I. LAFFONT-SCHWOB
H. FOLZER

Genotoxicology
Th. ORSIERE



Soil microbiology
A.M. FARNET DA SILVA



**Psychosocial
studies**
R. BERTOLDO

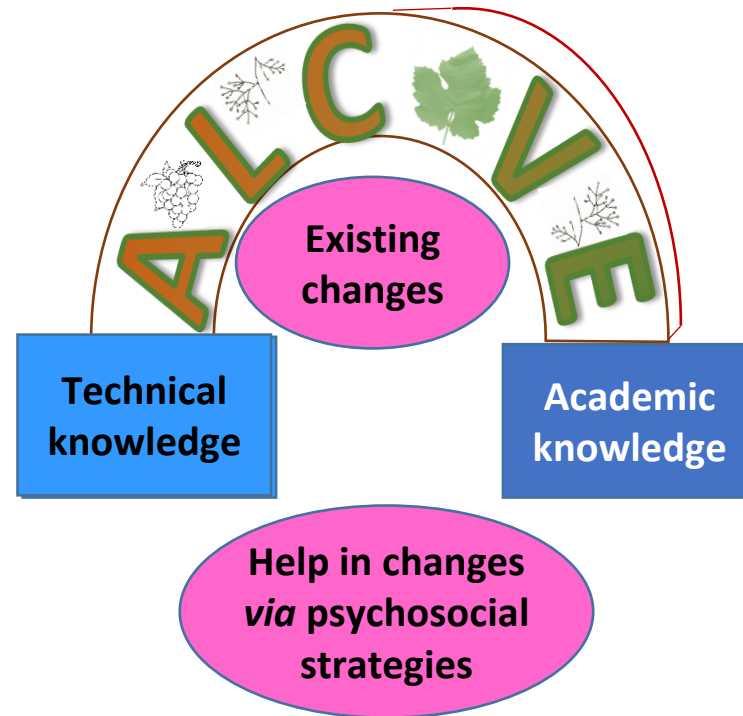
Geography
P. MINVIELLE

**Chemical
Analyses**

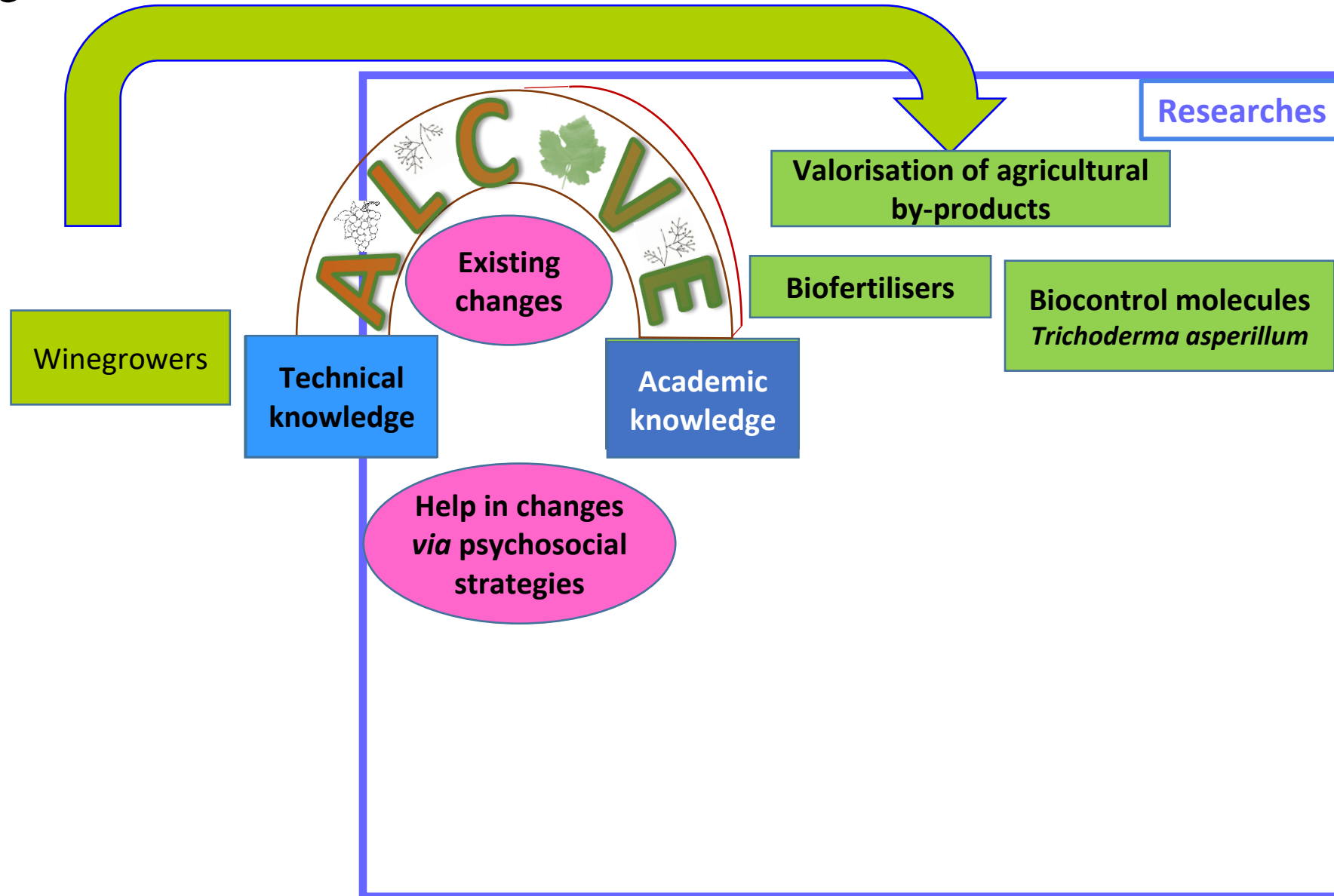
J.L. BOUDENNE
P. PRUDENT
J. MOLINET

Soil Sciences
Meso macrofauna
C. PELOSI
Y. CAPOWIECZ

AgricuLture bioContrôle biOfertilisant ViticolEs

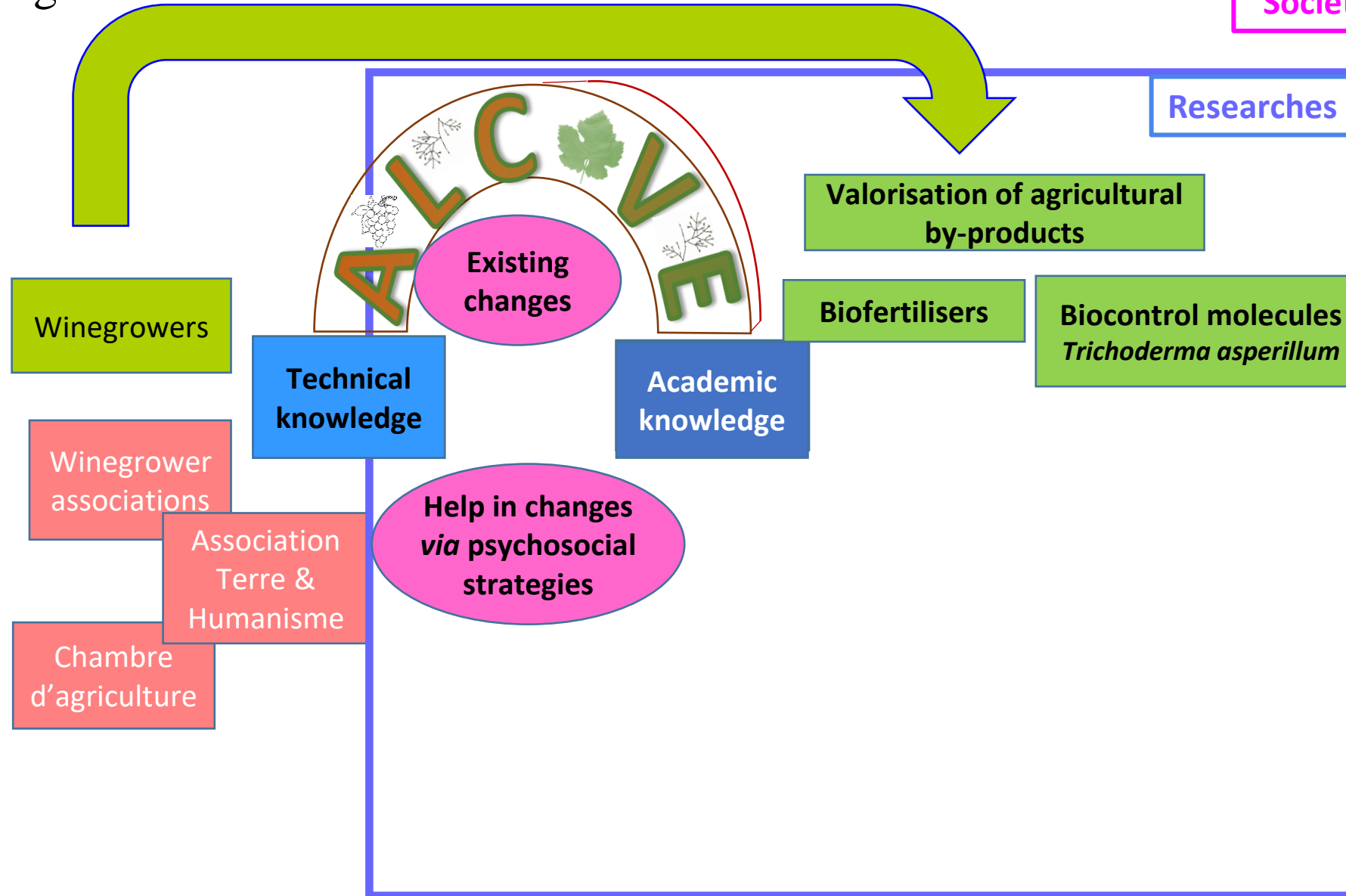


AgricuLture bioContrôle biOfertilisant ViticolEs



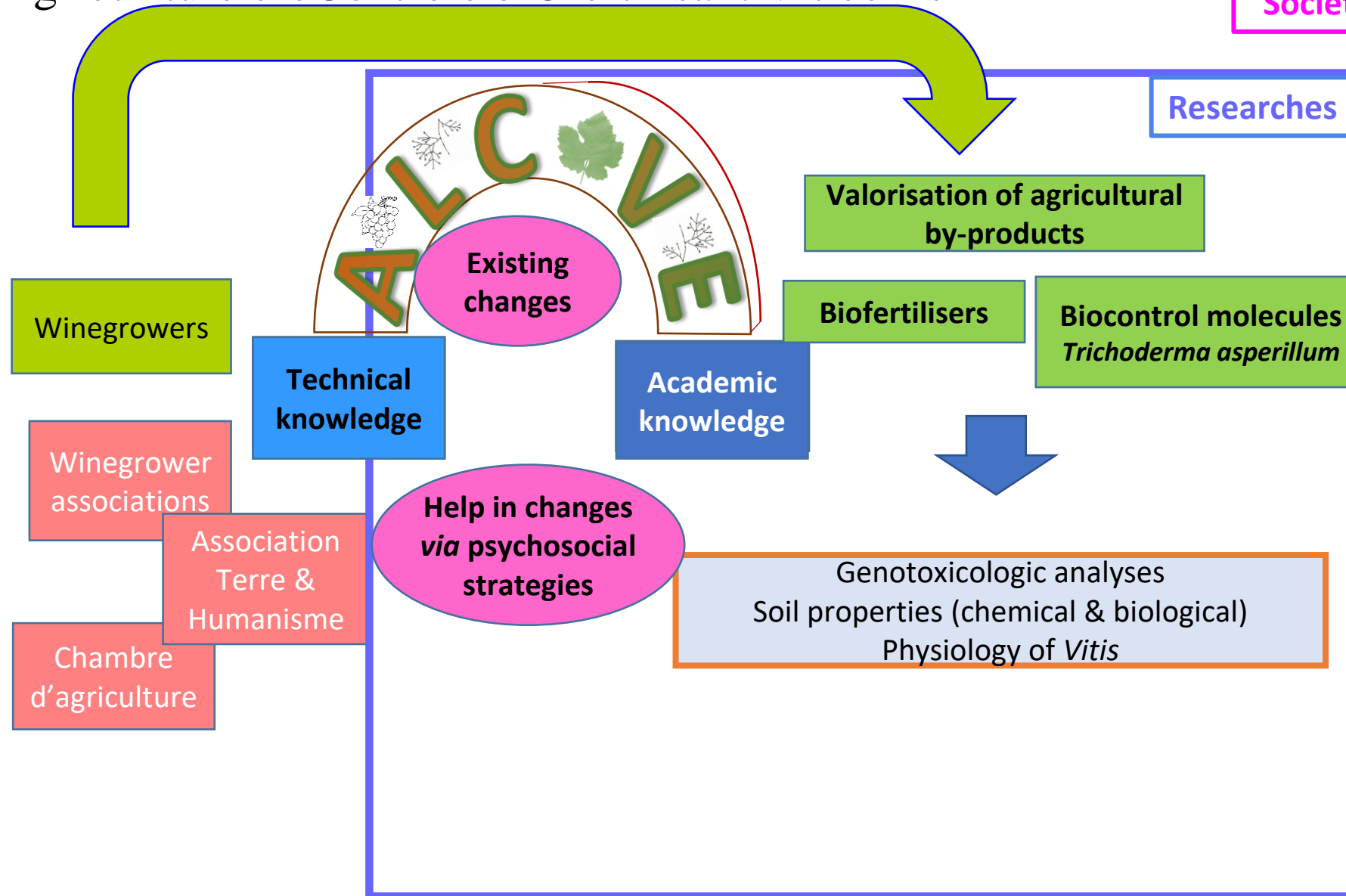
AgricuLture bioContrôle biOfertilisant ViticolEs

