SESSION 7

BIOCONTROL

K5 Challenging plants to find effective biocontrol agents: investigating late blight induced shifts in the potato microbiota
Mout De Vrieze (University of Fribourg, Switzerland)

O28 Combining antagonistic bacteria with copper to control late blight disease in potato plants
Fanny Germanier (University of Fribourg, Switzerland)

O30 New Challenges for Biological Active Substances. *T. asperellum*, T34 an EU authorized plant protection product soon against *Rhizoctonia* for potato in Central zone
Isabelle Trillas-Gay (Universitat de Barcelona, Spain)

O31 Determination of antagonist activity origin of Pseudomonas PA14H7 against Pectobacteriaceae
Euphrasie Lépinay (inov3PT, France)

O32 Laboratory and field evaluation of bioinsecticides for Colorado potato beetle control
Primož Žigon (Agricultural Institute, Slovenia)

P29 Microbial diversity associated with potato tuber blemishes
Karima Bouchek-Mechiche (inov3PT/INRAE-IGEPP, France)

P30 Antifungal evaluation of plant extracts as alternative fungicides for the management of early and late blight in potato crops
Armand Grillon (Agroscope, Switzerland)

P34 Antifungal activity and post-harvest control of blemish diseases by plant extracts
Josep Massana-Codina (Agroscope, Switzerland)

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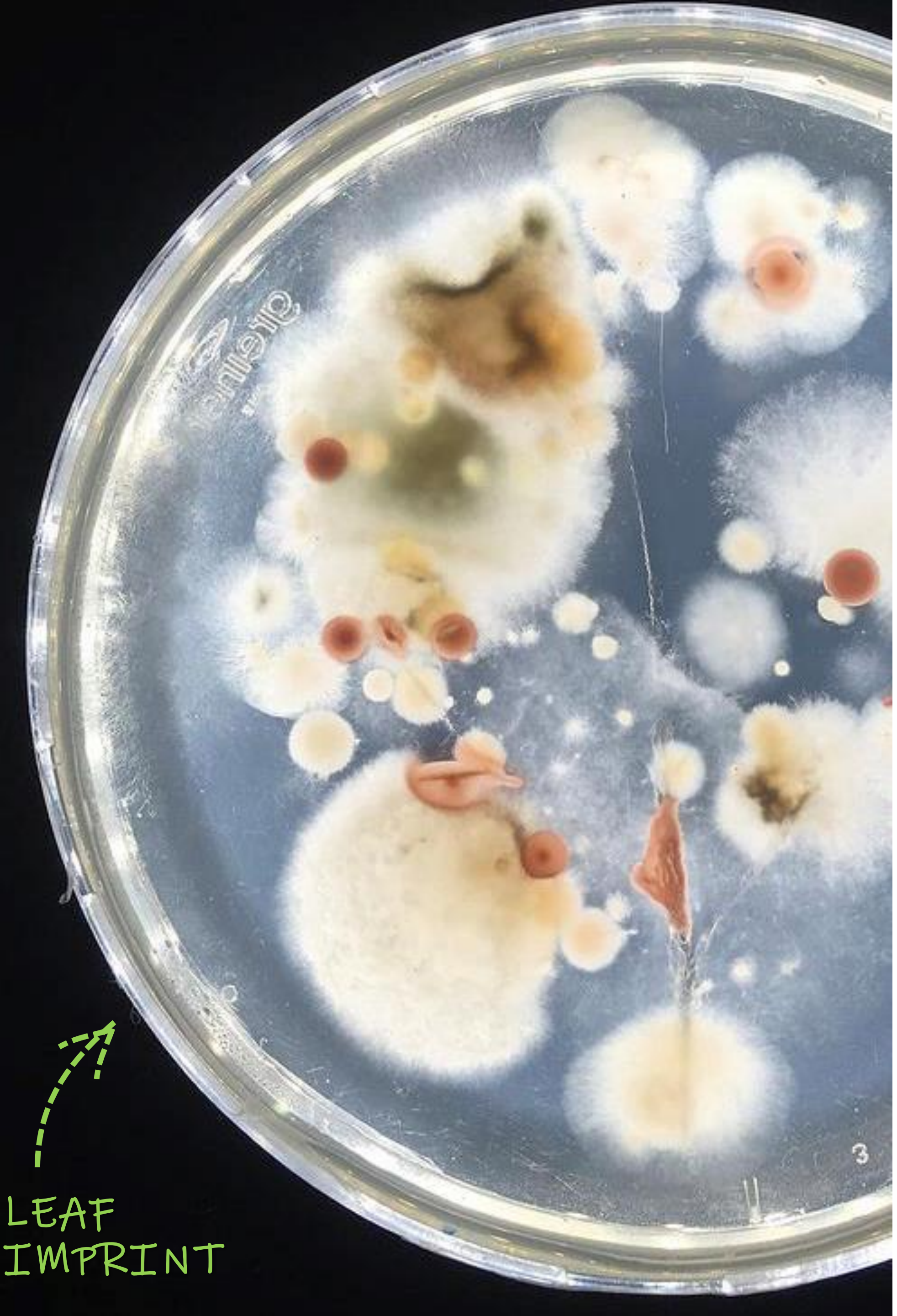
FR

Challenging plants to find effective biocontrol agents

Investigating late blight induced shifts in the potato microbiota

Mout De Vrieze
University of Fribourg



THE PLANT MICROBIOTA

The plant microbiota encompasses all the microorganisms that colonize plants as epi- or endophytes

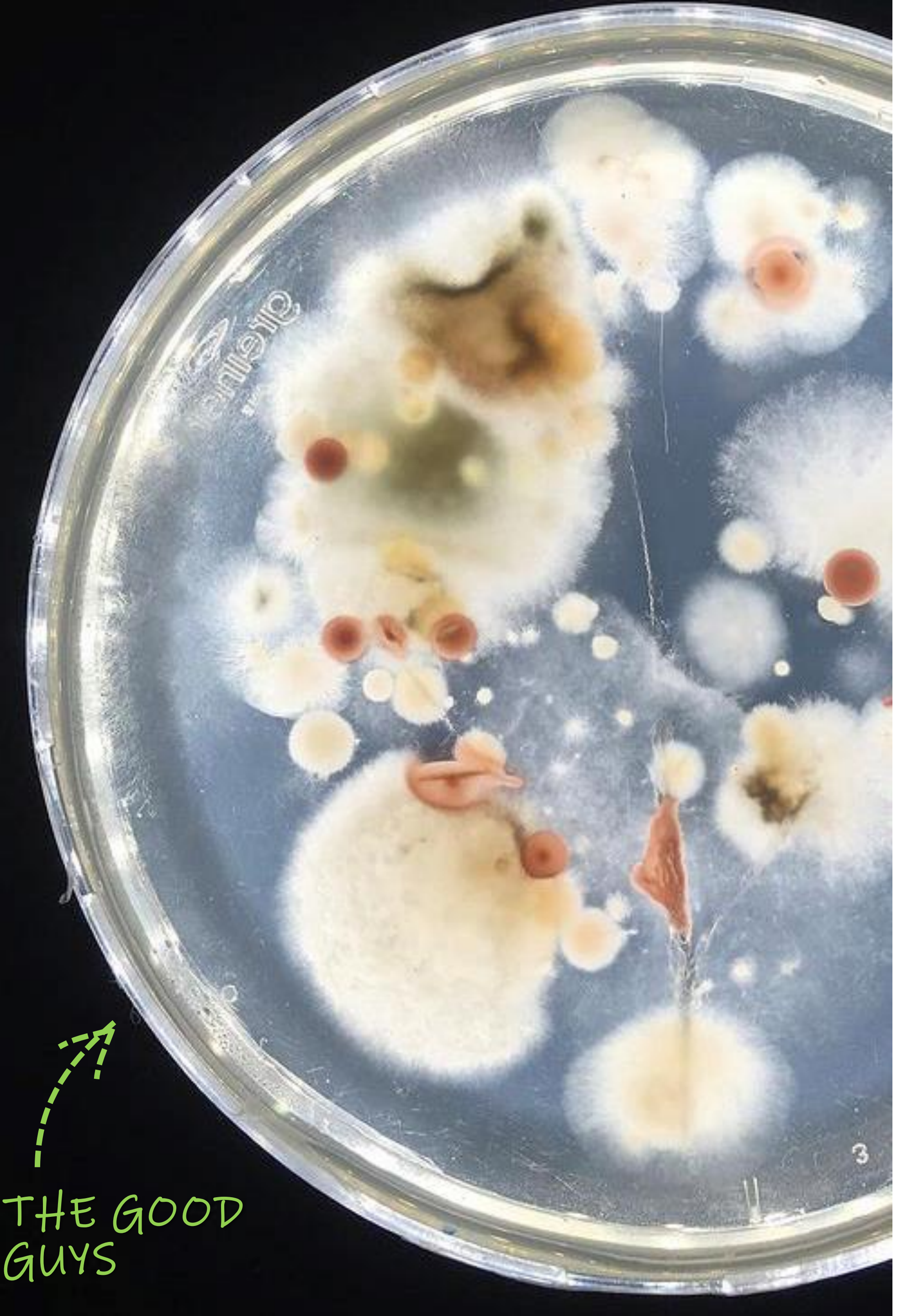
✓All plant compartments have their own microbiota

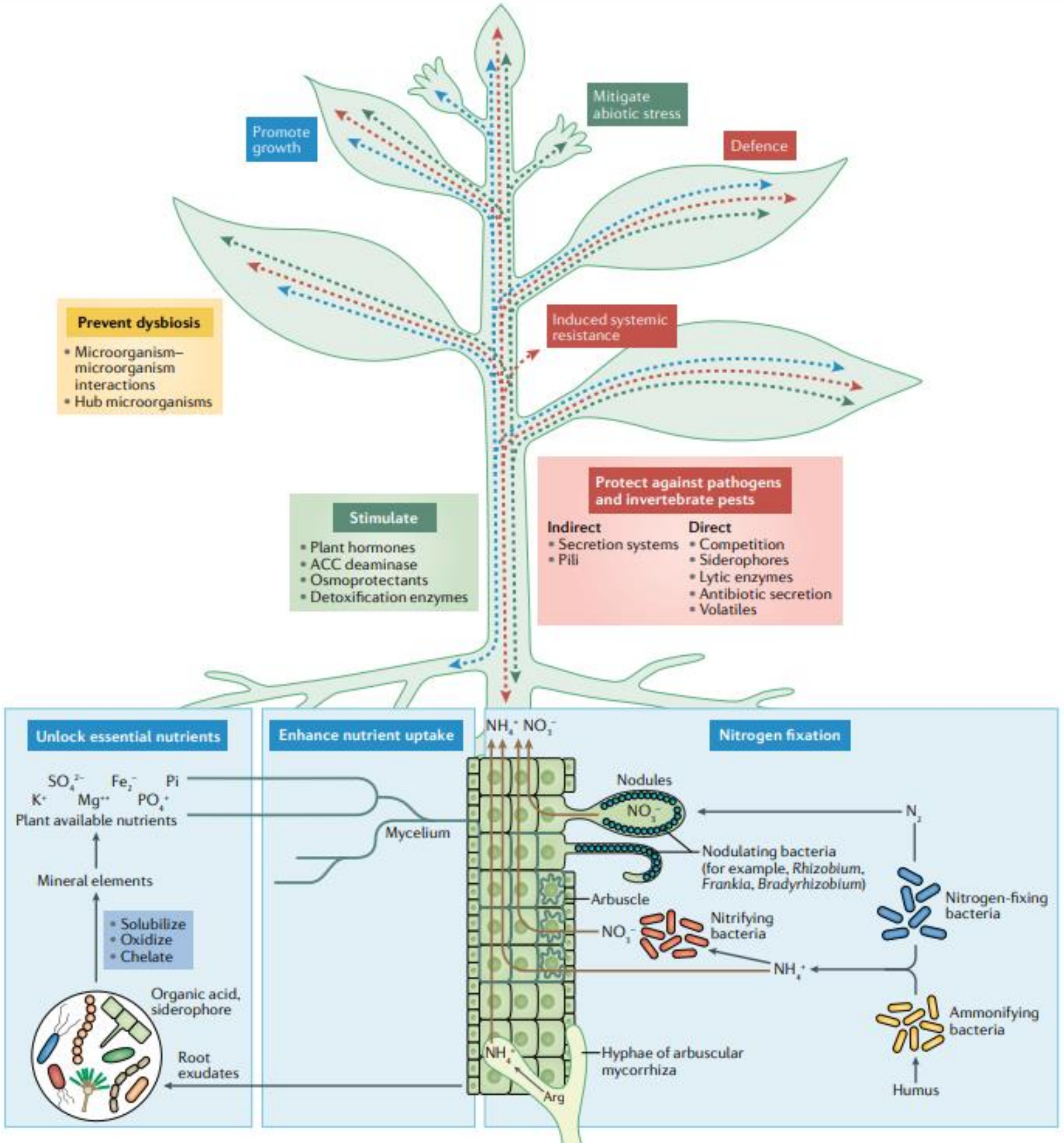
✓Dynamic environment

✓Fitness advantages

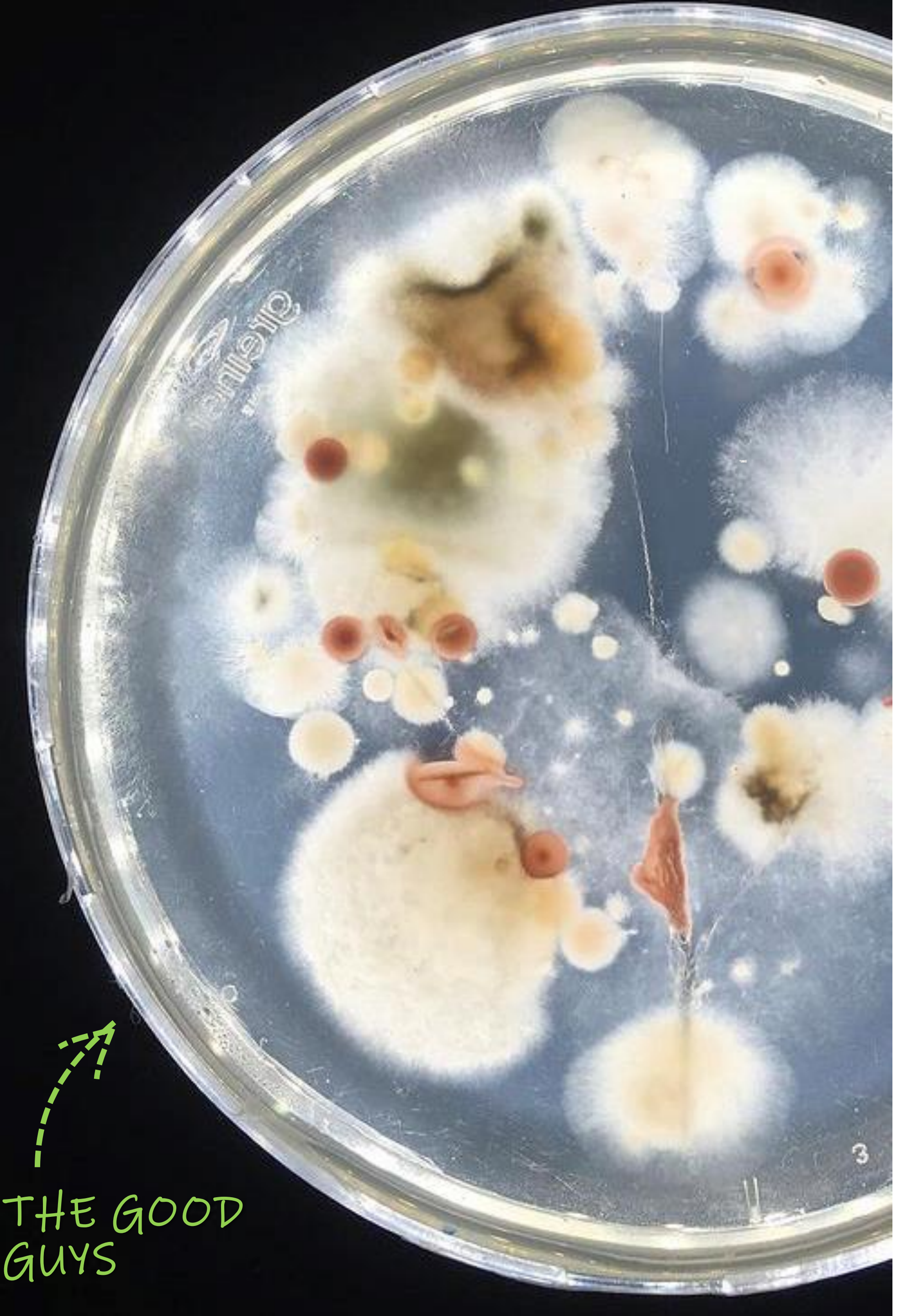
Trivedi et al., 2020

THE PLANT MICROBIOTA



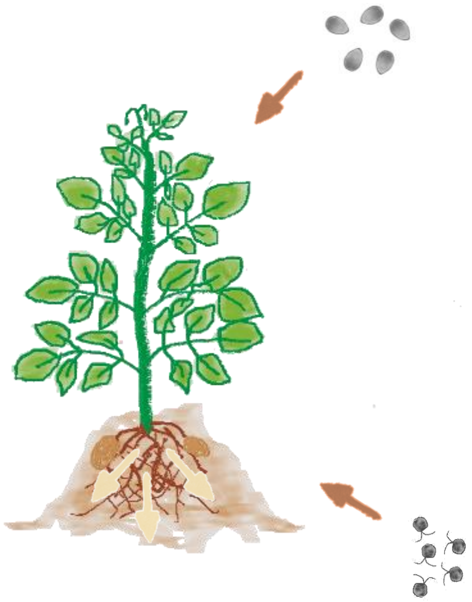


Trivedi et al., 2020



SELECTION OF MICROORGANISMS **BY THE PLANT**

The cry for help hypothesis



Attack by pest or pathogen

↓

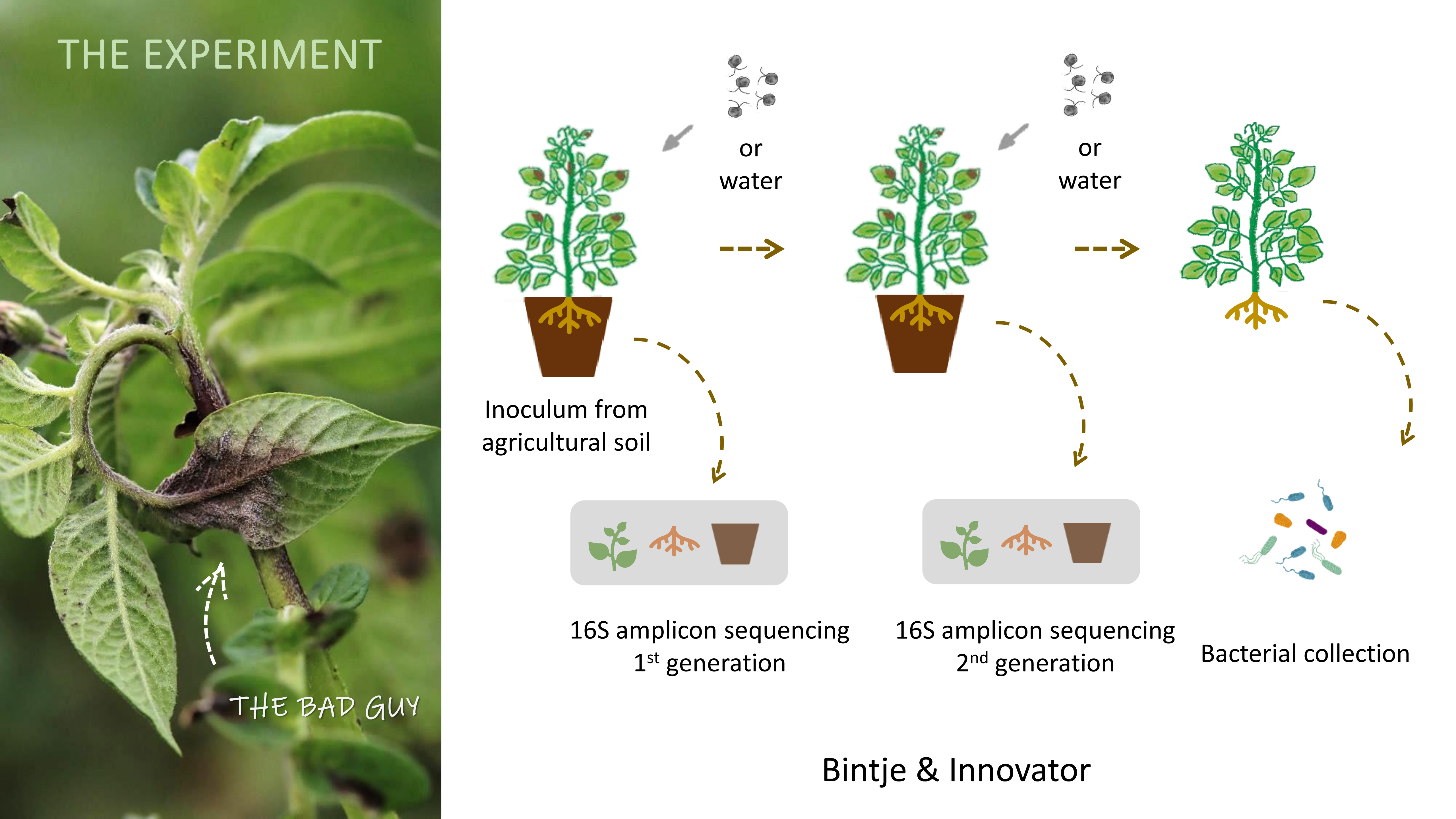
Changes in root exudation profiles

↓

Selection/recruitment of beneficial microorganisms

Adapted from Rolfe et al., 2019

Can potato plants recruit beneficial microorganisms upon pathogen infection?


THE EXPERIMENT
THE BAD GUY
or
water
or
water
Inoculum from agricultural soil
16S amplicon sequencing
1st generation
16S amplicon sequencing
2nd generation
Bacterial collection
Bintje & Innovator

The microbiota analysis approach

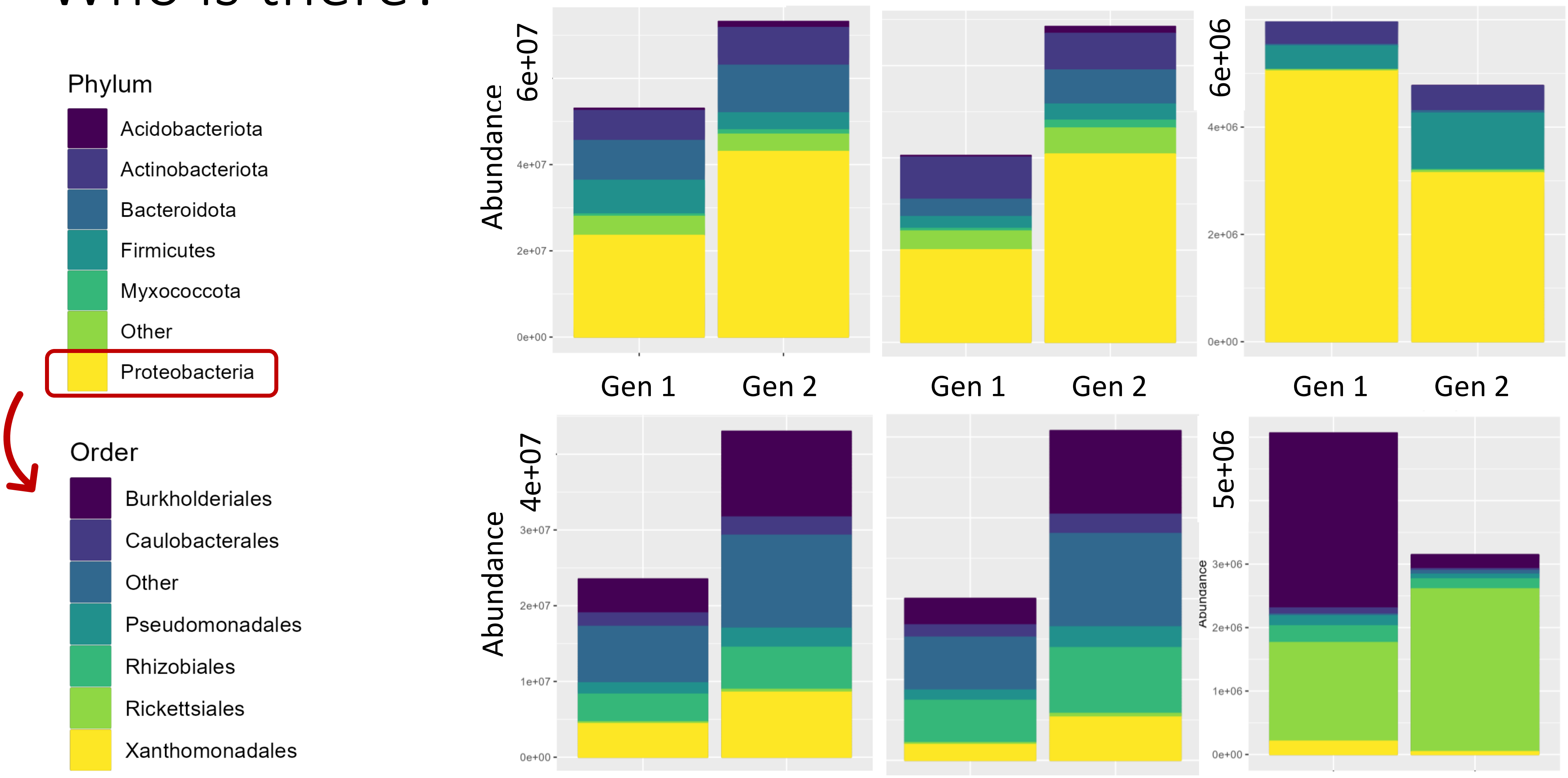
Microbiota sample collection and analysis

---> DNA extraction ---> Illumina Miseq 16S amplicon sequencing ---> ASV table

Amplicon Sequence Variant (ASV)

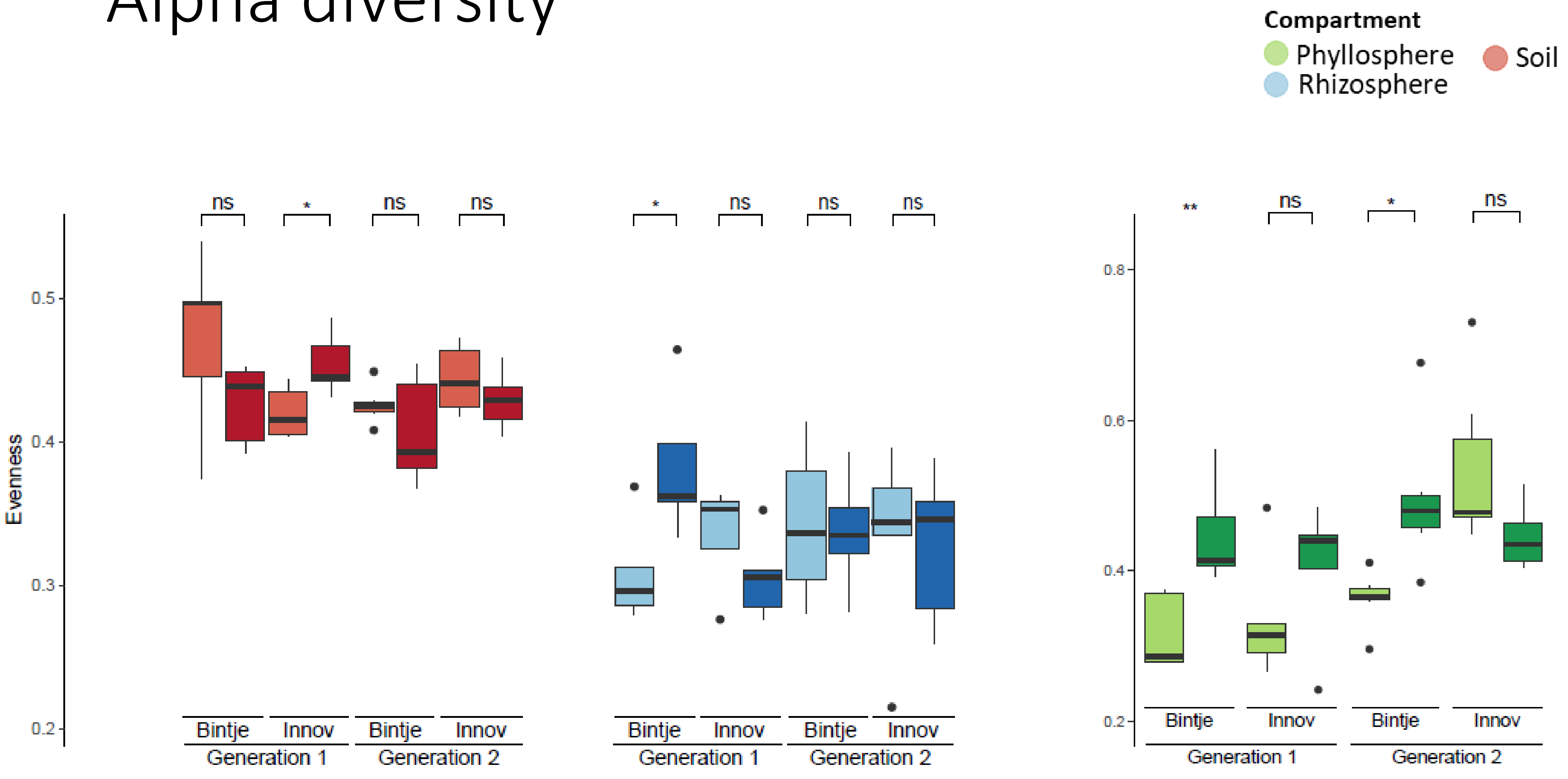
The ASV approach identifies **single, exact sequences that are statistically supported as being** present in the sample

Who is there?

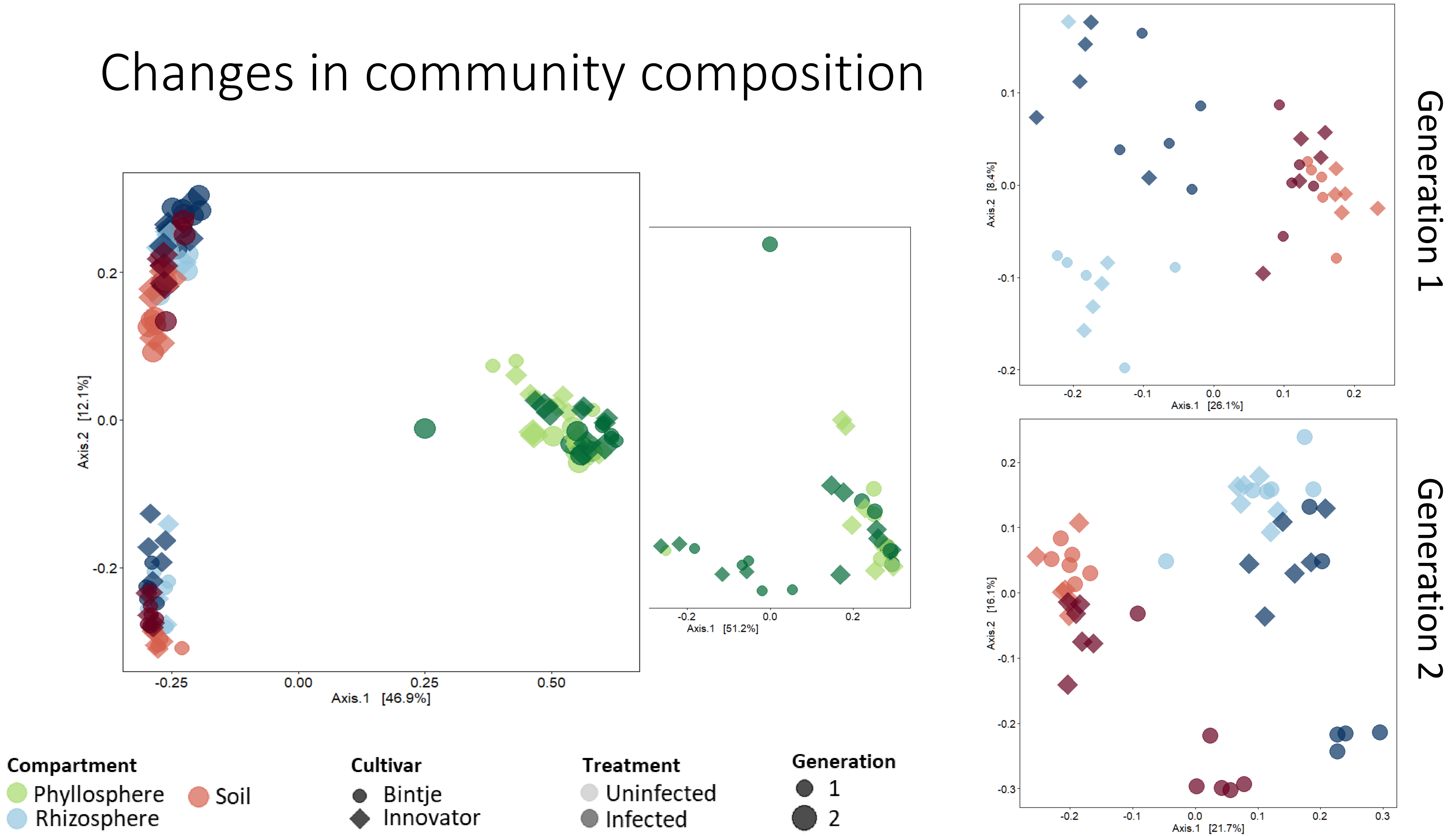


Phylum
Acidobacteriota
Actinobacteriota
Bacteroidota
Firmicutes
Myxococcota
Other
Proteobacteria
Order
Burkholderiales
Caulobacterales
Other
Pseudomonadales
Rhizobiales
Rickettsiales
Xanthomonadales
RHIZOSPHERE
SOIL
PHYLLOSPHERE
Abundance
6e+07
4e+07
2e+07
0e+00
4e+06
2e+06
6e+06
Gen 1
Gen 2
Gen 1
Gen 2
Gen 1
Gen 2
Abundance
4e+07
3e+07
2e+07
1e+07
0e+00
5e+06
3e+06
2e+06
1e+06
0e+00

