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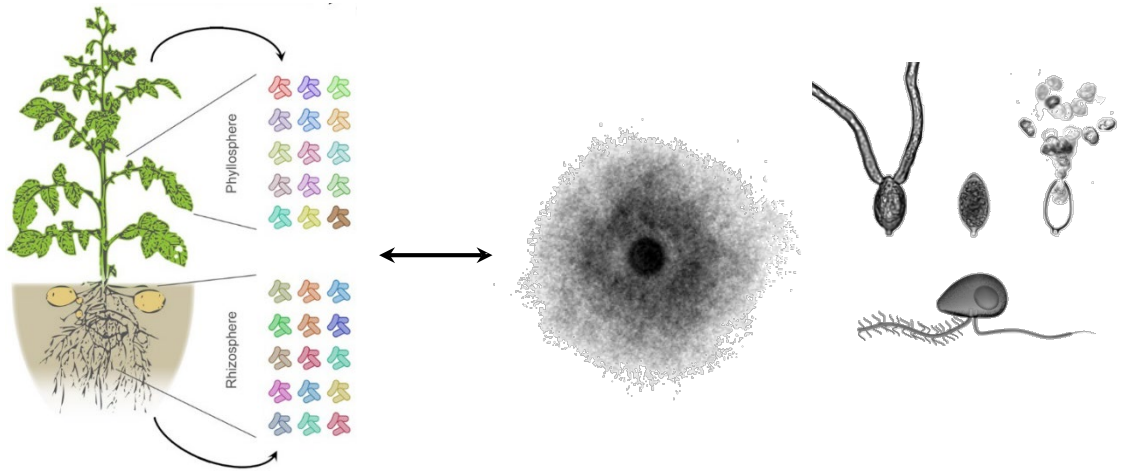
The potential of potato-associated bacteria and their metabolites for late blight control