Society



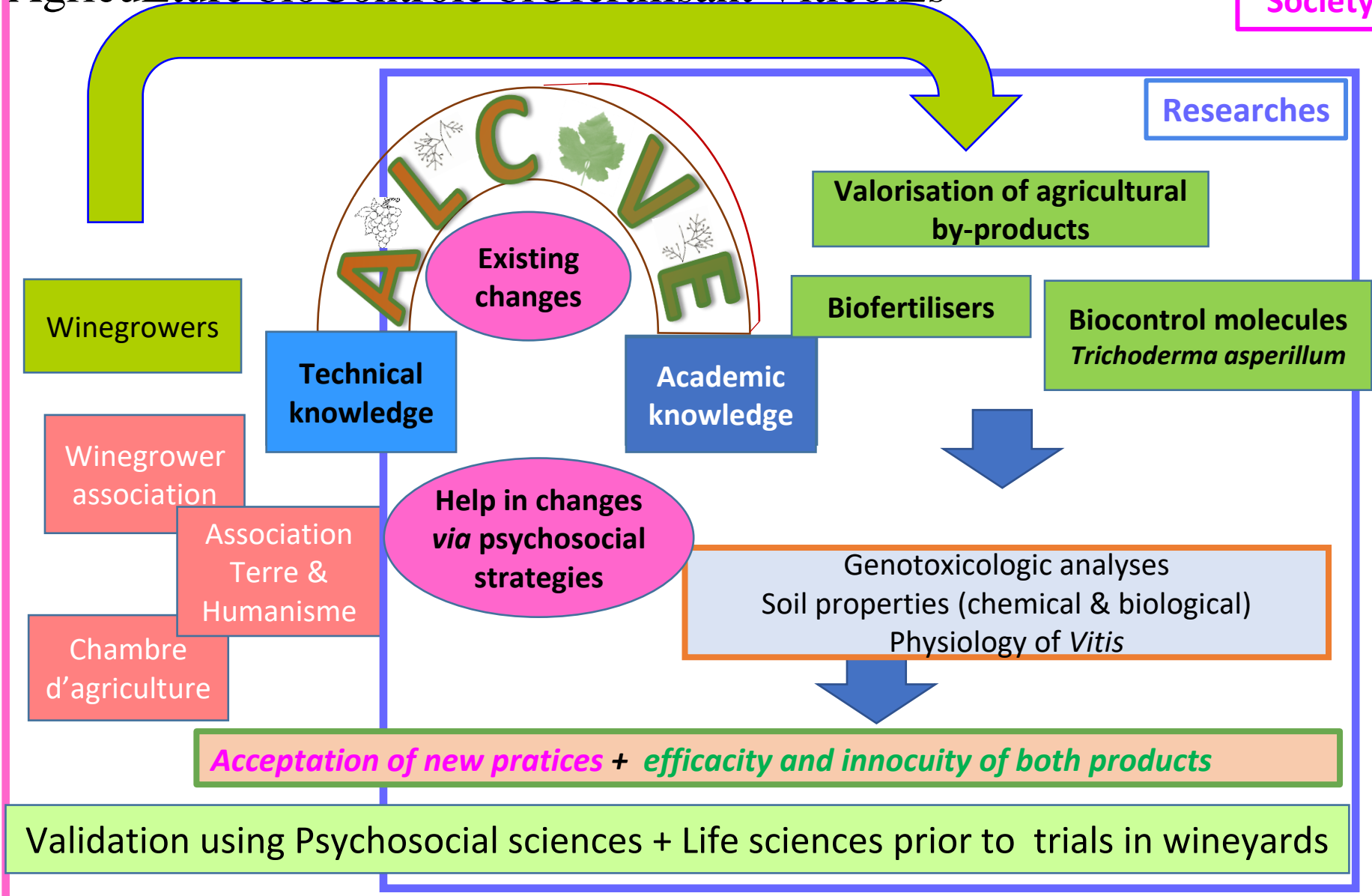
AgricuLture bioContrôle biOfertilisant ViticolEs

Society

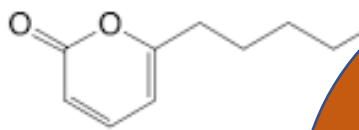


AgricuLture bioContrôle biOfertilisant ViticolEs

Society



6 pentyl α pyrone

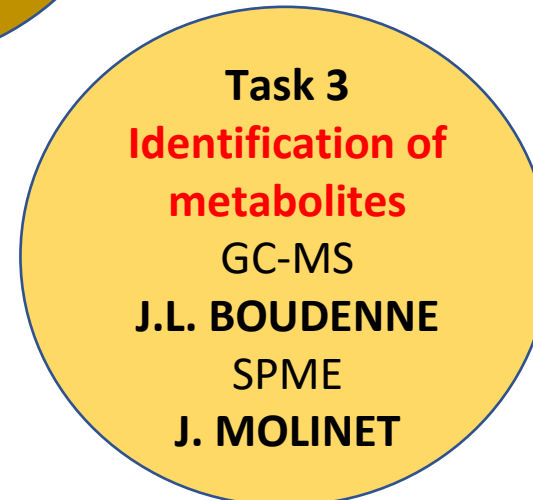
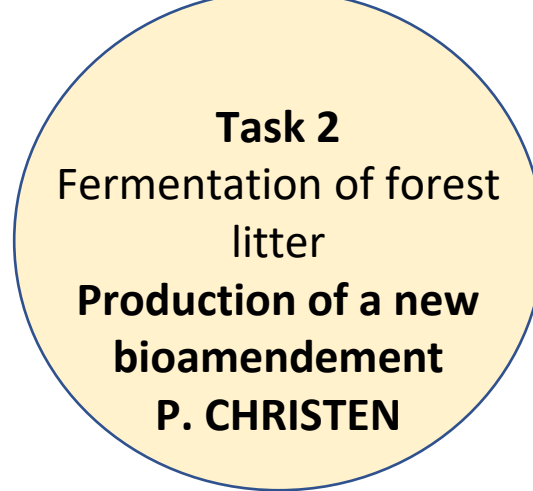
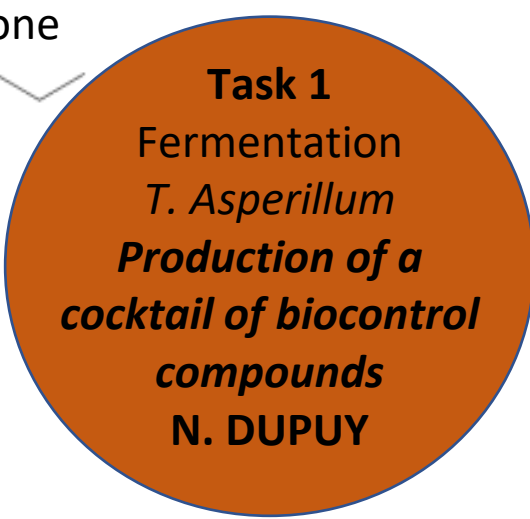
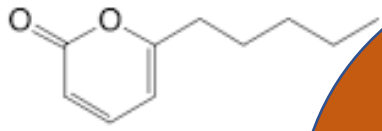


Task 1
Fermentation
T. asperillum
Production of a cocktail of biocontrol compounds
N. DUPUY

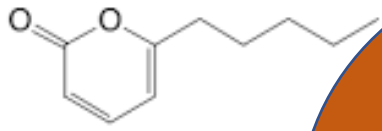
Task 2
Fermentation of forest litter
Production of a new bioamendment
P. CHRISTEN

Management of the project
A.M. FARNET DA SILVA
Coordinator
I. LAFFONT-SCHWOB

6 pentyl α pyrone



6 pentyl α pyrone



Task 1
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T. Asperillum
Production of a cocktail of biocontrol compounds
N. DUPUY

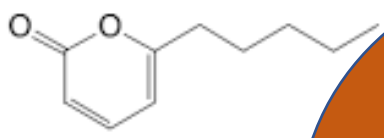
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P. CHRISTEN

Task 4
Genotoxicology
Th. ORSIERE

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Coordinator
I. LAFFONT-SCHWOB

Task 3
Identification of metabolites
GC-MS
J.L. BOUDENNE
SPME
J. MOLINET

6 pentyl α pyrone



Task 1
Fermentation
T. Asperillum
Production of a cocktail of biocontrol compounds
N. DUPUY

Task 2
Fermentation of forest litter
Production of a new bioamendment
P. CHRISTEN

Task 5
Plant physiology
I. LAFFONT-SCHWOB

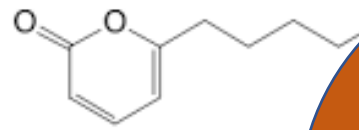
Task 4
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Task 6
Soil biology
(μ org, meso/macrofauna) and chemical analyses
A.M. FARNET DA SILVA
Y. CAPOWIECZ

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6 pentyl α pyrone



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Task 7
Psychosocial studies
R. BERTOLDO
S. BIENVILLE (M2)
Geographical approaches
P. MINVIELLE

Task 3
Identification of metabolites
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SPME
J. MOLINET

Human Ressources



PhD student : Flor REGUS => *start in February 2021*

* **DIPLOME D'INGENIEUR ISA LILLE • LILLE-FRANCE**

SEPTEMBER 2018-FALL 2020 : DIPLOME D'INGENIEUR IN ENVIRONMENTAL SCIENCE & SUSTAINABLE MANAGEMENT OF POLLUTION TOPIC INTERNSHIP : Proposed phyto- management plan for contaminated soil to treat a former wood processing plant contaminated with Cu, by using amendements and tolerant plants (to Cu).

* **R&D TECHNICIAN • NITTO INC. • NEW JERSEY • USA • APRIL 2015-JUNE 2018**

* **BACHELORS OF SCIENCE AUGUST 2009-AUGUST 2013 • UNIVERSITY OF ARIZONA • ARIZONA- USA**
Bachelor's degree in Environmental Science with a focus on Environmental Biology

Human resources

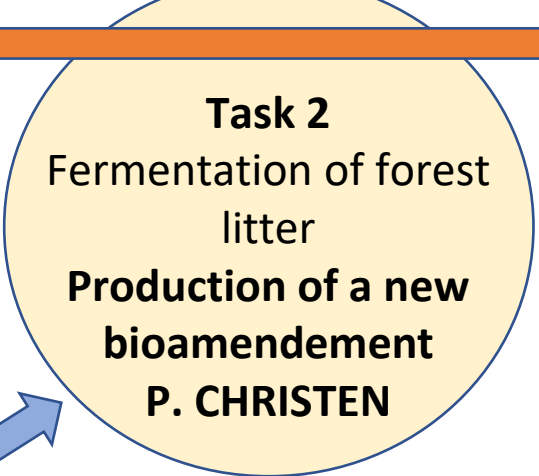
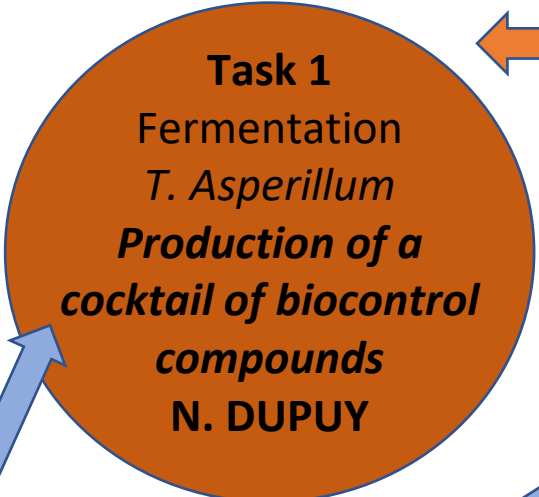
Post Doctorant : Rayhane Hamrouni=> February 2021



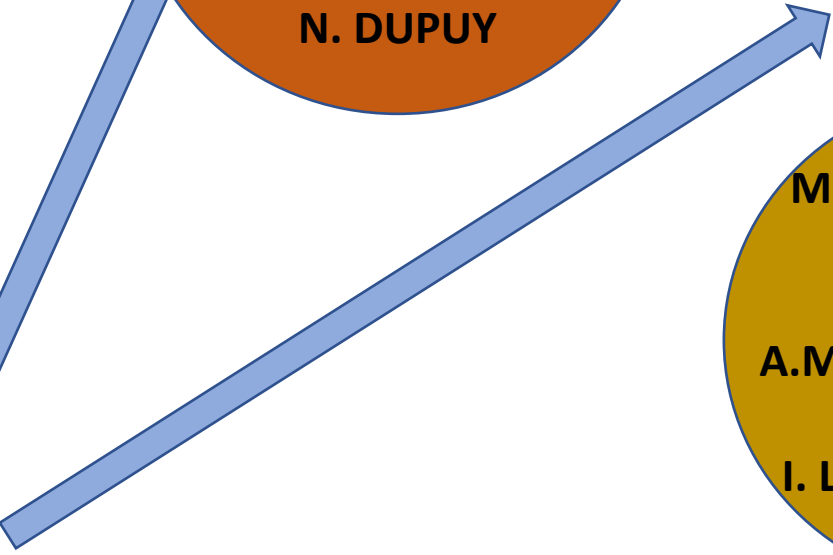
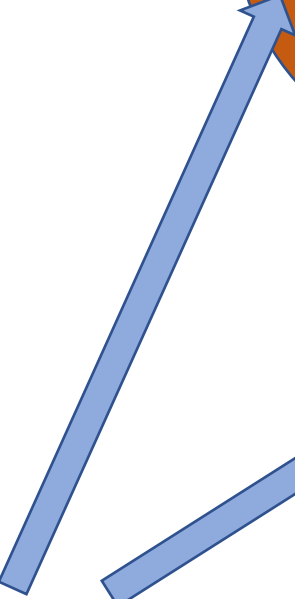
2019- 20 ATER :Institut des Neurosciences de Montpellier (INM), Institut Européen des Membranes (IEM).