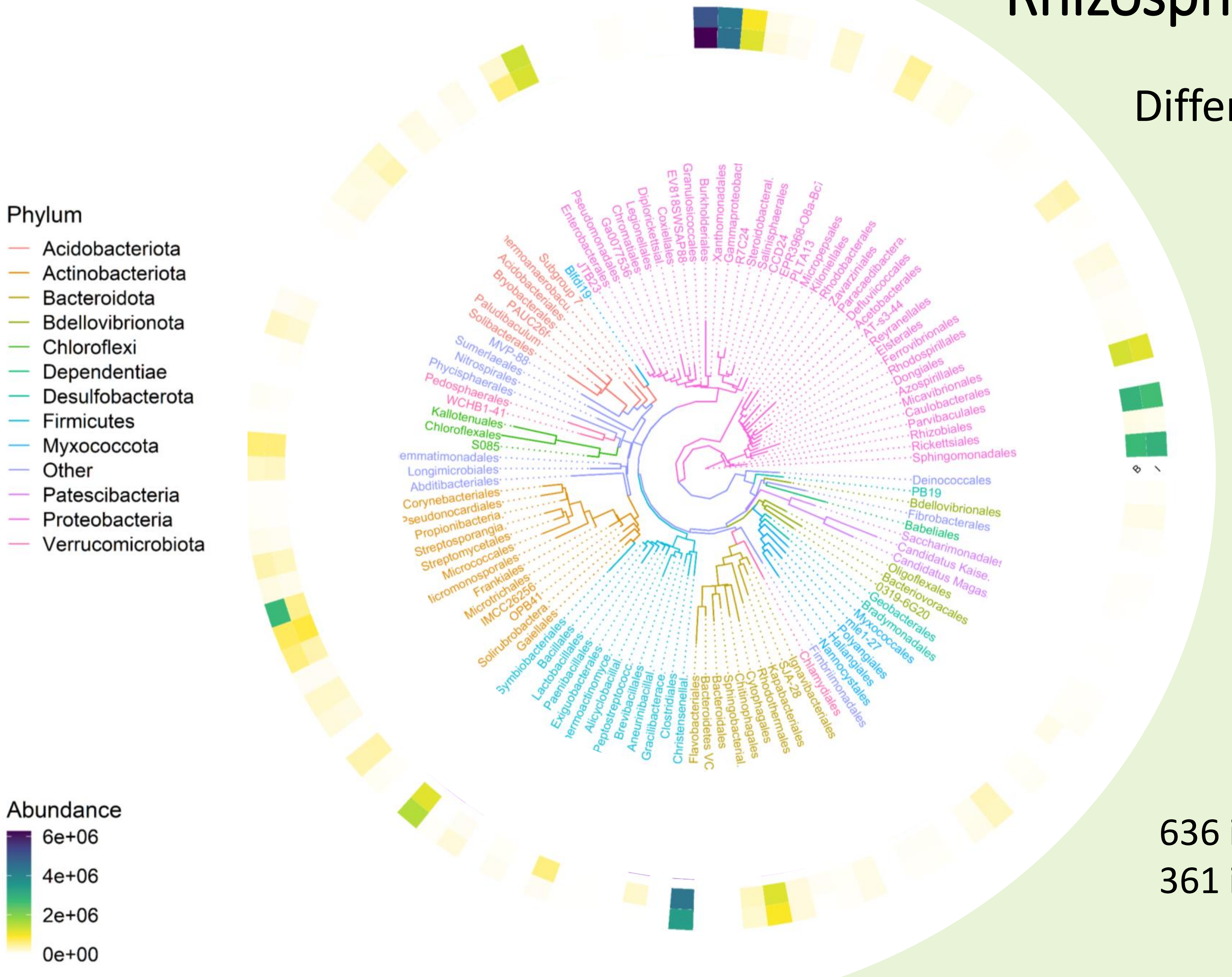
Alpha diversity



Changes in community composition



Rhizosphere 2nd generation



Differentially abundant ASVs

894 out of 5939 ASVs

15 % ASVs

24 % read counts

560 ↗ upon infection

343 ↘

636 in Bintje

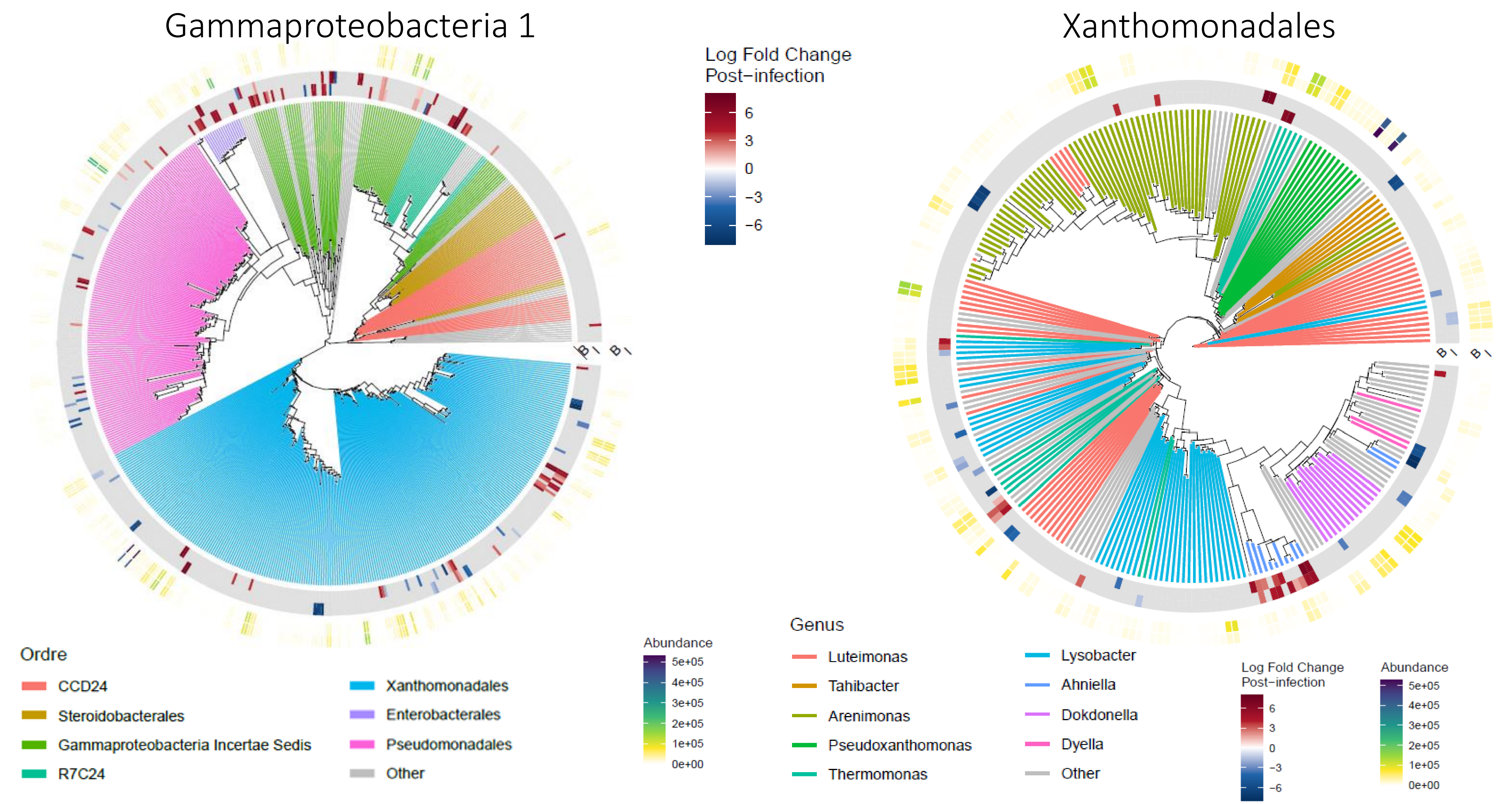
361 in Innovator

103 in common

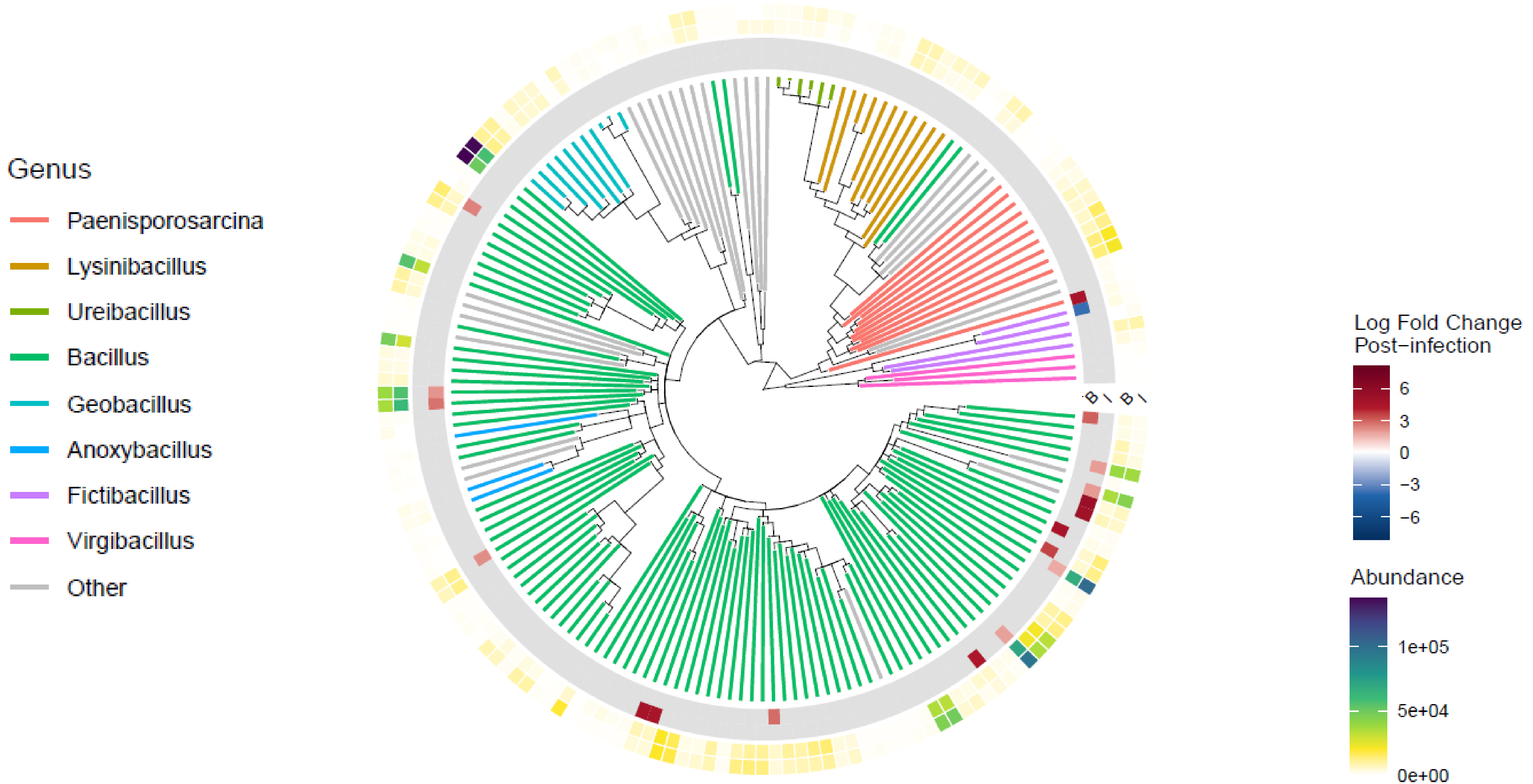
68 ↗

16 ↘

9 ↔


Gammaproteobacteria 1
Xanthomonadales
Log Fold Change
Post−infection
6
3
0
−3
−6
B
B
Ordre
CCD24
Steroidobacterales
Gammaproteobacteria Incertae Sedis
R7C24
Xanthomonadales
Enterobacterales
Pseudomonadales
Other
Abundance
5e+05
4e+05
3e+05
2e+05
1e+05
0e+00
Genus
Luteimonas
Tahibacter
Arenimonas
Pseudoxanthomonas
Thermomonas
Lysobacter
Ahniella
Dokdonella
Dyella
Other
Log Fold Change
Post−infection
6
3
0
−3
−6
Abundance
5e+05
4e+05
3e+05
2e+05
1e+05
0e+00

Bacillales



Genus
Paenisporosarcina
Lysinibacillus
Ureibacillus
Bacillus
Geobacillus
Anoxybacillus
Fictibacillus
Virgibacillus
Other
B
I
B
I
Log Fold Change Post−infection
6
3
0
−3
−6
Abundance
1e+05
5e+04
0e+00

The microbiota analysis approach

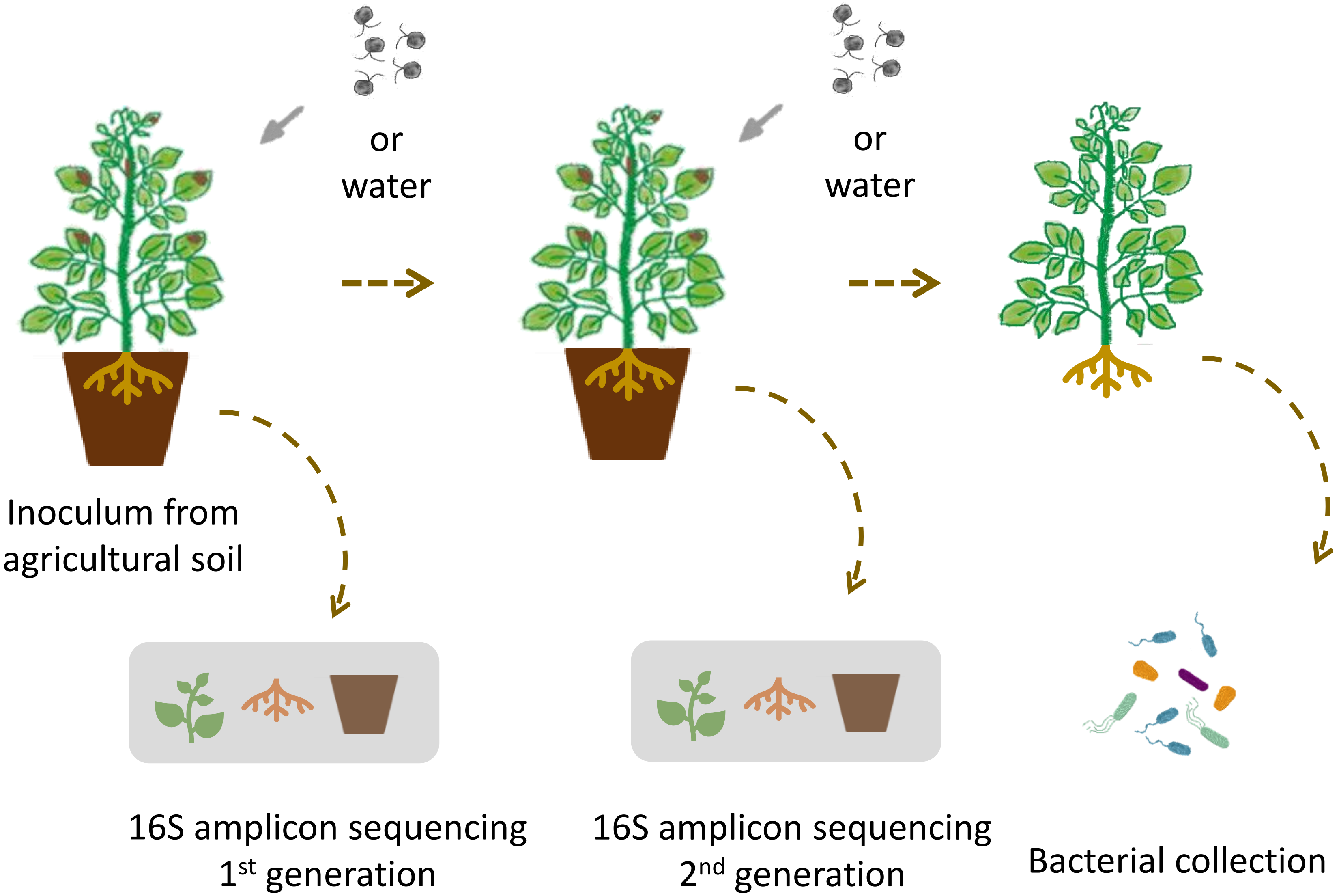
- Richness and evenness are overall hardly affected by an infection with *P. infestans*
- The community composition of the phyllosphere, rhizosphere and soil are affected by an infection
- Bintje shows a stronger reaction

Can potato plants recruit beneficial microorganisms upon pathogen infection?

- Differentially abundant ASVs found in and scattered across all phyla
- In most cases, effect is ASV specific or specific to small groups

The combined approach

THE EXPERIMENT

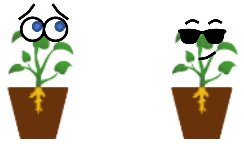


Bintje & Innovator

Differentially abundant strains

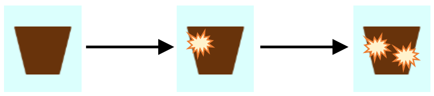
Infected vs non-infected plants

Cultivars



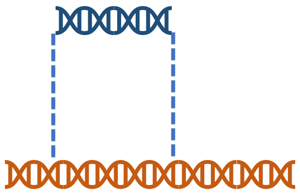
Compartiments

Generations

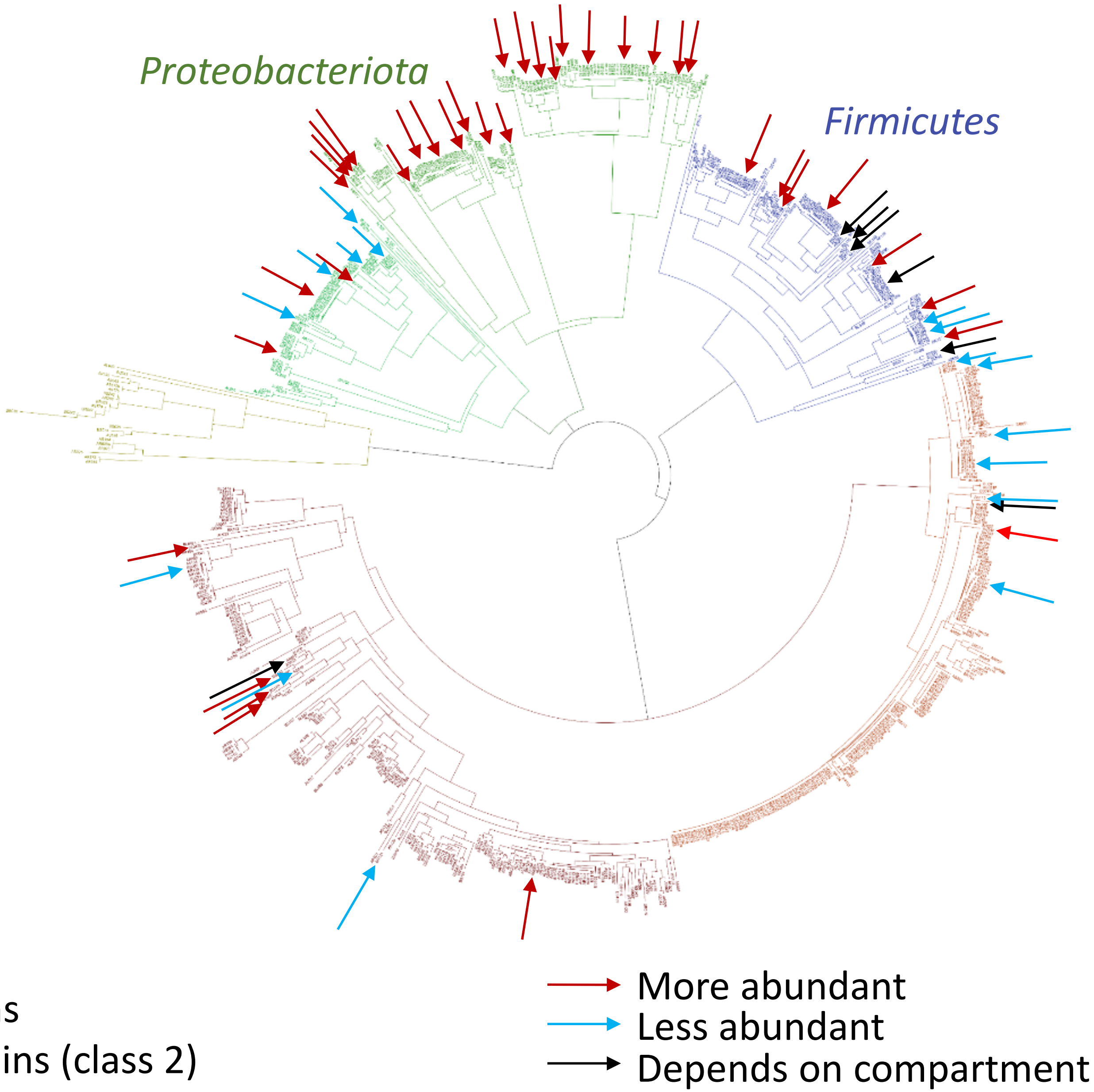


4731 differentially abundant strains

636 isolated strains

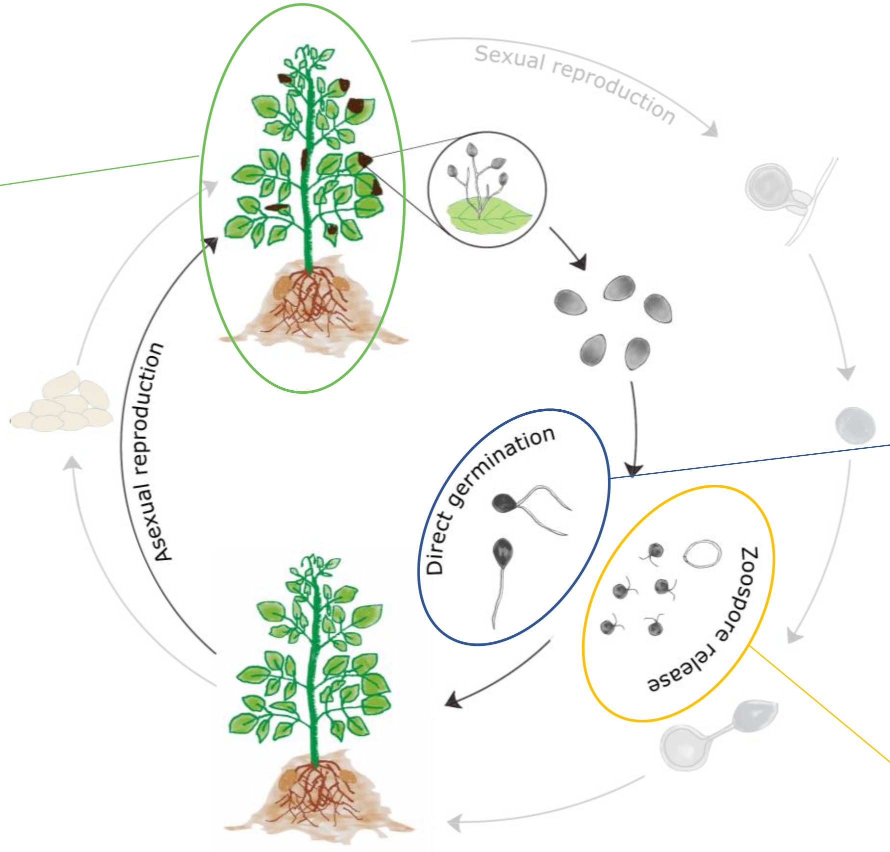


170 strains
- 31 strains (class 2)



Testing the strains of interest

Effect on symptom development on **plants**

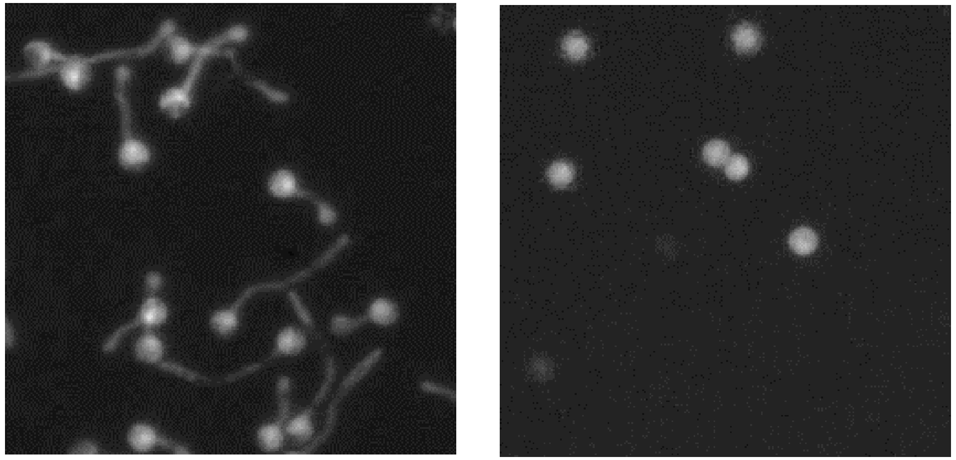


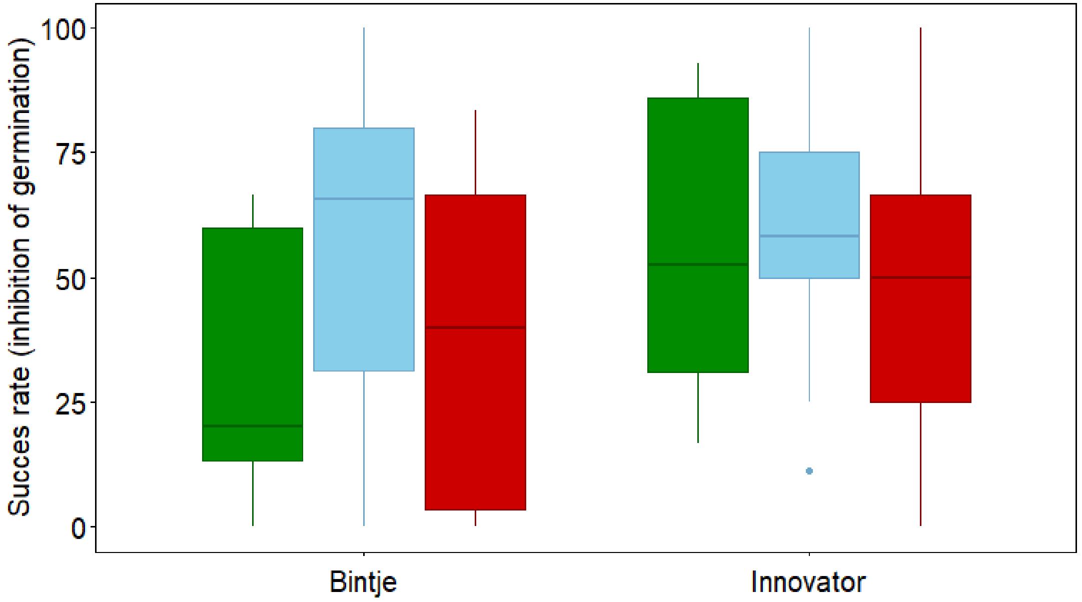
Effect on **sporangia** germination and germ tube development

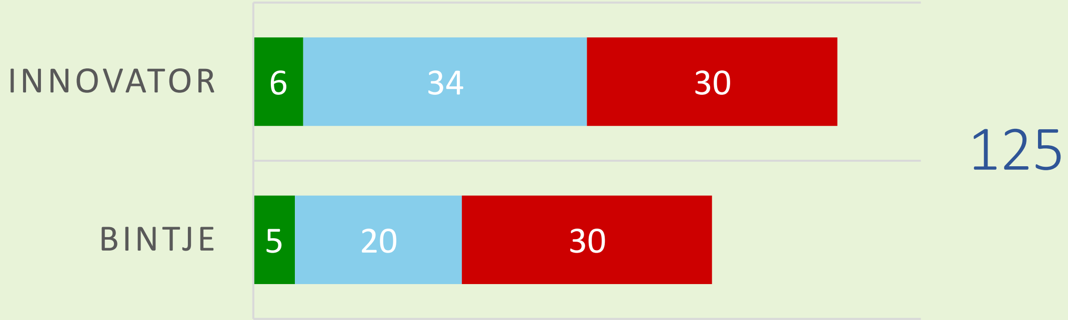
Effect on **zoospore** release, and germination

Testing the strains of interest

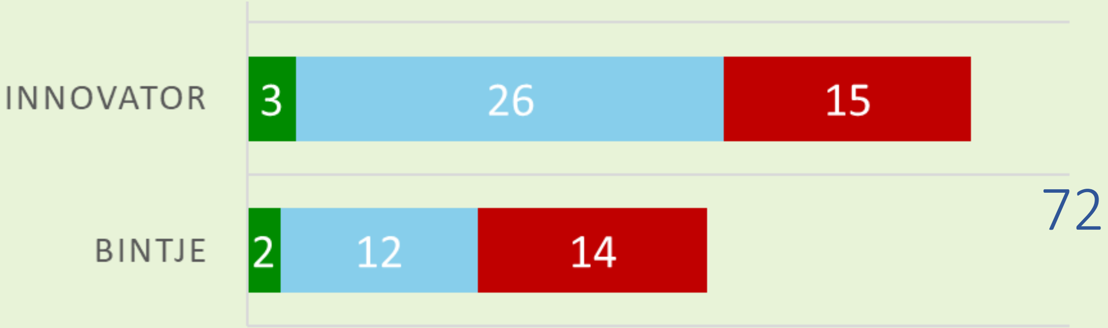
Zoospore germination



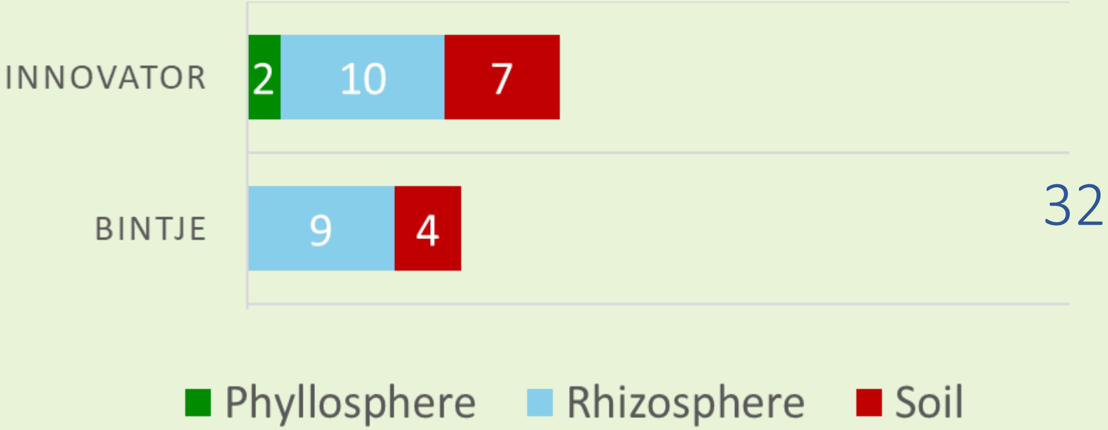




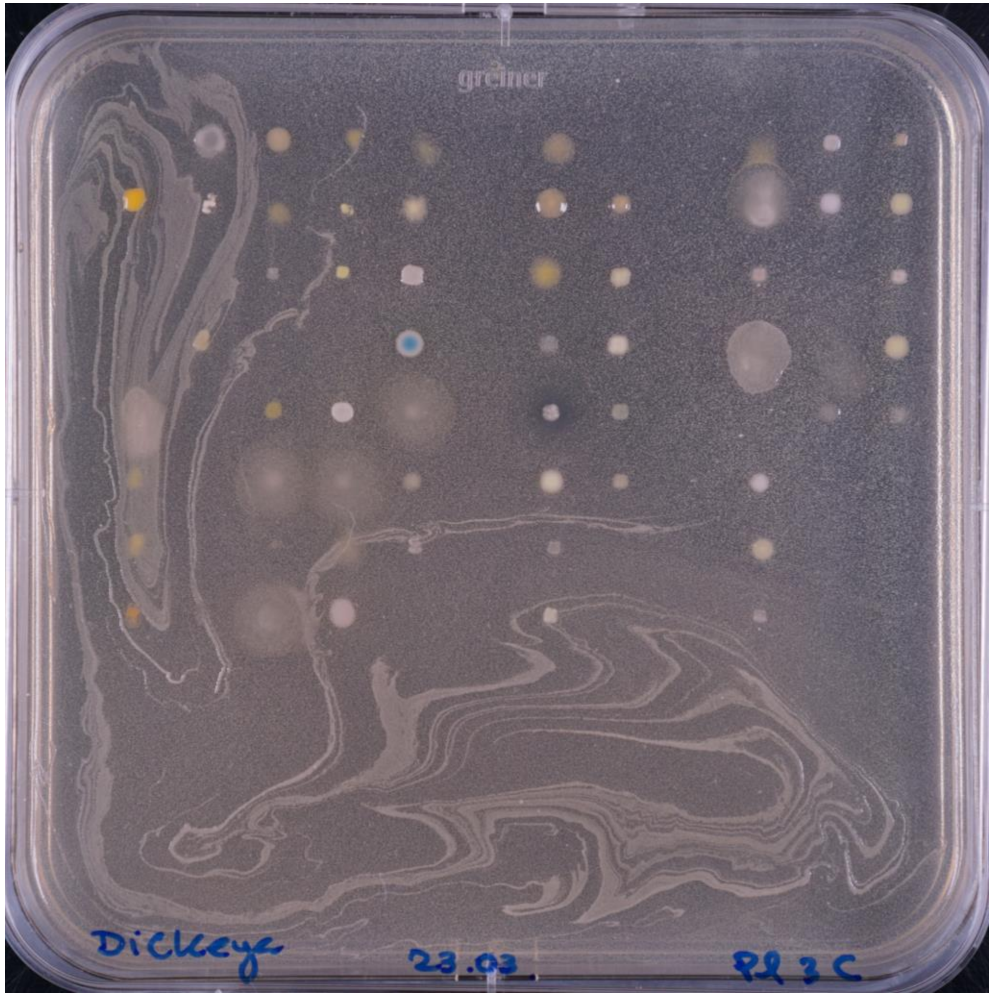
68 % of the strains have a succes rate > 50 %

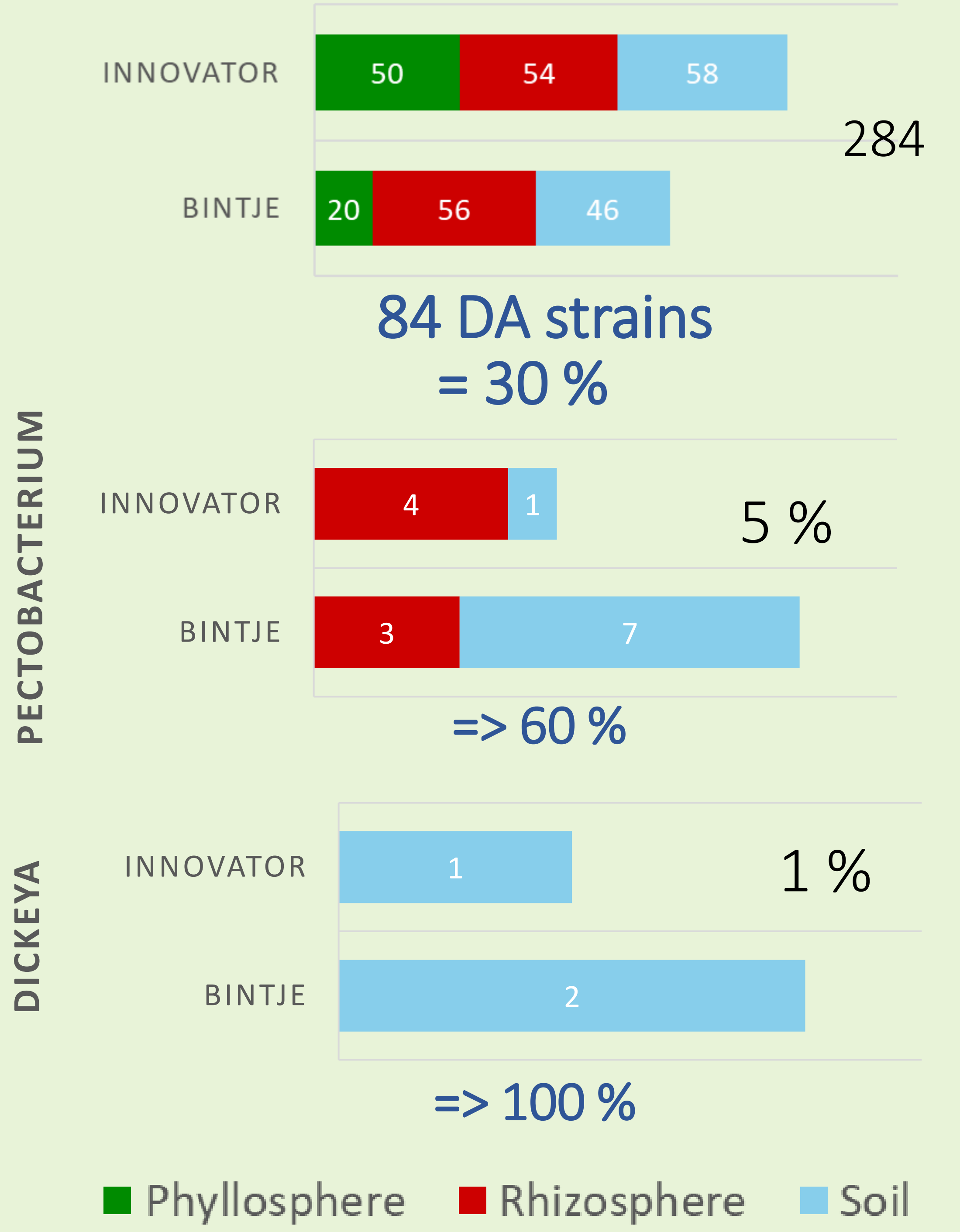


25 % of the strains have a succes rate > 75 %



What about other pathogens?