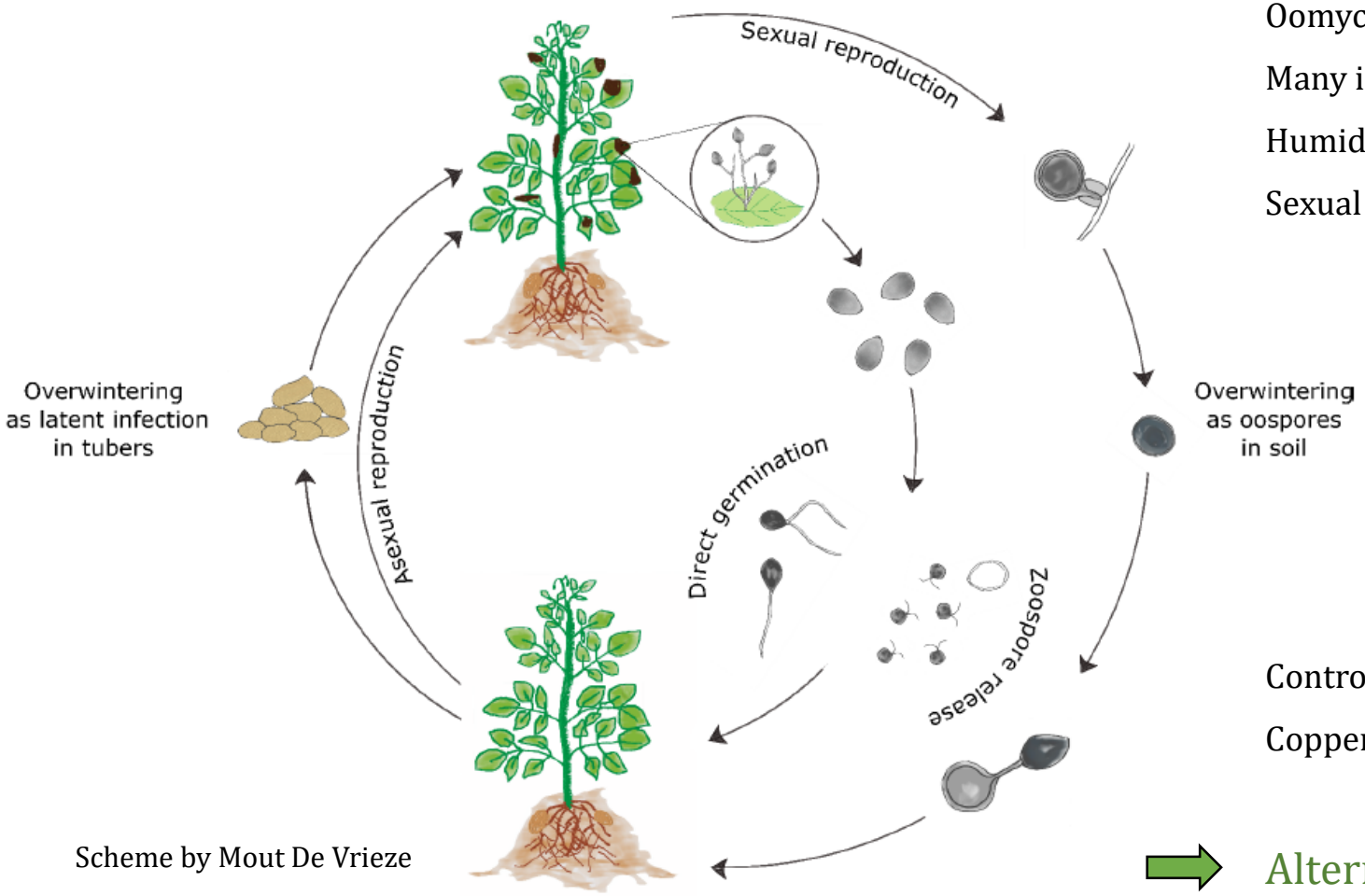


Laure Weisskopf

EAPR meeting, Neuchâtel, 02.09.2019

Our model phytophathogen: *Phytophthora infestans*

Life cycle of potato late blight



Scheme by Mout De Vrieze

Oomycete

Many infection cycles/season

Humidity and 10-25 °C

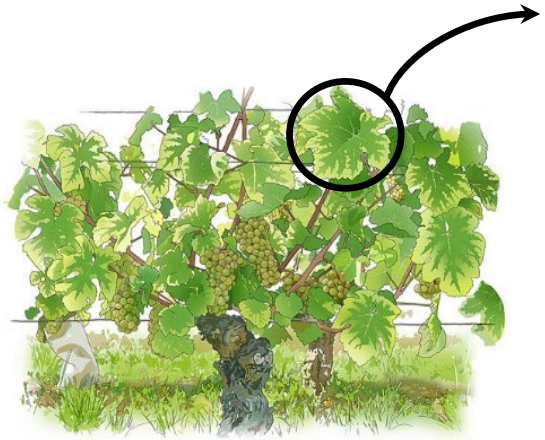
Sexual reproduction

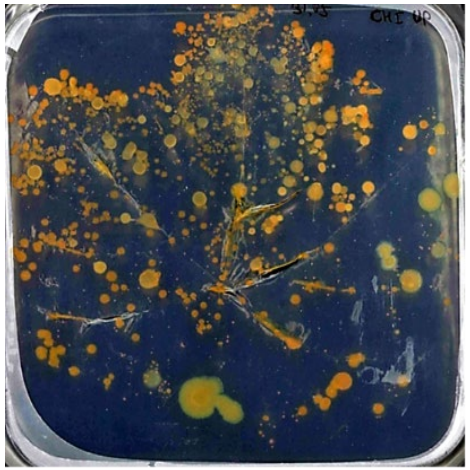
Controlled by synthetic fungicides

Copper used in some countries

Alternative control measures?

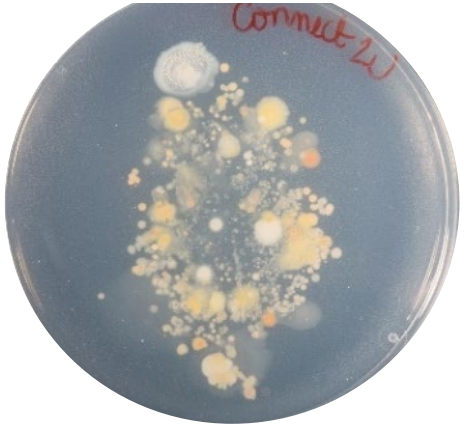
The plant microbiome: a source of natural antagonists ?





Potato leaf microbiome

resistant cultivar



Susceptible cultivar

Microbes can help plants to:

- **Eat** e.g. supplying nitrogen or phosphorus
- **Relax** e.g. lowering ethylene, i.e. stress levels
- **Detox** e.g. breaking down methanol
- **Resist** *e.g. occupying the niche of pathogens*
  e.g. inducing plant resistance
  e.g. producing antimicrobial compounds
- **...**

Non volatile compounds

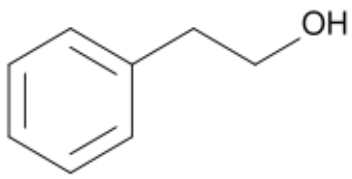
Volatile compounds

Bacterial volatiles: diverse scents and chemical structures

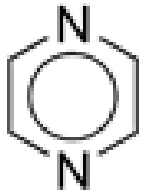
2-undecanone

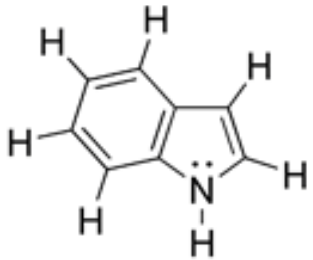
2,3- butanediol

2-phenylethanol



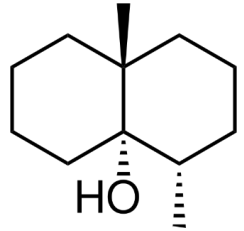
Pyrazine



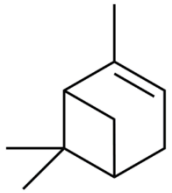


Indole

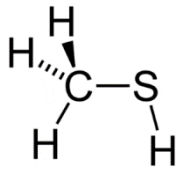
Geosmin
(4,8a-dimethyldecalin-4a-ol)