2018-19 ATER: AMU UMR BBF (Biodiversité et Biotechnologie Fongiques).

2016-19 Thèse : IMBE AMU Production, purification et caractérisation des métabolites secondaires antifongiques (peptaibols « 6-pentyl- α -pyrone », Trichoharzianines) produits par *Trichoderma asperellum* en FMS



Flor, PhD



Rayhane,
post doctoral position

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Plant physiology
I. LAFFONT-SCHWOB



Flor, PhD

Task 4
Genotoxicology
Th. ORSIERE

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I. LAFFONT-SCHWOB

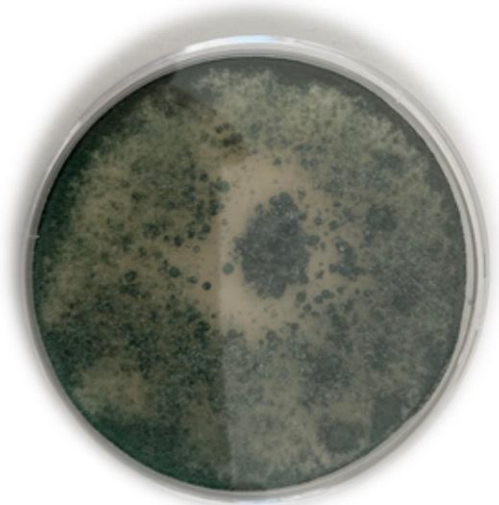
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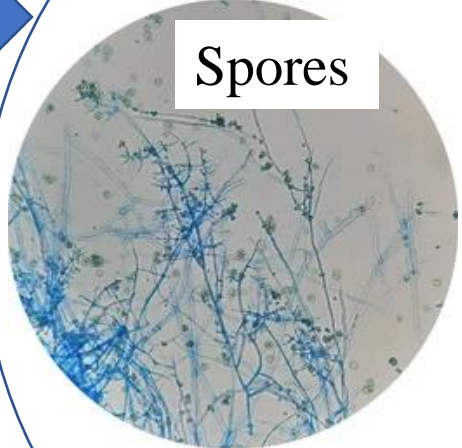
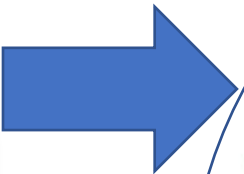


Rayhane,
post doctoral position

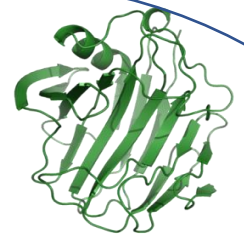
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N. DUPUY



Trichoderma asperillum **TF1**
IMBE-IRD

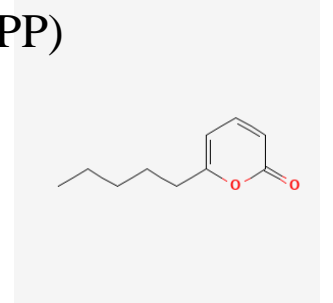


Spores



Enzymes

6-pentyl-alpha-pyrone
(6-PP)

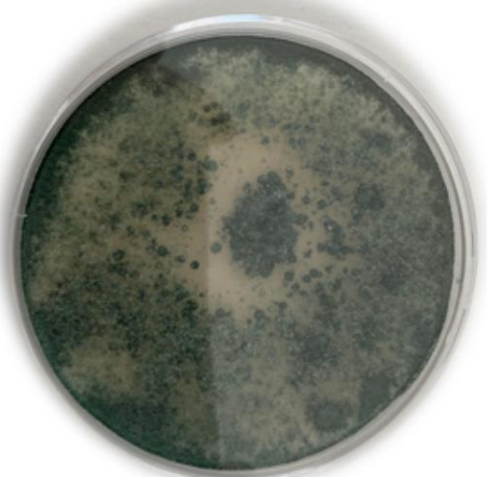


BCC Biocontrol compounds

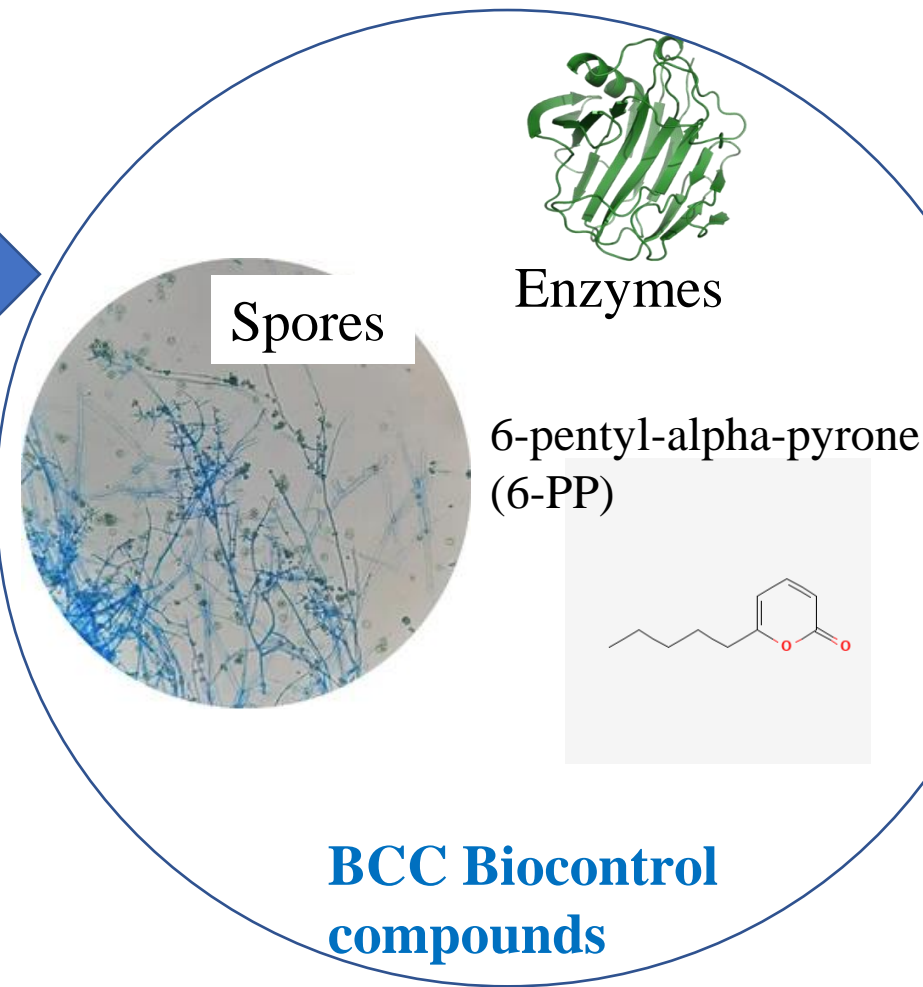
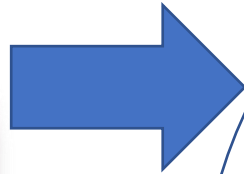
R. Hamrouni PhD

Substrates for solid-state fermentation SSF: wheat bran, jatropha, *Vitis* puring....

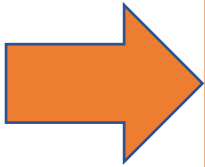
Task 1
Fermentation
T. Asperillum
Production of a cocktail of biocontrol compounds
N. DUPUY



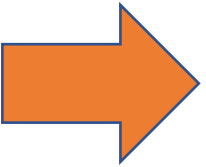
Trichoderma asperillum **TF1**
IMBE-IRD



R. Hamrouni PhD
Substrates for SSF: wheat bran, jatropha, *Vitis* puring....



Substrates for SSF with **local agricultural by products**: wheat bran, olive pomace, *Vitis* puring

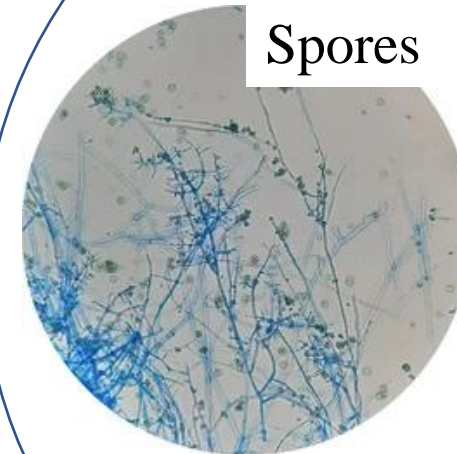
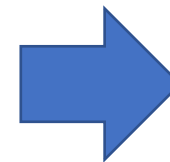


Experimental design

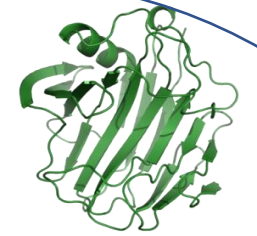
Task 1
Fermentation
T. Asperillum
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N. DUPUY

Optimised composition of the substrate

Variables	Pourcentage (%)
Wheat bran	18
Olive pomace	20
Oatmeal	24
Blent potato	23
Olive oil	14
Vitis puring	40
Chitin	10

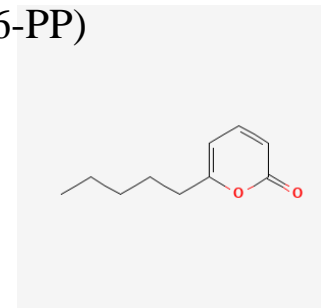


Spores



Enzymes

6-pentyl-alpha-pyrone
(6-PP)



BCC Biocontrol compounds



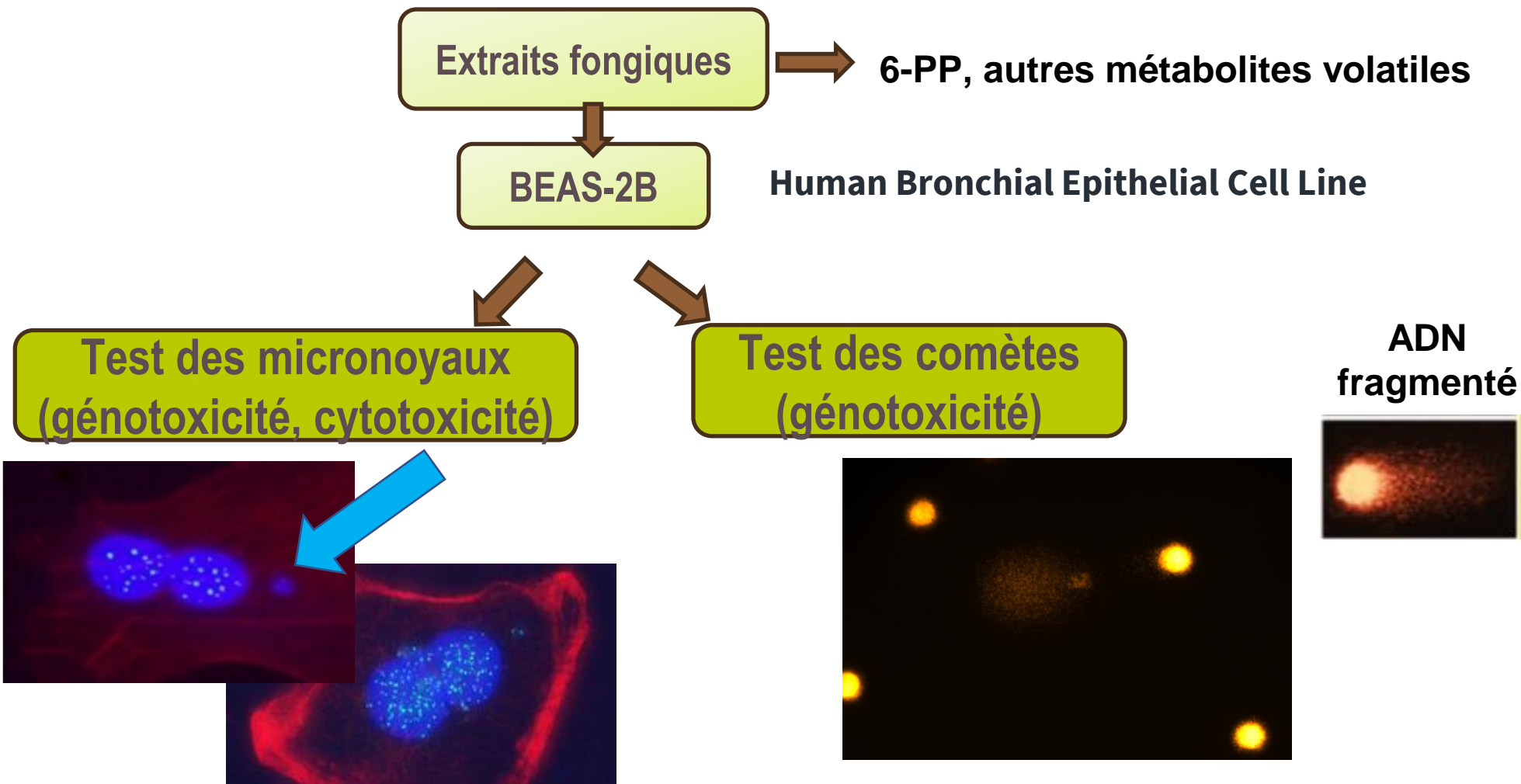
Publication in prep ... J. of Env. Management

“Statistical experimental design and solid-state fermentation: a new approach to optimize the mixture proportions of solid medium for induced sporulation and fungal bioactive compounds”

Objectif: Evaluation de la génotoxicité des produits de fermentation

Task 3
Genotoxicology
R. Hamrouni post doctoral position
Th. ORSIERE

Task 2
Identification of metabolites
GC-MS
J.L. BOUDENNE
R. Hamrouni post doctoral position