The combined approach

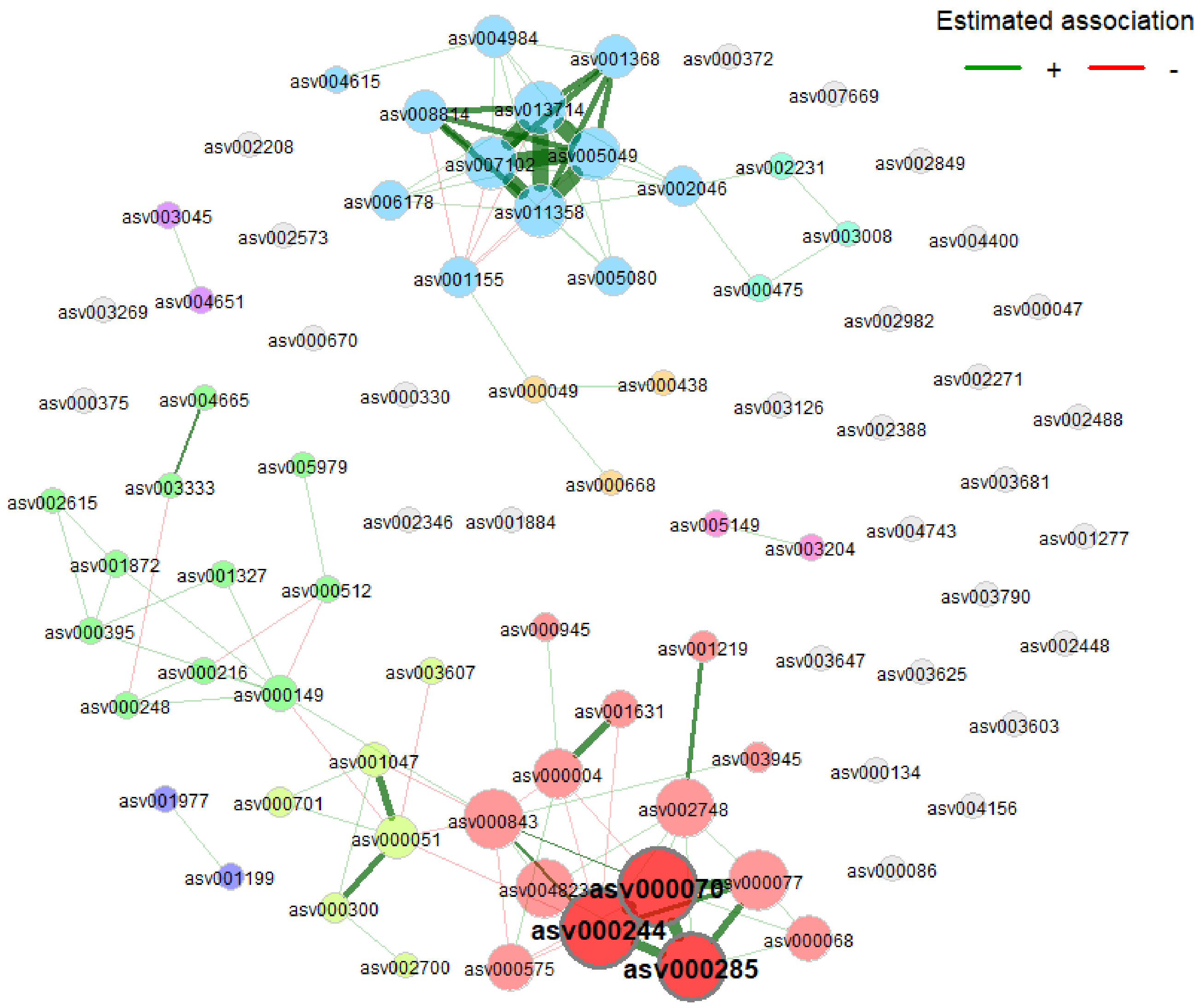
- Enriched strains can be found in all compartments and in both cultivars

Can potato plants recruit beneficial microorganisms upon pathogen infection?

- Antagonistic strains are found among enriched ASVs
- Activity is not necessarily pathogen-specific

The network analysis approach

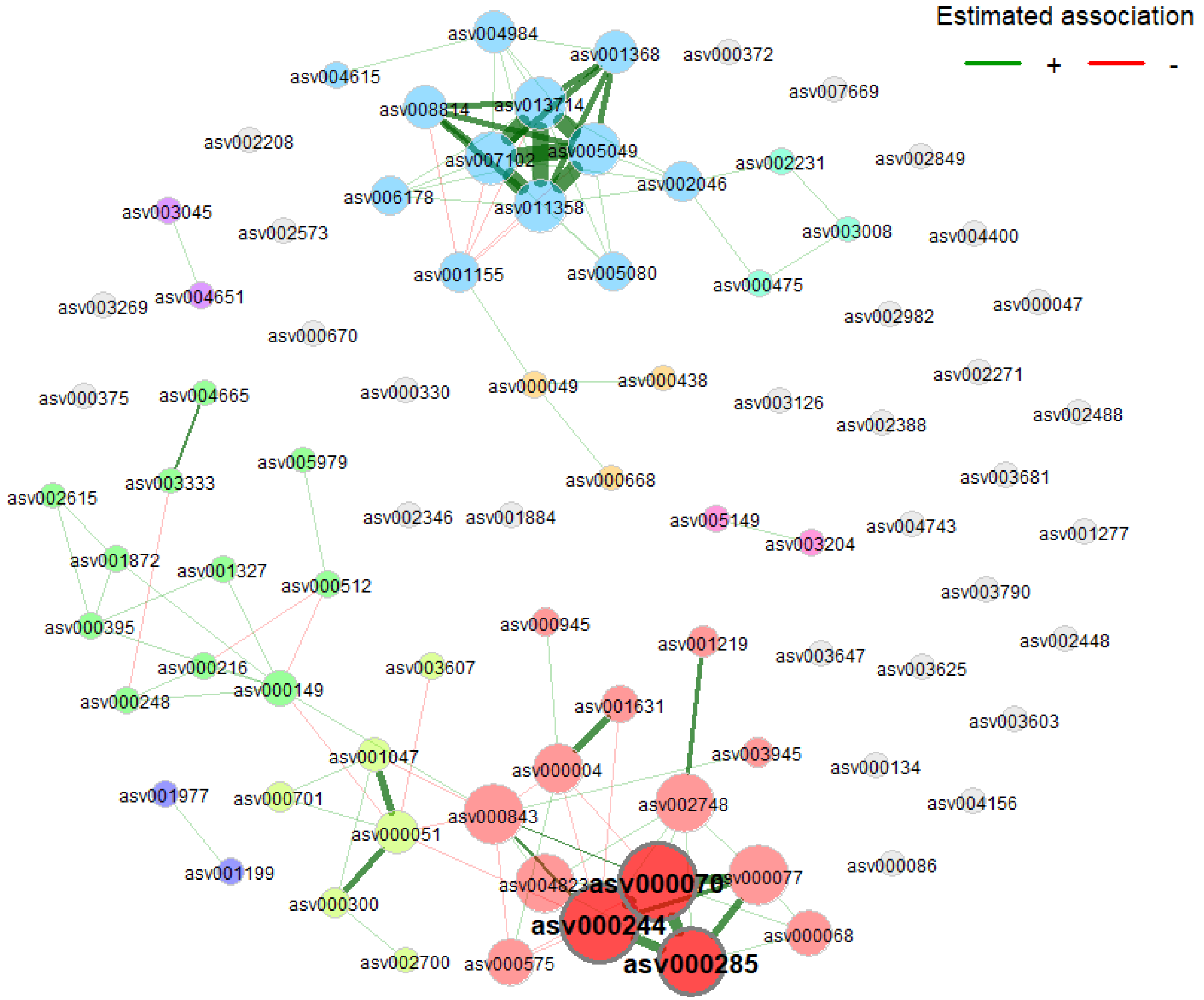
Co-occurrence networks

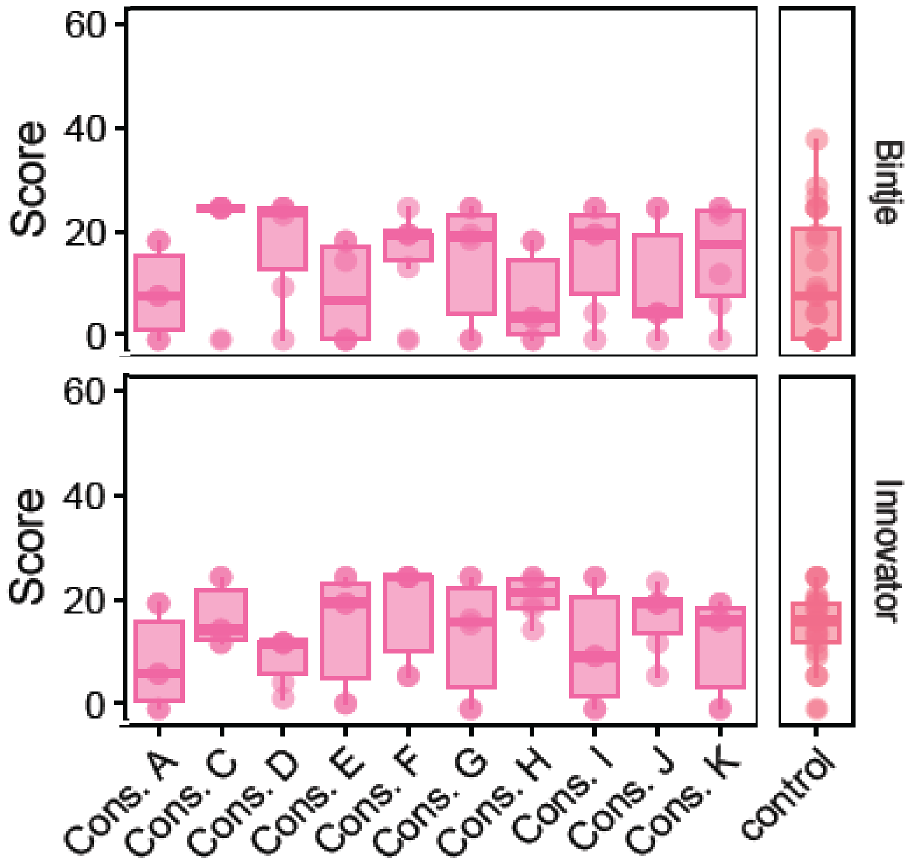


Different clusters detected:

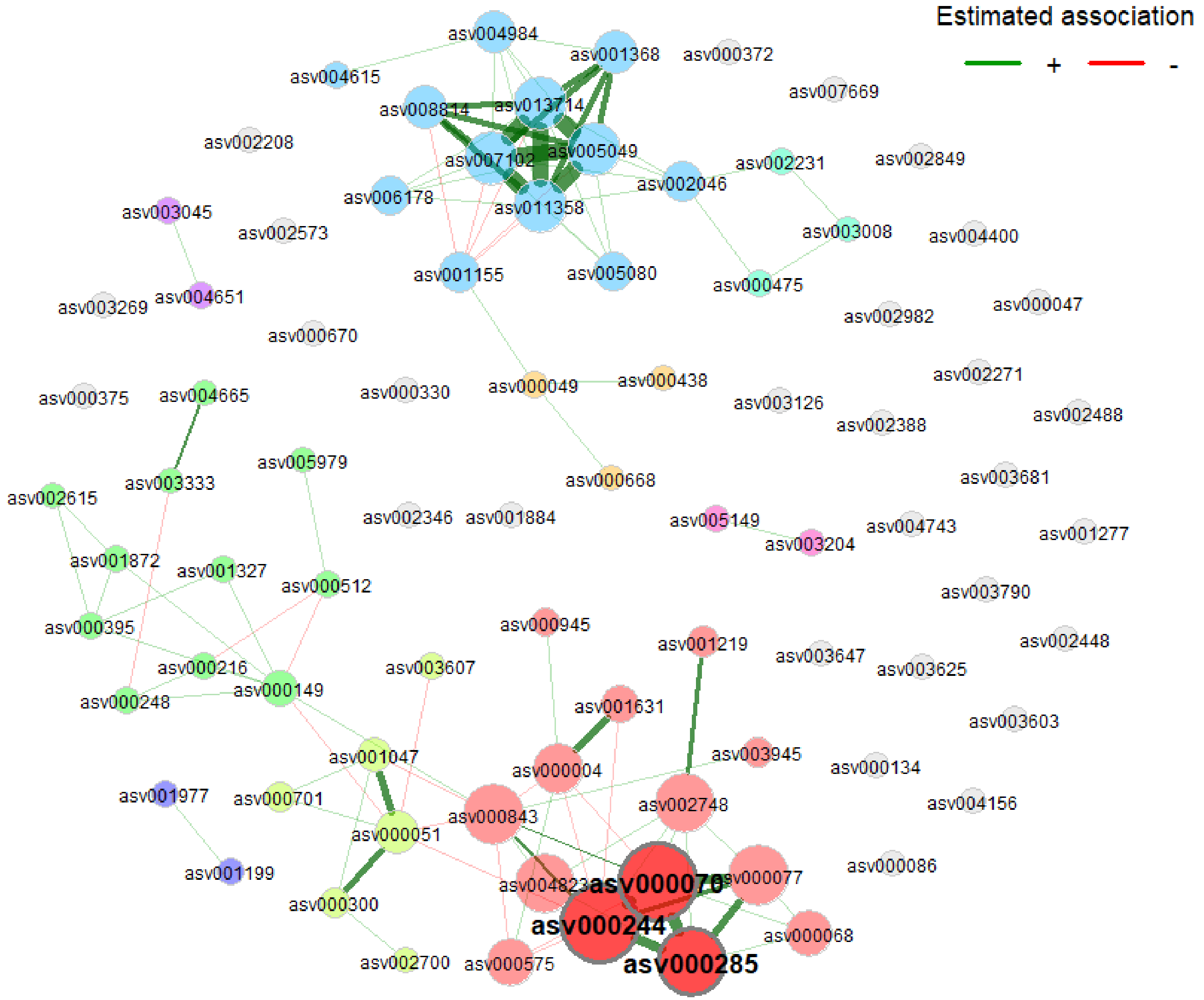
- Mixed cluster
  - *Bacillus*
  - *Microbacterium*
  - *Mesorhizobium,*
  - *Achromobacter*
- *Bacillus* cluster
- *Burkholderia* cluster
  - *Variovorax*
  - *Acidovorax*
- *Pseudomonas* cluster

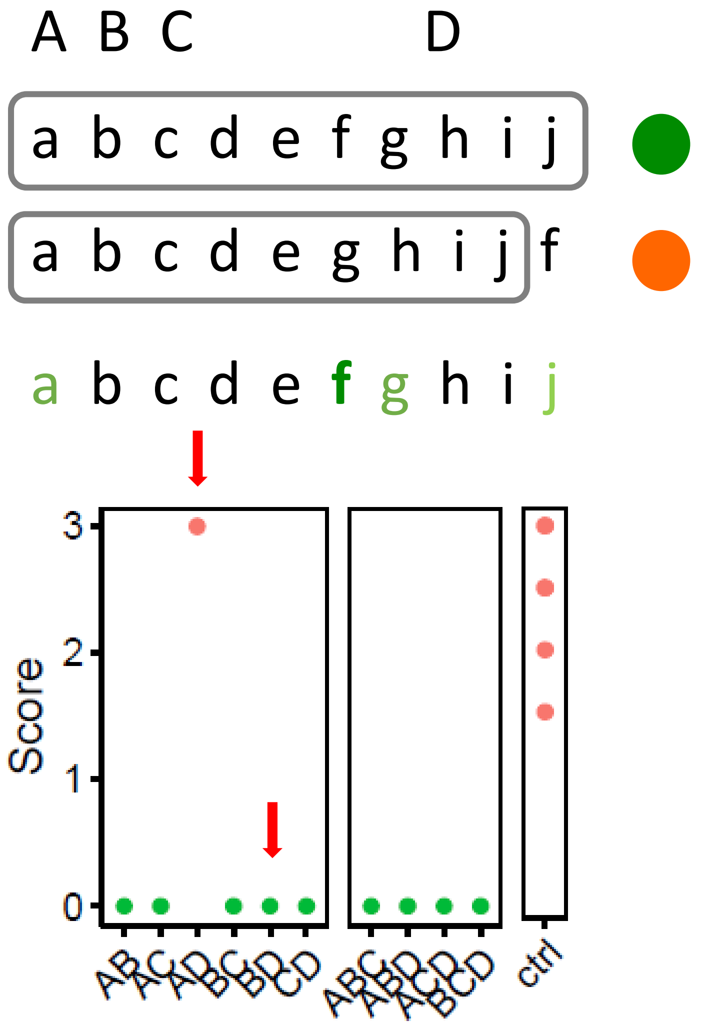
Can we make SynComs?





Can the strains help each other out?





Advantage for survival?

The network analysis approach

- Several clusters were detected involving single genera or a mixtures of genera

Can potato plants recruit beneficial microorganisms upon pathogen infection?

- Mixtures or strains are not necessarily better than single strains under controlled conditions
- Need to further investigate whether combinations of co-occurring strains offer an advantage in terms of survival and activity

Can microbiota studies help identify potential biocontrol strains?

Yes, but a better resolution is necessary

Thank you!

University of Fribourg
Prof. Laure Weisskopf
Prof. aurent Falquet

Eva Trutmann
Fanny Germanier
Carola Velti

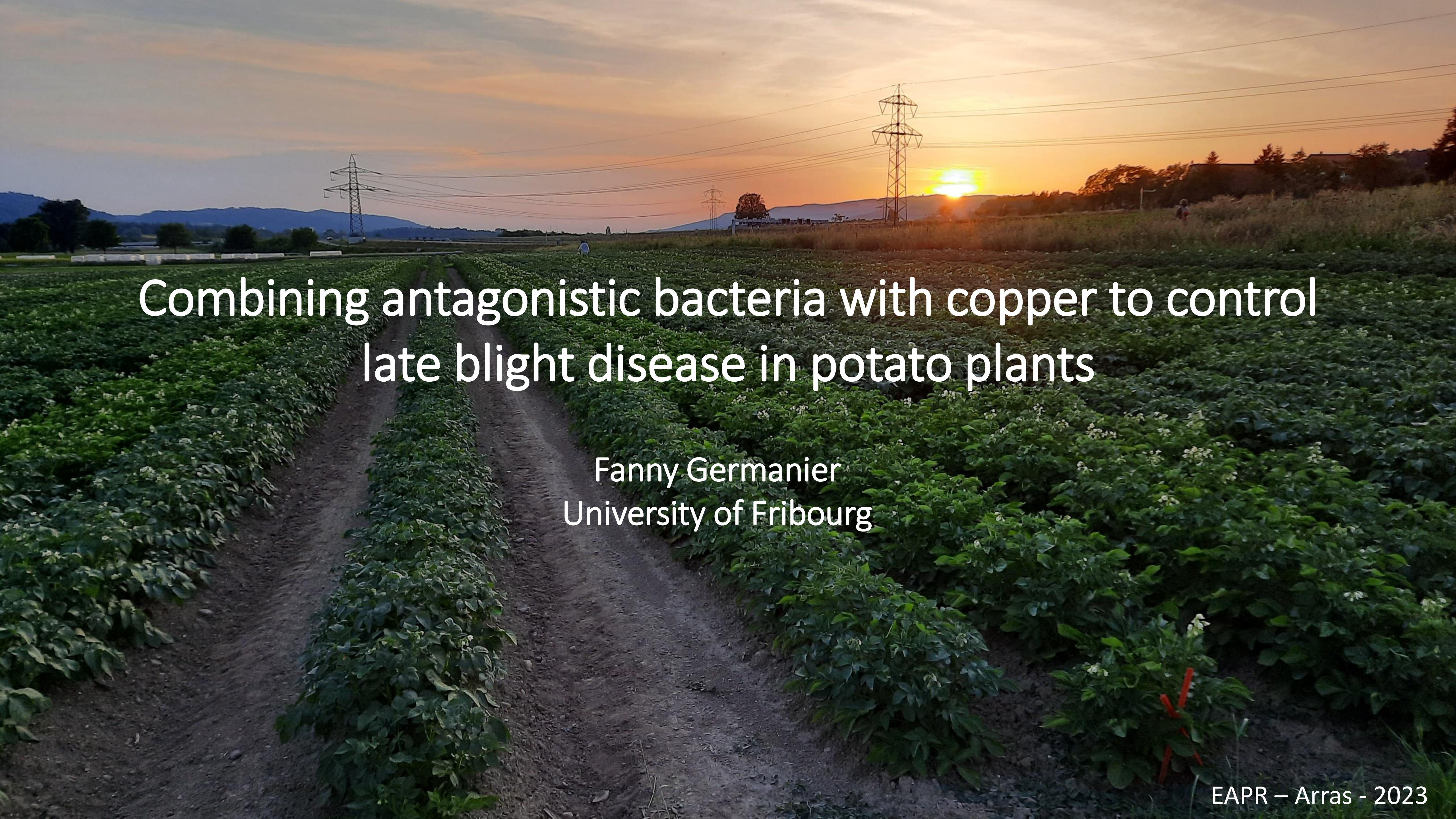
Floriane L'Haridon
Rares Cristea
Camila Morales
Aurélie Esseiva

FiBL
Natacha Bodenhausen

Agroscope
Brice Dupuis
Maud Tallant

WISSENSCHAFT. BEWEGEN
GEBERT RÜF STIFTUNG

… and you for your attention!



Combining antagonistic bacteria with copper to control late blight disease in potato plants

Fanny Germanier
University of Fribourg

EAPR – Arras - 2023

The project

Phytophthora infestans

- Tremendous losses

- Solutions:
  - → "Conventional" fungicides CHEMICALS
  - → COPPER-based fungicides
    - 4kg/ha/year of Cu

Our main goal

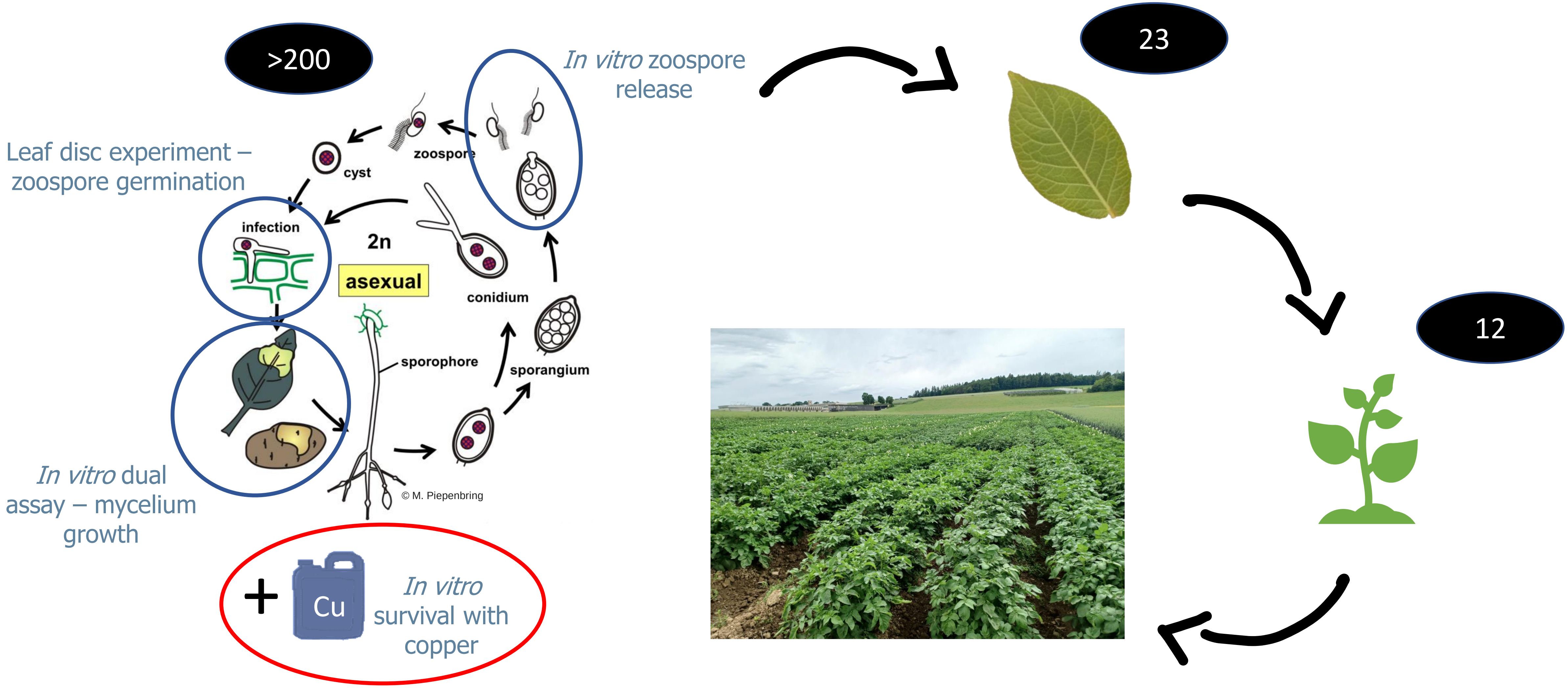
- To reduce the use of copper in the fields

➢ By combining copper and antagonistic bacteria

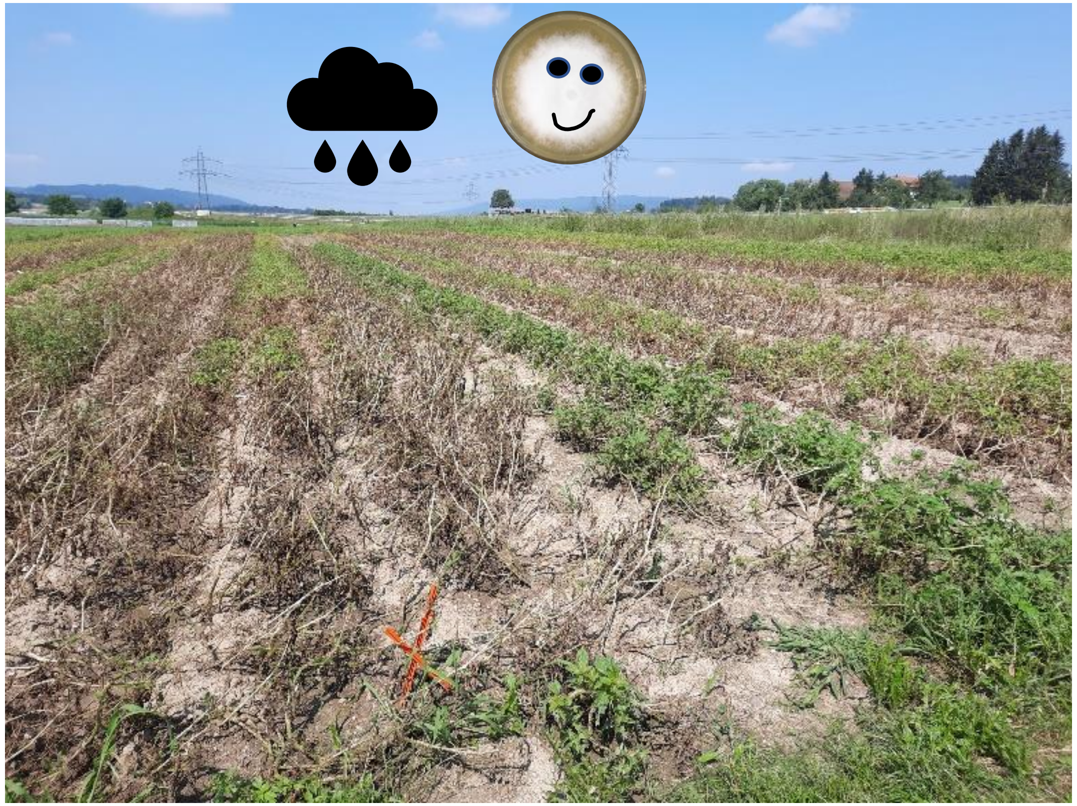
Can bacteria be combined with copper to fight against *Phytophthora infestans* in potato fields?

How does copper influence the physiology and activity of bacterial strains against *Phytophthora infestans*?

Can bacteria be combined with copper to fight against *Phytophthora infestans* in potato fields?



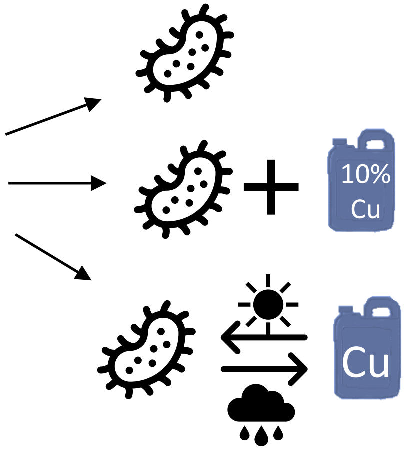
Field 2021 - Zürich



Field 2022 - Fribourg

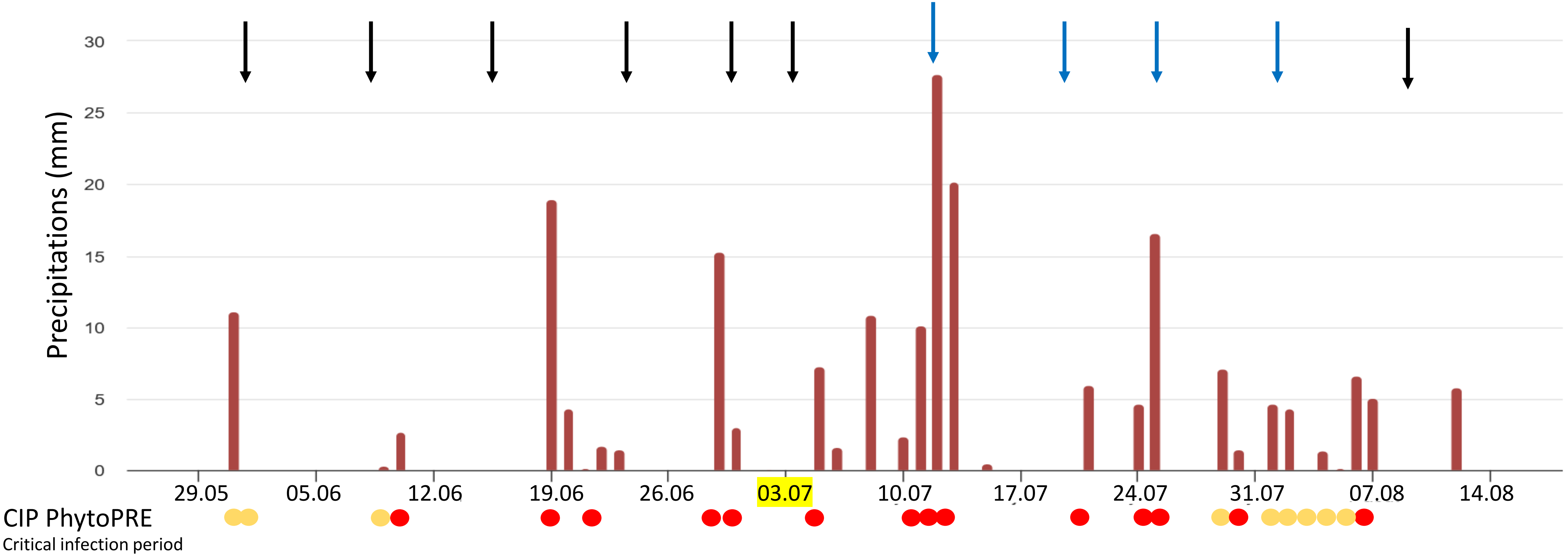
Field trial 2023 Fribourg

Bacillus sp.
Streptomyces sp.



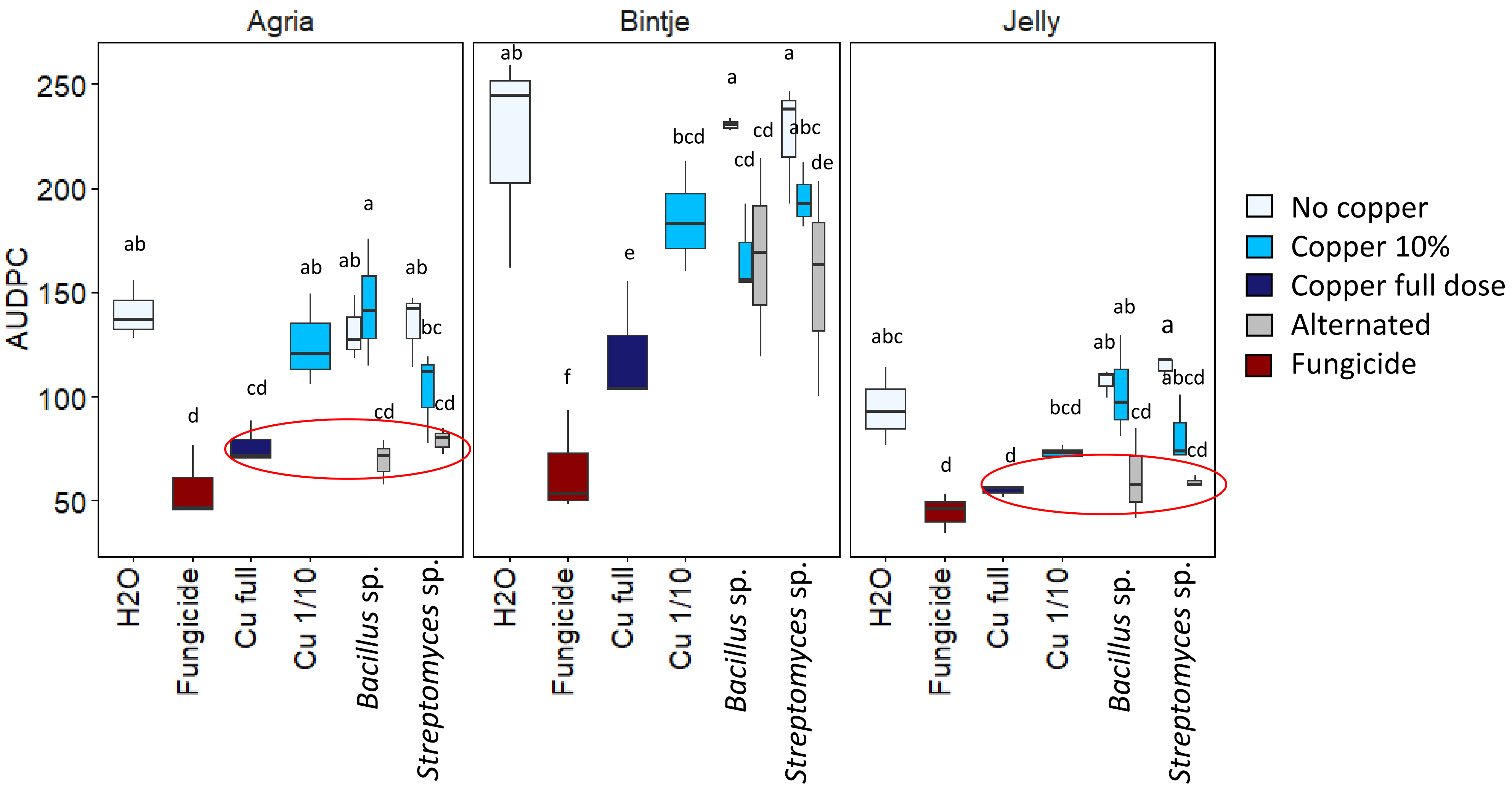
Controls:

- H_2O
- Copper 10%
- Copper Full
- Fungicide

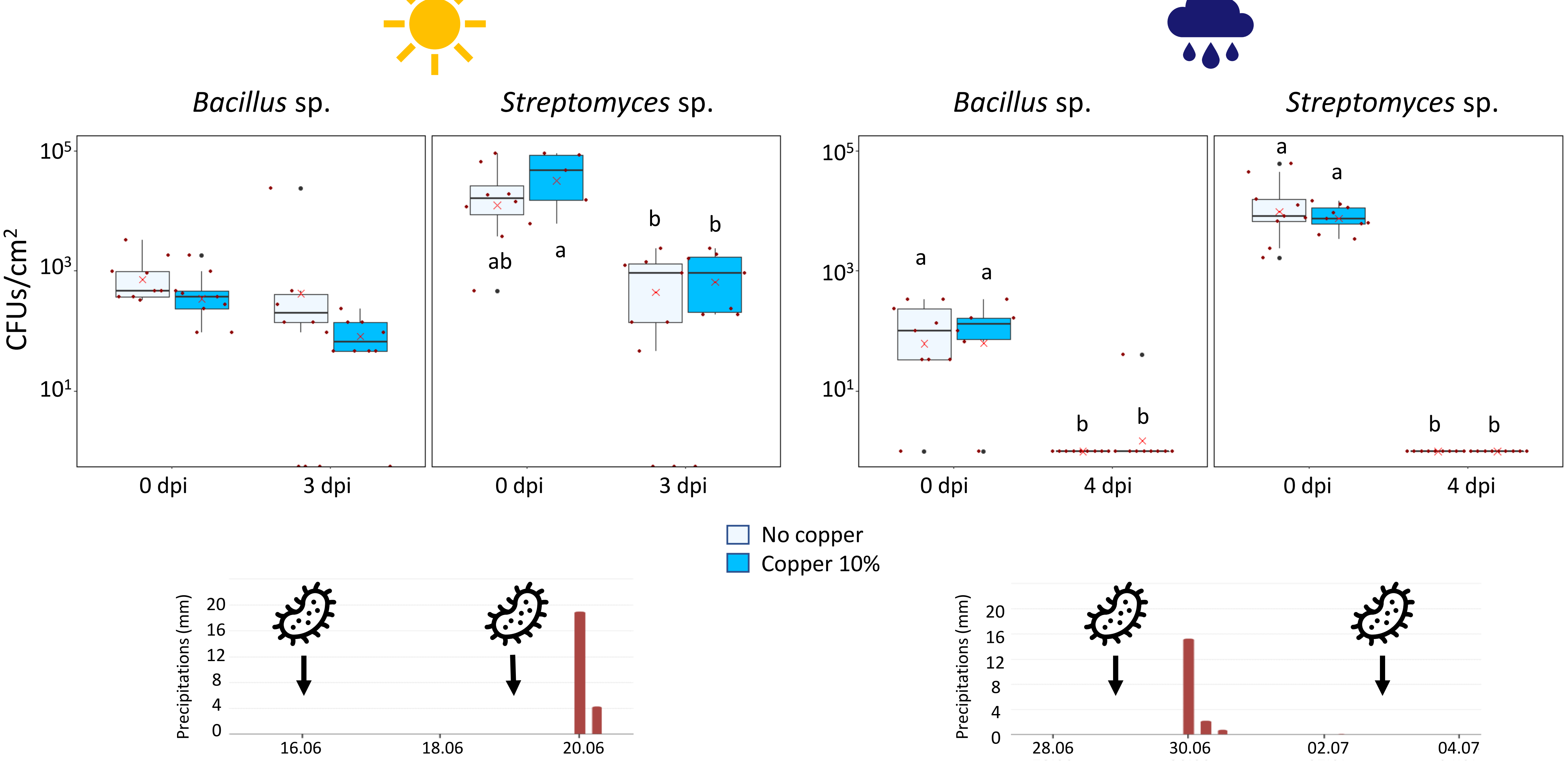


Infection – field

Foliar treatments



Survival – field (Bintje)

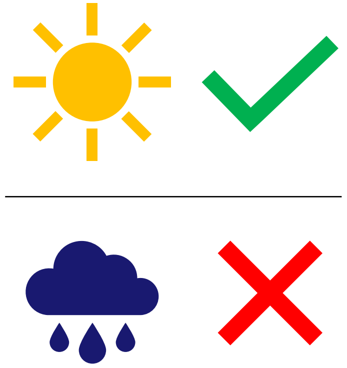


Can bacteria be combined with copper to fight against *Phytophthora infestans* in potato fields?