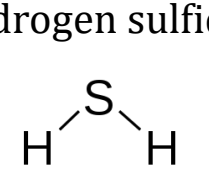
Alpha-pinene



Methanethiol

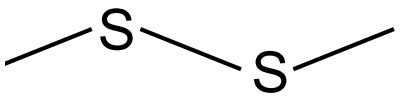


Hydrogen sulfide

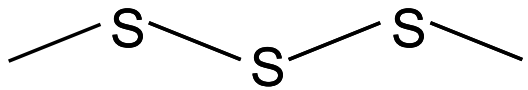


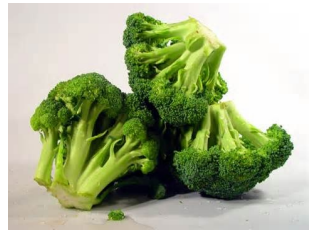
Dimethyl sulfide

Dimethyl disulfide

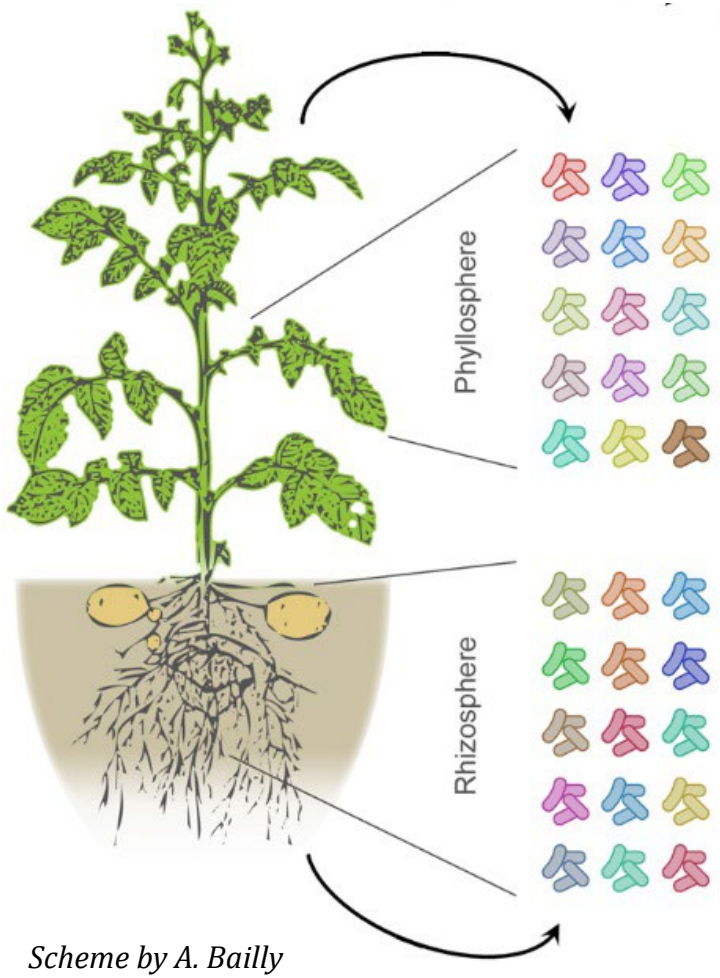


Dimethyl trisulfide





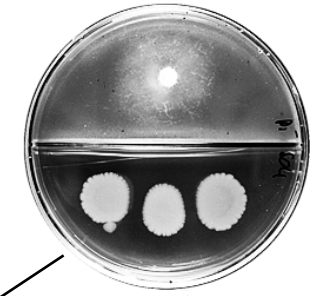
Isolation and screening of the potato microbiome



Scheme by A. Bailly

***In vitro* screen**
for volatile- mediated activity
towards five potato pathogens

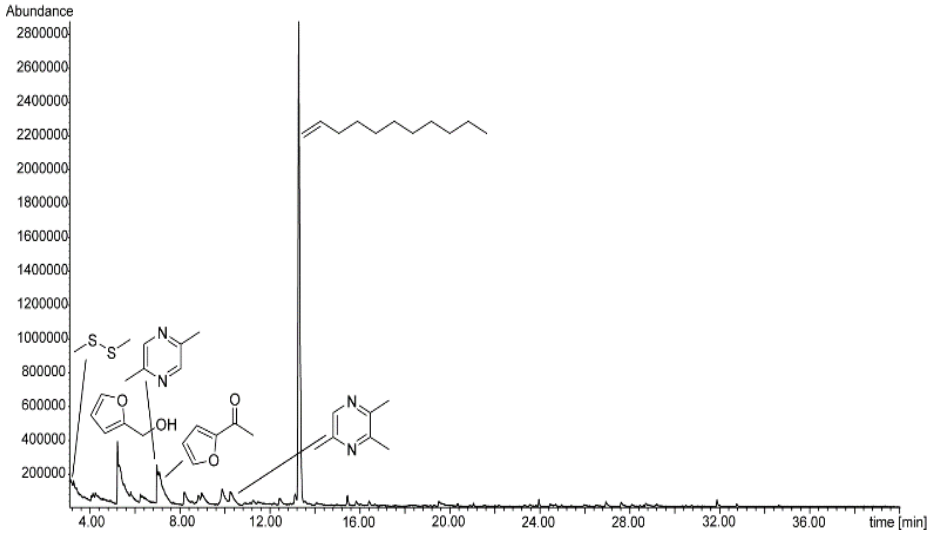
Selection of active strains:
mostly *Pseudomonas*



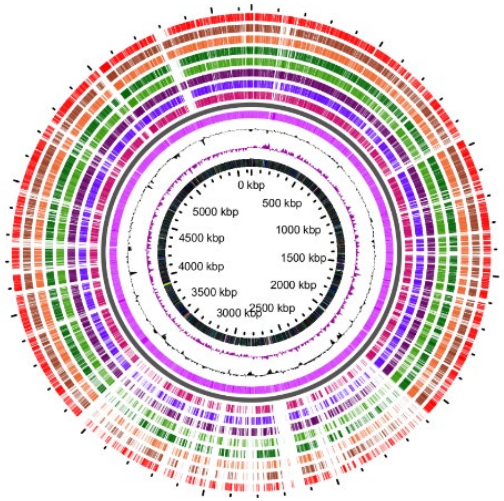
16 strains

10 strains

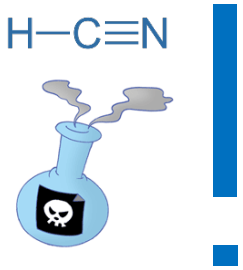
Volatile analysis



Genome sequencing



Phytophthora's delicate nose



Bacterial isolates		*Phytophthora*	*Helminthosporium*	*Rhizoctonia*	*Fusarium*	*Dickeya*
R32	*Pseudomonas vranovensis*	**0**	**53**	76	88	**41**
R47	*Pseudomonas chlororaphis*	**0**	**59**	96	99	**38**
R84	*Pseudomonas marginalis*	**0**	**40**	**87**	97	**37**
R82	*Pseudomonas marginalis*	**0**	71	**73**	102	**53**
S50	*Pseudomonas moraviensis*	**3**	**39**	109	98	**52**
S49	*Pseudomonas fluorescens*	**3**	**55**	104	101	**56**
R76	*Pseudomonas fluorescens*	**13**	**50**	**69**	98	**70**
R01	*Pseudomonas moraviensis*	**0**	**33**	92	100	**39**
S24	*Pseudomonas frederiksbergensis*	**21**	**70**	**86**	99	100
S35	*Pseudomonas marginalis*	**30**	72	90	97	113
R54	*Bacillus subtilis*	64	85	108	98	123
S01	*Streptomyces sp.*	98	**65**	95	101	133
S06	*Pseudomonas frederiksbergensis*	**49**	**81**	**77**	**98**	121
S27	*Arthrobacter sp.*	**50**	86	95	**97**	106
S04	*Pseudomonas frederiksbergensis*	**58**	77	**59**	101	77
S22	*Pseudomonas syringae*	**86**	**42**	110	101	**136**
S19	*Pseudomonas frederiksbergensis*	**62**	**63**	**83**	98	**164**
S34	*Pseudomonas jessenii*	76	87	98	97	121
R29	*Bacillus sp.*	**32**	**39**	91	101	99
S46	*Curtobacterium flaccumfaciens*	108	**62**	103	103	128
R60	*Arthrobacter sp.*	**75**	**59**	98	103	113
R02	*Pseudomonas veronii*	**61**	93	**82**	96	91
R96	*Flavobacterium sp.*	**57**	90	98	101	93
R95	*Pseudomonas lini*	87	**60**	92	99	121
S25	*Curtobacterium sp.*	96	100	**82**	101	91
R74	*Pseudomonas frederiksbergensis*	**35**	79	**77**	99	105
R73	*Bacillus subtilis*	**79**	80	109	101	128
R75	*Pseudomonas frederiksbergensis*	**15**	73	91	**96**	**151**
R42	*Microbacterium sp.*	77	**39**	102	101	145
R31	*Sporosarcina sp.*	**82**	**56**	97	101	106
R61	*Arthrobacter oryzae*	**66**	**72**	**115**	100	123
R85	*Rhodococcus sp*	**75**	89	96	99	**181**

Growth inhibition

Growth promotion

Yuck, can't breathe!!