**Task 3
Genotoxicology**

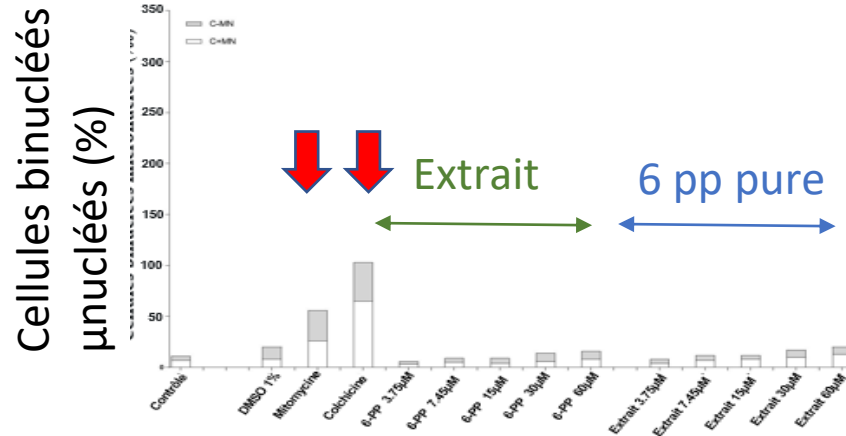
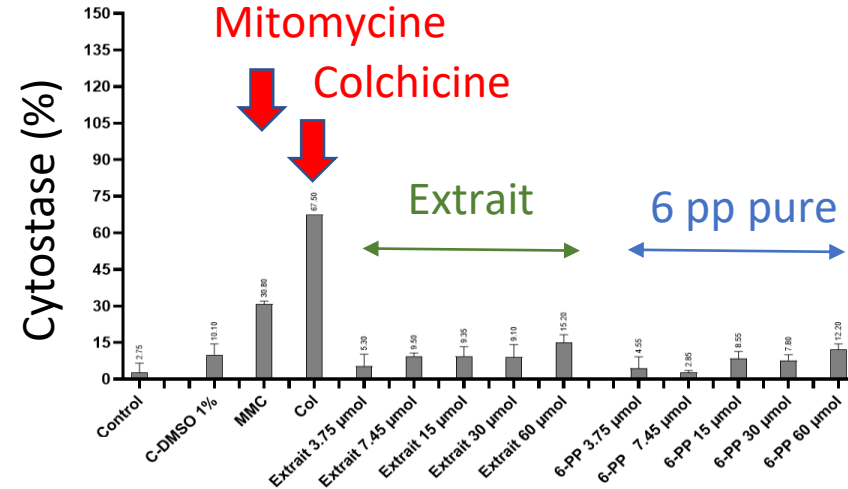
**R. Hamrouni post
doctoral position
Th. ORSIERE**

**Task 2
Identification of
metabolites
GC-MS
J.L. BOUDENNE**

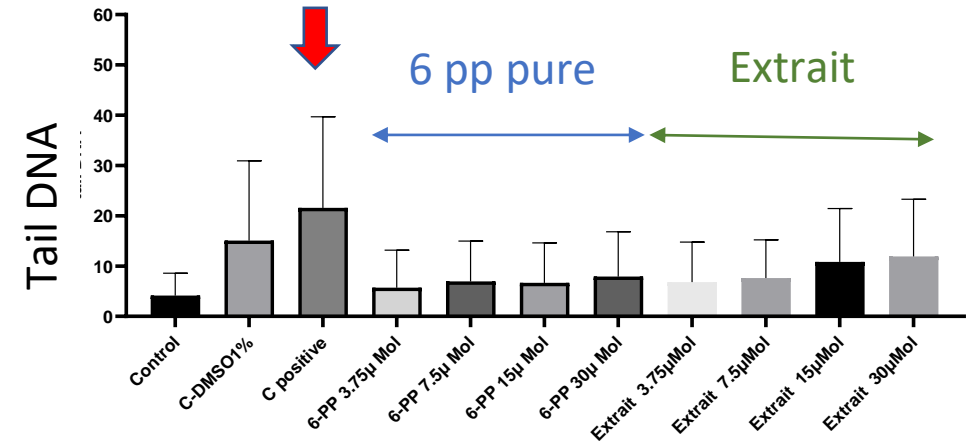
**R. Hamrouni post
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Objectif: Evaluation de la génotoxicité des produits de fermentation

Test des micronoyaux



Test des comètes



**Task 3
Genotoxicology**

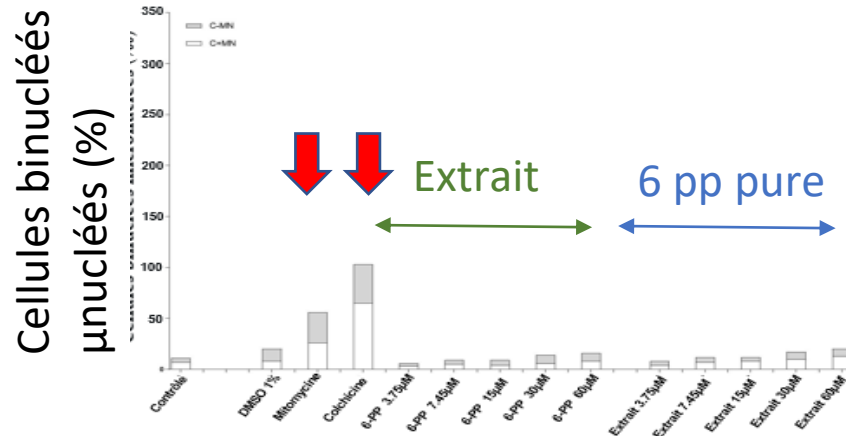
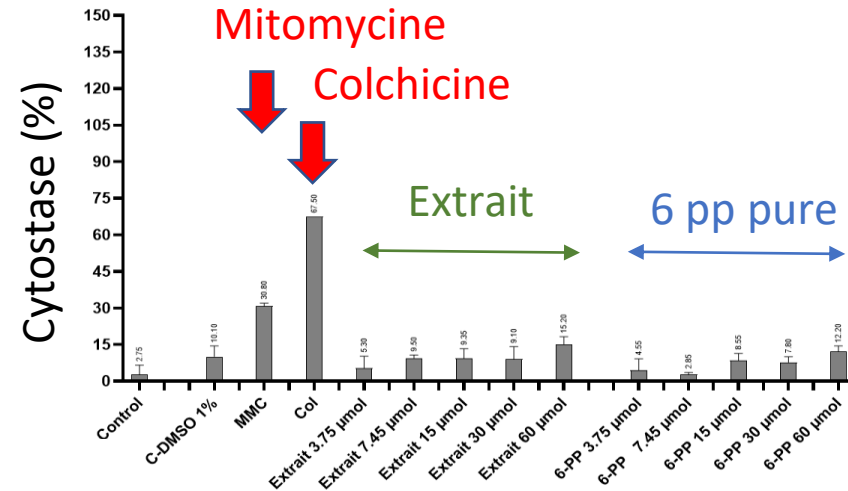
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**Task 2
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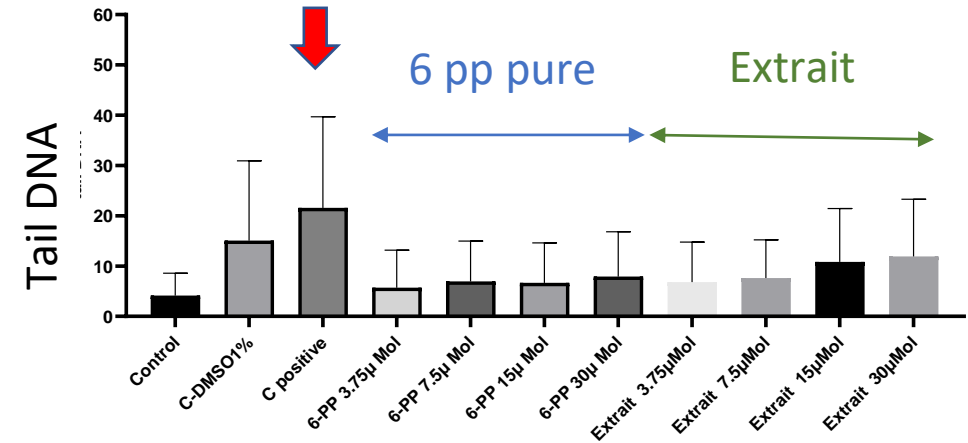
**GC-MS
J.L. BOUDENNE
R. Hamrouni post
doctoral position**

Objectif: Evaluation de la génotoxicité des produits de fermentation

Test des micronoyaux



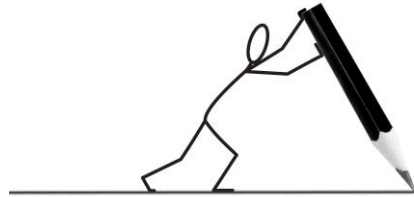
Test des comètes



la 6-PP et l'extrait de BCC semblent être non cytotoxiques et non génotoxiques aux concentrations testées.

PhD student : Flor REGUS

*** BIBLIOMETRIC REVIEW**

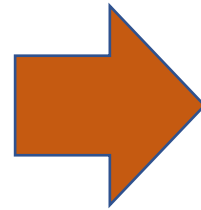


Regus, F., Schwob-Laffont, I., Hamrouni, R., Dupuy, N., Farnet Da Silva A.M., Using bibliometrics to analyze the state of art of pesticide use in vineyard agrosystems: A review, under review in Environmental Science and Pollution Research, (JIF: 4.2)



Task 2
Fermentation of forest
litter
**Production of a new
bioamendement**
P. CHRISTEN

Task 1
Fermentation
T. Asperillum
**Production of a
cocktail of biocontrol
compounds**
N. DUPUY

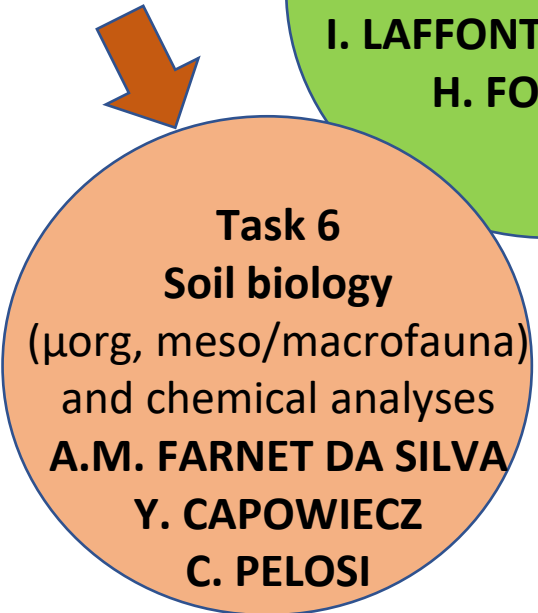
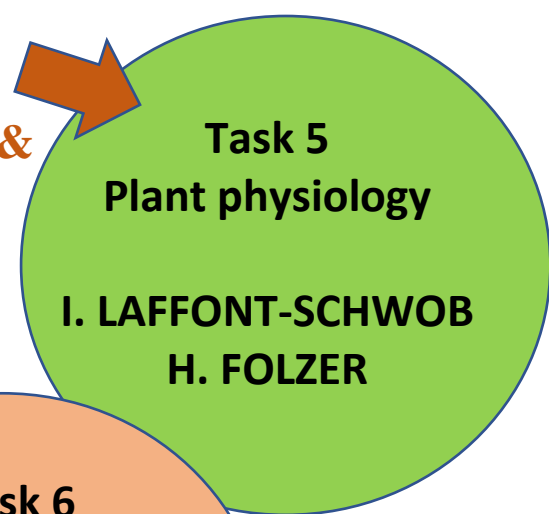


Task 5
Plant physiology
I. LAFFONT-SCHWOB
H. FOLZER

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Y. CAPOWIECZ
C. PELOSI

BCC

Biocontrol
compounds &
Fermented
forest litter



In vitro experiments using experimental design

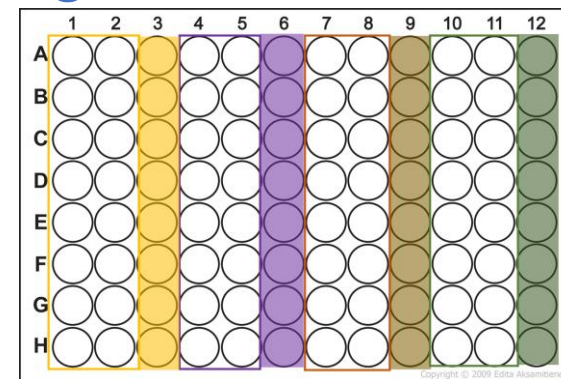
Factors tested

BCC,
6PP,
 CuSO_4 ,
Bouillie bordelaise



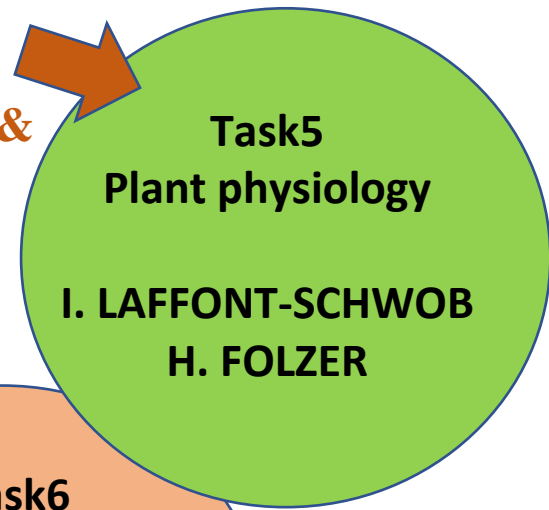
Responses

Spore germination
Hyphae growth



BCC

Biocontrol compounds & Fermented forest litter



In vitro experiments using experimental design

Factors tested

BCC,
6PP,
 CuSO_4 ,
Bouillie bordelaise



Responses

Spore germination
Hyphae growth

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

Mesocosm experiments

Factors tested:

BCC

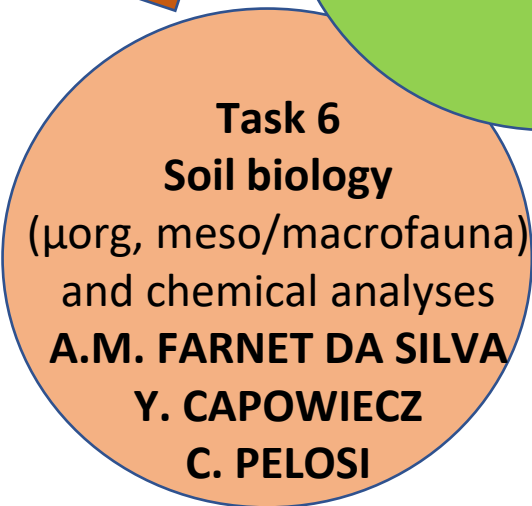
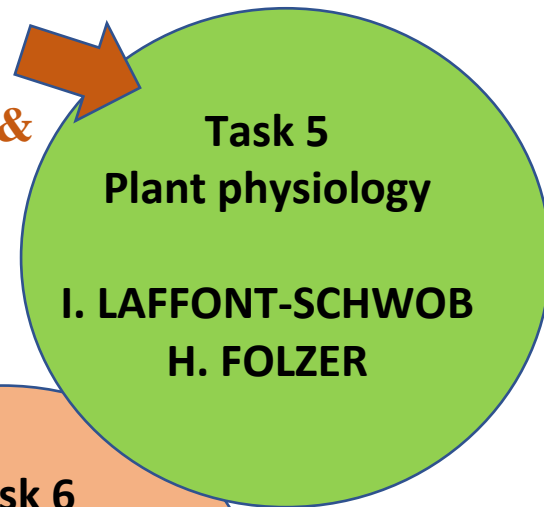
CuSO_4

Bouillie bordelaise

Fermented forest litter amendment

With or without earthworms





Mesocosm experiments



Plant responses

Non-destructive

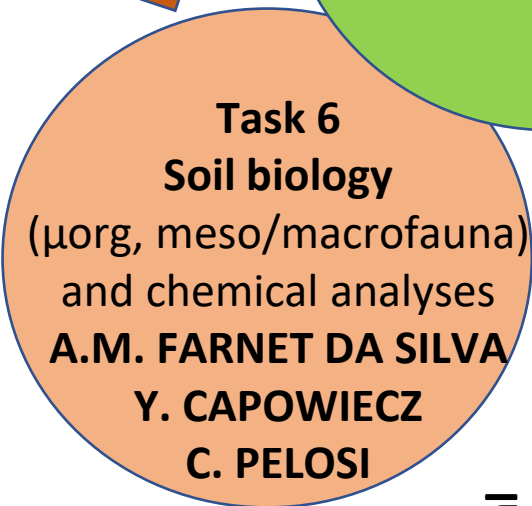
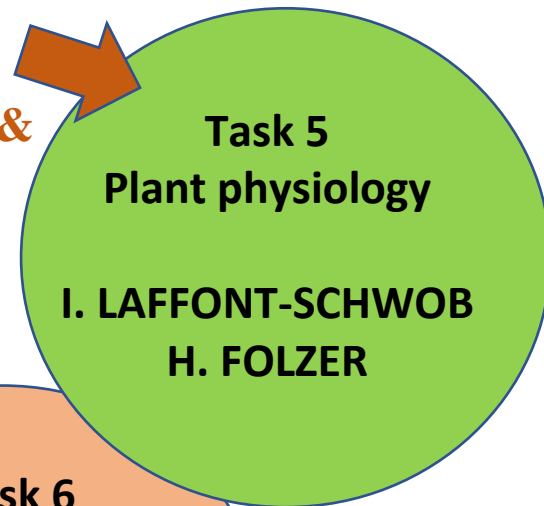
- Phytometabolites (multiplex force A)
- Surface, perimeter and thickness of the leaves
- Phytopathogen growth (spots of fungal growth)
- Measurement of stem
- Copper content in roots and aerial organs

Destructive

- Plant biomass (Aerial and root)
- Copper content in roots
- Phytometabolites (NMR, IR)
- Measurement of stem and root
- Copper speciation

BCC

Biocontrol
compounds &
Fermented
forest litter



Mesocosm experiments



Soil responses

Physico-chemical

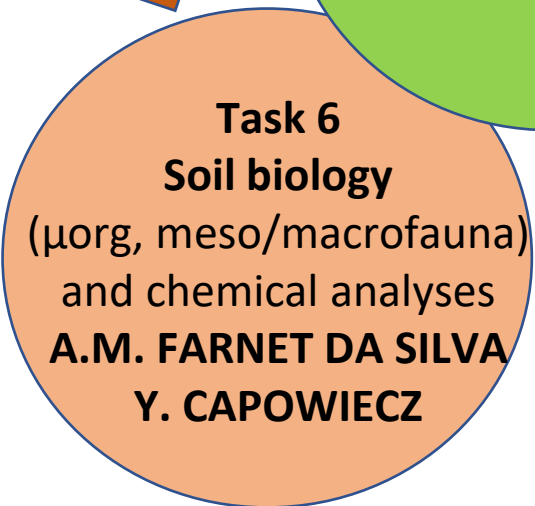
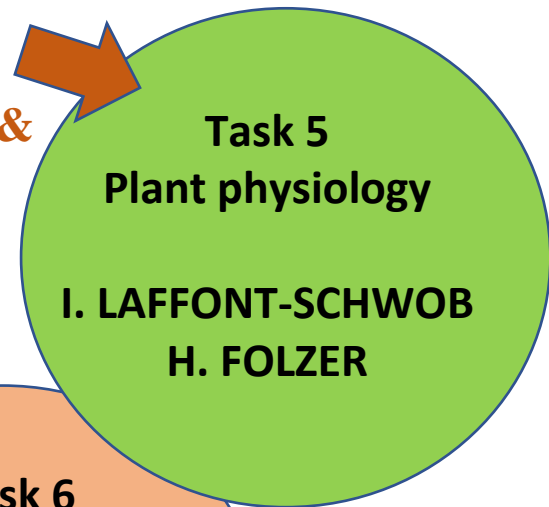
- Granulometry
- Cation exchange capacity (CEC)
- Copper content (ICP-AES)
- pH
- Nutrient content (P & N₂)
- Organic Matter content (IR & NMR)
- Water holding capacity

Microbiological

- Microbial respiration and biomass (by substrate-induced respiration)
- Lignocellulolytic activities, proteases, ureases, phosphatases, catabolic diversity

BCC

Biocontrol compounds & Fermented forest litter



In vitro experiments using experimental design

Factors tested

BCC,
6PP,
CuSO₄,
Bouillie bordelaise



Responses

Spore germination
Hyphae growth

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

Mesocosm experiments

Factors tested:

BCC

CuSO₄

Bouillie bordelaise

Fermented forest litter amendment

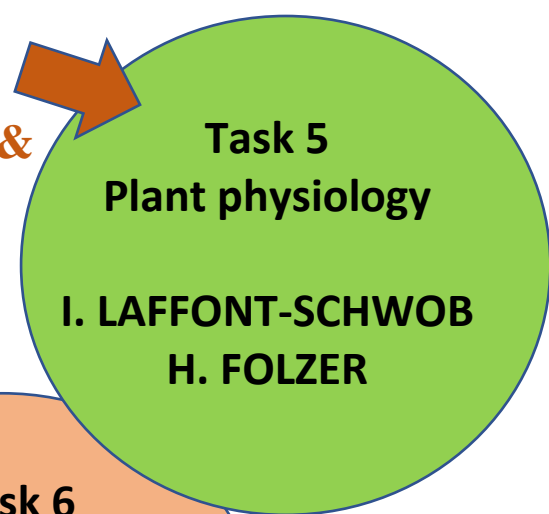
With or without earthworms



Experiments started in April , work in progress ...

BCC

Biocontrol compounds & Fermented forest litter



Task 5
Plant physiology

I. LAFFONT-SCHWOB
H. FOLZER

Task 6
Soil biology

(μ org, meso/macrofauna) and chemical analyses

A.M. FARNET DA SILVA

Y. CAPOWIECZ

C. PELOSI

In vitro experiments using experimental design

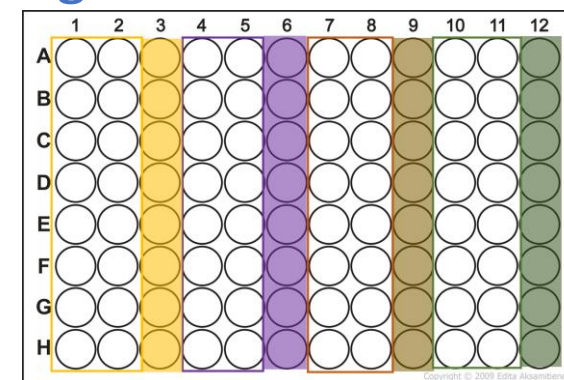
Factors tested

BCC,
6PP,
 CuSO_4 ,
Bouillie bordelaise



Responses

Spore germination
Hyphae growth



Mesocosm experiments

Factors tested:

BCC

CuSO_4

Bouillie bordelaise

Fermented forest litter amendment

With or without earthworms



In natura experiments

Factors tested : type of practices

Massive organic amendments

Organic practices

« HVE » practices (Haute Valeur Environnementale)



CÔTES DE PROVENCE

sainte-victoire

Association des vignerons de la Ste
Victoire (Jean-Jacques Balikian)

Apports de la psychologie sociale et environnementale

- Collaboration CPIE

Task7
Psychosocial studies
R. BERTOLDO
S. BIENVILLE (M2)

Thématiques	Objectif de la thématique	Questions générales	Questions de relance
<p><i>Thématique 5 : Pratiques agricoles, Insertion collective et connaissances de la réglementation en lien avec les pratiques</i></p>	<p><i>L'insertion collective correspond aux logiques de gestion collective dans le cadre par exemple des labels ou des signes de qualité (HVE, IGP, AOP, ...)</i></p> <p><i>(Exemple : les agriculteurs dans un label vont avoir des logiques / réglementations communes concernant les pratiques agricoles)</i></p> <ul style="list-style-type: none"> ➤ <i>Connaître le niveau de connaissance des réglementations en vigueur</i> ➤ <i>Connaître les méthodes et pratiques de l'agriculteur</i> ➤ <i>Connaître le degré d'assimilation et/ ou de prise d'initiative par rapport à ces réglementations</i> ➤ <i>Déterminer un parcours collectif ou individuel chez l'agriculteur</i> 	<p>Quel est le statut officiel de votre exploitation ? (conventionnel, bio,raisonné, ...)</p> <p>Quel type de réglementation devez-vous suivre ?</p> <ul style="list-style-type: none"> - (Exemples de structures à l'origine des réglementations : PAC, coopératives, charte riverain, contrats d'agriculture durable, mesures, taxes, redevances...) <p>Qu'est-ce que cela implique dans votre pratique ?</p>	<ul style="list-style-type: none"> ➤ Quand et comment se font les traitements ? ➤ Dans quel cas utilisez-vous des PPP ? et pour quelles raisons ? ➤ Quels produits utilisez-vous / Quelles techniques utilisez-vous ? Pourquoi ? ➤ Vos pratiques culturales ont-elles évolué au cours du temps / pour quelles raisons ? <p>(Contexte climatique, nouvelle demande des consommateurs, passage à l'agriculture biologique et autres...)</p>

Apports de la psychologie sociale et environnementale

Task7

Psychosocial studies

R. BERTOLDO

S. BIENVILLE (M2)

- Collaboration CPIE

Thématiques	Objectif de la thématique	Questions générales	Questions de relance
<i>Thématique 6 : Adaptation et initiatives individuels</i>	<p>➤ <i>Comprendre comment les agriculteurs s'adaptent à la réalité du terrain et vers quel type de solutions ils se tournent individuellement. Cette question nous donne également un indicateur : ouvert à d'autres pratiques, adaptabilité, ...</i></p>	<p>Quelles initiatives individuelles mettez-vous en place sur votre exploitation pour faire face à ces difficultés ?</p> <p>(Exemple : Enherbement, apport de matière organique, méthodes d'arrosage, haies, paillis, laine de mouton ...)</p>	<p>➤ Vers quels types de solutions vous tournez-vous ? Quelles techniques utilisez-vous ? Pourquoi ?</p> <p>➤ Vos pratiques culturelles ont-elles évolué au cours du temps / pour quelles raisons ?</p> <p>(Contexte climatique, nouvelle demande des consommateurs, passage à l'agriculture biologique et autres...)</p>



DEUXIEME JOURNEE VITICULTURE MEDITERRANEENNE ET ENVIRONNEMENT

Journée de rencontre entre les chercheurs universitaires du Consortium ALCOVE et les organisations professionnelles viticoles.

Le programme ALCOVE réunit des chercheurs de plusieurs laboratoires rattachés à ITEM, l'Institut Méditerranéen pour la Transition Environnementale dans une optique interdisciplinaire. Des écologues, chimistes, génotoxicologues, agronomes, pédologues s'associent pour évaluer l'action de produits de biocontrôle et d'un nouveau biofertilisant. Géographes et psychologues collaborent au projet afin de mieux comprendre les dynamiques de changement des pratiques en agriculture durable.

Un partenariat actif avec les organisations professionnelles viticoles est souhaitable pour mener à bien ce programme. L'objectif de cette journée est d'ouvrir un dialogue entre chercheurs et vignerons afin de partager connaissances, expertises et expériences de terrain.

Journée organisée à La Maison de la Sainte Victoire le 24 mars 2022 de 9h à 16h30
par Paul Minvielle (laboratoire TELEMME) et Jean-Jacques Balikian (Association des Vignerons de la Sainte Victoire).