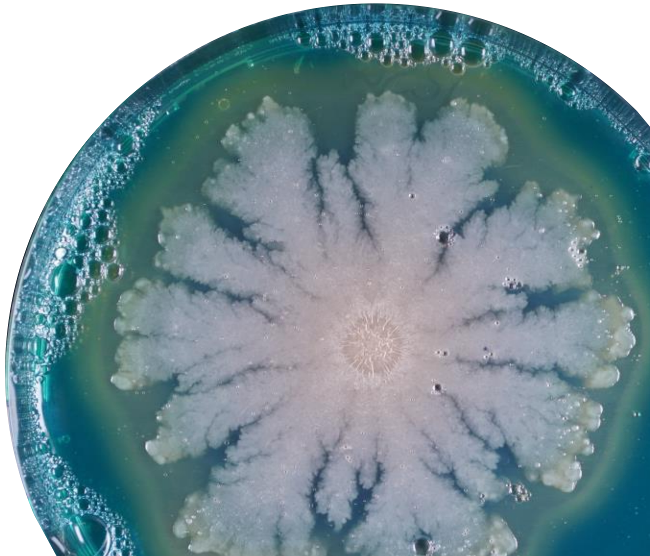
- Field results did not show a protective effect of neither the strains alone nor mixed with copper

  → But strategic treatments (alternated) depending on the disease pressure seemed like a good approach

- Both strains were able to survive under sunny conditions for at least 3 days post inoculation
- Heavy rain probably washed the strains which were not retrieved after 4 days post inoculation



How does copper influence the physiology and activity of bacterial strains against *Phytophthora infestans*?



Copper impaired the motility of bacterial strains

Motility

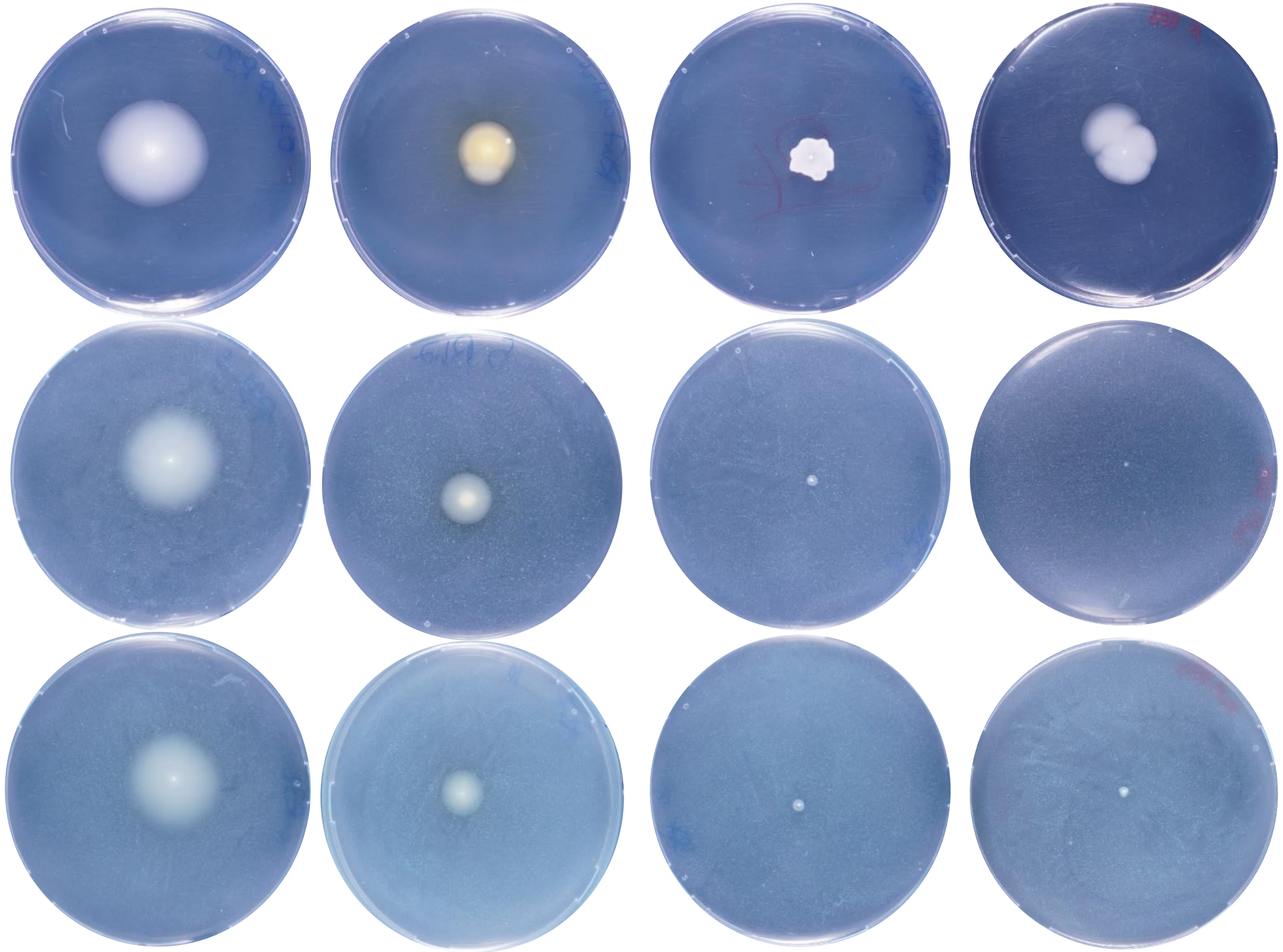
Pseudomonas sp. | *Pseudomonas* sp. | *Rhodococcus* sp. | *Pseudomonas* sp.

Cu

0%

5%

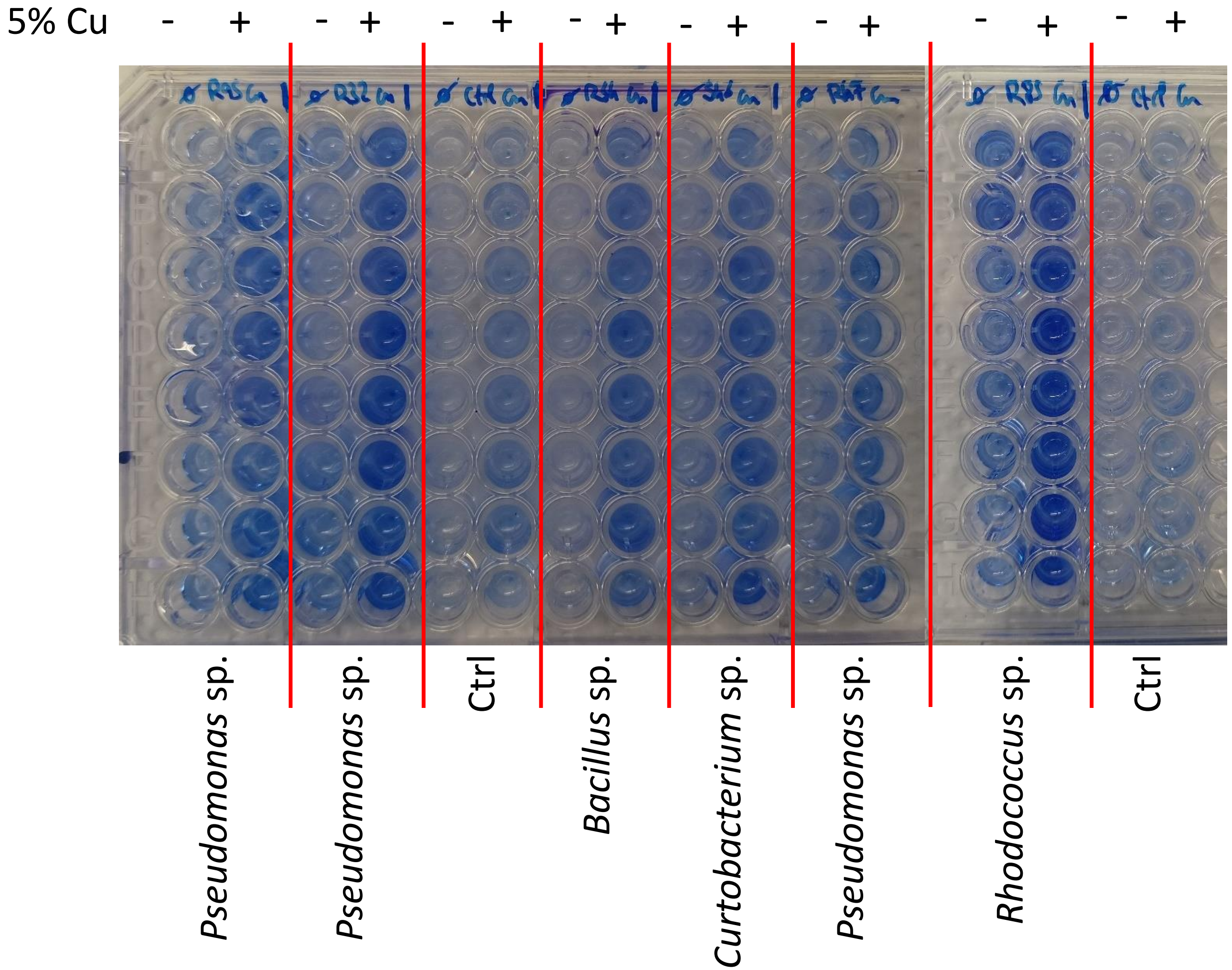
10%



Copper promoted the biofilm formation of the bacterial strains

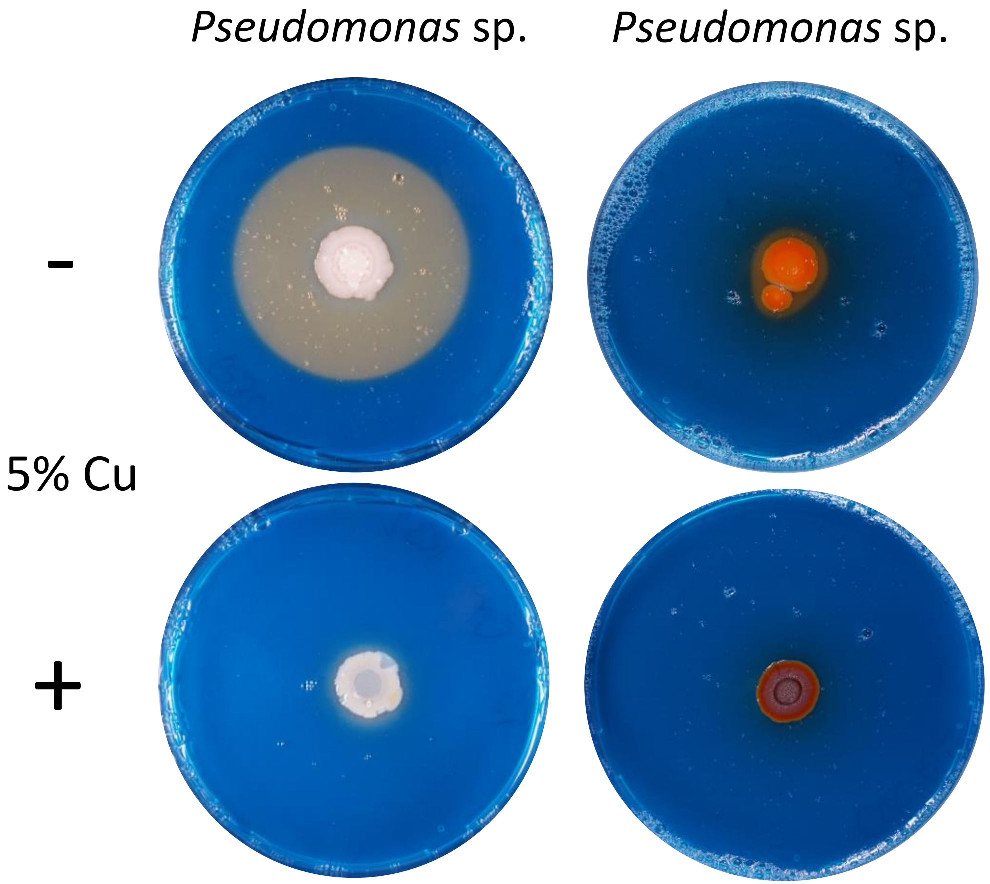
Motility

Biofilm



Copper inhibited the siderophore production of bacterial strains

Motility	↘
Biofilm	↗
Siderophore	↘

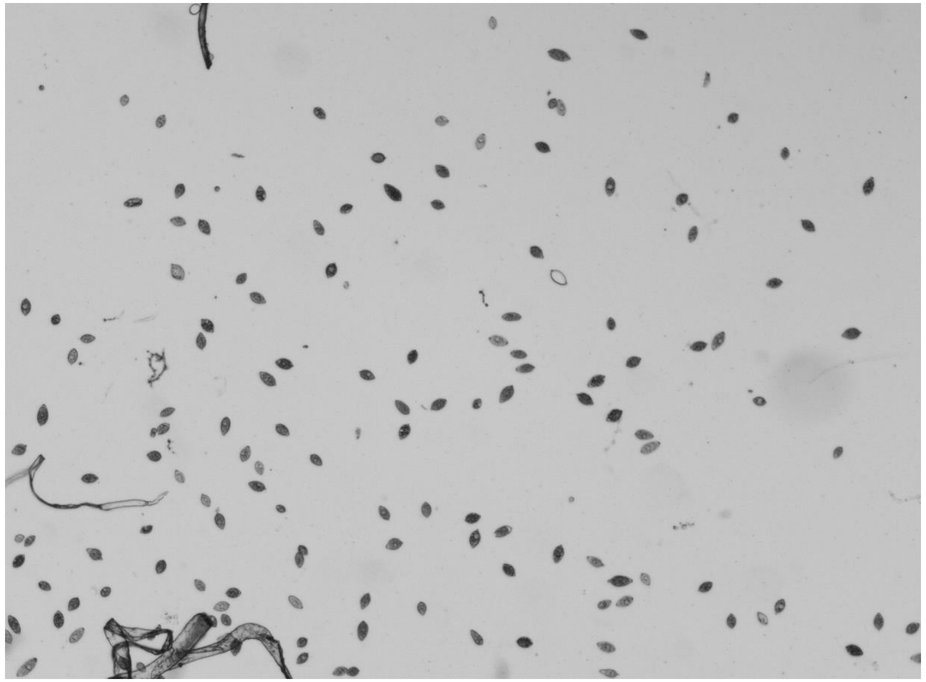


Copper did not impair the production of metabolites inhibiting (or not) the zoospore release, except for one strain

Motility	↘
Biofilm	↗
Siderophore	↘
Zoospore release inhibition	= ↘

Pseudomonas sp.

- Cu



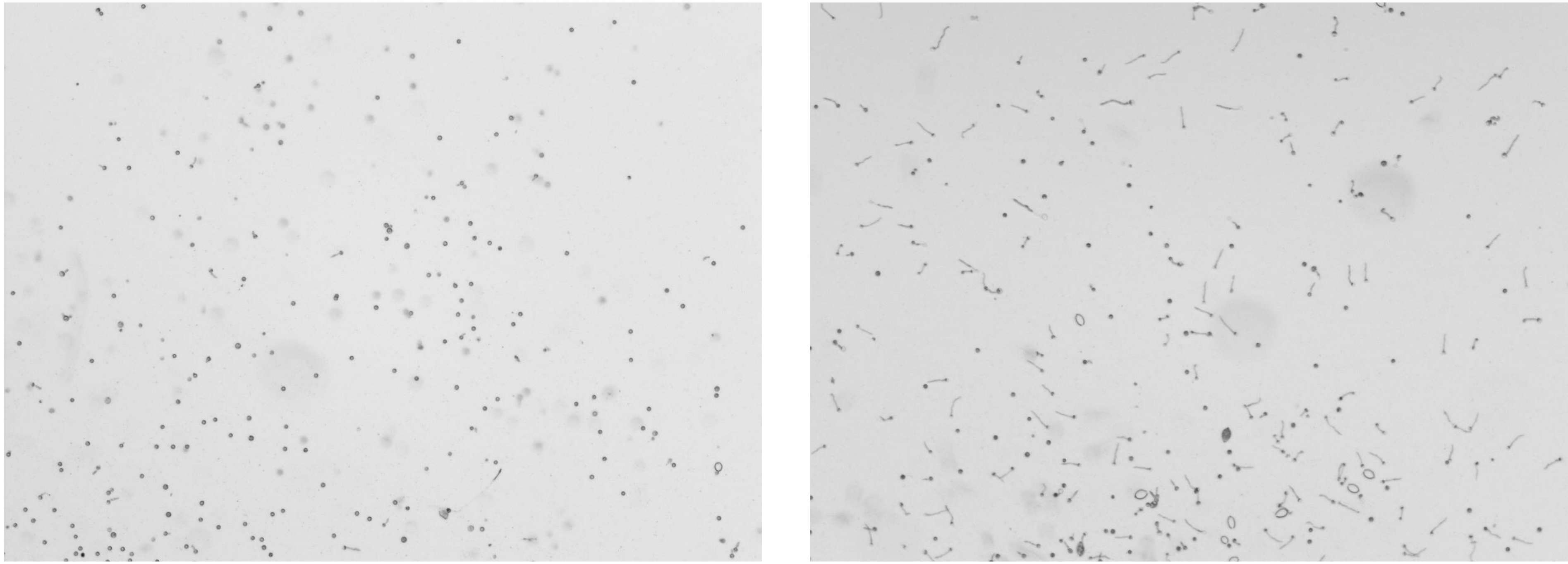
+ Cu

Copper did not impair the production of metabolites inhibiting (or not) the zoospore germination, except for one strain

Motility	↘
Biofilm	↗
Siderophore	↘
Zoospore release inhibition	= ↘
Zoospore germination inhibition	= ↘

Bacillus sp.

- Cu + Cu



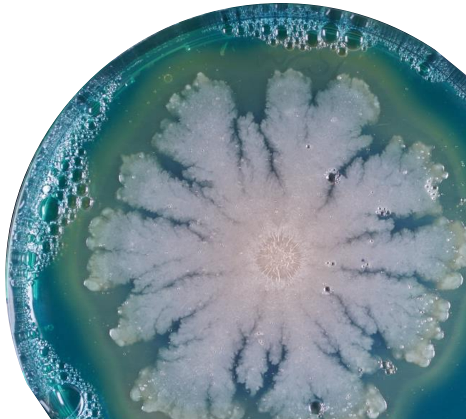
How does copper influence the physiology and activity of bacterial strains against *Phytophthora infestans*?

- Copper promoted the biofilm formation of every tested strains but diminished the motility of the strains

- Siderophore production was inhibited by copper

- Copper had overall a neutral impact on the production of active molecules against *P. infestans*

- In two cases, copper impaired the inhibition potential of the strains



Thank you for your attention!

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Mout De Vrieze
Laure Weisskopf
Floriane L'Haridon
Noémie Poli
Ola Abdelrahman
Vincent Glais
Carola Velti
Vivien Pichon
Alisson Gillon
Sébastien Bruisson
Alissa Deschamps
Maxime Staedler
Livia Jerjen
Camila Morales Undurraga
Aline Altenried

Agroscope

Brice Dupuis
Maud Tallant
Gaëtan Riot

ETAT DE FRIBOURG
STAAT FREIBURG
Institut agricole de Grangeneuve

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Fundings:

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Office fédéral de l'agriculture OFAG

New Challenges for Biological Active Substances.
T. asperellum, T34 an EU authorized plant protection product soon against *Rhizoctonia* for potato in Central zone.

M.I. Trillas [1], E. Casanova [2], E. Fernández [2], G. Segarra [1,2]

[1] Universitat de Barcelona. Faculty of Biology. Plant Physiology Section
[2] Biocontrol Tecnologies, S.L. spin-off Universitat de Barcelona

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1. Introduction

Trichoderma asperellum strain T34:
from laboratory to commercialisation

Challenges for Biological Active Substances

1. Introduction

Trichoderma asperellum strain T34: from laboratory to commercialisation

More than 20 years working for the biological control of plant diseases

FUNDATION

Creation of Biocontrol Technologies, S.L.

The company was founded in 2004 as Spin-off of Univerity of Barcelona.

EU AUTHORITZATION

Authorized at EU.

The active substance was included in Annex I of Regulation (EC) 1107/2009 on plant protection products.

UNIVERSITAT DE BARCELONA

BIOCONTROL TECHNOLOGIES

Chair on Microorganisms for Agriculture

CHAIR UB

University of Barcelona Chair.

2023 was the year in which Chair UB of Microorganism for Agriculture-Biocontrol Technologies was created.

2002 — *Trichoderma asperellum* strain T34 **Patent.**

2004 — **Biocontrol Technologies SL** creation.

2011 — T34 Biocontrol® is **accepted as plant protection product at USA.**

2013 — *Trichoderma asperellum* strain T34 **authorized at EU.**

2016 — Sales permit at **6 new european countries.**

2018 — Sales permit at **5 new european countries.**

2019 — T34 Biocontrol®'s new marketing agreement **in Italy.**

2020 — Extension of uses in the USA **+200 crops.**

2021 — First subsidiary in Mexico to market **T34 Biocontrol®.**

2023 — Chair UB **Microorganisms for Agriculture - Biocontrol Technologies.**

Our Professional Background

1. Introduction

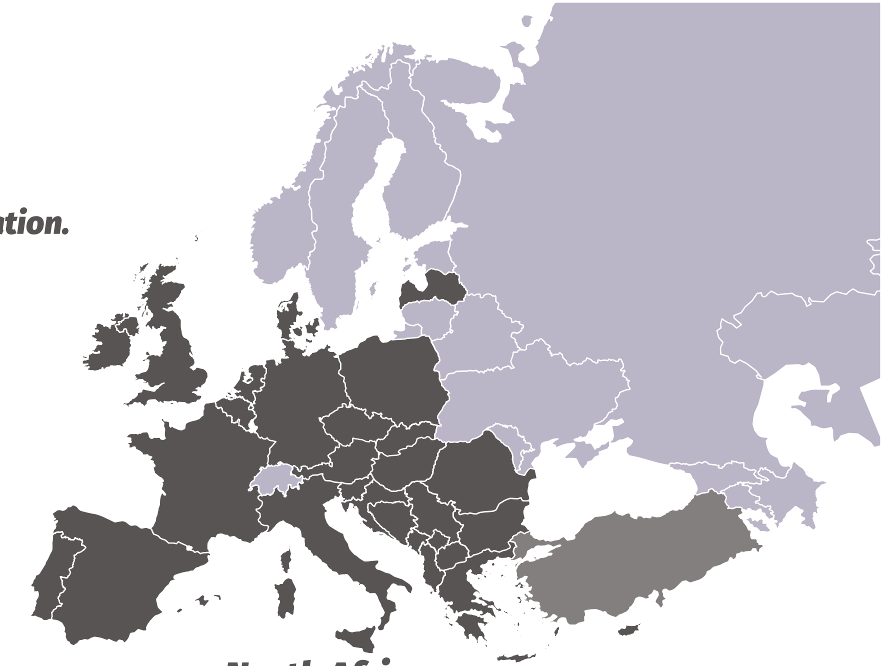
Trichoderma asperellum strain T34: from laboratory to commercialisation

Authorisations as biological Plant Protection Product

Authoritzation Number	
EUROPE	EU Nº1238/2012
THE NETHERLANDS	15135N y 15212N
BELGIUM	10481P/B
FRANCE	AMM 2160492
SPAIN	ES-00283
PORTUGAL	AV 00898
ITALY	16734
IRELAND	PCS 05620
HUNGARY	04.2/2074-1/2018 NÉBIH
RUMANIA	350PC/29.11.2017
POLAND	R-43/2018 / R-22/2019
LATVIA	0809 (2022)
GREECE	61060
UNITED KINGDOM	MAPP 17290
UNITED STATES	87301-1
CANADA	30229
DOMINICAN REP.	7163
PERU	066-SENASA-PBA-ACBM
EGYPT	1718 (2013)
MOROCCO	F.10-9-005 (2022)
TUNISIA	F.19-20

In process of
Registration** & **Commercialization.

TURKEY
+
COLOMBIA
ECUADOR
MEXICO



North Africa

EGYPT *MOROCCO* *TUNISIA*

INPUTS ECOCERT Approved by Ecocert · CAAE INSUMOS UNE 315500 · OMRI LISTED For Organic Use · Skal BIO CONTROLE · OF&G ORGANIC · JAS

Organic Certifications

1. Introduction

Challenges for Biological Active Substances.

EU Regulatory System:

Cost and **Time**

Time of Registration for a new active substance

- According Dunham Trimmer (2023) **the average time is 8 years .**

Time of Registration for approved active substances (new uses) under low risk statement

- Our experience is very variable between EU countries:
  - ✓ **France** is a very good choice (very efficient and not the most expensive).
  - ✓ Our **experience with potato in Germany**, it was applied to Oundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) on September 2021 and now (September 2023) it is finishing.

2. GEP Studies

Control of Rhizoctonia solani

Black scurf - Stem canker

Field GEP Studies

2018 - 2019

2. GEP Studies. *Characteristics*

Rhizoctonia solani. **Black scurf - Stem canker**

GEP Field studies

(15) different EU countries

x2 x2 x5 x4 x2

Years of experiment: 2018-2019

A. Experimental design:

✓ **4 Blocs (experimental units), size 15-48 m^2**

All studies were evaluated at harvest: 100 tubers/block, total of 400 tubers.

B. Potato varieties:

Balatoni rózsa, Kondor, Milva, Solara, Eurustarch, Taifun, Abelia, Laura, Hermus, Glorietta, Quarta, Charlton, Hansa and Desiree.

C. Treatments: in furrow or tuber at planting

All treatments were applied according the dosage recommended in the label, except for:

T34 *T. asperellum* at three doses: 125 g, 250 g and 500 g/Ha.

- ✓ **Chemicals:**
  - ✓ **Monceren**® (Pencicuron)
  - ✓ **Moncut**® (Flutolonil)
  - ✓ **Rialto**™ (Chlorothalonil)
  - ✓ **Allstar**® (Fluxapyroxad)
  - ✓ **Emesto**® **Silver** (Penflufen + Prothioconazole)
  - ✓ **Amistar**® (Azoxystrobin + Difenoconazole)
- ✓ **Biological:**
  - ✓ **Proradix**® *Pseudomonas*, spp. strain DSMZ 13134
  - ✓ **RootDei**®, *T. asperellum* strain T34

GOOD EXPERIMENTAL GEP PRACTICES CERTIFICATION

2. Control of *Rhizoctonia solani*

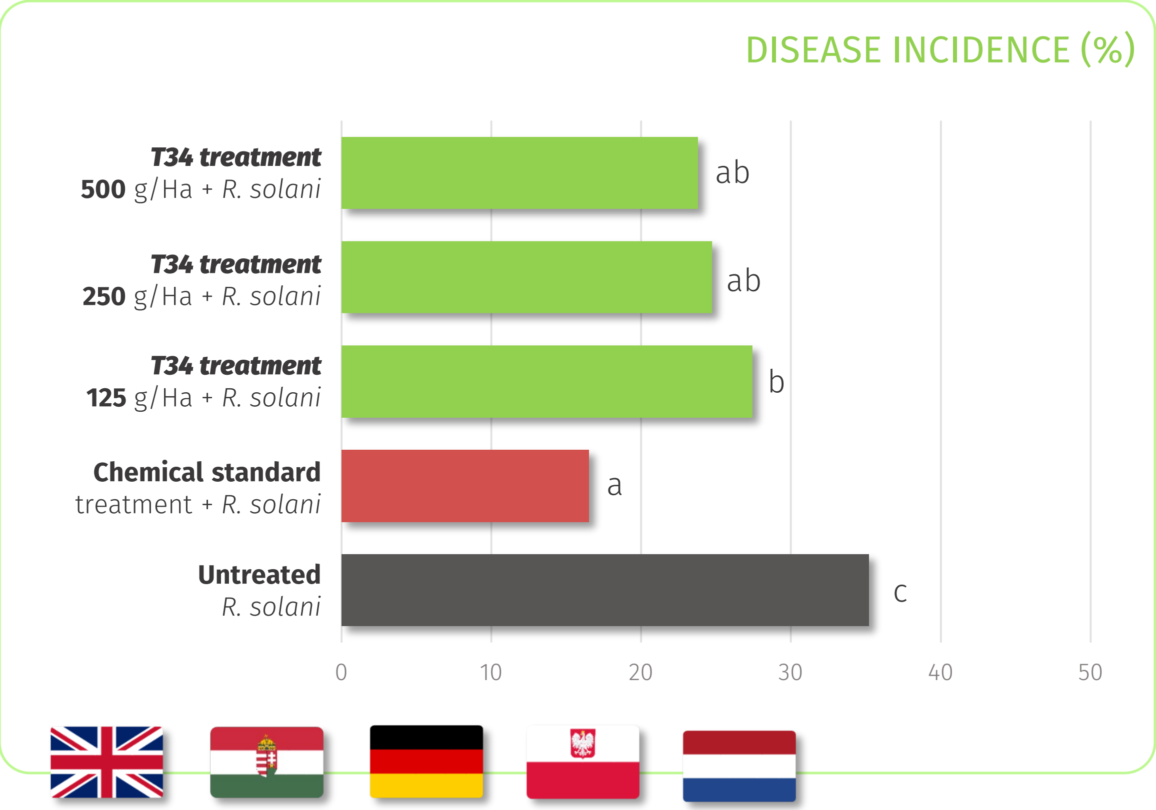
Black scurf - Stem canker

15 out of 15 GEP studies.