Don't like it!

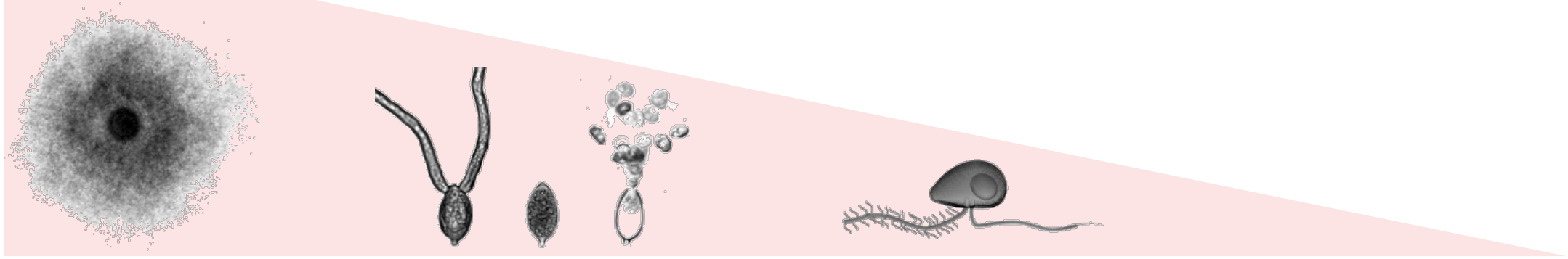
I've seen worse...

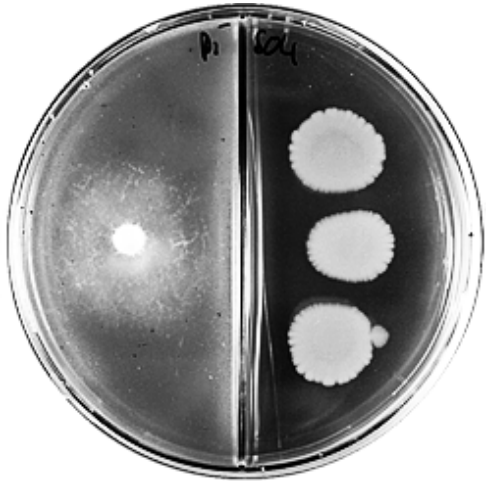
Do you smell anything?

Yummy!!

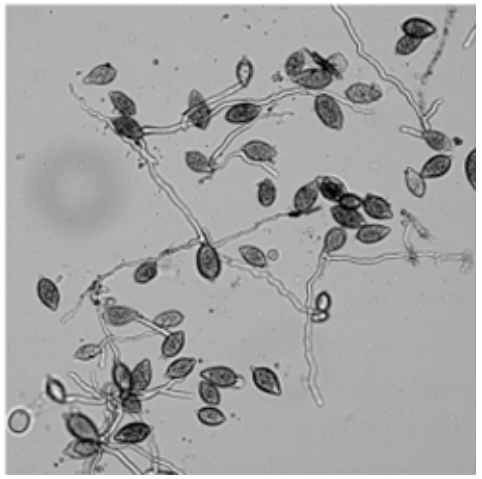
Searching for anti-*Phytophthora* volatiles