ALCOVE ITEM 2020-2024



Merci de votre attention !

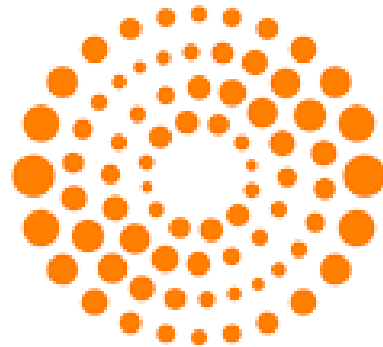
BIBLIOMETRIC REVIEW

The use of statistical methods to analyze publication metadata

Viticulture, grape,
wine, vineyard,
pesticides, copper,
soil, phytopathogen,
plant health

Time frame: 1990-2020

Database



WEB OF SCIENCE™

1317 records:

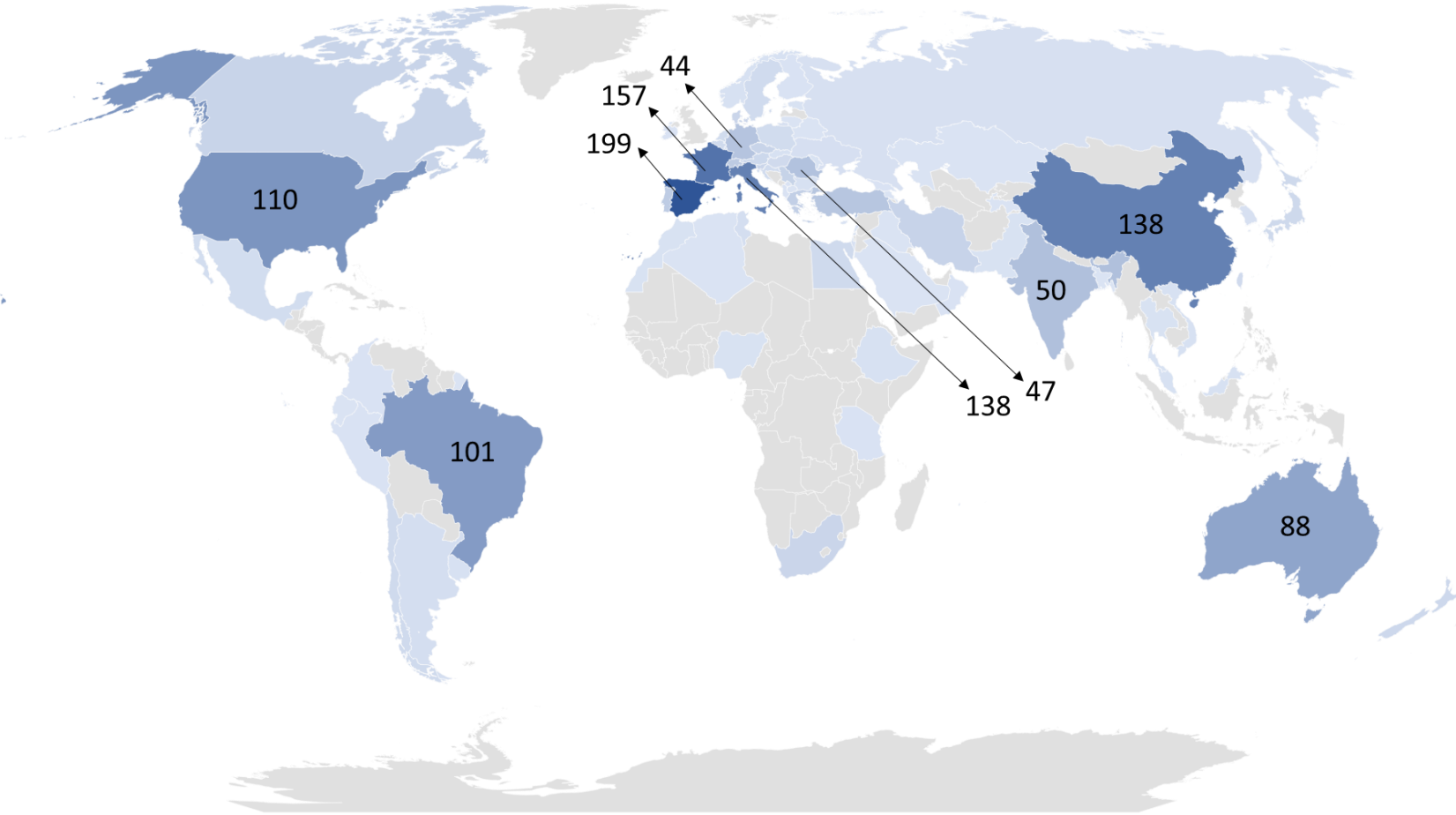
Scientific articles, Review, Book chapters

Export Metadata: Keywords, authors, year,
journal, country of affiliation

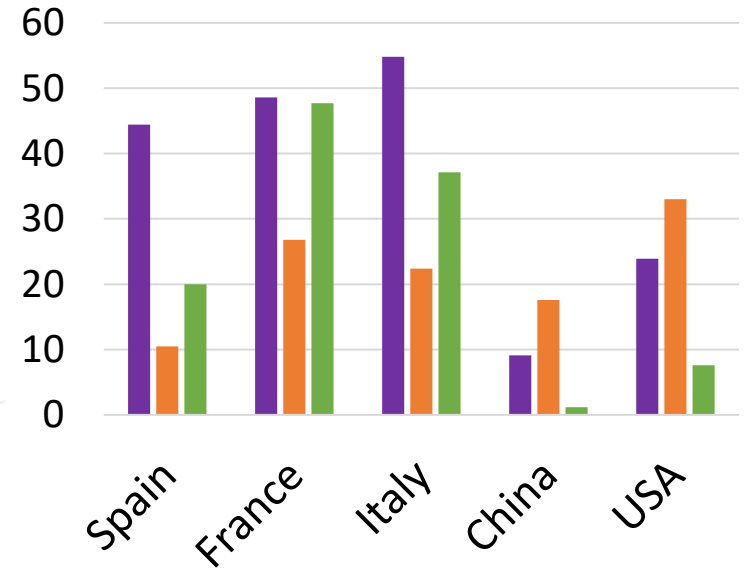
Analysis of metadata using






BIBLIOMETRIC REVIEW



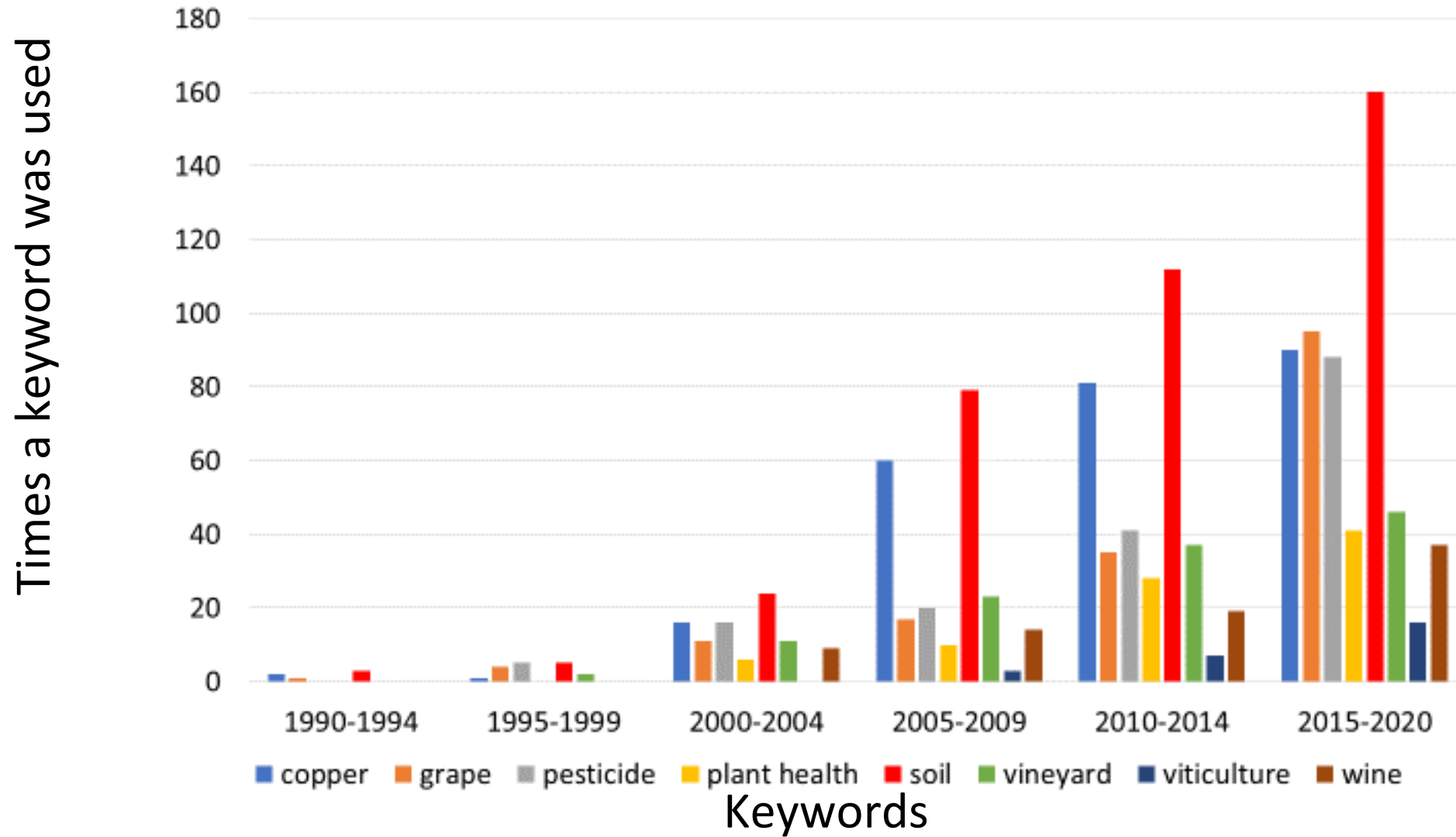
1  199
Number of publications



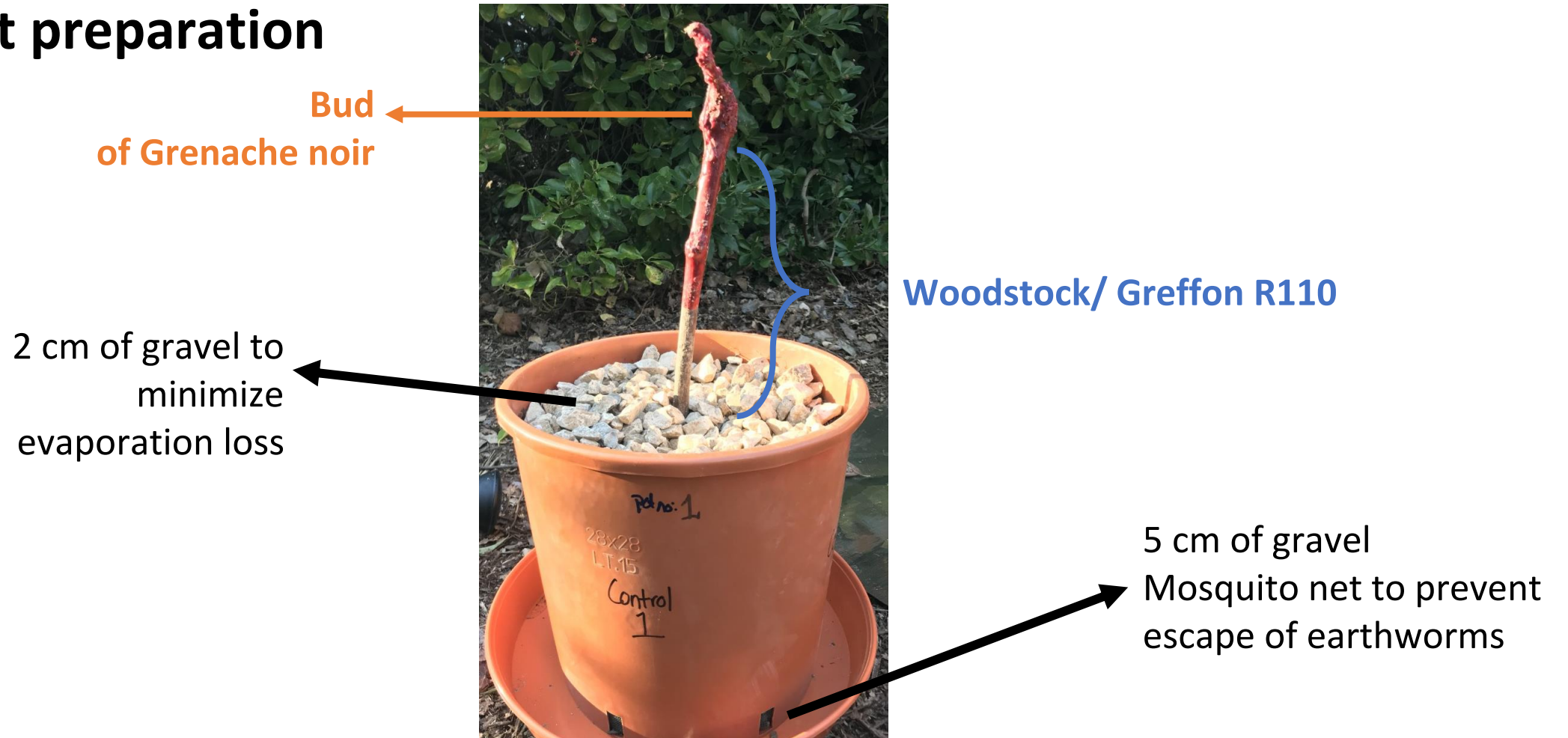
	Production (million hectoliters)
	Consumption (million hectoliters)
	Consumption (L /per capita)

Avec Bing
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BIBLIOMETRIC REVIEW



Soil pot preparation



MESOCOSM EXPERIMENT TO TEST THE EFFECTS OF BCC ON *BOTRYTIS CINEREA*



Mesocosm set up in the botanical garden of St Jérôme

Experiments started in April, work in progress ...