2 United Kingdom, 2 Hungary; 5 Germany; 2 Poland; 4 The Netherlands

DATA from overall studies

- ✓ The average **Disease Incidence** (untreated tubers) from all 15 studies was **35,2%.**
- ✓ The average **disease reduction** of all evaluated chemicals was **53%.**
- ✓ The average **disease reduction** of the medium dosage studied (**250 g/Ha**) **of RootDei®** was **30%.**

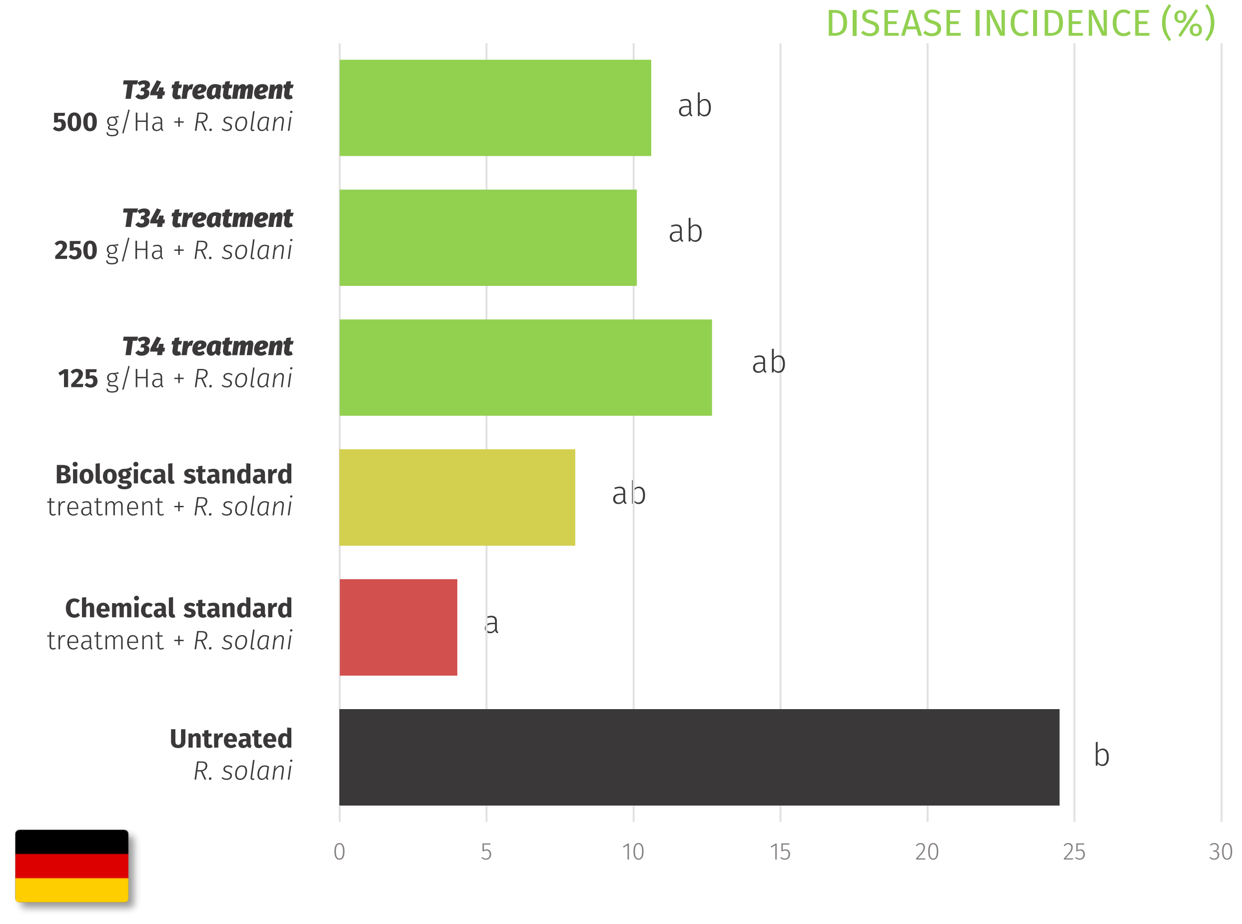


2. Control of *Rhizoctonia solani*
Black scurf - Stem canker

3 out of 3 GEP studies.
3 Germany

DATA from the 3 studies using a biological standard

- ✓ The average **Disease Incidence** (untreated tubers) from all 3 studies was **24,5%.**
- ✓ The average **disease reduction** of the standard chemicals was **84%.**
- ✓ The average **disease reduction** of the standard biological was **63%.**
- ✓ The average **disease reduction** of the medium dosage studied (**250 g/Ha**) **of RootDei®** was **59%.**

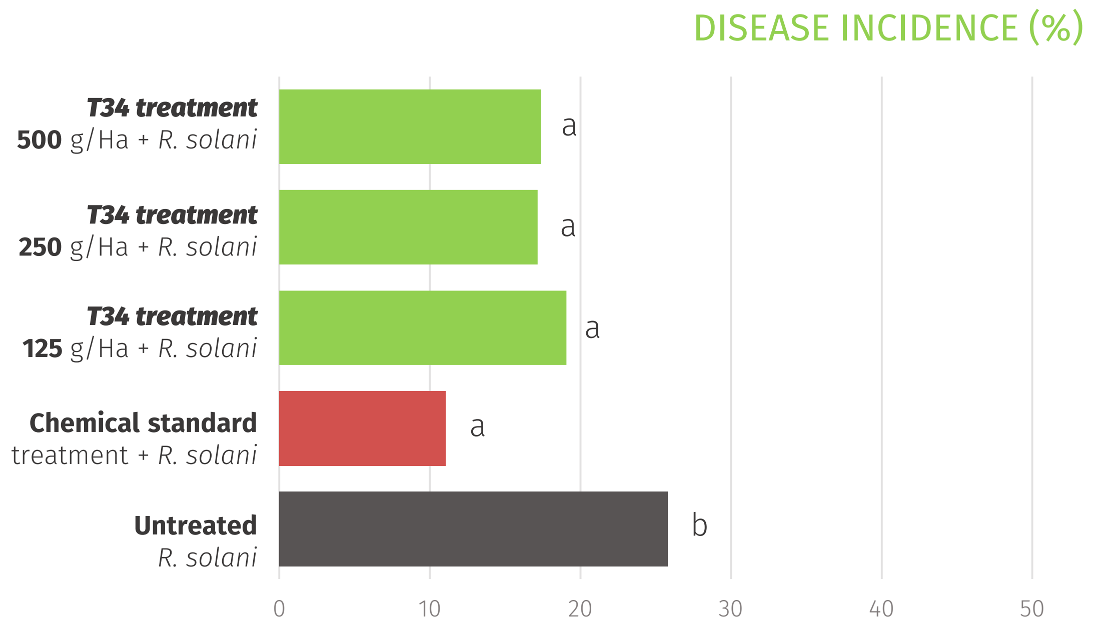


2. Control of *Rhizoctonia solani*
Black scurf - Stem canker

10 out of 11 GEP studies
Maritime climatic EPPO zone (UK, NL, DE)

DATA from Maritime climatic EPPO zone

- ✓ The average **Disease Incidence** (untreated tubers) was **25,78%.**
- ✓ The average **disease reduction** of chemical treatments was **57%.**
- ✓ The average **disease reduction** of the medium dosage studied (**250 g/Ha**) **of RootDei®** was **33%.**



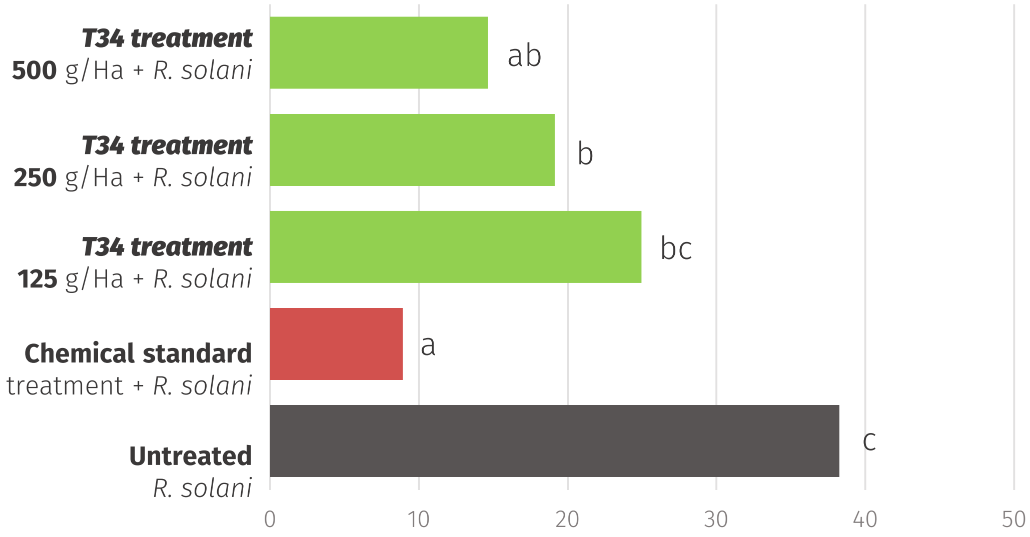
2. Control of Rhizoctonia solani
Black scurf - Stem canker

2 out of 2 GEP Studies
North-East Climatic EPPO Zone (PL)

DATA from North-East climatic EPPO zone

- ✓ The average **Disease Incidence** (untreated tubers) was **38,25%.**
- ✓ The average **disease reduction** of chemical treatments was **77%.**
- ✓ The average **disease reduction** of the medium dosage studied **(250 g/Ha) of RootDei®** was **50%.**

DISEASE INCIDENCE (%)



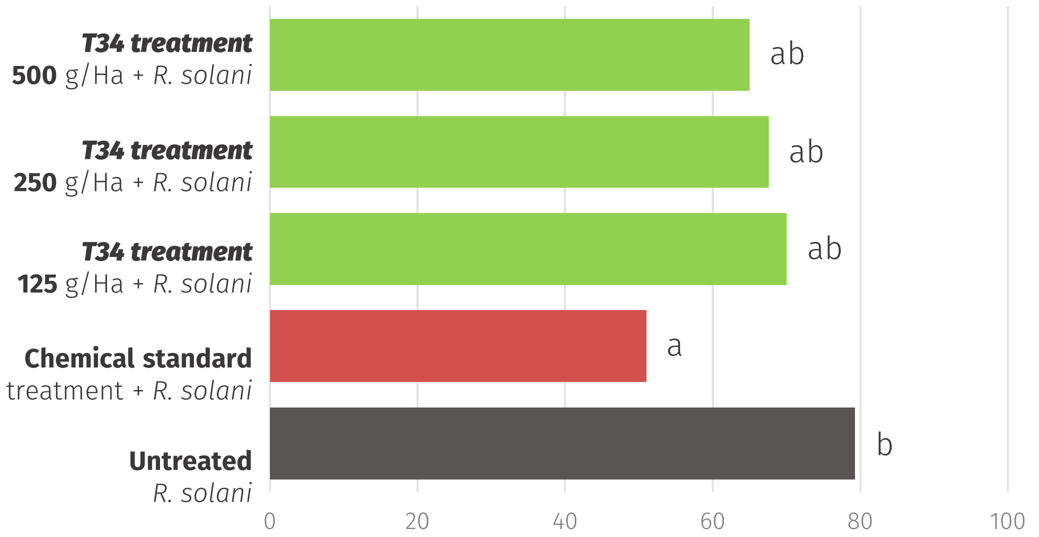
2. Control of *Rhizoctonia solani*
Black scurf - Stem canker

2 out of 2 GEP Studies
South-East climatic EPPO zone (HU)

DATA from South-East climatic EPPO zone

- ✓ The average **Disease Incidence** (untreated tubers) was: **79,25%**.
- ✓ The average **disease reduction** of chemical treatments was: **35%.**
- ✓ The average **disease reduction** of the medium dosage studied (**250 g/Ha) of RootDei®** was: **15%.**

DISEASE INCIDENCE (%)



3. University Studies

Control of *Sclerotium rolfsii*

Stem rot / Southern blight

Control of *Pectobacterium carotovorum* or *Dikeya solani*

Tuber soft rot / Black leg

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3. Control of *Sclerotium* rolfsii (syn. *Athelia rolfsii*)

Potato Stem rot

FIELD LOCATION:

El Pino Agricultural holding. Carmona, Sevilla.

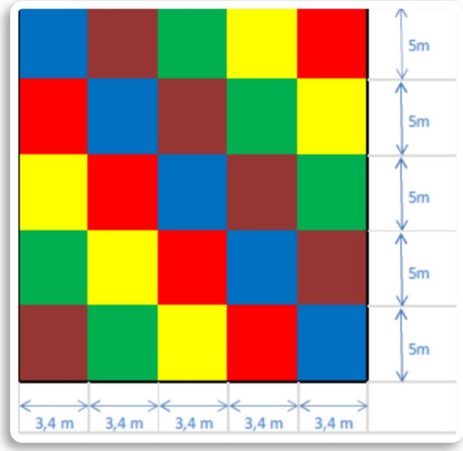
Years of experiment 2016-2017

Experimental design:

- ✓ Natural infected field.
- ✓ 5 plots (5m x 3,4 m), distance between plots 0,85 m

Treatments (drip irrigation)

- **Ortiva**: Azoxystrobin (1 L/ha)
- **Ranman Top**: Cyano-imidazole (0.5 L/ha)
- **Serenade® Max**: *B. subtilis* QST 713 (4 Kg/ha)
- **Rootdei®:** *T. asperellum* T34 (0.6 Kg/ha)
- **Untreated control**



All of them had 2 applications:
5th and 19th May 2017

Evaluation at harvest:
20 plants/ experimental unit = 100 tubers

Potato cv. Challenger

3. Control of *Sclerotium* rolfsii (syn. Athelia rolfsii)

Potato Stem rot

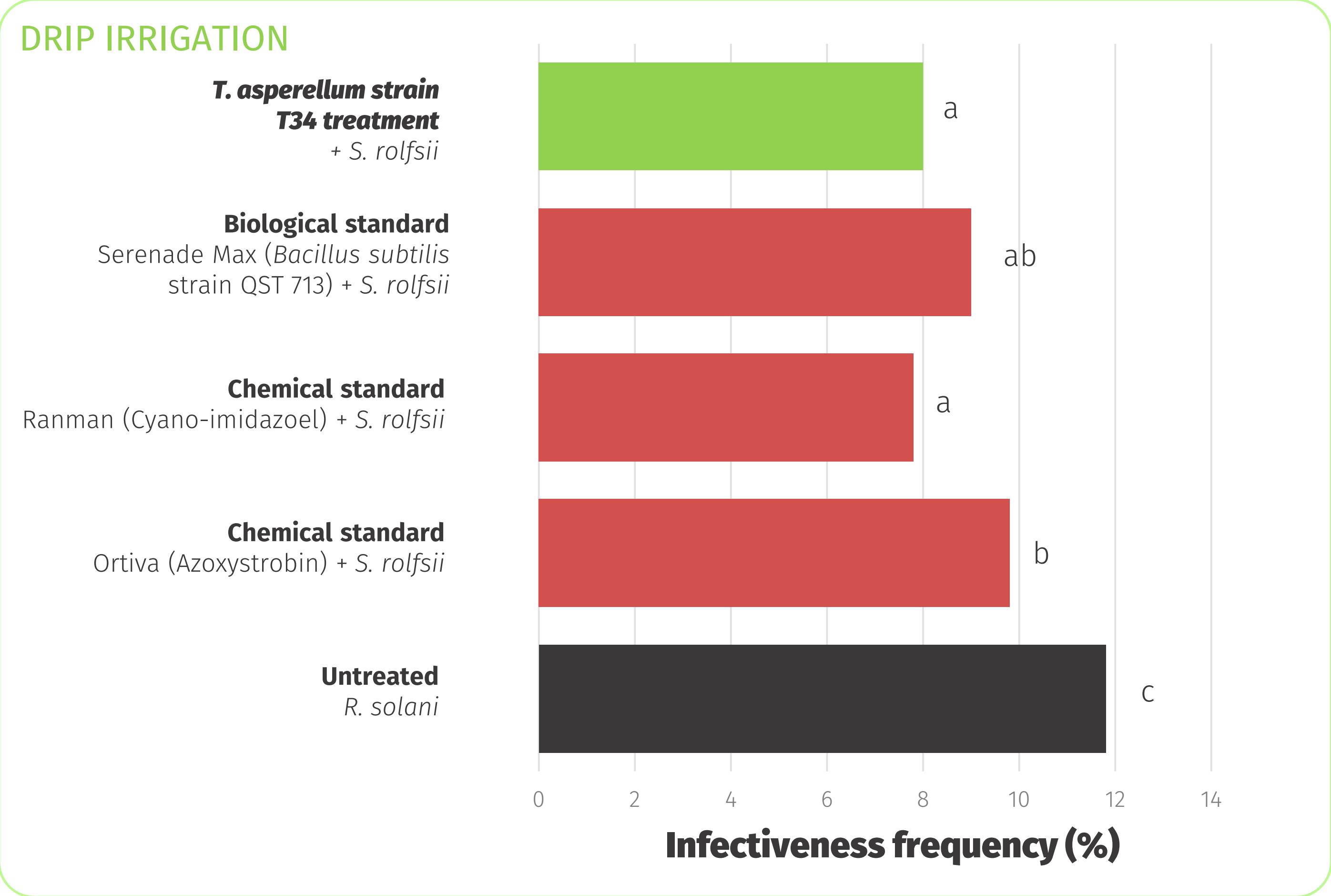
Conclusions

The average **Disease Incidence** (measured as infectiveness frequency) of untreated tubers was **11,8%.**

✓ The lowest disease incidence was for:

- Ranman (7.8% disease) **efficacy 34%.**
- T. *asperellum* T34 (8.0% disease) **efficacy 31.4%**

both were statistically significant different from untreated and Ortiva and Serenade Max had an intermediate behaviour.



3. *Dikeya / Pectobacterium*

Southern blight/wilt/stem rot

Its was studied the effect of *Trichoderma asperellum* strain T34.

- ✓ **T34 applied at two concentrations:** 10^3 cfu/ml or 10^5 cfu/ml in (50 µl) solution.
- ✓ At different times: **6, 9** or **12 h before the pathogen.**
- ✓ **Potato tubers** different varieties Agata (very susceptible), Monalisa (slightly susceptible) or Picobello (moderately susceptible).
- ✓ **Inoculated with either *Dickeya* solani or *Pectobacterium carotovorum*** (50 µl) OD_{600} = 0.1 ml (10^8 cells/ml) or 0.01 ml (10^7 cells/ml).

Part of the PhD Thesis of Rachid Ladjouzi.
Université Abderrahmane Mira. Béjaia, Algérie.

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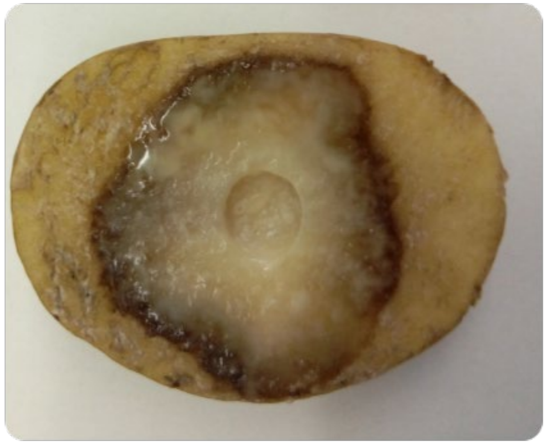
12 h before the pathogen



***Dickeya solani* –**
***T. asperellum* T34**

***Dickeya solani* +**
***T. asperellum* strain T34**
applied 10^3 cfu/ml.

12 h before the pathogen

***Pectobacterium carotovorum* –**
***T. asperellum* T34**

***Pectobacterium carotovorum* +**
***T. asperellum* T34**
applied 10^3 cfu/ml.

3. Dikeya / Pectobacterium
Southern blight/wilt/stem rot

Potato variety (Agata)
inoculated with *Dickeya solani* (50 µl) OD_{600} = 0.01 ml equivalent to 10^7 cells/ml and:

a) T34 untreated

b) T34 treated ***6 h before the pathogen***

c) T34 treated ***9 h before the pathogen***

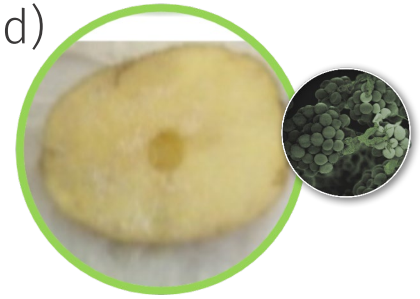
d) T34 treated ***12 h before the pathogen***

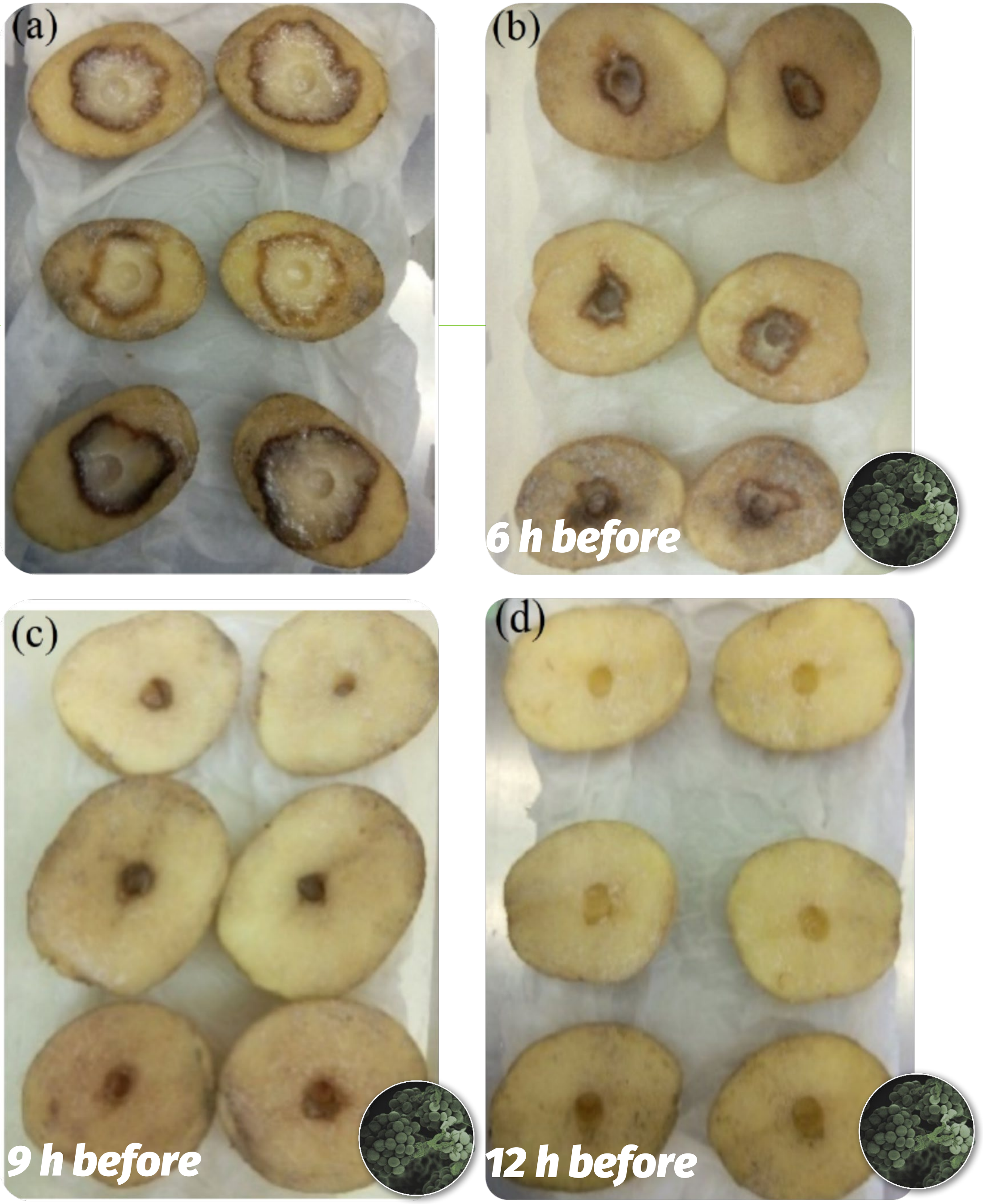
Trichoderma asperellum **strain T34 was applied 50 µL at 10^5 cfu/ml solution**

Part of the PhD Thesis of Rachid Ladjouzi. Université Abderrahmane Mira. Béjaia (Algérie)

a)

d)



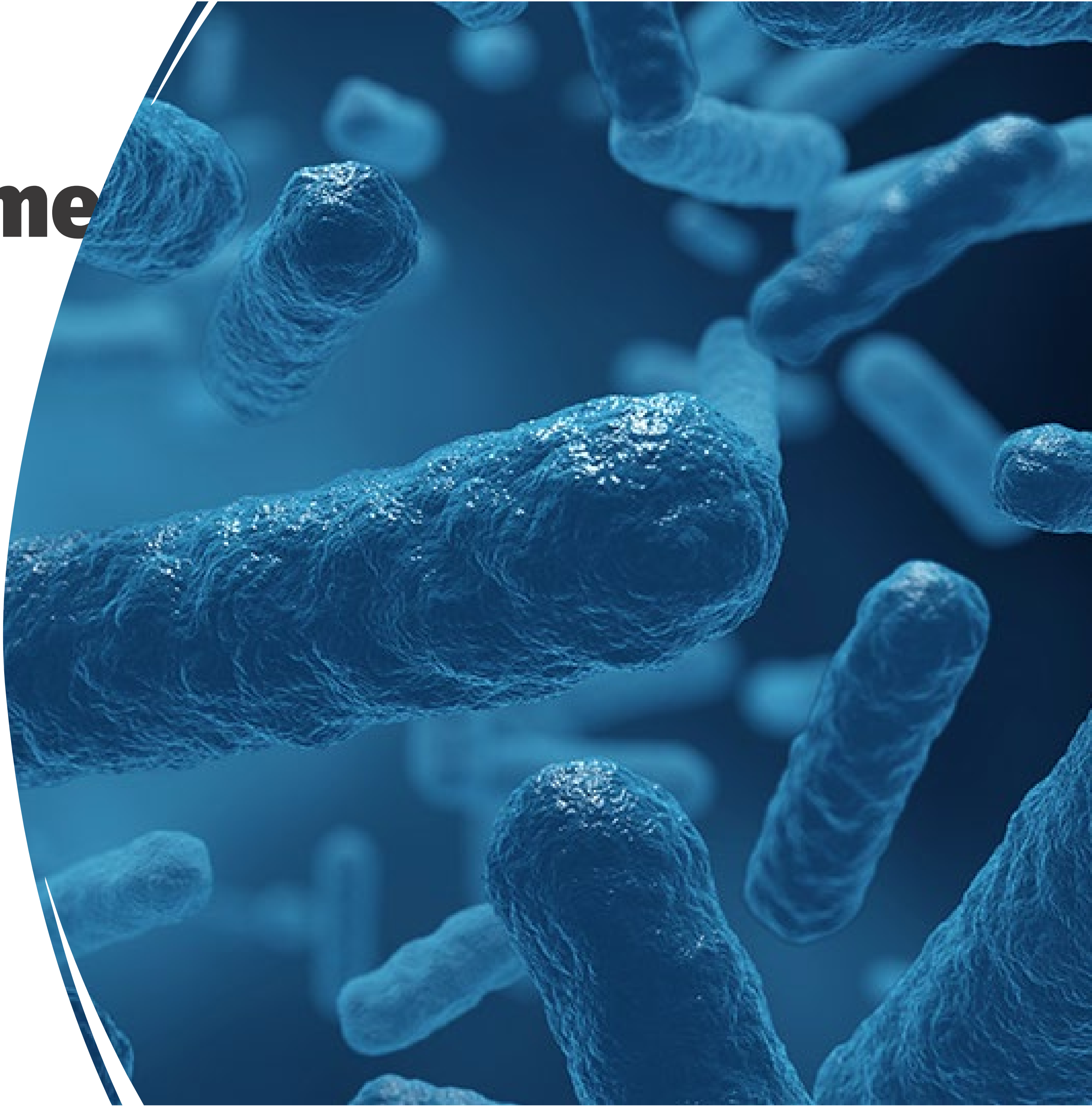


4. Plant-Microbiome services

Parallelism between human Gut & plant Rhizosphere

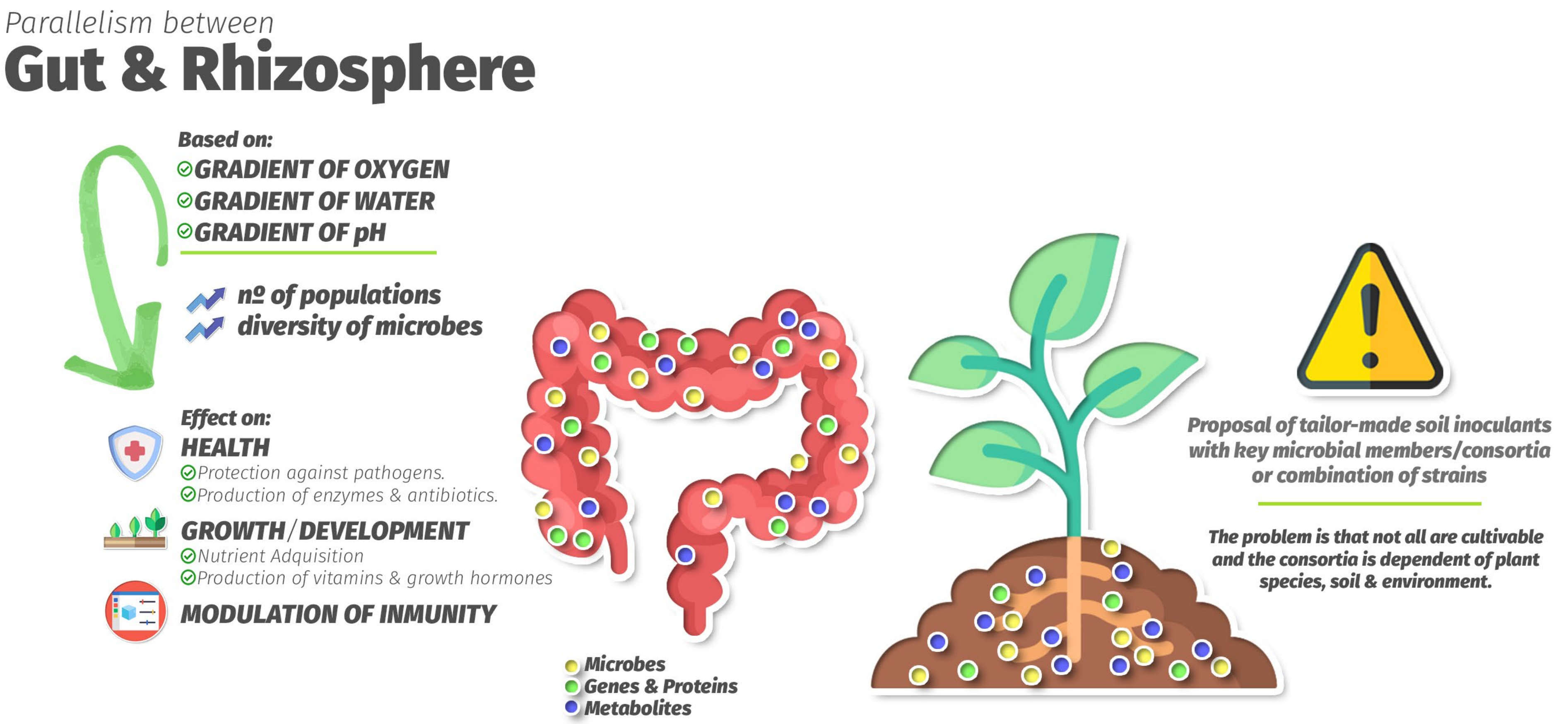
Mechanisms of Action

Scientific Publications



4. T. asperellum strain T34.

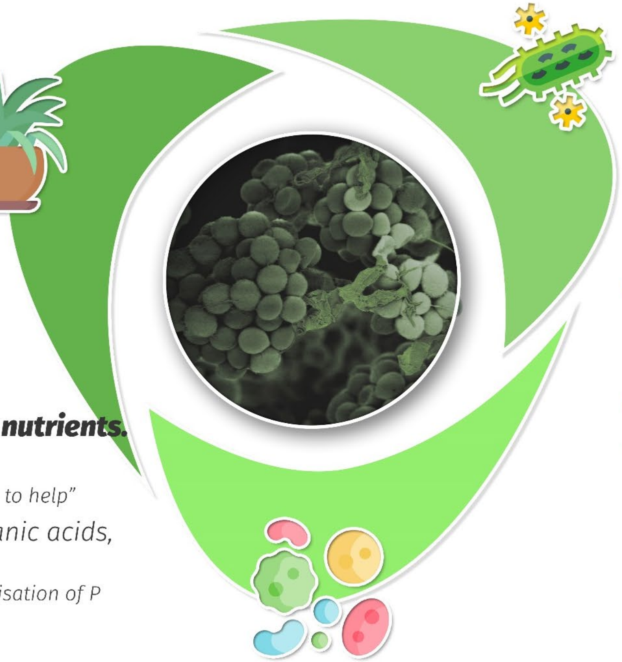
Plant-Microbiome Services



Mendes and Raaijmakers 2015 *The ISME Journal* 9:1905–1907

4. *T. asperellum* strain T34.

Plant-Microbiome Services



Plants

- **Activation on plant defences/immunity.**
  · Biotic + Abiotic Stress.
- **Reciprocal exchange of nutrients.**
  · Plant, sugar exhudates
  - recruitment of beneficials "cry to help"

  · Microbial, release of organic acids, protons, enzymes, ….
  - absorption of Fe, Ca, … solubilisation of P

Pathogen

- **Parasitism of resting strucutres**
  (Sclerotia, mycelia,…)
  Microbial relase of enzymes & other substances.
- **Competition for Space & Nutrients.**
- **Multiple Mechanisms of Action:**
  - No Resistance.
  - Action against different pathogens.
  - EU Authorized.
  - 0 Residue.

0 RESIDUE

Soil Microbiome

- **Compatibility with other biological products**
- **It keeps the balance of microbial diversity**
  While reduces pathogens population.

4. T. asperellum strain T34.
Scientific publications

Studies from other Universities & Research Centres

2017. **Plant Cell** & **Environment** 10/3042
2018. **Bioscience Research** 15(2): 602-609
2019. **Növényvédelem** 80:10 429-438
2019. **Egyptian Journal of Biological Pest Control** 29:88
2020. **Egyptian Journal of Biological Pest Control** 30:61
2020. **Frontiers in Microbiology** 10/3042
2021. **Journal of Phytopathology and Pest Management** 8: 46-63
2021. **Physiologia Plantarum** 172:1950-1965
2023. **Plant Disease** doi.org/10.1094/PDIS-07-22-1593-RE

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2002. **Soil Biology** & **Biochemistry** 34: 467-476
2006. **Biological Control** 39:32-38
2006. **Phytochemistry** 67:395-401
2007. **Proteomics** 7:3943-3952
2008. **Journal of Plant Pathology** 90 (S3):42
2009. **Plant Biology** 11:90-96
2009. Book. **Plant Innate immunity**. Chapter 8 Elsevier/Academic Press
2009. **Soil Biology** & **Biochemistry** 41: 2453-2459
2010. **Biological Control** 53:291-296
2010. **Microbial Ecology** 59:141-149
2011. **Plant** & **Soil** 342: 97-104
2012. **Plant Pathology** 61: 132-139
2013. **Phytopathologia Mediterranea** 52: 77-83
2013. **Journal of Plant Nutrition and Soil Science** 176: 867-875
2013. **Soil Biology** & **Biochemistry** 57: 598-605
2014. **Biological Control** 78: 77-85
2015. **Agriculture and Food Science** 24: 249-260
2016. **Biological Control** 95:31-39
2016. **Journal of Plant Nutrition and Soil Science** 179: 454-465
2016. **Journal of Plant Nutrition and Soil Science** 000, 1-12
2017. **Plant Pathology** 66: 1110-1116
2018. **Journal of Plant Pathology** 101: 121-127
2018. **J. Soils Sediments** 18:727-738
2020. **Planta** 252:8
2020. Book. **Progress in Biological Control**. Chapter 18. Springer

New Challenges for Biological Active Substances.