Robustness

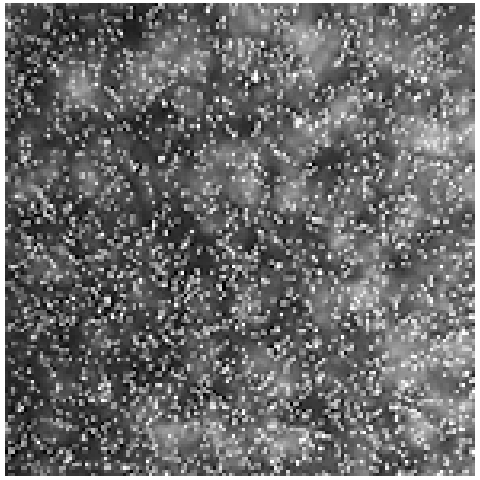




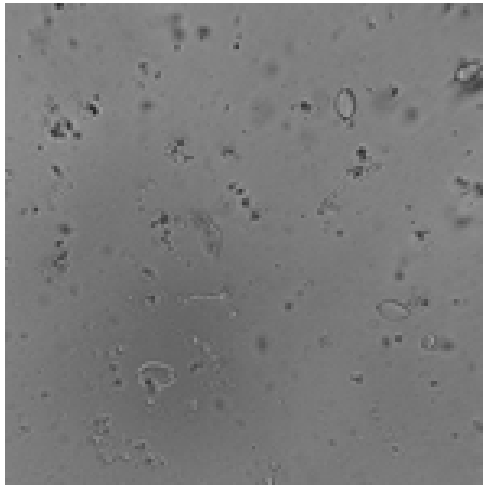
Mycelial growth



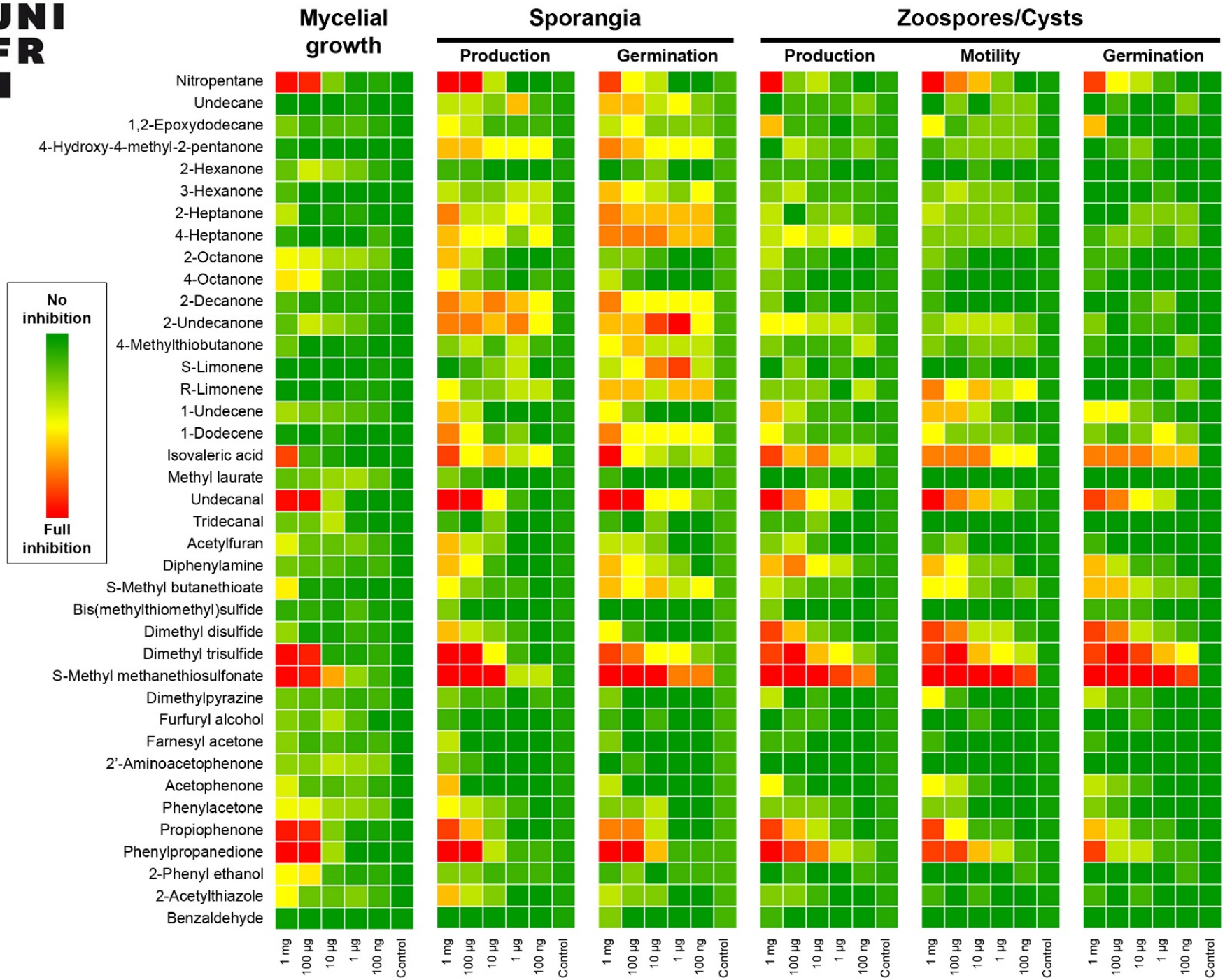
Sporangia germination



Zoospore release

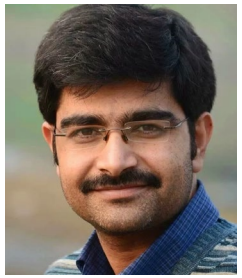


Zoospore motility, encystement, germination, formation of appressorium



Aurélien Bailly

Mout De Vrieze