Treatment	
Control	
BB	BB: Bouillie Bordelaise
BCC	BCC: Biocontrol compound
FFL	FFL: Fermented forest litter
BCC+BB	MF: Macro/meso fauna
BCC+FFL	
BCC+BB+FFL	
BB+FFL	
Control (MF)	
BB (MF)	
BCC (MF)	
FFL (MF)	
BCC+BB (MF)	
BCC+FFL (MF)	
BCC+BB+FFL (MF)	
BB+FFL (MF)	

MESOCOSM EXPERIMENT TO TEST THE EFFECTS OF BCC ON *BOTRYTIS CINEREA*

Parameters followed



Weekly:

- pH
- Temperature
- % Humidity
- Conductivity
- pH
- Assessment of infection

Monthly:

- PAR (Photosynthetically active radiation)
- Respiration
- Plant biomass (Length of stem, leaf areas)
- Phytometabolites (Multiplex A)
- Cu content of pruning
- Collection of fresh leaves before FALL?**

Experiments started in April, work in progress ...

Task 1 : To produce biocompounds with high antifungal activities against phytopathogens of *Vitis* (N. Dupuy)

Rapid description of the knowledge

The strains of *Trichoderma asperellum* TV104, *T. asperellum* TF1 and *T. asperellum* QT22046 from the IRD/IMBE fungi collection were used previous study. These strains were selected after a screening and identification of *Trichoderma* strains for 6-PP and conidia production (23 strains)

For these strains Optimisation was performed using Adamard design

Best conditions : Mixture containing 30% of vine shoots, 20% of potatoes flour, 20% of jatropha, 20% of olive pomace and 10% of olive oil are optimal conditions for 6PP production

From flasks to single used bioreactor: Scale-up of solid state fermentation process for metabolites and conidia production by *Trichoderma asperellum*.

Task 2 To produce a bioamendment from fermented forest litter (P. Christen)

Rapid description of the method of production of fermented forest litter (solid FFL)

Substrates and inoculum

Agro-industrial by-products

ground vineyard leaves or vine shoots -> Cellulose
Whey -> Proteins + lactose + lactic bacteria
Molasse -> Oligo saccharides



Biofertilizer (FFL)

+ Forest litter -> Microorganism (biodiversity)

+ Water

1 month batch process in anaerobic conditions

Mixing



Airtight vessel



FFL



Task 2 To produce a bioamendment from fermented forest litter (P. Christen)

- Activation of solid FFL in water
- Dilution for use in cultures
- For olive cultures, ground olive tree cutting and leafs ground could be used, (oil cakes ?) as C-substrates.
- Little is known about the mode of action of FFL in the soil, microorganisms and plant interactions.
- In order to explore the microbial community evolution during the fermentation process, as well as its fate in the soil, metabarcoding studies are undergone in IMBE labs.

Task 3 : To test the innocuity of both BCCs and bioamendment (JL Boudenne, Th. Orsière)

- Extractions of BCC using different protocoles

- UHPLC-Fluo coupled with a fraction collector; each fraction collected analyzed by LC-MS/MS and GC/MS
- Gel-permeation chromatography allowing to select and collect fractions (based on spectral properties? on **polarity properties?**)

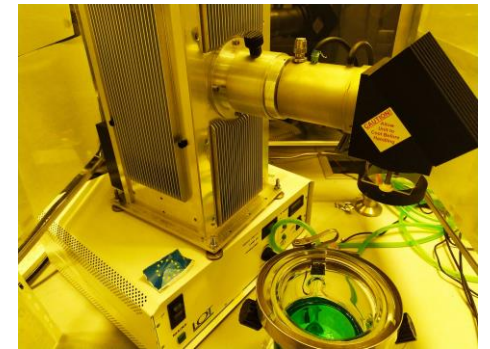
Fractions collected should be of sufficient volume to allow toxicity analyses and photo-oxidation assays

- Fate of identified molecules and/or collected fractions when submitted to solar irradiation (by use of a solar simulator)

= Analysis of by-products following same extraction protocoles described above



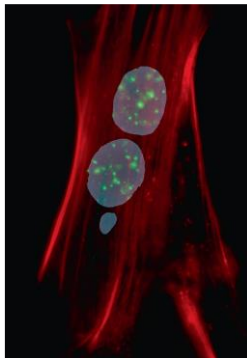
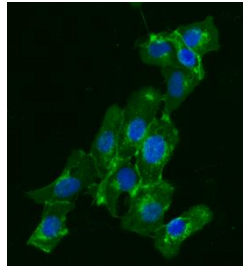
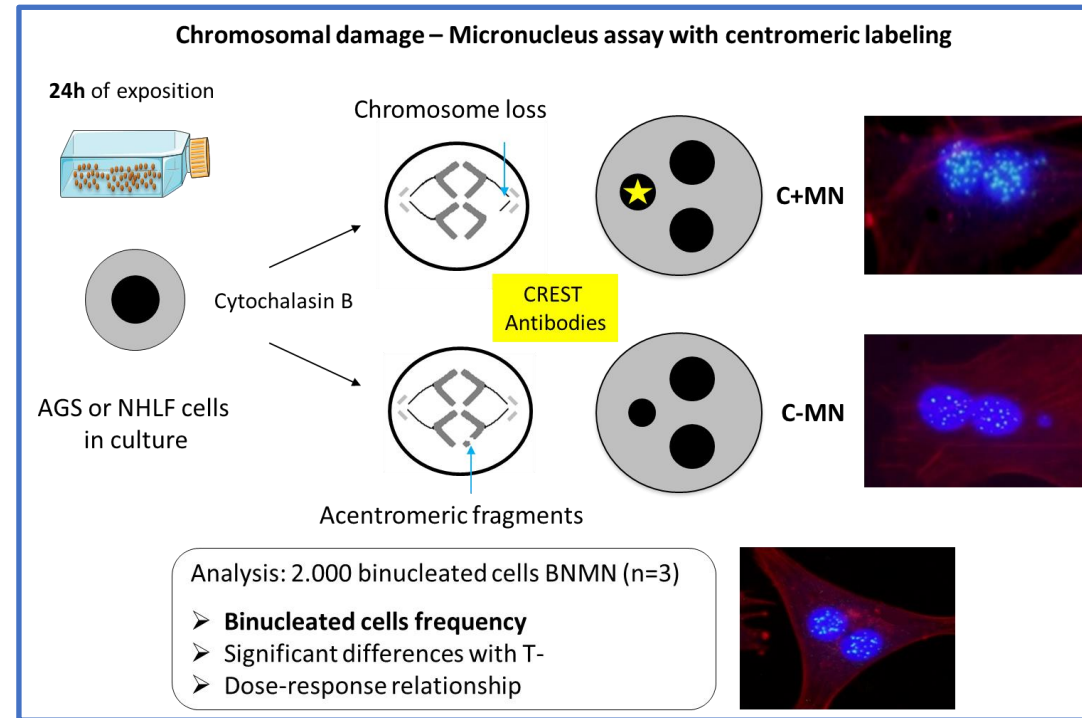
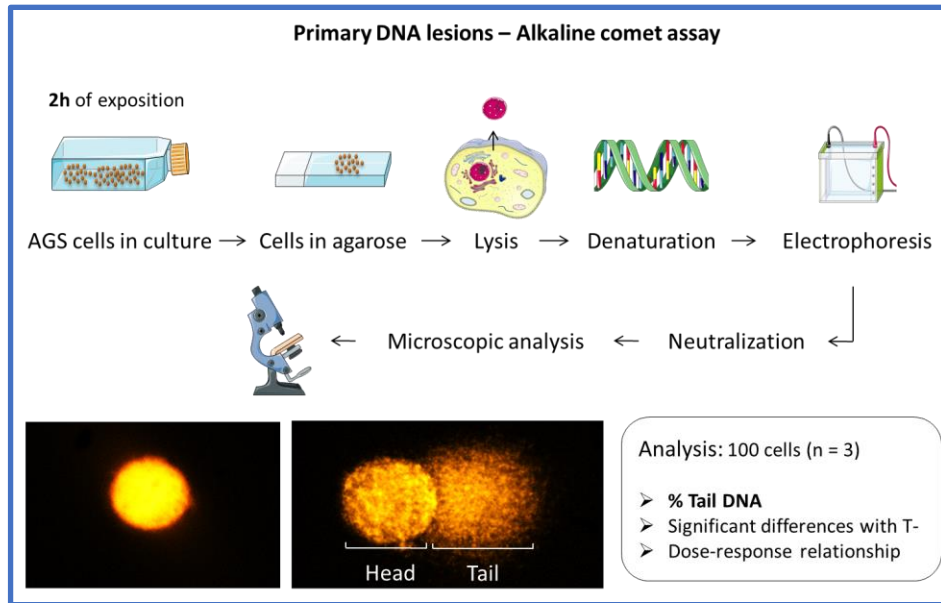
Fractionation / Identification



Photooxidation

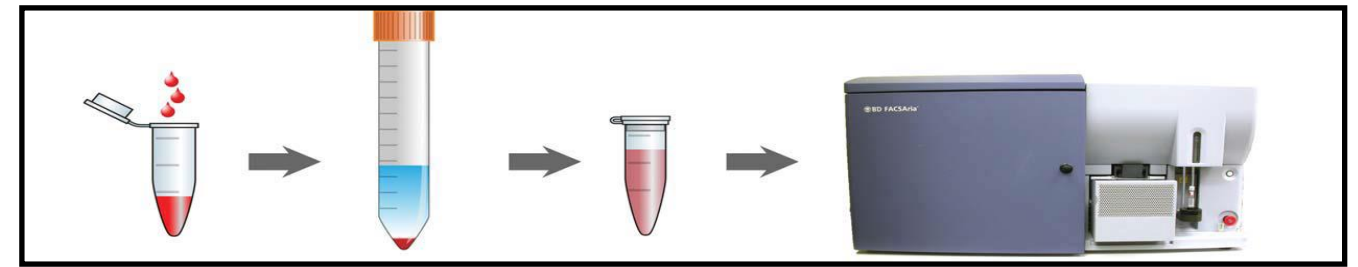
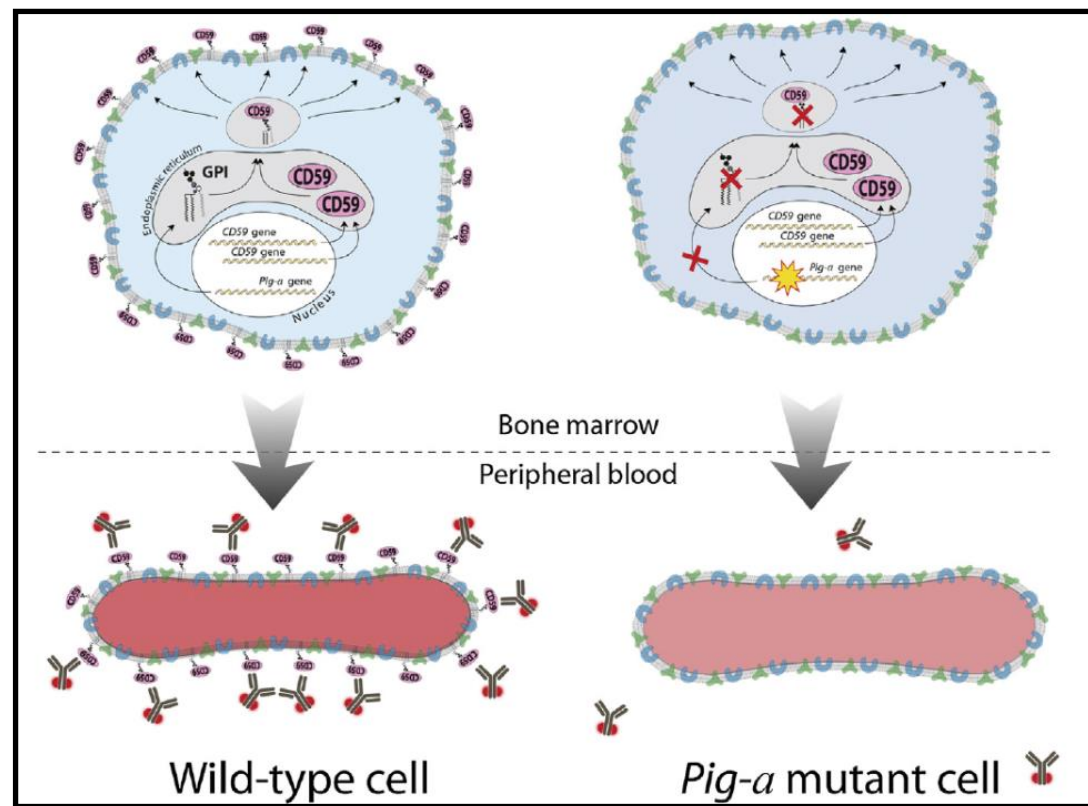
Task 3 : To test the innocuity of both BCCs and bioamendment (JL Boudenne, Th. Orsière) 2/3)

- **Cyto-genotoxicity assessment of biocontrol products fractions will be performed by the use of the comet assay, the cytokinesis blocked micronucleus assay, and if possible by the PIG-a Assay on human cell lines (BEAS-2B and spermatogonia GC-6spg)**