T. asperellum, T34 an EU authorized plant protection product soon against *Rhizoctonia* for potato in Central zone.

THANK YOU FOR YOUR ATTENDANCE

mtrillas@ub.edu

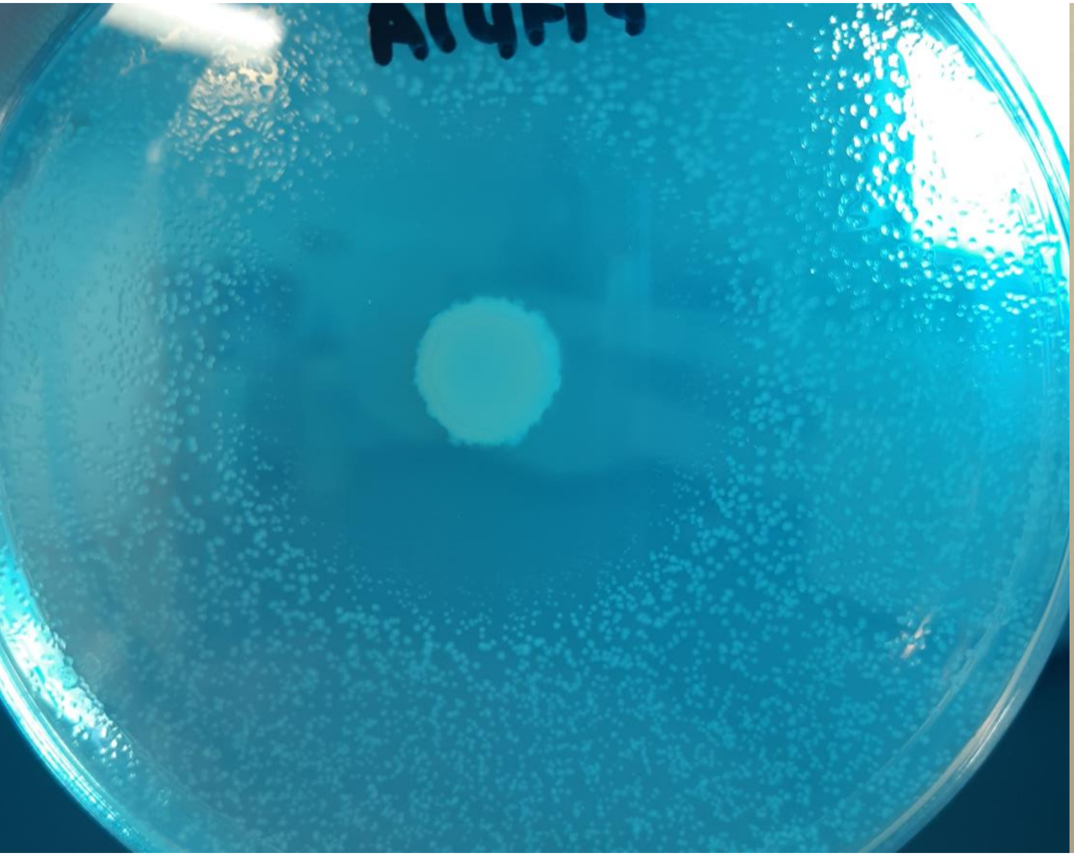
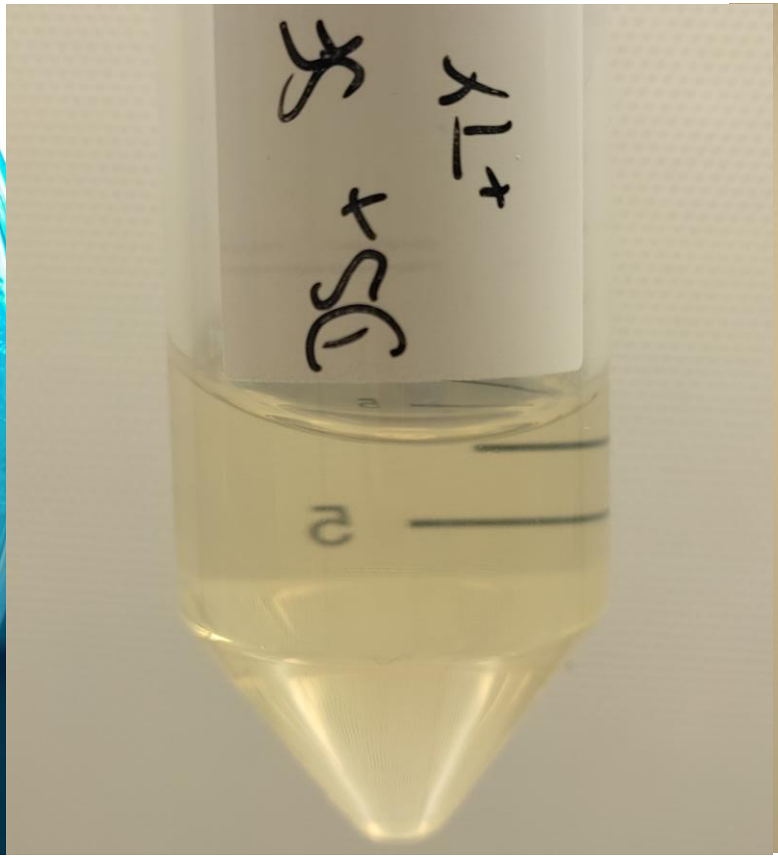
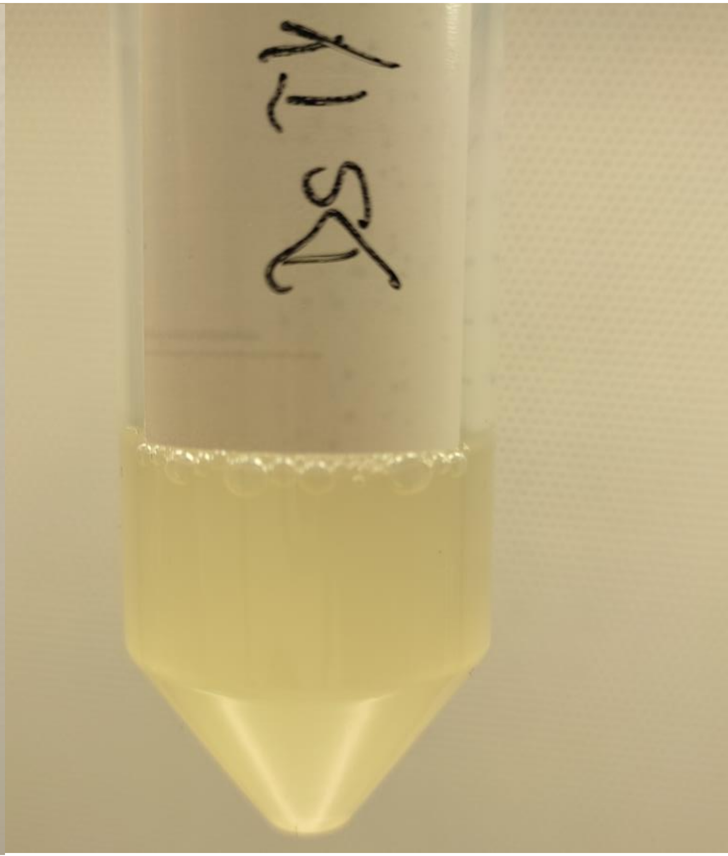
Universitat de Barcelona
Chair on Microorganisms for Agriculture

BIOCONTROL TECHNOLOGIES

RootDei Biocontrol®

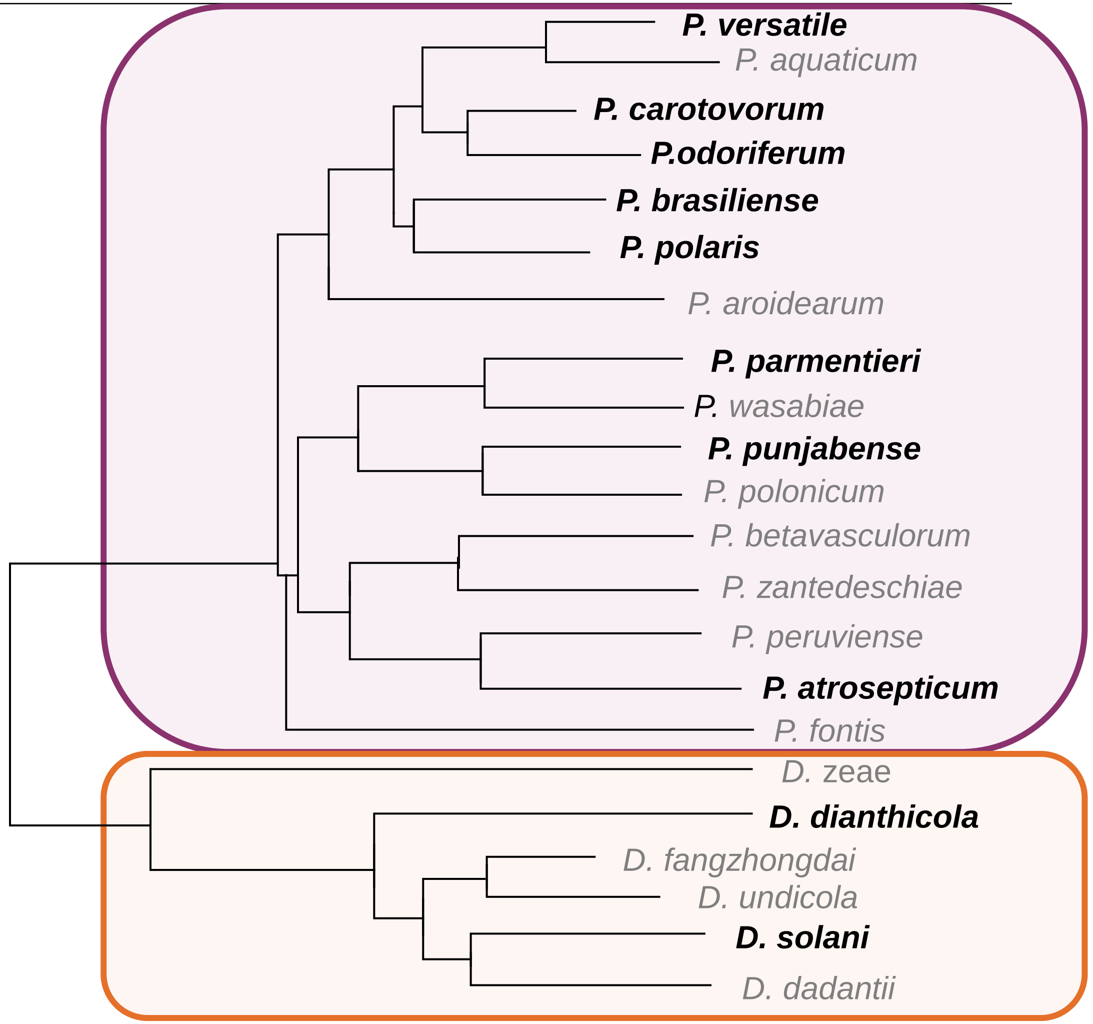
Determination of antagonist activity origin of Pseudomonas PA14H7 against Pectobacteriaceae

Euphrasie Lépinay, Coline Amaro, Denis Faure, David Mathiron, Mounia Khelifa, Sylvain Laclef, Serge Pilard.

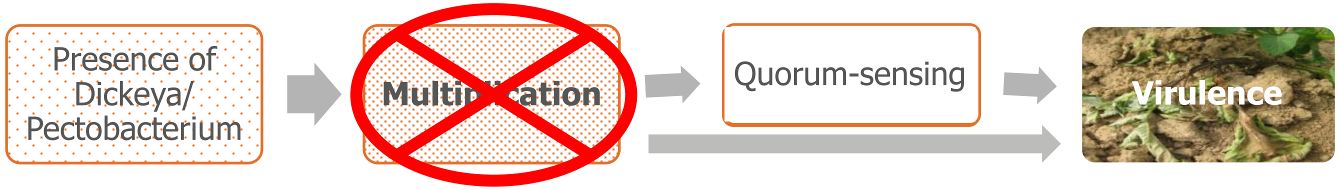
CONTEXT

- Bacterial disease
  - Pectobacteriaceae : ***Pectobacterium*** & ***Dickeya***
    - ***Observed on potato, in France***
  - Blackleg (plant)/soft rot (tuber)
    - *No treatment solution available on the market*

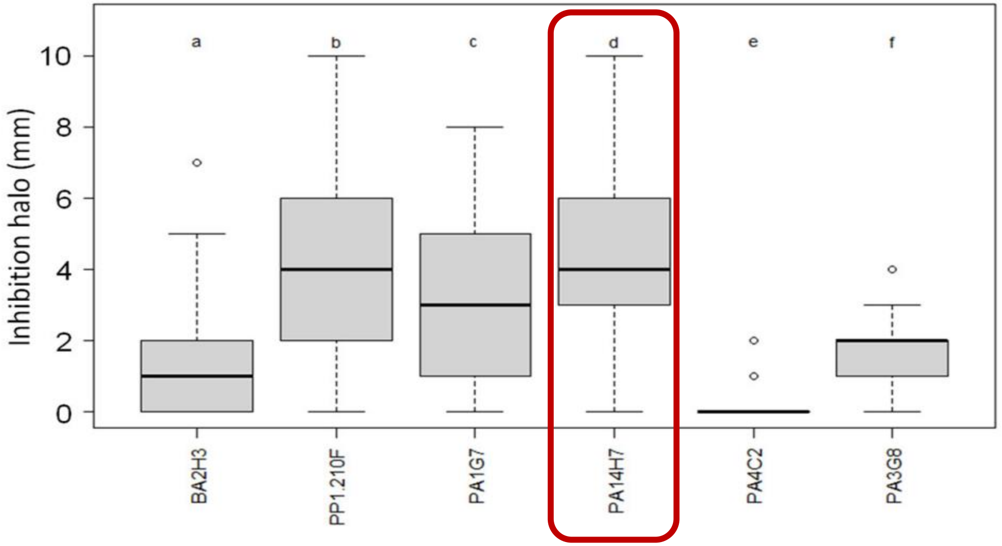


BACTERIA/BACTERIA INTERACTION

- **Previous strategie : Quorum-Quenching** Beury, A. et *al., Appl. Environ. Microbiol., 2012*



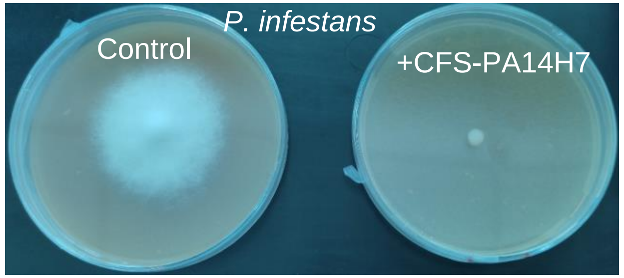
Cigna, J. et *al., Microorganism,* 2023

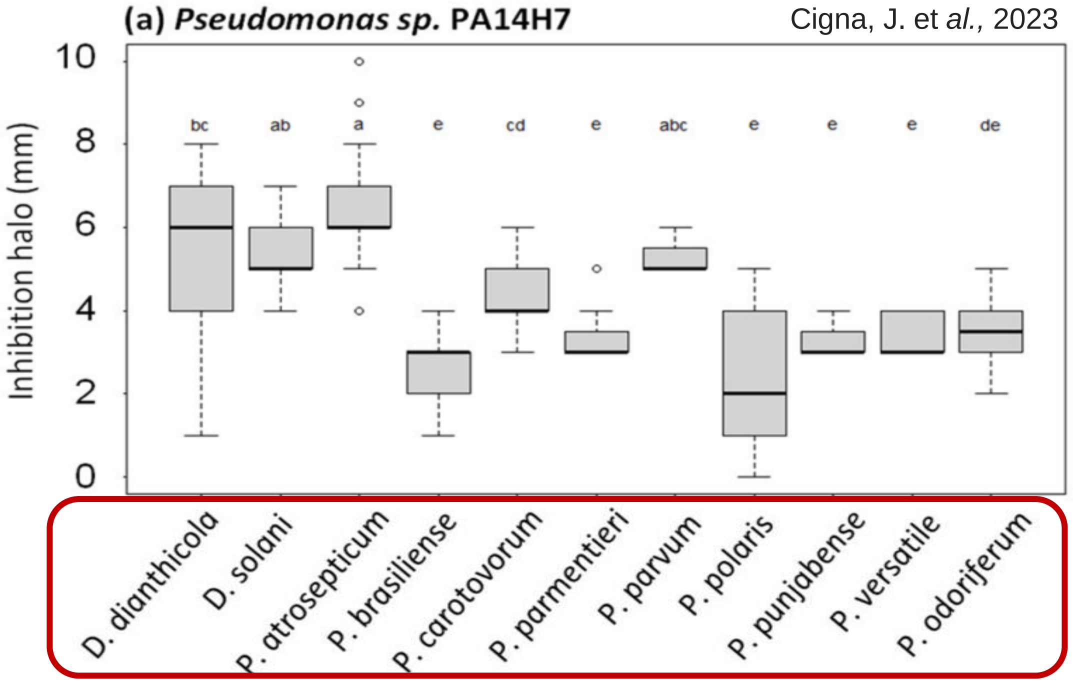


- **Other strategy : Antibiosis**
- **6 antagonists**
- **Choice of PA14H7 !**
  - **Bacteria/bacteria** confrontation test → one of the most efficient

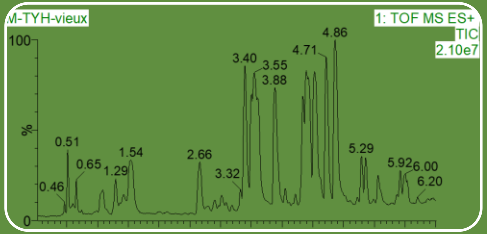
BACTERIA/BACTERIA INTERACTION

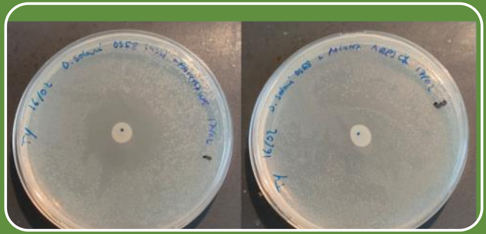
- **Specificity of PA14H7**
  - Large range of species
  - Other pathogens
    - *P. infestans (late blight)*
    - *R. solani (black scurf)*





→ Study of PA14H7 mode of action in order to develop biocontrol methods





Analytic

- Molecule Identification

Genetic

- Mutant construction

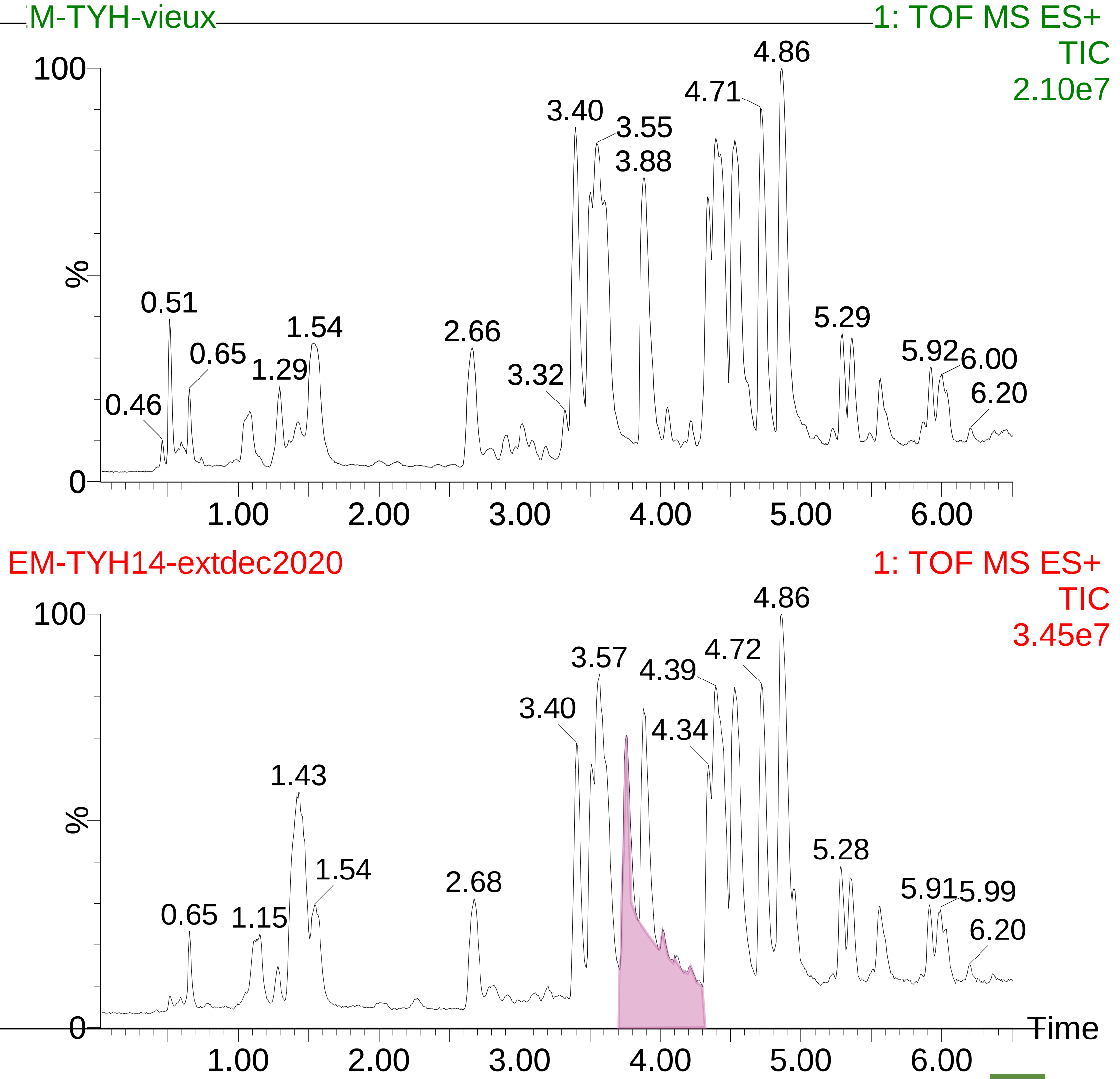
Identification and characterization of metabolite(s) responsible of PA14H7 antagonist activity against Pectobacteriaceae

Analysis of the Cell-Free Supernatant (CFS)

COMPARISON OF TY AND CFS-PA14H7 UPLC-MS PROFILES

Munier-Lépinay, E. et *al.*, *Molecules.*, *2023*

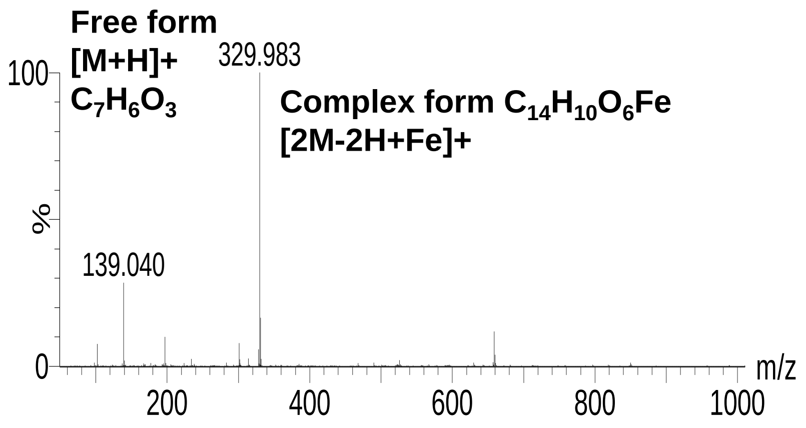
- Extraction of CFS (chloroform or ethyl acetate, pH2 or 7)
  - Antagonist activity → only **organic phase**
- UPLC-MS profile of **organic phase** (chloroform, ph 7)
  - *Column Waters CSH C18, 1,7 µm, 2,1X100 mm*
  - *Gradient water/methanol (0,1 % formic acid)*
- Spectrum of the control TY
  - Medium rich = highly charged spectrum
- Spectrum of CFS-PA14H7
  - Focus on disappearance/appearance
    - *Major difference between 3,8-4,4 min (<!>superposition with another peak)*

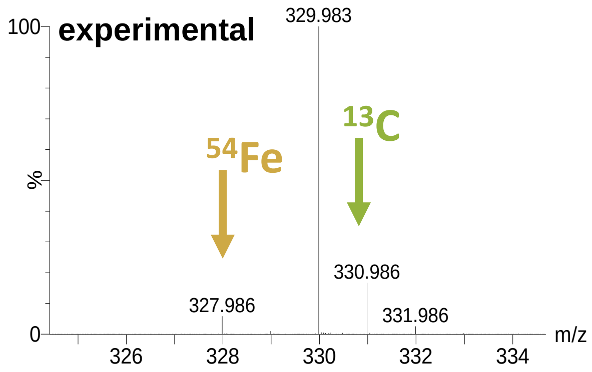


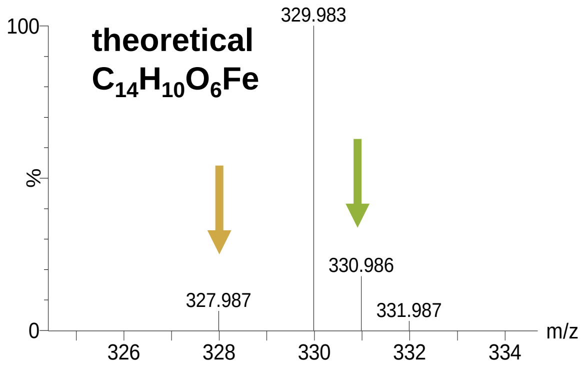
IDENTIFICATION BY HRMS OF THE MAJOR PEAK PRESENT IN CFS-PA14H7 CHROMATOGRAM

- Large peak (3,80-4,40 min) = complex
- LC-HRMS: elemental composition of each ion
  - Organic molecule alone: $C_7H_6O_3$
  - Complexed form: $C_{14}H_{10}O_6Fe$

→ $C_7H_5O_3FeC_7H_5O_3$, m/z 329.983







inov3PT
SEED POTATO
FOR THE FUTURE

ANALYSIS OF PURIFIED FRACTION BY GC-MS AND NMR

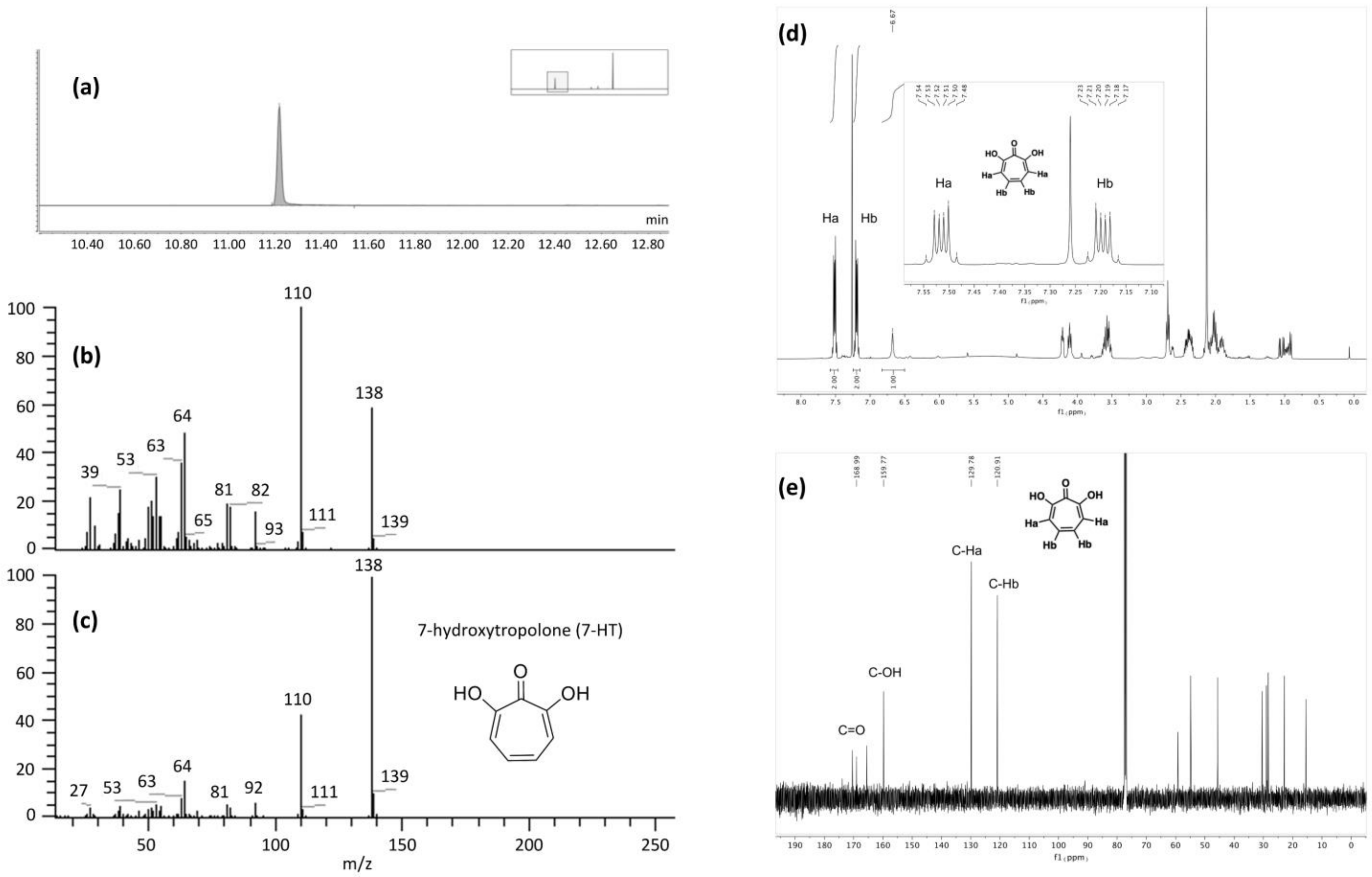
- Flash chromatography
  - Verification of collected fraction by LC-MS
  - Biological activity checking tests
  - Characterization of structure of the organic part of the complex $C_7H_5O_3FeC_7H_5O_3$:
    - *GC-EI*
    - *NMR ^1H and ^{13}C*
- Identification of the molecule

  → **7-hydroxytropolone** (7-HT)

  - $C_7H_6O_3$, MM=138 g.mol^{-1}
- Confirmation after synthesis of the 7-HT

  Directed by Sylvain Laclef based on Winter, N.; Trauner ; D. *J. Am. Chem. Soc.* **2017**, *139*, 11706-11709 et Takeshita, H.; Mori, A. *Synthesis* **1985**, 578-579.

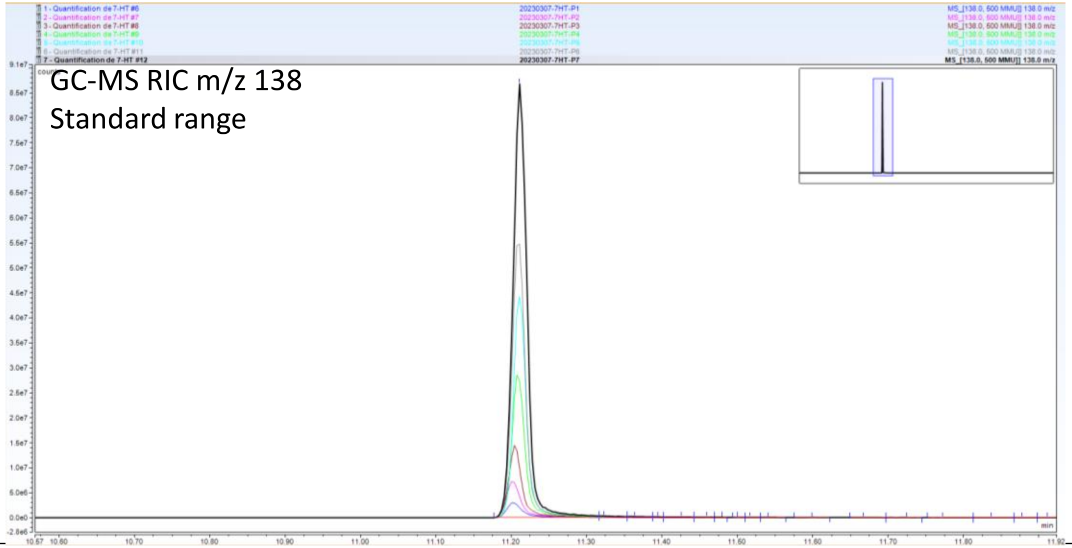
Munier-Lépinay, E. et *al.*, *Molecules.*, *2023*



QUANTIFICATION OF THE 7-HT IN THE CFS-PA14H7

Quantification using different methods:

- Direct UV measurement at DO_{327nm} in CFS
  - Measured concentration: 14.2 mg/L
  - Non-specific method
- Measure after CFS-extraction : LC-UV, LC-MS, **GC-MS**
  - Average concentration: 9 mg/L



Munier-Lépinay, E. et *al., Molecules., 2023*

Table 4. 7-HT measured concentrations in CFS-PA14H7 (mg/L) for each extraction condition, which were processed using LC-UV (λ 320 nm), LC-MS (*m/z* 329.983), and GC-MS (*m/z* 138).

	Analytical Method				
	Extraction Condition		LC-UV	LC-MS	GC-MS
Extraction of CFS PA14H7 [1]	Ethyl acetate	pH 2	8.9	8.5	12.4
		pH 7	5.7	6.0	7.3
	Chloroform	pH 2	7.9	8.2	10.0
		pH 7	10.3	8.5	8.9
Synthetic 7-HT in water (9.6 mg/L)	Ethyl acetate	pH 2	n.d.	8.9	9.7
	Chloroform	pH 7	n.d.	11.3	9.2

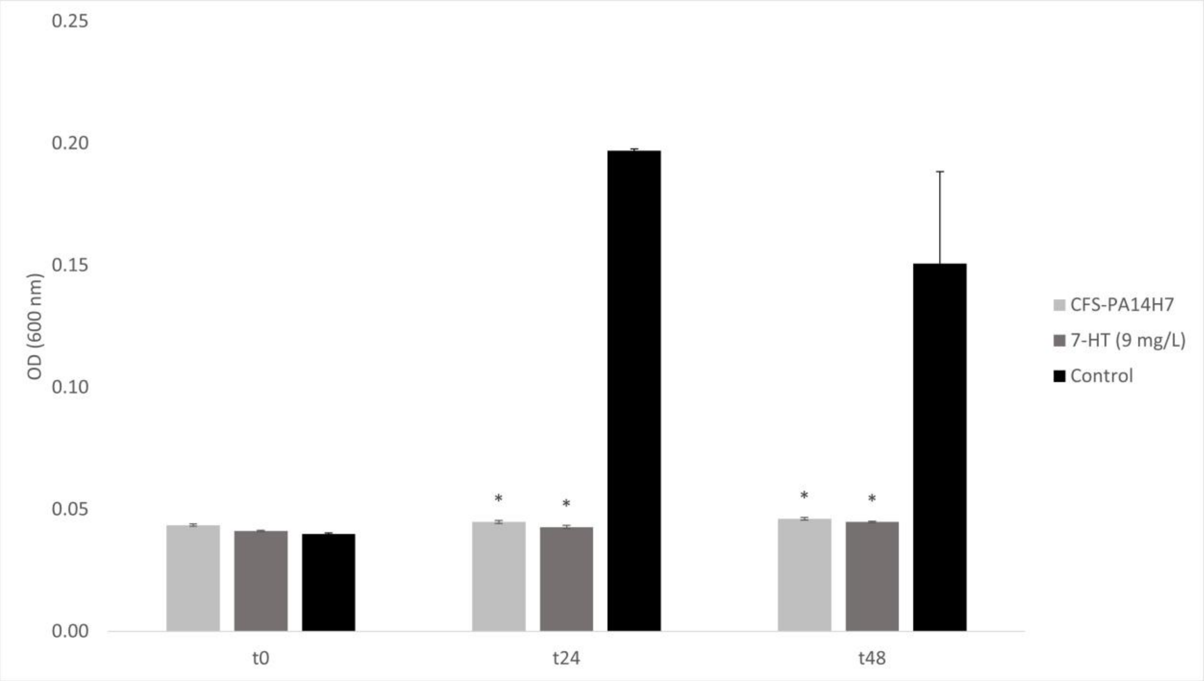
[1] Extraction and quantification were conducted on CFS-PA14H7 obtained from 1 L of PA14H7 culture in TY after 48 h incubation (values expressed in mg/L are a mean value obtained from three replicates). n.d.—not determined.