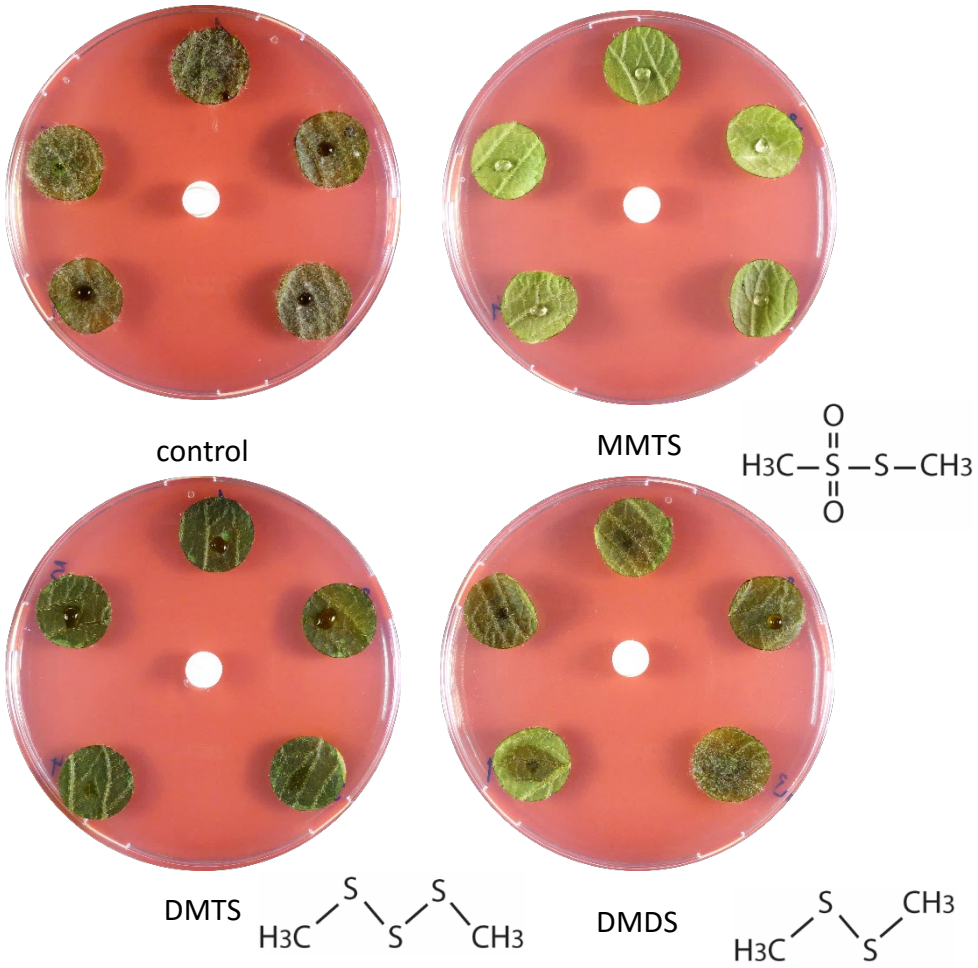


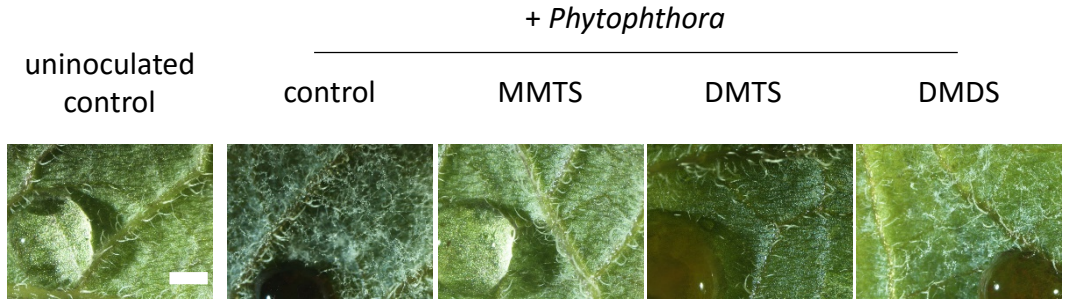
Piyush Pandey

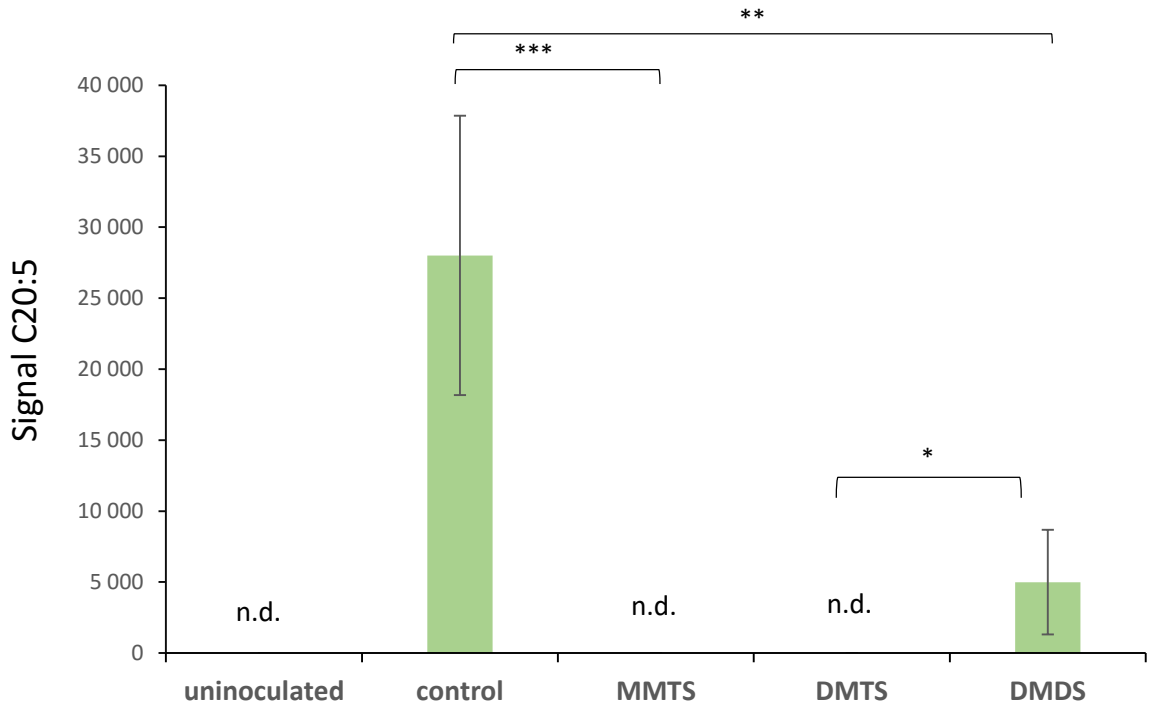
De Vrieze et al., 2015, Front Microb

Effect of S-containing VOCs on late blight disease in leaf discs

Delphine Chinchilla





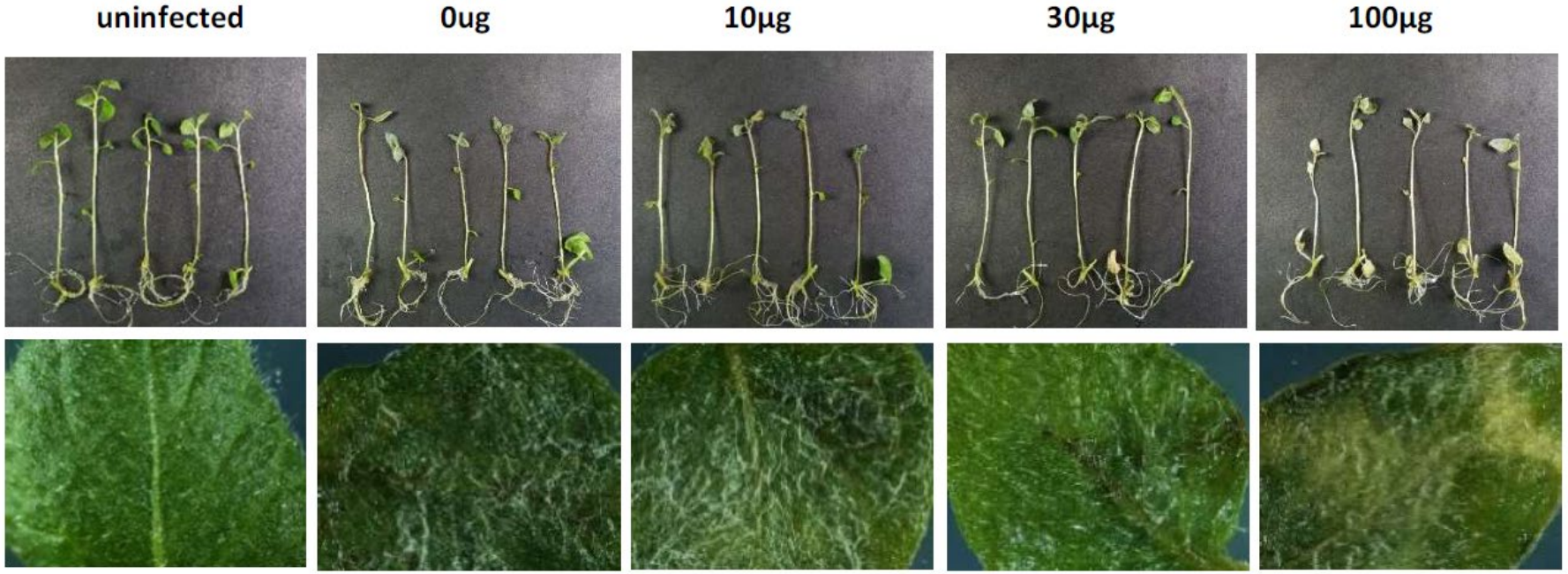


Chinchilla et al., submitted