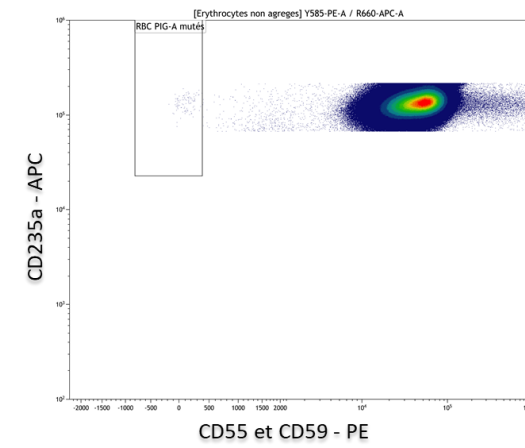


Task 3 : To test the innocuity of both BCCs and bioamendment (JL Boudenne, Th. Orsière) 3/3

- Cyto-genotoxicity assessment of biocontrol products fractions will be performed by the use of the comet assay, the cytokinesis blocked micronucleus assay, and if possible by the PIG-a Assay on human cell lines (BEAS-2B and spermatogonia GC-6spg)



Cell exposure (24h) Cell collection) Immunostaining for two GPI-anchored proteins and cell viability Flux cytometry analysis of $> 5 \times 10^6$ cells



Example of data obtained on human erythrocytes

Task 4 : To test their activities using *in vitro* conditions and under mesocosm conditions (AM Farnet Da Silva, I. Laffont Schwob, Y. Capowiez, P. Prudent)

What is the protective effects of non-toxic compounds (probably a cocktail) against *Botrytis*, *Oidium* and mildew: an **in vitro assay approach** (Schnee et al., 2013)

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Vitis vinifera Canes, a New Source of Antifungal Compounds against *Plasmopara viticola*, *Erysiphe necator*, and *Botrytis cinerea*

Sylvain Schnee,[†] Emerson F. Queiroz,[‡] Francine Voinesco,[†] Laurence Marcourt,[‡] Pierre-Henri Dubuis,[†] Jean-Luc Wolfender,[‡] and Katia Gindro^{*,†}

Post doc

PhD

Post doc + PhD

1

Fermented forest litter
FFL (solid)

1

Fermentation for BCC
production (solid)

1

Activated FFL
(liquid)

2

BCC extracts (H₂O, H₂O/ethanol,
H₂O/methanol,)+ fractionation

1

Genotoxicity
assays

→

toxicity of fractions with
high antifungal potential

3

In vitro assays to test anti fungal
activities

yes

no

Safe and antifungal-active extracts & Safe
activated FFL

4

Mesocosm experiment

1,2, 3, 4!

Communication with winegrowers about the project,
participation to field schools

2021

	Feb-March	April –June	July-Sept	Oct-DEc
Task 1.1 production of BCC and fractionation of extracts	Production	Fractionation	Fractionation	Fractionation
Task 1.2 production of bioamendement				
Task 2 antifungal activities in vitro				
Task 3 Genotoxicity		bioamendement	BCC	BCC
Task 4 mesocosm study				
Task 5 psychosociology geography	Master?			
Task Meetings	April	June : Prep consortium meeting	September	November : prep consortium meeting
Consortium meeting			July	December
Meeting with winegrowers		Presentation of the project		Presentation of first results
Field Schools			September? Master?	

2022

	Feb-March	April –June	July-Sept	Oct-DEc
Task 1.1 production of BCC and fractionation of extracts	Production Fractionation <i>Article writing</i>	Production Fractionation <i>Article writing</i>	Production Fractionation <i>Article writing</i>	
Task 1.2 production of bioamendment				
Task 2 antifungal activities in vitro				
Task 3 Genotoxicity	<i>Article writing</i>			
Task 4 mesocosm study	Mesocosms set up	Online monitoring	Online monitoring	Online monitoring
Task 5 psychosociology geography	Master			
Task Meetings	April	June : Prep consortium meeting	September	November : prep consortium meeting
Consortium meeting			July	December
Meeting with winegrowers		Meeting students/ wine growers/ scientists		Meeting students/ wine growers/ scientists
Field Schools				

2023

	Feb-March	April –June	July-Sept	Oct-DEc
Task 1.1 production of BCC and fractionation of extracts	Production Fractionation <i>Article writing</i>	Production Fractionation <i>Article writing</i>	Production Fractionation <i>Article writing</i>	
Task 1.2 production of bioamendement				
Task 2 antifungal activities in vitro				
Task 3 Genotoxicity	<i>Article writing</i>			
Task 4 mesocosm study	Online monitoring	Final analyses	<i>Article writing</i>	<i>Article writing</i>
Task 5 psychosociology geography	Master			
Task Meetings	April	June : Prep consortium meeting	September	November : prep consortium meeting
Consortium meeting			July	December
Meeting with winegrowers		Meeting students/ wine growers/ scientists		Meeting students/ wine growers/ scientists
Field Schools				

Task 4 : To test their activities using *in vitro* conditions and under mesocosm conditions (AM Farnet Da Silva, I. Laffont Schwob, Y. Capowiez, P. Prudent)

What is the protective effects of non-toxic compounds (probably a cocktail) against Botrytis, Oïdium and mildew: an **in vitro assay approach** (Schnee et al., 2013)

* **Germination rate** of spores of *Erysiphe necator* (oïdium) and of *Botrytis cinerea*

Control (+) : commercial fungicide Thiovit (wetable sulfur, Syngenta) at 4 mg/mL for oïdium, Switch (37.5% Cyprodinil and 25% Fludioxonil, Syngenta) for *Botrytis*

Aqueous/ethanol extracts

Cultures in microplates for *Botrytis* spores, microscopic observations for *Erysiphe* spores

* **Mobility** of zoospores of *Plasmopara viticola* (mildew)

Control (+) : commercial fungicide (Melody Combi, 9% iprovalicarb + 56% folpet, Bayer) used at 2 mg/mL.

* **Electron Microscopy to detect cytotoxicity effects**

Cells stained with uranyl acetate and lead citrate

Control (+) : as described above.

* **Elicitation experiments**

Task 4 : To test their activities using *in vitro* conditions and under mesocosm conditions (AM Farnet Da Silva, I. Laffont Schwob, Y. Capowiez, , P. Prudent)

Mesocosm study

Treatments (5 replicates) :

- (1) control no treatment
- (2) BCC (200 mg/l equivalent 6PP),
- (3) CuSO_4 as “bouillie bordelaise” (using conventional concentration),
- (4) Bioamendment
- (5) BCC (200 mg/l equivalent 6PP) + CuSO_4
- (6) Bioamendment+ BCC (200 mg/l equivalent 6PP)
- (7) Bioamendment + CuSO_4
- (8) Bioamendment + BCC (200 mg/l equivalent 6PP) + CuSO_4

*CuSO₄ addition is needed since this practice will
may be used for a while in complementation
with BCC by the winegrowers*

*With or without macro fauna (earthworm =
Aporrectodea caliginosa and mesofaune (= *Enchytraeus albidus*)
Summer drought stress
2 additions under favorable conditions
5kg/mesocosme*

160 mesocosmes = 5 replicates x 8 treatments x with/without meso/macrofauna

x with or without inoculation of both mildew /oidium

Task 4 : To test their activities using *in vitro* conditions and under mesocosm conditions (AM Farnet Da Silva, I. Laffont Schwob, Y. Capowiez, , P. Prudent)

Mesocosm study

Plant

markers followed throughout the incubation:

- traits (number of stems, twigs etc...)
- phytometabolites (Multiplex, Force A)
- phytopathogen growth (spots of fungal growth on leaves, Schnee et al, 2013) (or using SEM).

At the end of the experiment (1.5, 2 years?)

- Aerial and root plant biomass (winrhizo)
- Chemical analyses of plant organs (phytometabolites, NMR and Infra red spectroscopy, copper in roots and aerials organs by ICP-AES after acidic mineralisation + Mg^{2+} , K^+ , Ca^{2+})

Soil

markers followed throughout the incubation:

- respiration=> we could ask for an equipment used for field schools

At the end of the experiment (1.5, 2 years?)

- survival rate of meso and macrofauna (state of development)
- Physico-chemical (pH H_2O , pH KCl, C, N, cations by ICP-MS, density, water holding capacity, organic matter quality by NMR and Infra red spectroscopy) + Copper in soil (chemical speciation : pseudo-total fractions (by ICP-AES after aqua regia mineralisation), phytoavailable and water lixiviable or exchangeable fractions (BCR protocols).
- microbiological properties (microbial respiration and biomass by substrate-induced respiration, lignocellulolytic activities, proteases, ureases, phosphatases, catabolic diversity).

Task 5 : To help the winegrowers to accept the changes in their agricultural practices (R. Bertoldo, P. Minvielle, Association T&H, Ch. Agriculture PACA F. Groëll)

- Viticulture is heavily influenced by social elements, local history and social identities.
- Locally adopted practices are sometimes at odds with scientific expertise.
- Given the experimental character of the present project, we will use **qualitative methods (interviews & observations)** to understand how these participants respond to new biocontrol alternatives.
- This task will build on the fieldwork started by the PhD student and the post-doc researcher.
- **2 master thesis [2022]**
- Study 1 - Social identities and the adoption of new agricultural practices: how social identities support the uptake or resistance to new biocontrol methods
- Study 2 – “I know better than you”: How do farmers understand scientific knowledge in relation to their local know-how [are they capable of merging these knowledge or they resist them based on perceived legitimacy?]

Task 6 : Management of the interdisciplinarity of the project (AM Farnet Da Silva & I. Laffont Schwob)

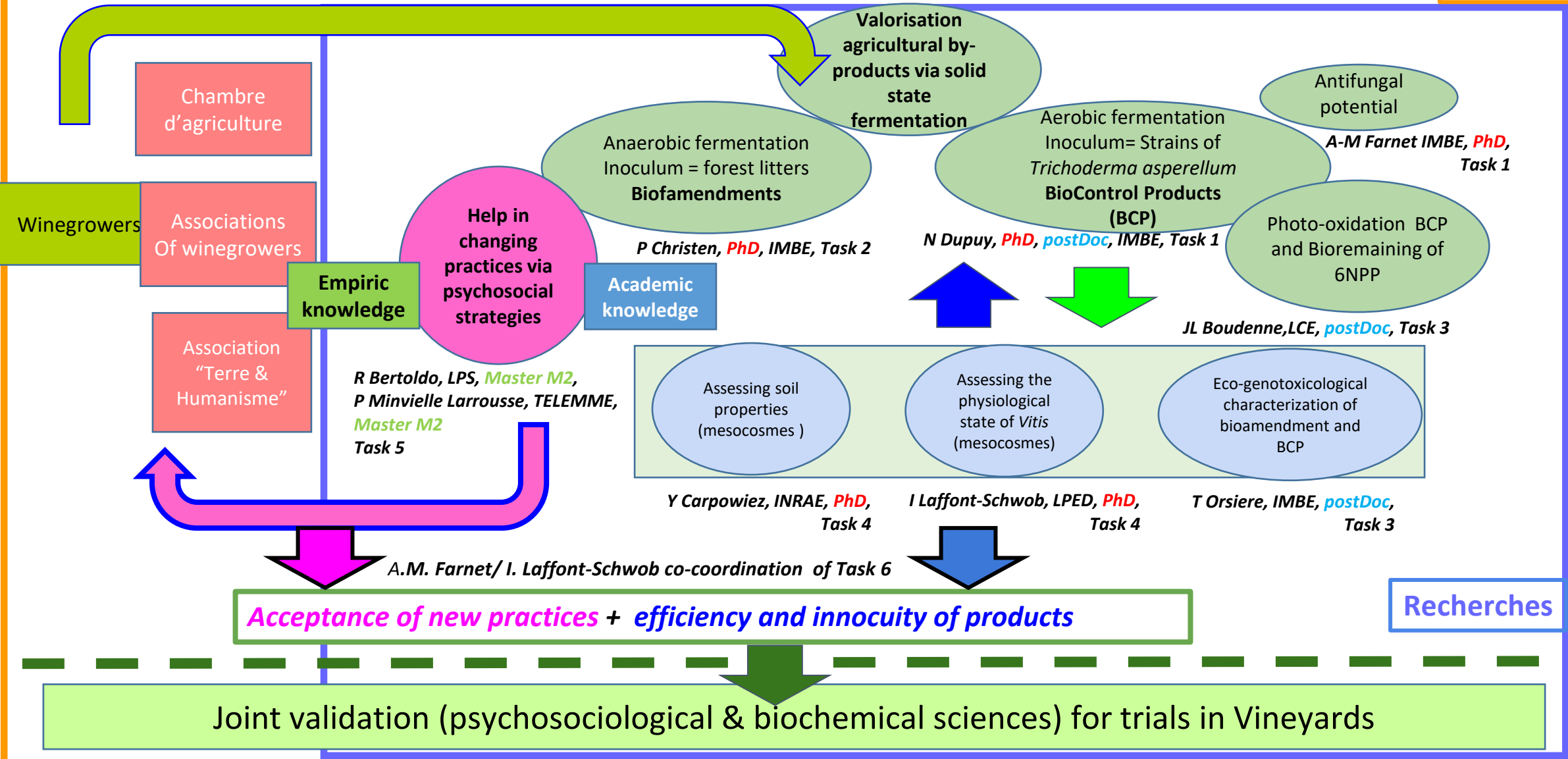
General organisation /Interactions between the different partners

between scientists, between field partners and researchers, students from Masters...

Shared documents in the cloud of OSU PYTHEAS, e-mailing list => each partner has to be pro-active !

Planning of meetings :

- **Task meetings** (each 3 months) => brief document (1 page, 2 pages max) as a report of each meeting, main results + issues
- **Consortium meetings** (each 6 months) => document presenting our results, matching/no matching with main milestones of the project.
- **Meetings with winegrowers**
- **Field school**



Green Deal

SARDOS project

More than 40 partners...thus from our side and for the moment only two partners
AMU and the Chambre Régionale d'Agriculture PACA.
Other partners (and funding!) in case of success via conventions (INRAE).

For ALCOVE : an opportunity to transfer our knowledge in the field....!