BIOLOGICAL TEST OF SYNTHETIC 7-HT VS. CFS-PA14H7

- *In vitro* test conducted in microplate :
  - *Dickeya solani*
  - 7-HT at 9 mg/L in water solution
  - CFS-PA14H7
  - Control: water

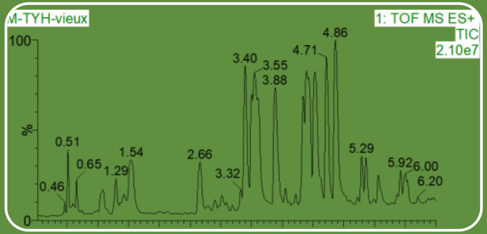
- Results after spreading on Petri dishes
  - Bacteriostatic effect

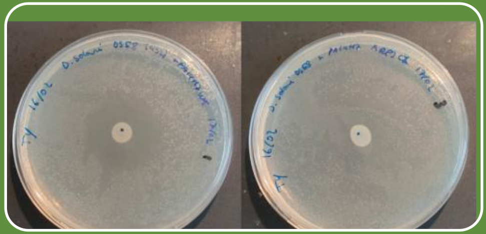
Munier-Lépinay, E. et *al.*, *Molecules.*, *2023*



Dickeya solani growth in TY media containing CFS-PA14H7, 7-HT, or water (control) measured using optical density. Bars are the mean value for three biological assays and the standard errors are represented. (*) Significant difference between the mean values compared to the control according to the Kruskal–Wallis test at $p < 0.05$.

inov3PT SEED POTATO FOR THE FUTURE





Analytic

- Molecule Identification

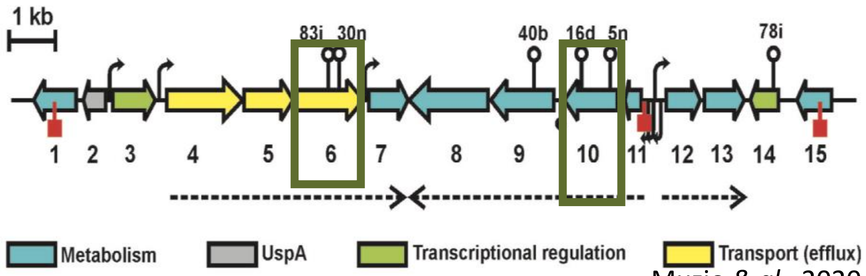
Genetic

- Mutant construction

Validation of PA14H7 antagonism origin using a mutant deficient in 7-HT biosynthesis pathway

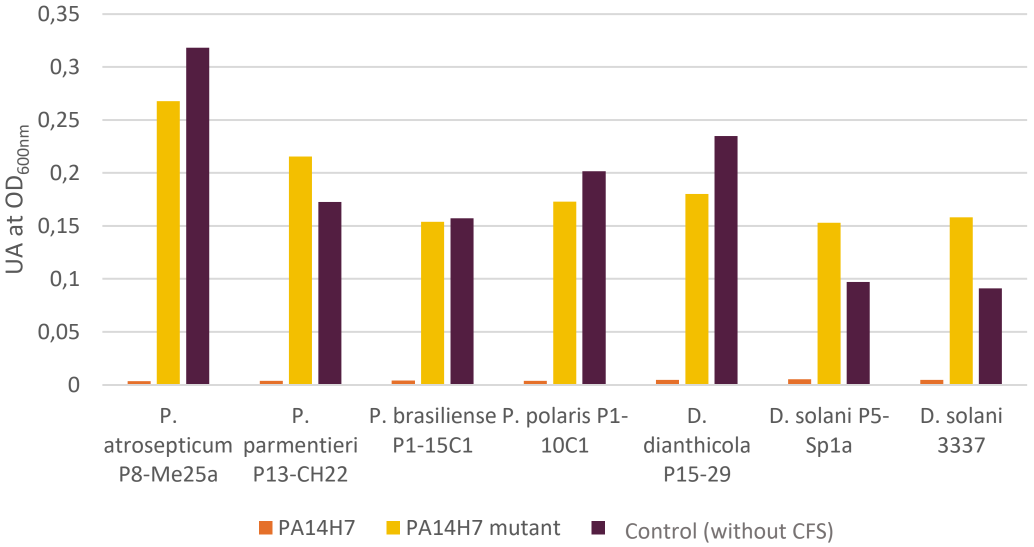
CONSTRUCTION OF A MUTANT OF PA14H7

- Construction of a PA14H7 mutant KO in 7-HT
  - Coline Amaro (01-07/23), M2 inov3PT trainee,
    - *based at Gif-sur-Yvette*
    - *supervised by Denis Faure and Tatiana Timtchenko (I2BC, université Paris-Saclay)*
  - *Pseudomonas* PA14H7 genome includes:
    - ***Biosynthesis of 7-HT pathway (gene 10)***
    - *Transport pathway (gene 6)*
  - Bacteria/bacteria confrontation test
    - *Lost of inhibition of the mutant against D. solani 0432.1 vs. PA14H7 WT*
- *In vitro* test of CFS
  - ***Lost of antagonism effect with CFS of the mutant***



Muzio & *al.*, 2020

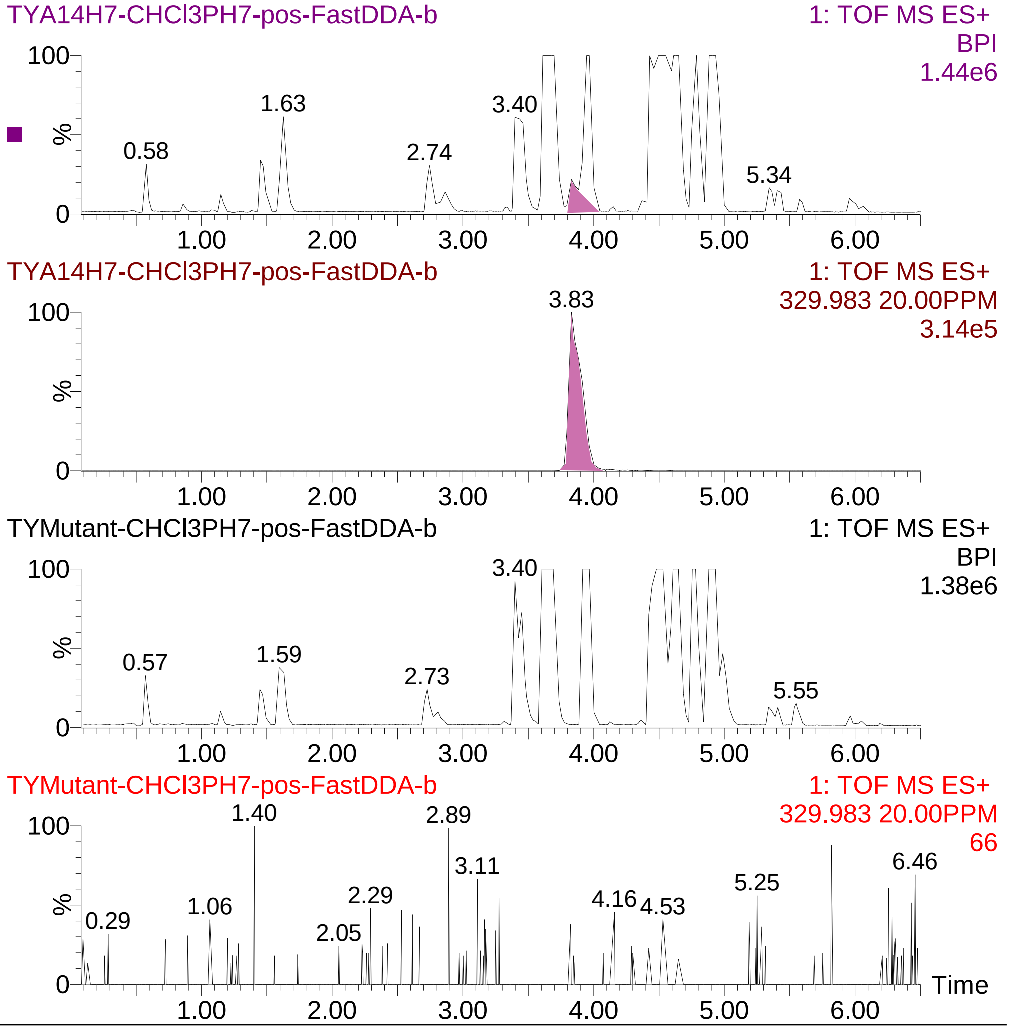
Growth of Pectobacteriaceae depending on the presence of CFS-PA14H7, CFS-mutant in biosynthesis of 7-HT pathway, or water.

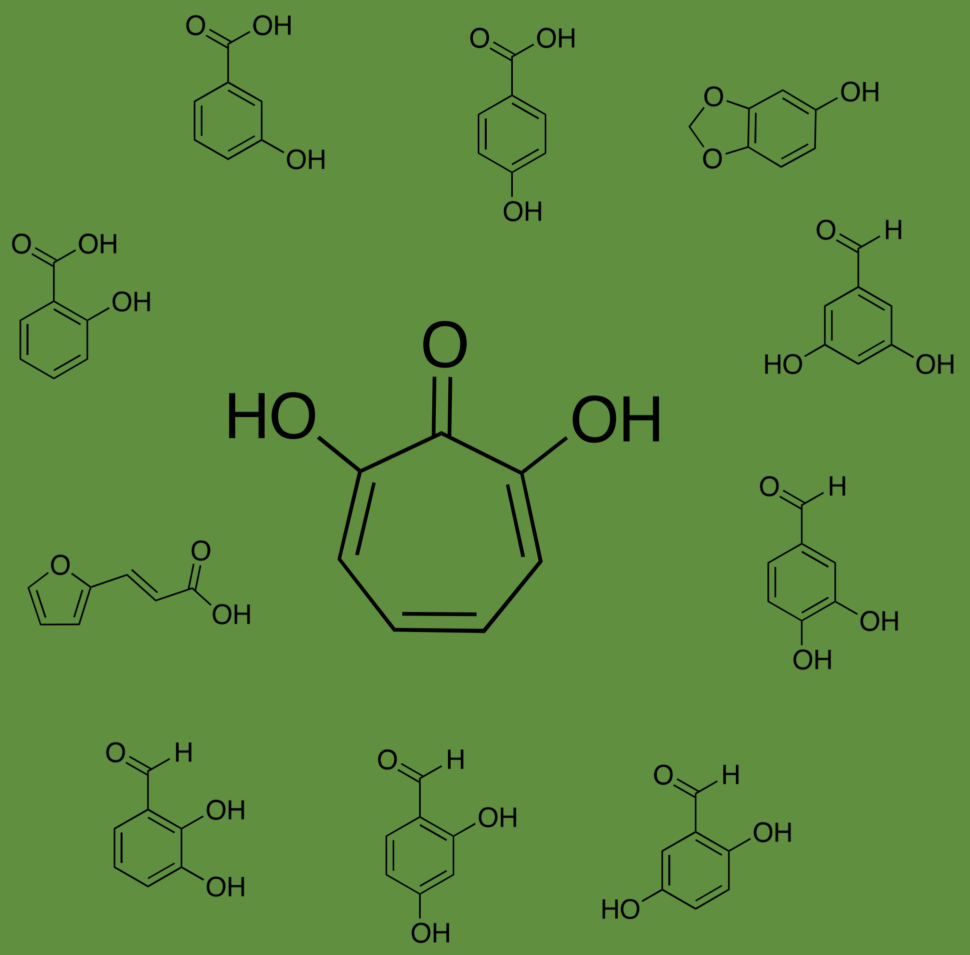


VALIDATION ANTAGONSIM ORIGIN BY ANALYTICAL METHODS

- Extraction of PA14H7 and mutant CFS
- Comparison of LC-MS chromatogram of CFS-PA14H7 vs. CFS-mutant
    - *BPI*
    - *RIC m/z 329,983*
  - Absence of 7-HT as complex iron form in CFS-mutant
- Absence of free 7-HT was confirmed in GC-MS

→ The lost of antagonism activity is linked to the lost of 7-HT production





PERSPECTIVES

- *Increase 7-HT biosynthetic pathway*
- *Comparison with analogue molecules*

THANKS

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Serge Pilard
David Mathiron

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Mounia Khelifa
Jérémy Cigna
Peggy Colson

Coline Amaro
Denis Faure

Anthony Quéro

This work is part of my thesis project (2022-2024). More information: Munier-Lépinay *et al.*, *Molecules*, 2023. https://doi.org/10.3390/molecules28176207

molecules
MDPI

Article

Pseudomonas PA14H7: Identification and Quantification of the 7-Hydroxytropolone Iron Complex as an Active Metabolite against Dickeya, the Causal Agent of Blackleg on the Potato Plant

Euphrasie Munier-Lépinay [1,2,3], David Mathiron [2], Anthony Quéro [4], Mounia Khelifa [1], Sylvain Laclef [3,*] and Serge Pilard [2,*]

1 inov3PT—Recherche Développement Innovation des Producteurs de Plants de Pomme de Terre, 43-45 Rue de Naples, 75008 Paris, France; euphrasie.lepinay@inov3pt.fr (E.M.-L.); mounia.khelifa@inov3pt.fr (M.K.)
2 Plateforme-Analytique (PFA), Institut de Chimie de Picardie FR 3085, Université de Picardie Jules Verne, 33 Rue Saint Leu, 80039 Amiens, France; david.mathiron@u-picardie.fr
3 Laboratoire de Glycochimie, des Antimicrobiens et des Agroressources (LG2A), Institut de Chimie de Picardie FR 3085, Université de Picardie Jules Verne, 33 Rue Saint Leu, 80039 Amiens, France
4 UMRT INRAE 1158 BioEcoAgro, UFR de Pharmacie, Université de Picardie Jules Verne, 1 Rue des Louvels, 80037 Amiens, France; anthony.quero@u-picardie.fr
* Correspondence: sylvain.laclef@u-picardie.fr (S.L.); serge.pilard@u-picardie.fr (S.P.); Tel.: +33-695-937-647 (S.L.); +33-322-828-854 (S.P.)

Citation: Munier-Lépinay, E.; Mathiron, D.; Quéro, A.; Khelifa, M.; Laclef, S.; Pilard, S. *Pseudomonas* PA14H7: Identification and Quantification of the

Abstract: Soft rot *Pectobacteriaceae* (SRP), such as *Pectobacterium* and *Dickeya*, are phytopathogenic agents responsible for blackleg disease on several crops, such as potatoes, affecting the yield and depressing the seed production quality. However, neither conventional nor biocontrol products are available on the market to control this disease. In this study *Pseudomonas* PA14H7, a bacteria isolated from potato rhizosphere, was selected as a potential antagonist agent against *Dickeya solani*. In order to understand the mechanism involved in this antagonism, we managed to identify the main active(s) molecule(s) produced by PA14H7. Cell-free supernatant (CFS) of PA14H7 cultures were extracted and analyzed using LC-MS, GC-MS, and NMR. We further correlated the biological activity against *Dickeya solani* of extracted CFS-PA14H7 to the presence of 7-hydroxytropolone (7-HT) complexed with

Laboratory and field evaluation of bioinsecticides for Colorado potato beetle control

Žigon P., Petek M., Gruden K., Praprotnik E., Modic Š., Dolničar P., Razinger J.

Agricultural Institute of Slovenia
Primož Žigon
primoz.zigon@kis.si

EAPR Pathology & Pests Section Meeting,
Arras FR, September 4-6 2023

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Grant agreement No 771367

Introduction

- Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), is one of the most notorious insect pests of potatoes.
- Larvae and beetles feed on plant tissue causing leaf plant defoliation and stunting.
- The most damaging pest of potato plants since its introduction to Europe in 1922.

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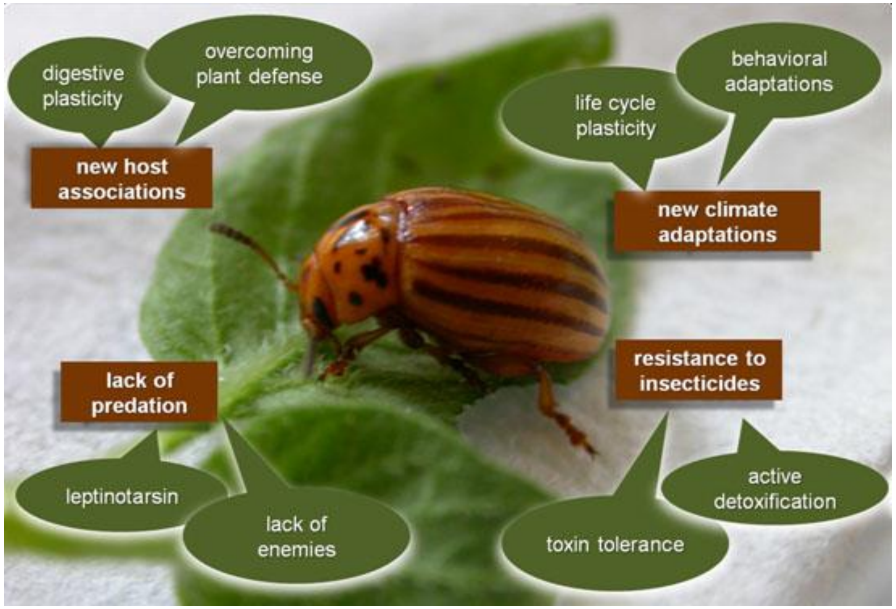
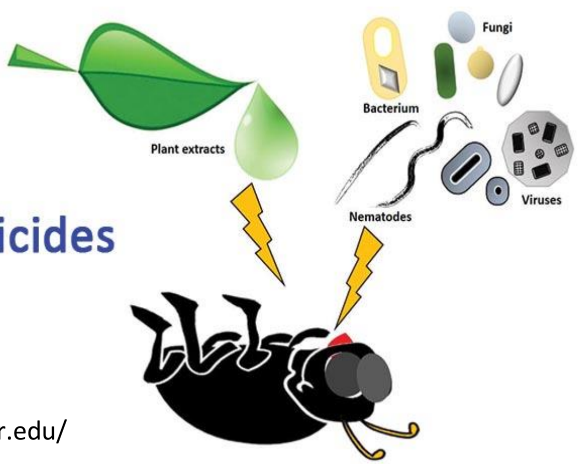
Introduction



Cingel et al., 2016

Biopesticides



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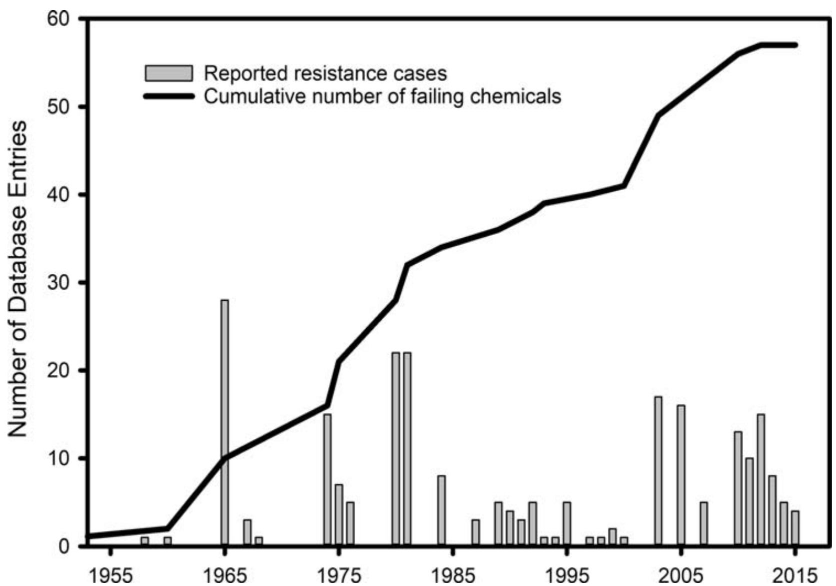

Mota-Sanchez and Wise, 2021

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Research aims and methods

- Alternative IPM strategy on the basis of low-risk plant protection methods.
- Laboratory and field testing of established bioinesticides and novel non-chemical control measures.
- Evaluation of local entomopathogenic fungal isolates against CPB.
- Studies of interactions and potential synergistic effects of biopesticide mixtures.

Treatment	Active substance
Neemazal - T/S	azadirachtin A (1 g/L)
Laser plus	spinosad (480 g/L)
Laser plus 0,2 dose	spinosad (480 g/L)
B. bassiana	*Beauveria bassiana* (KIS isolates 2300 and 2121)
***B. bassiana* + Laser plus 0,2 dose**	*Beauveria bassiana* (KIS isolates 2300 and 2121) + spinosad (480 g/L) 0,2 dose
***B. bassiana* + Neemazal – T/S**	*Beauveria bassiana* (KIS isolates 2300 and 2121) + azadirachtin (1 g/L)
RNAi	RNAi (dsMESH)
Novodor FC	*Bacillus thuringiensis* var. *tenebrionis* (20 g/L 10000 BTTU/g)

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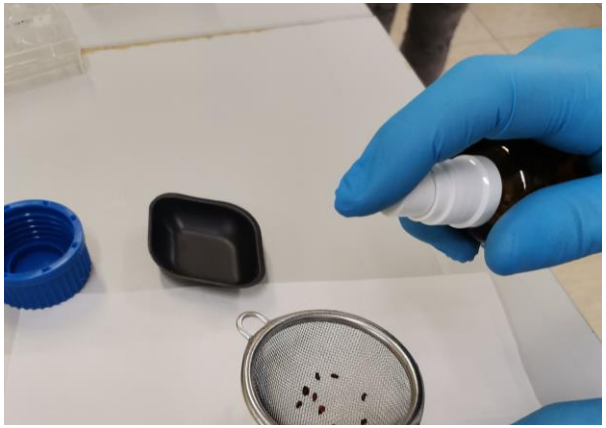

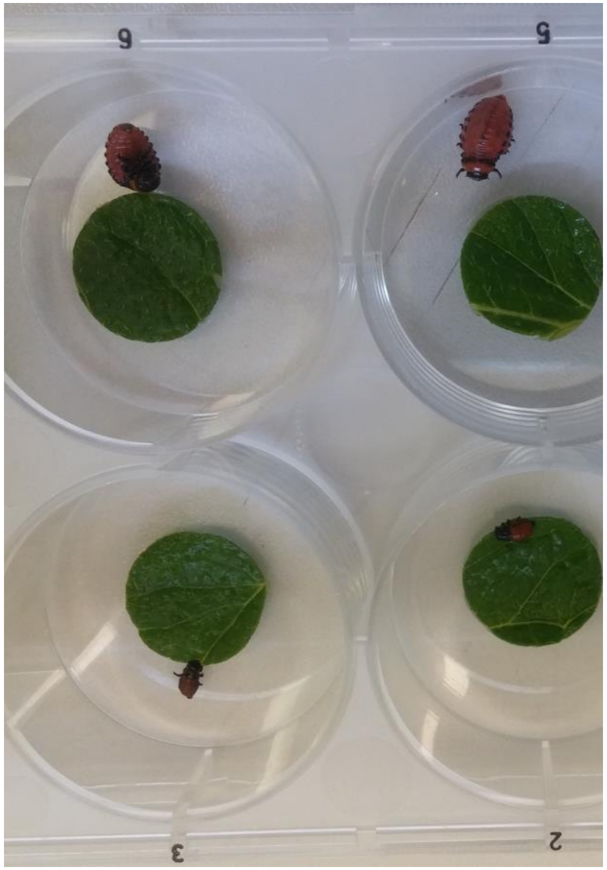

Methodology – laboratory tests

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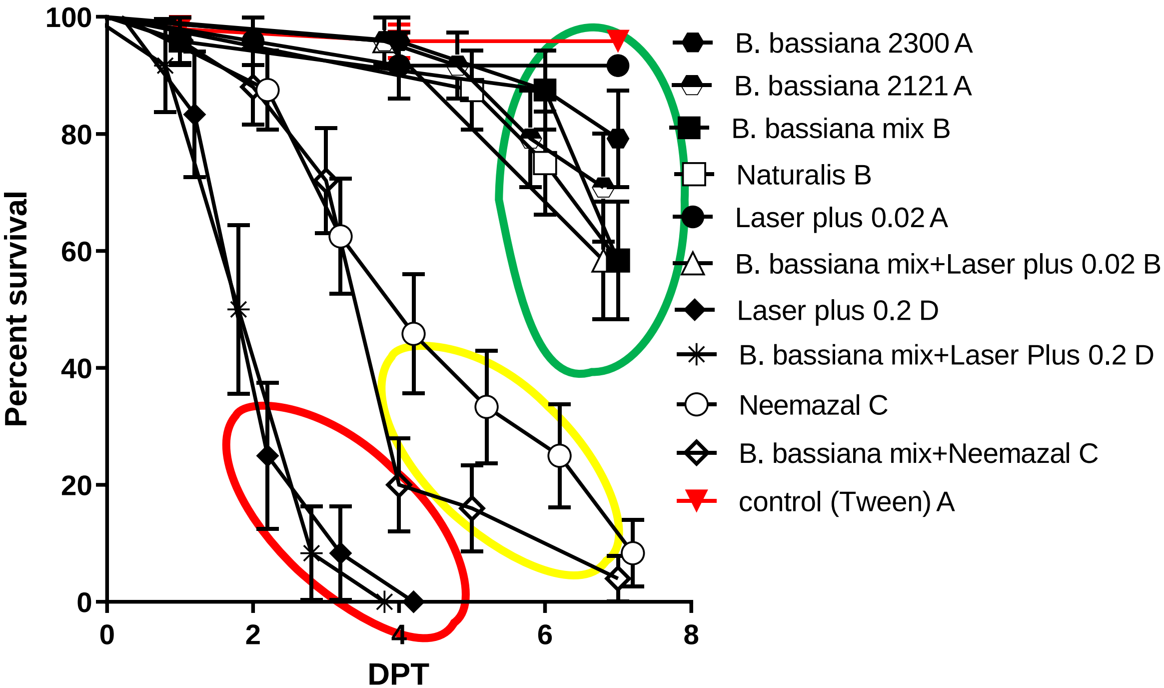
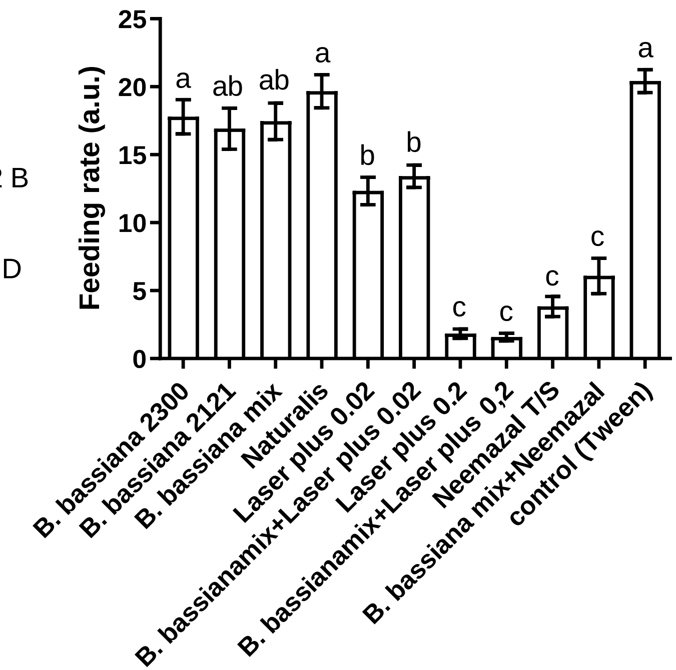
Results – laboratory tests

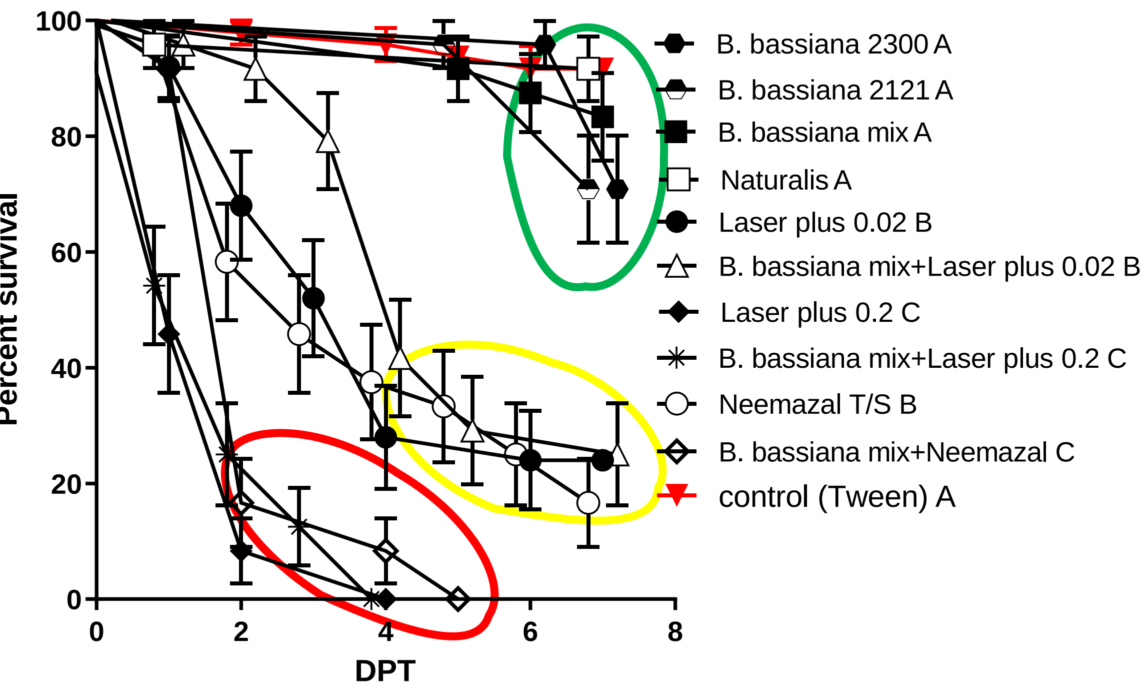
1. Results of larval direct exposure to biopesticides.

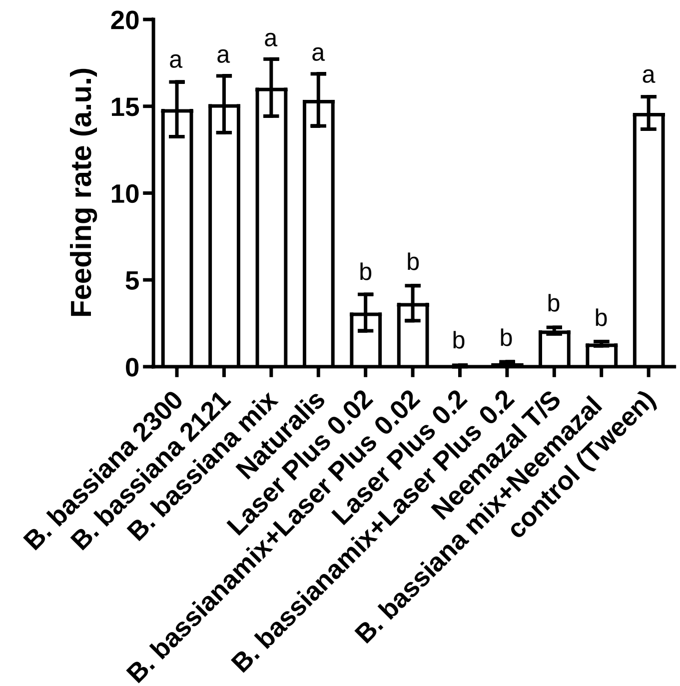




Results – laboratory tests

2. Results of larval indirect exposure to biopesticides.





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Methodology - field tests

- 2020-2022: field experiments conducted in potato field (organic production) at Agricultural Institute of Slovenia, Infrastructure Centre Jablje
- Slovenian potato variety KIS Kokra
- Randomized block design with 6 replicates
- 30 plants/plot = 3 rows with 10 plants/row

- Testing the efficacy of 7 bioinsecticides (and their combinations) against CPB larvae:
    - Laser plus (spinosad)
    - *Beauveria bassiana* (isolates 2300 and 2121)
    - Laser plus + *B. bassiana*,
    - Neemazal T/S (azadirachtin),
    - azadirachtin + *B. bassiana*,
    - RNAi
    - Novodor FC (*B. t.* var. *tenebrionis*)

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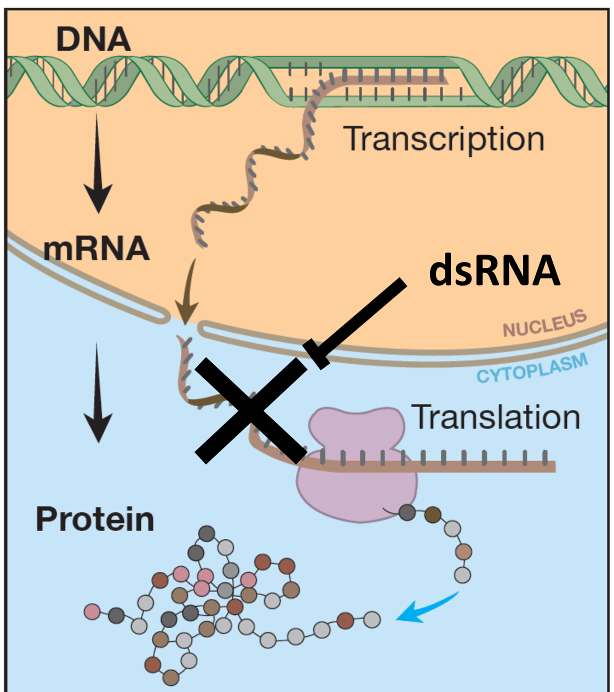
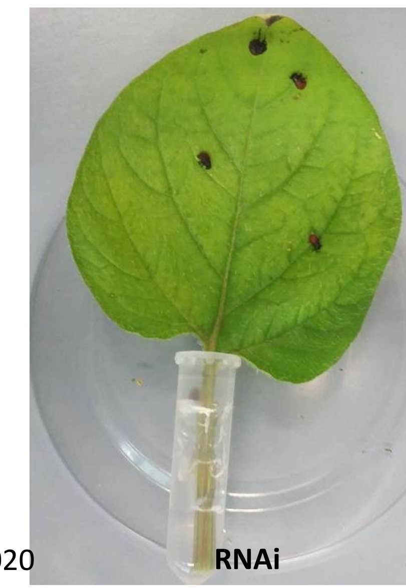
Methodology - RNAi

- Post-transcriptional gene silencing mechanism whereby target gene messenger RNA (mRNA) is neutralized by double-stranded RNA (dsRNA) homologous to the mRNA sequence.
- Use of specific dsRNA to silence CPB mesh gene (dsMESH).



nobelprize.org

Petek et al., 2020



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Metodology – field tests

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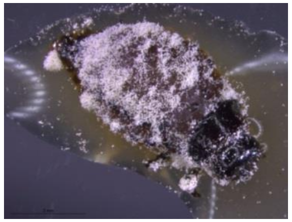
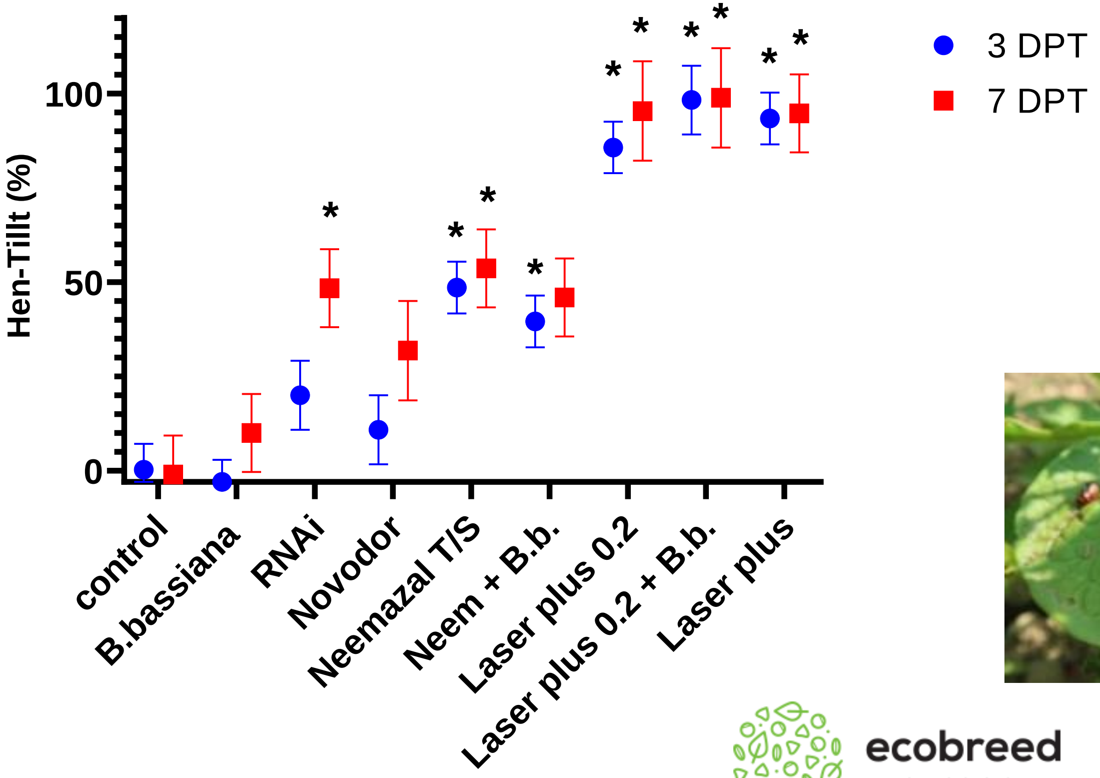
Results – field tests

Effectiveness of individual bioinsecticide expressed as a reduction in number of larvae (3 and 7 days post treatment).