From leaf discs to *in vitro* plants

Silvan Meyer

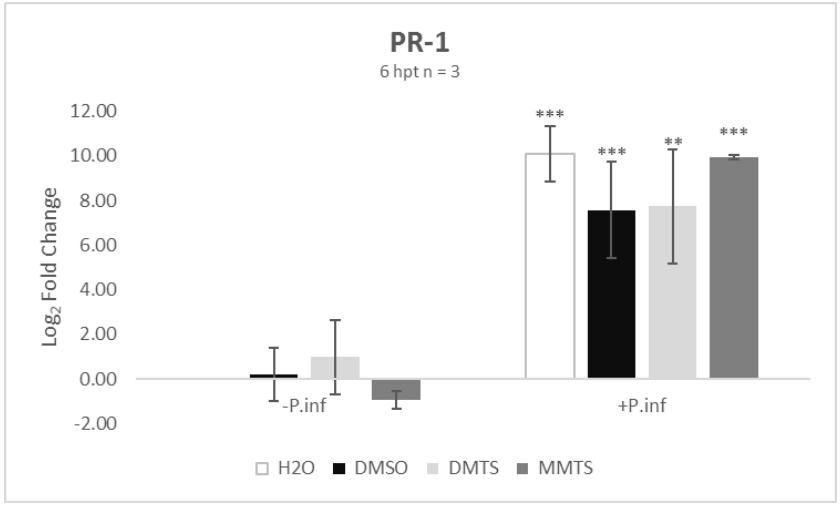
DMTS

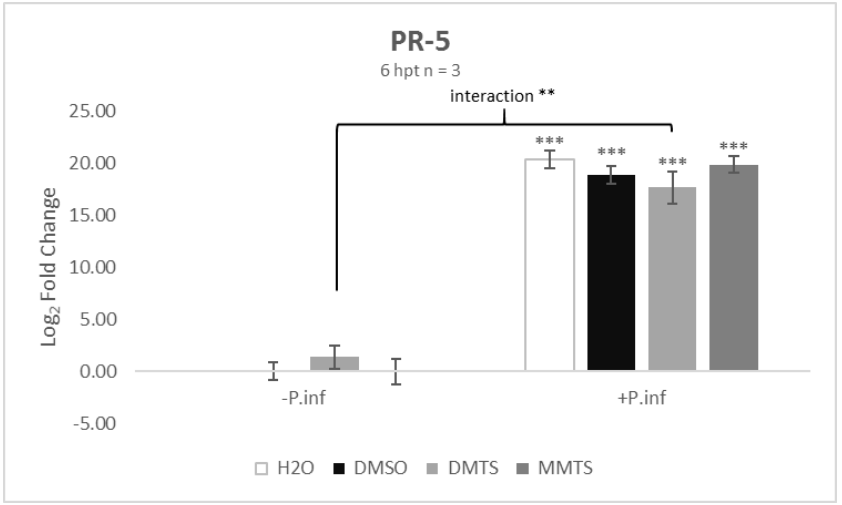


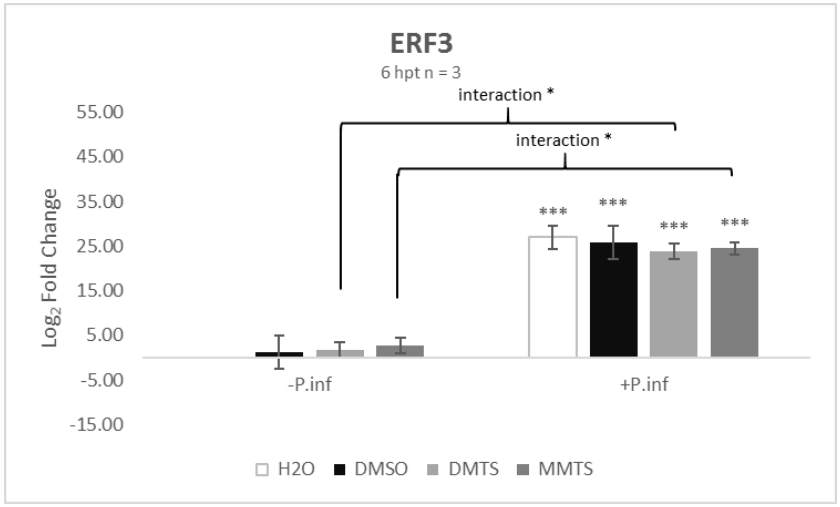
MMTS

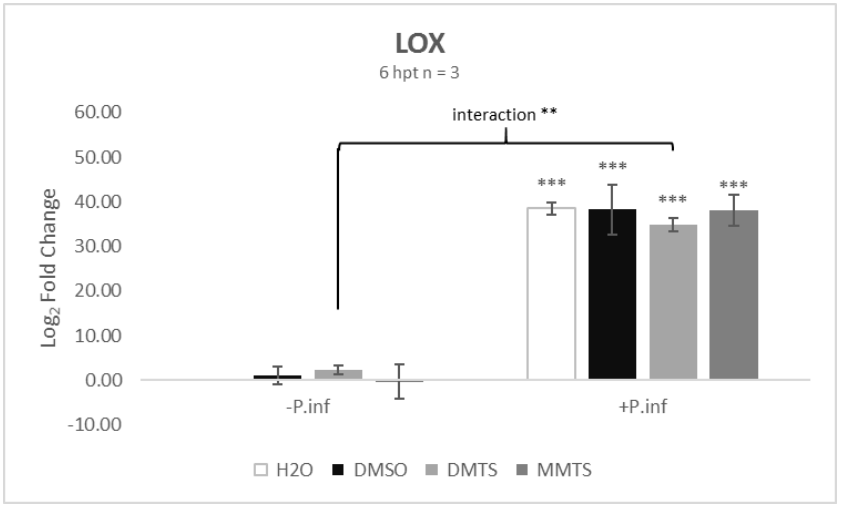
Chinchilla et al., submitted

Are S-VOCs inducing plant defenses?

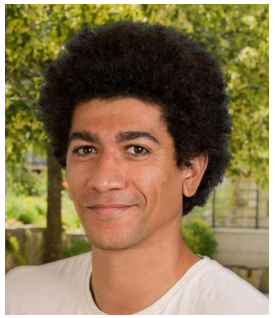