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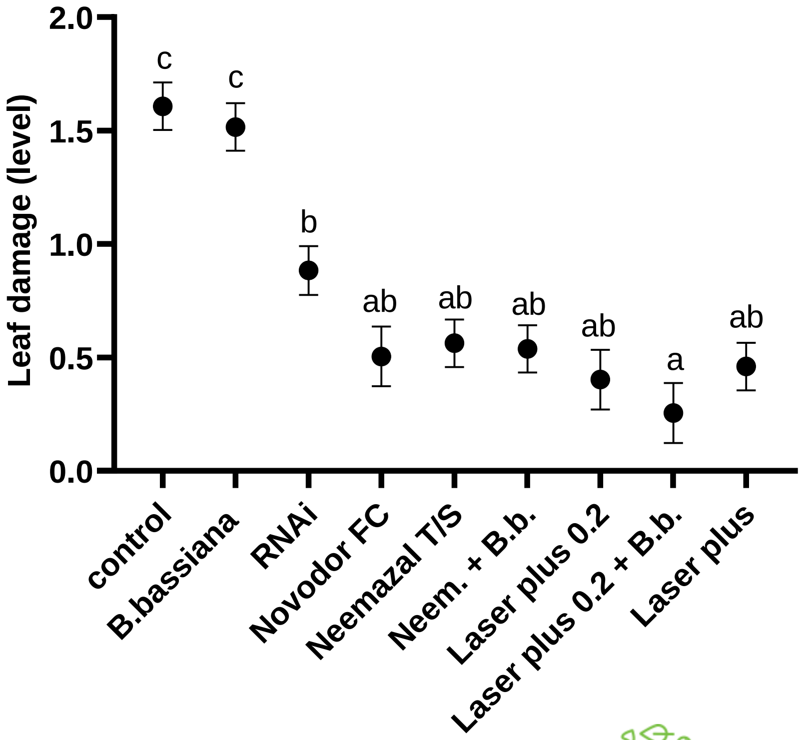
Results – field tests

Effectiveness of individual bioinsecticide expressed as a difference in plant defoliation (7 days post treatment).



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Conclusions

- Treatments with spinosad (Laser plus, Laser plus 0.2 dose and *B. bassiana* + Laser plus) provided significantly better control of larval population compared to all other insecticide treatments.
- A mix of both *B. bassiana* isolates outperformed individual isolates and, combined with a 2% recommended dose of spinosad or 100% dose of azadirachtin outperformed those bioinsecticides alone, in laboratory assays.
- The effectiveness of tested bioinsecticides under field conditions was limited.
- Low direct efficiency -> indirect effect on reduced feeding and leaf damage.

Acknowledgements

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Agricultural Institute of Slovenia

Marko PETEK, Kristina GRUDEN

NACIONALNI INŠTITUT ZA **BIOLOGIJO**
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Grant agreement No 771367

Slovenian Research and Innovation Agency

Microbial diversity associated with potato tuber blemishes

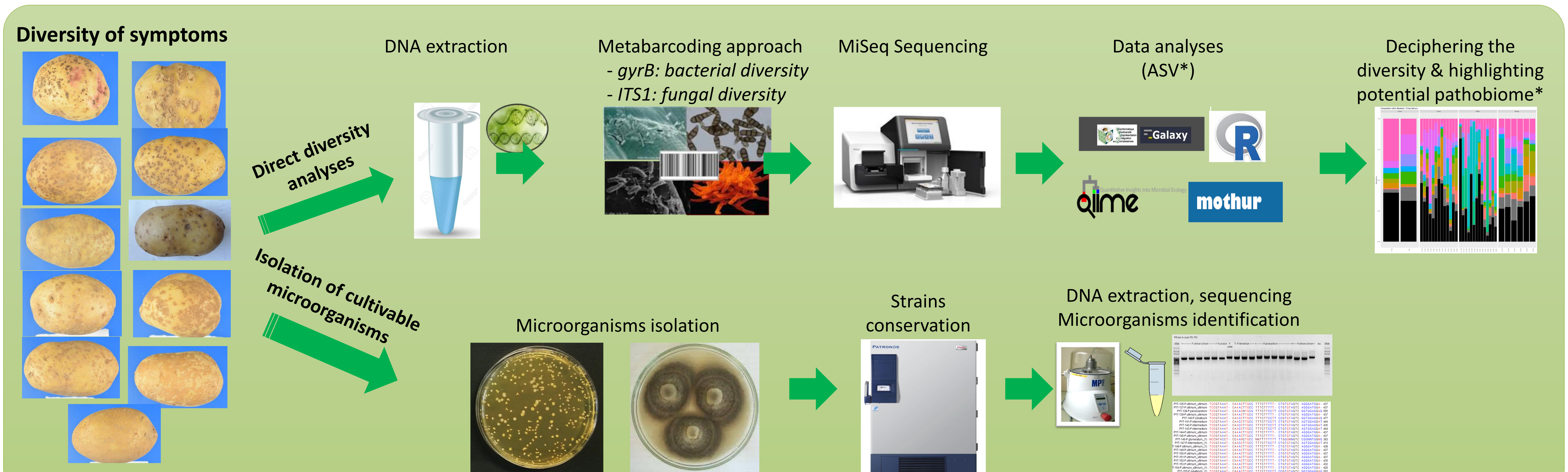
Samir Rezki & Karima Bouchek-Mechiche*

*inov3PT, INRA-IGEPP, domaine de la Motte, Le Rheu, France, karima.bouchek@inov3pt.fr

Potato tubers may be affected by a large range of blemishes among which several are well-studied with clearly identified causal pathogens. However, a number of others we referred to them as atypical blemishes have less clear origin. The objective of this study is to assess the microbiome associated with different blemish symptoms (typical and atypical) using NGS-DNA barcoding method. Cultivable microorganisms (fungi and bacteria including *Streptomyces*) were isolated from different blemishes for further studies.

Methodology approach

Potato lots with typical symptoms (common scab, black scurf, silver scurf, black dot) and atypical symptoms (Rhizoscab, corky lesions, discolorations, elephant hide, etc…) as well as symptomless tubers of each lot were included in these study.

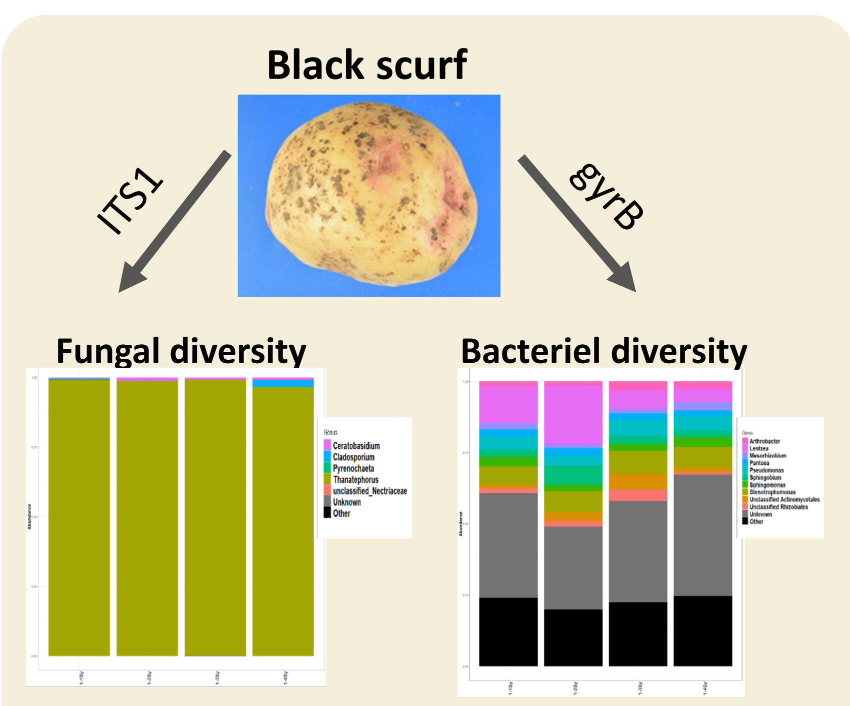


* ASV : Amplicon Sequence Variant = Molecular taxon with sequence clustering of 100% identity

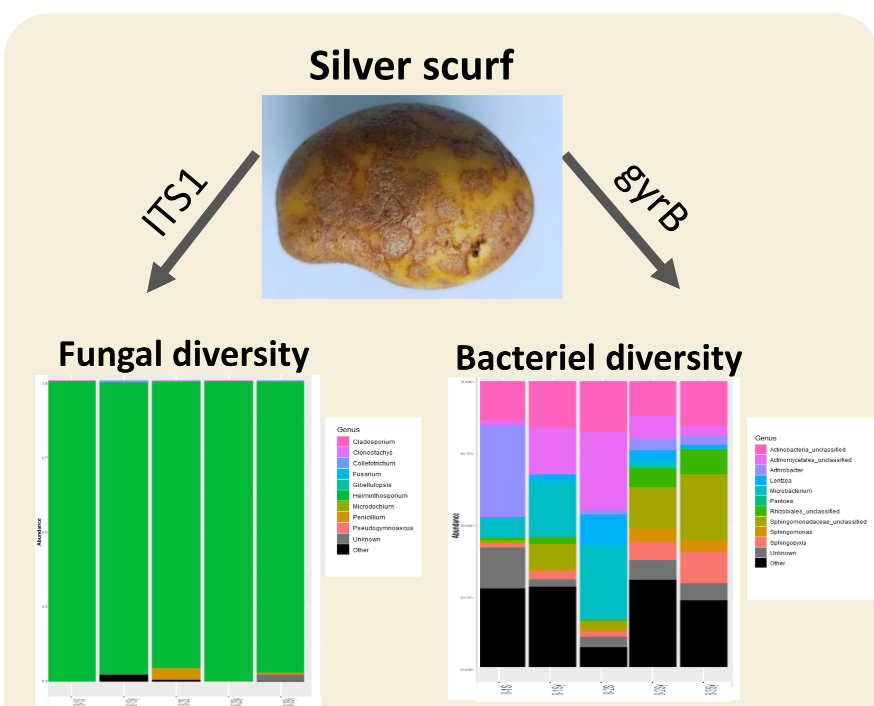
* Pathobiome : pathogenic microorganisms within microbial community associated to a specific environment

Results of microbial diversity using NGS-DNA barcoding

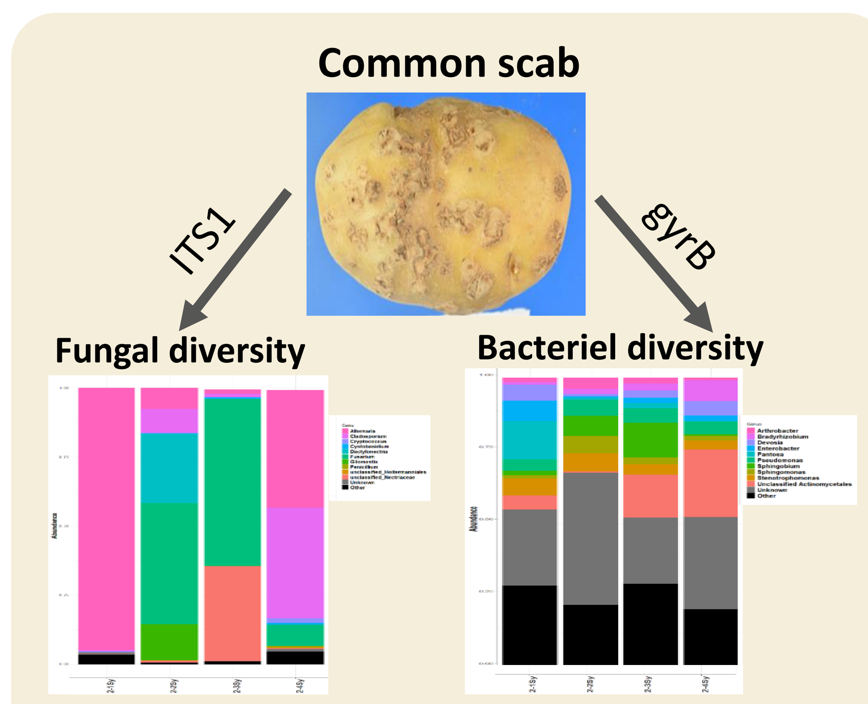
I. Typical symptoms : some examples



- *Thanatephorus (Rhizoctonia) represented 97 to 99% of fungal diversity*
- *High bacterial diversity was associated to black scurf*



- *Helminthosporium solani* represented 96 to 99% of fungal diversity
- High bacterial diversity was associated to silver scurf



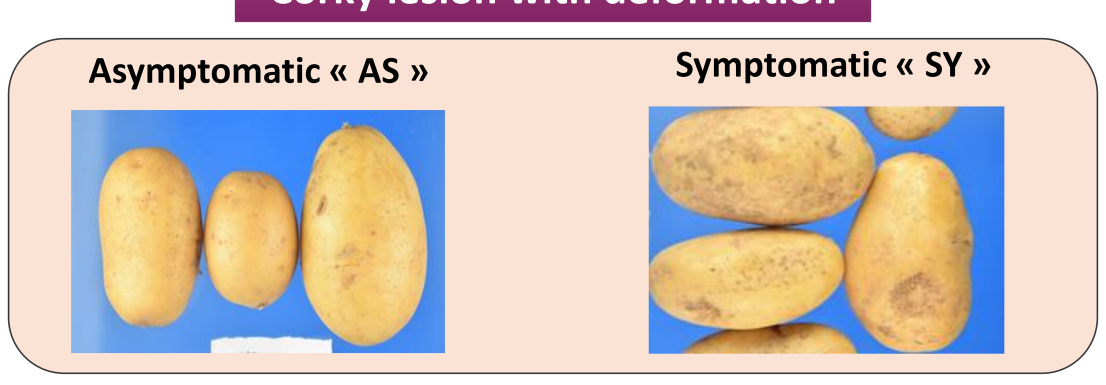
- Bacterial composition contains Actinomycetales which might *be Streptomyces*
- Fungal composition is dominated by *Alternaria, Fusarium* and *Cladosporium*

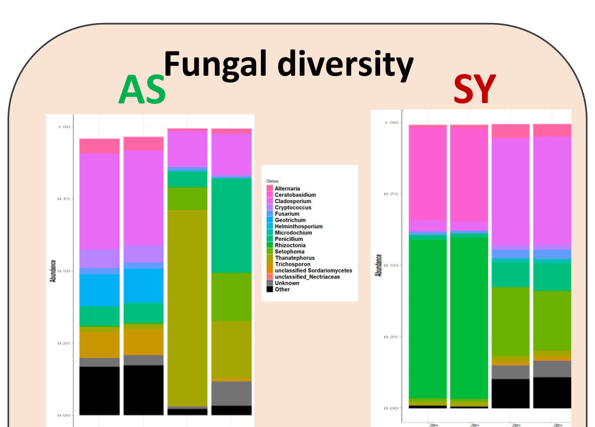
❖ Fungal diseases (Black scurf, black dot & silver scurf) were associated with lower fungal diversity and high abundance of the target pathogen and bacterial diversity

❖ Common scab symptoms were associated with bacterial diversity among which actinomycetal that might contain *Streptomyces* involved in the symptoms

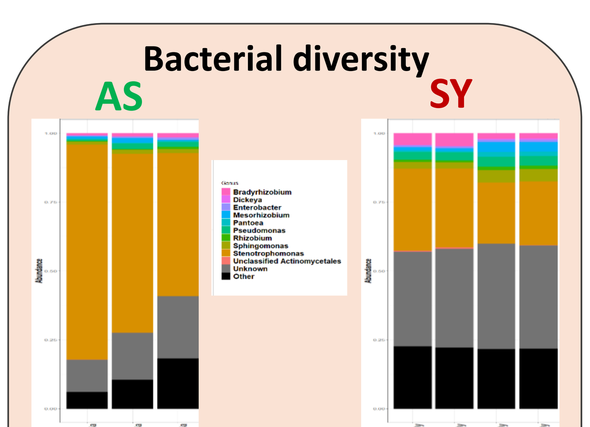
II. Atypical symptoms: some examples

Corky lesion with deformation



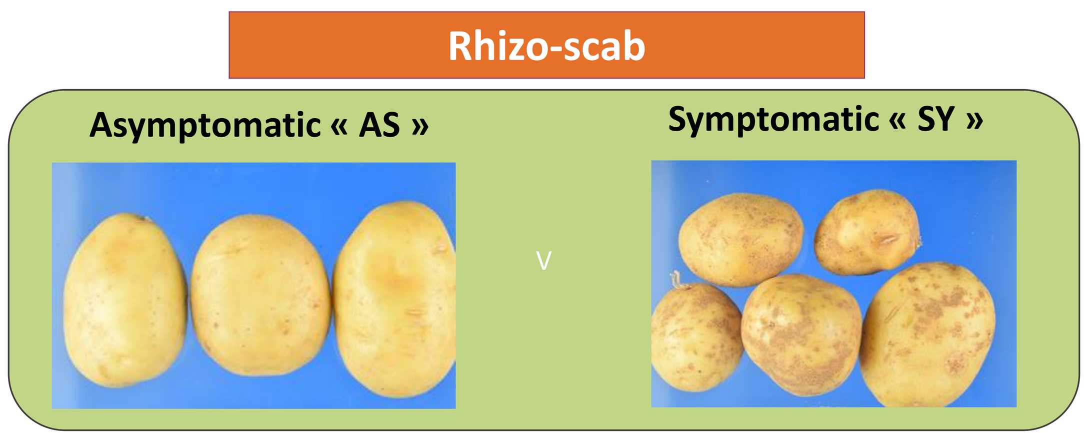


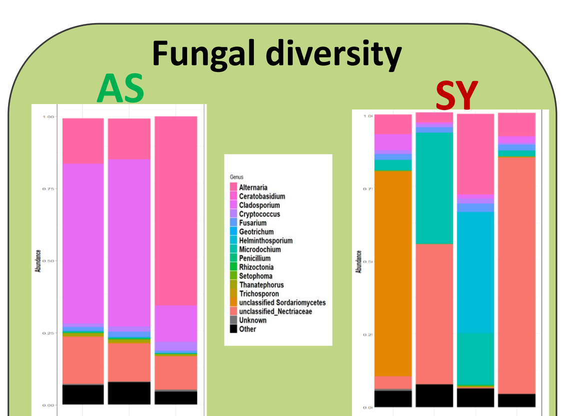
- **AS** : abundance of ***Cladosporium, Cryptococcus***
- **SY** *:* abundance of ***Rhizoctonia*** which might be involved in the symptoms
- Heterogeneity between tubers



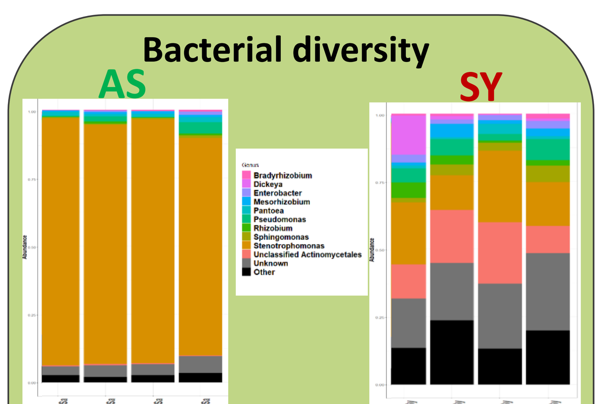
- **AS**: high abundance ***Stenotrophomonas***
- **SY** : higher diversity with lower Stenotrophomonas and abundance of unknown taxa

Rhizo-scab



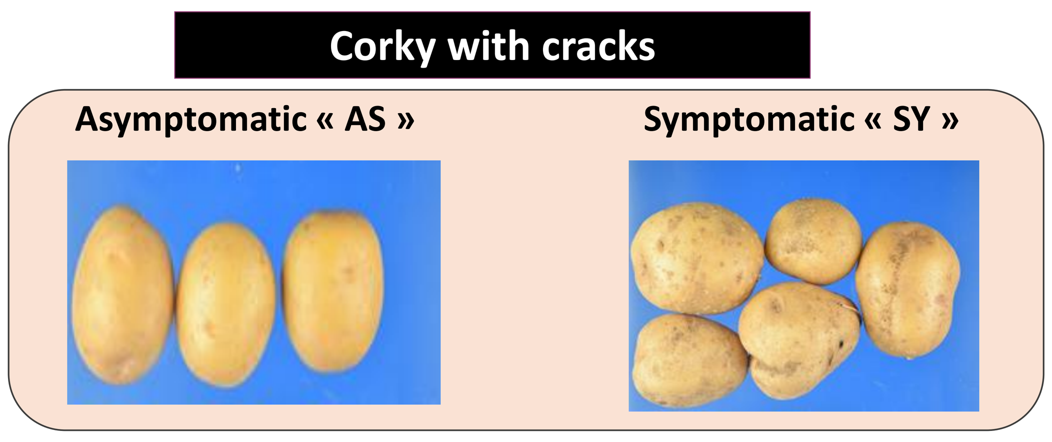


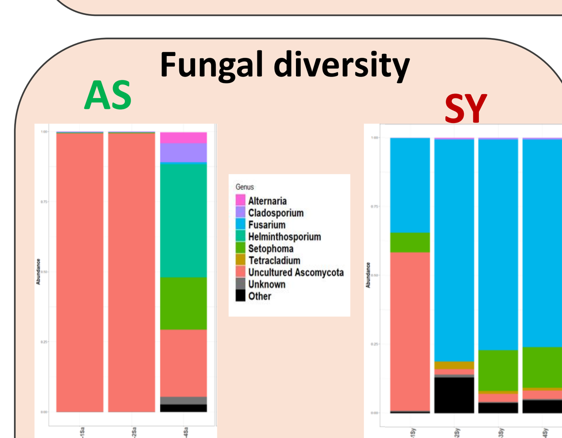
- **AS**: high abundance of ***Alternaira*** and ***Cladosporium***
- **SY**: The fungal composition is diverse and different from that of AS tubers



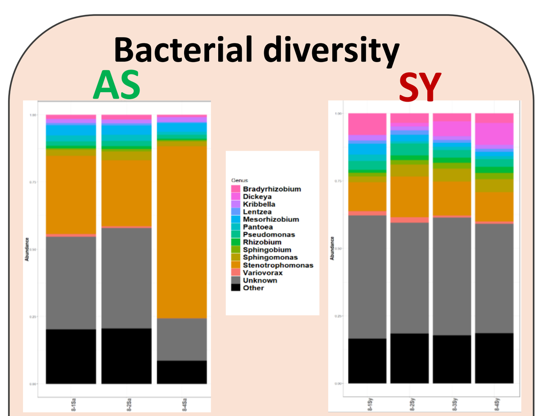
- **AS**: High abundance of ***Stenotrophomonas***
- **SY**: The bacterial composition is diverse with abundance of ***Actinomycetales*** which might be pathogenic *Streptomyces*

Corky with cracks





- **AS** : High abundance of non cultivable *Ascomycota*
- **SY** : ***Fusarium*** is the more abundant genus on symptomatic tubers



- **AS**: high abundance of ***Stenotrophomonas***
- **SY** : higher abundance of other taxa at the expense of ***Stenotrophomonas***

❖ *Alternaria*, *Fusarium*, *Cladosporium* , *Stenotrophomonas*, *Enterobacter* and *Pantoea* were found in abundance on different lots
❖ The microbial composition is different in the presence of symptoms
❖ The comparison between symptomatic and asymptomatic tubers of some atypical blemishes provides hypothesis of potential pathogens
❖ Asymptomatic tubers harboured high abundance of some taxa witch may be potential biocontrol agents (e.g. *Stenotrophomonas*)
❖ Microbial community is not structured according to blemish types (data not shown), it may be linked to geographical origin of the soils or to the varieties
❖ To confirm these results, the isolates collected from different samples will be sequenced and interesting candidates will be inoculated in order to reproduce the symptoms

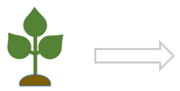
EAPR Pathology and Pests Section Meeting, 3-6 September 2023, Arras (France)

Antifungal evaluation of plant extracts as alternative fungicides for the management of early and late blight in potato crops

Grillon Armand, Gindro Katia, Chevalley Clara, Massana Codina Josep
Plant Protection Division, Mycology Group, Agroscope, Nyon, Switzerland, www.agroscope.ch

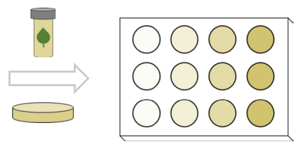
Objectives — The present project aims to investigate the potential of natural extracts from 24 plant species as efficient and environmentally friendly antifungal agents against predominant fungal diseases affecting potato crops, namely early blight (*Alternaria sp.*) and late blight (*Phytophthora infestans*). The antifungal potential of aqueous and methanolic plant extracts has been tested through a series of *in vitro* and *in vivo* analyses within laboratory, greenhouse and field trials.

Plant material extraction



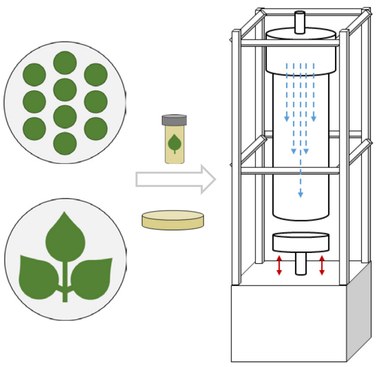
Cold aqueous and methanolic extraction of plant material (i.e. *leaf, root, flower, rhizome, bark*).

In vitro bioassay

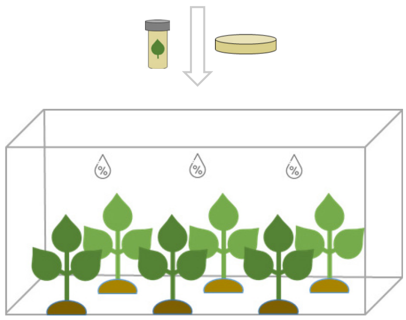


Inoculation of mycelium plugs in **12 multi-well plates** containing different concentrations of plant extracts (0, 5, 10 and 25 mg/ml).

In vivo bioassays

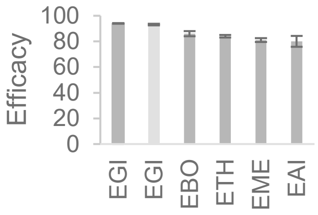


Treatment of **detached leaves** (discs or leaflets) with different concentrations of plant extracts and inoculation of the pathogen with spore suspension.

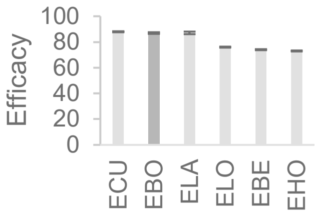


Treatment of **whole plants** in greenhouse under high humidity conditions and in field with plant extracts and inoculation of the pathogen with spore suspension.

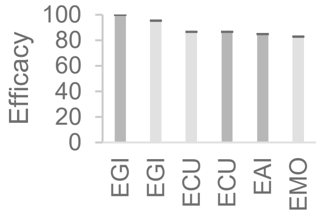
Alternaria solani



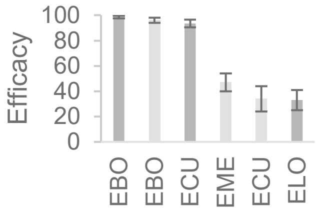
Phytophthora infestans



Alternaria solani



Phytophthora infestans



Preliminary results — Following the *in vitro* and *in vivo* bioassays, three plants (EBO, ECU, EGI) were found to be efficient against the two phytopathogens.

The nature of the extracts, whether aqueous (light grey) or methanolic (**dark grey**), does not appear to be the determining factor in the promotion of antifungal activity.

Further research – Induction of defense mechanisms by plant extracts will be studied in potato leaves using analytical methods.

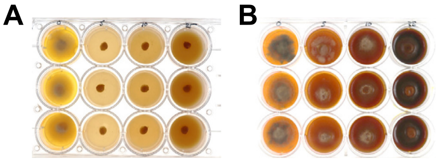


Fig 1 | *In vitro* bioassay results of *Alternaria solani* growth with different concentrations of EGI (A) and EBO (B) methanolic extract.

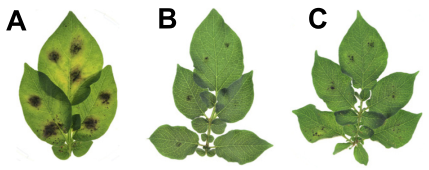


Fig 2 | *In vivo* bioassay results of *Alternaria solani* infection with water (A), commercial fungicide (B) and EGI methanolic extract (C).

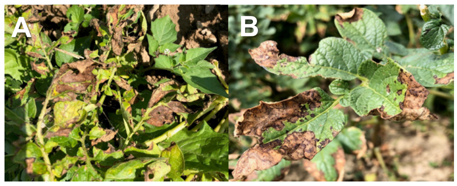


Fig 3 | *In vivo* bioassay symptoms of *Phytophthora infestans* (A) and *Alternaria solani* (B) infections on whole plants in field.

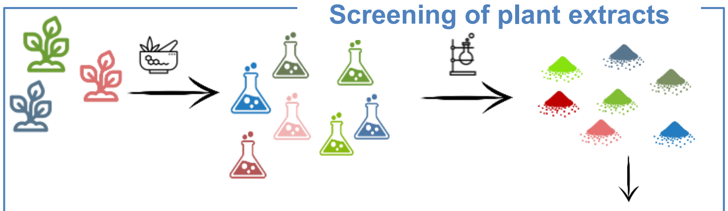
Antifungal activity and post-harvest control of blemish diseases by plant extracts

Stéphanie Schürch[α], Sylvain Schnee[α], Emilie Michellod[α], Clara Chevalley[α], Katia Gindro[α], Emerson Ferreira Queiroz[β], Laurence Marcourt[β], Jean-Luc Wolfender[β] and Josep Massana-Codina[α]

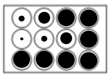
[α] Agroscope, 1260 Nyon, Switzerland; www.agroscope.ch; [β] Université de Genève, Section des Sciences Pharmaceutiques, 1211 Genève, Switzerland

Introduction – Black dot (*Colletotrichum coccodes*) and silver scurf (*Helminthosporium solani*) are diseases caused by two phytopathogenic fungi that induce important economic losses and food waste, specially during storage. Here, we present the results of a screening of plant extracts for their antifungal activity against both pathogens, the isolation of the main antifungal metabolites present in these extracts, and the characterization of their antifungal potential *in vivo*.

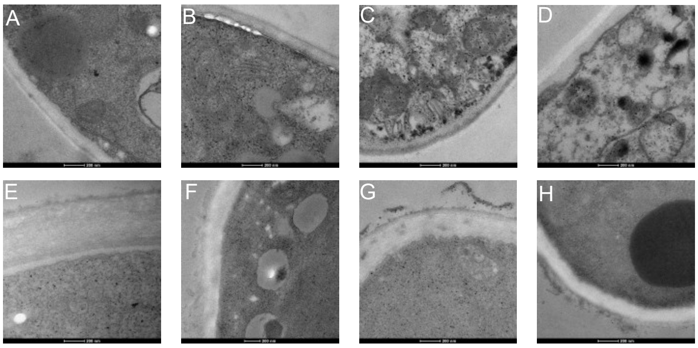
Screening of plant extracts



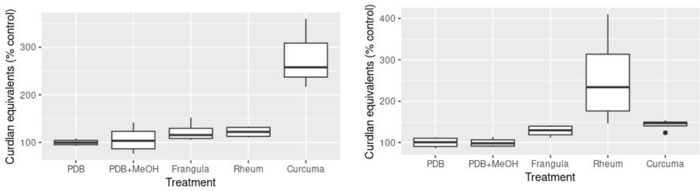
Plants are extracted with a diversity of hydroalcoholic solvents to increase chemodiversity. The extracts are then screened against phytopathogens *in vitro*.



Antifungal activity of plant extracts

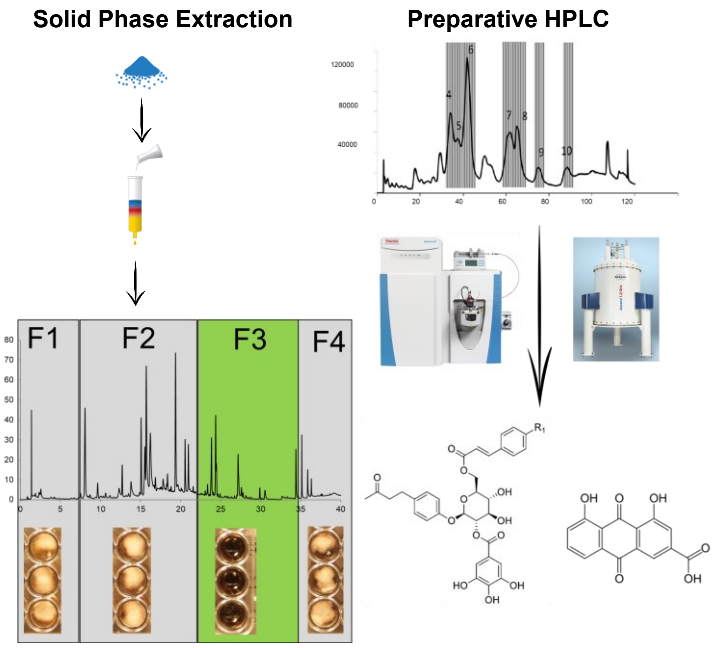


Electron microscopy pictures of *C. coccodes* (A – D) and *H. solani* (E – H) conidia treated with A), E) water, B), F) *F. alnus* aqueous extract (5g/L), C), G) *R. palmatum* methanolic extract (0.5g/L) and D), H) *C. longa* methanolic extract (0.1g/L) for 24 hours.



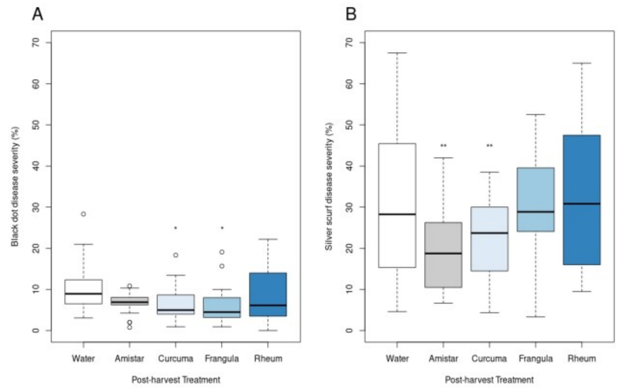
β-1,3-glucan quantification in A) *C. coccodes* or B) *H. solani* fungal propagules treated with PDB, *F. alnus* aqueous extract (5g/L), *R. palmatum* methanolic extract (0.5g/L) or *C. longa* methanolic extract (0.1g/L) for 72 hours. Data expressed as curdlan equivalents.

Bioguided isolation



Two-step purification (SPE + preparative HPLC) permitted the isolation of bioactive metabolites from three plant extracts, including anthraquinones, curcuminoids and phenolic compounds. The identity of the metabolites was confirmed by HRMS and NMR, and their antifungal activity determined *in vitro*.

Post-harvest treatment efficacy



Tubers with black dot and silver scurf symptoms at harvest were treated with the different plant extracts and stored at 6°C for 4 months. Disease severity was assessed after storage. The fungicide Amistar showed efficacy in controlling silver scurf during storage. The plant extract of *Curcuma longa* also showed efficacy against silver scurf. The extracts of *Frangula alnus* and *Curcuma longa* showed highest efficacy against black dot.

Summary and conclusions

- Anthraquinones, curcuminoids and phenolic derivatives isolated from *R. palmatum*, *C. longa* and *F. alnus* possess antifungal activity against *C. coccodes* and *H. solani.*
- Electron microscopy and biochemical analysis showed that the plant extracts affect the cell wall and cellular membranes of the fungal propagules.
- Post-harvest application of plant extracts on potato tubers shows potential in order to reduce the negative effects of black dot and silver scurf during storage

Acknowledgments: Swisspatat, Terralog, Fenaco, Andermatt Biocontrol, Omya AG, Steril Air AG, Bio-fresh, HAFL and CTI for their financial support