Delphine Chinchilla



Sébastien Bruisson

Direct effects on the pathogen rather than induced resistance

Chinchilla et al., submitted

What are the mechanisms of action of S-VOCs on *P. infestans*?

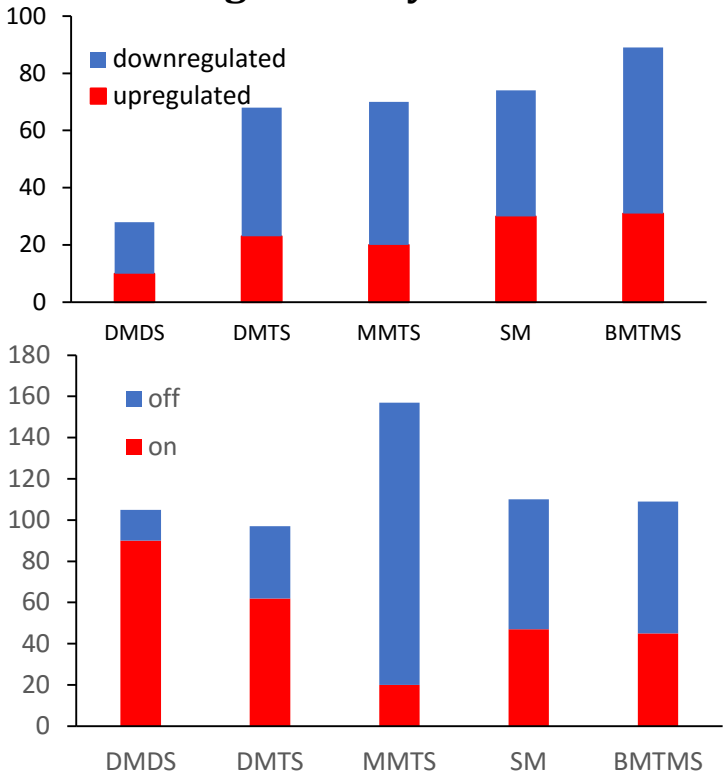
A proteomics approach

Delphine Chinchilla

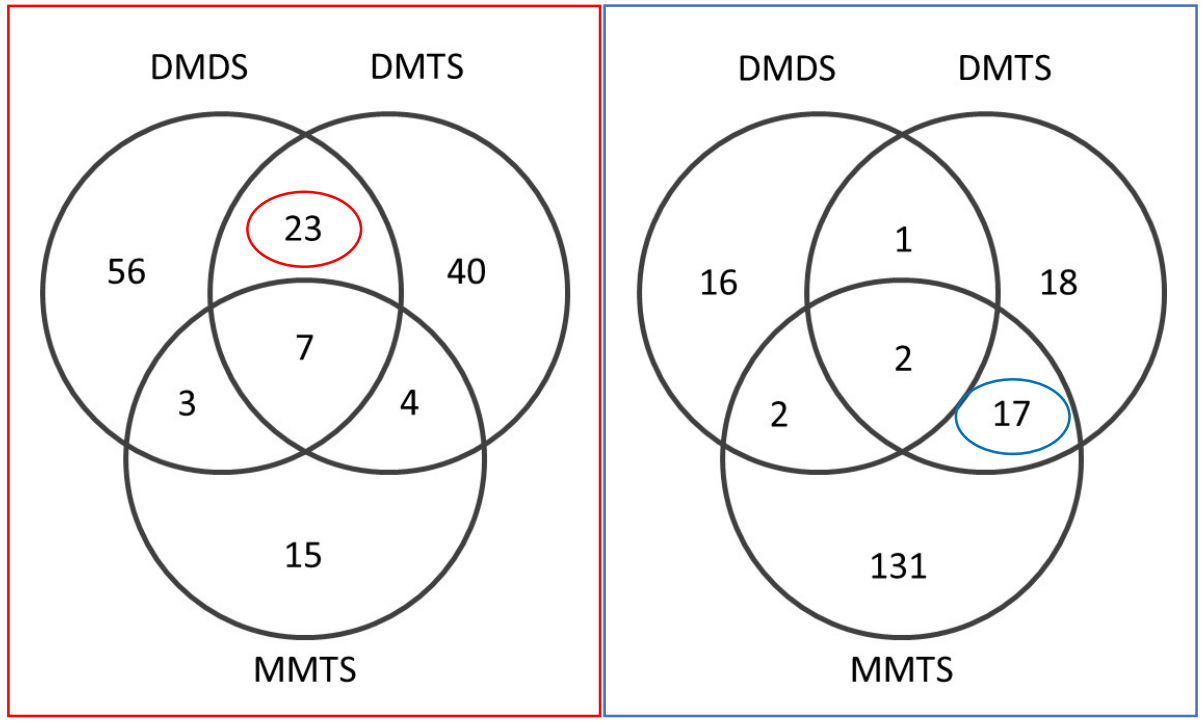
Kathrin Riedel

Daniela Zühlke

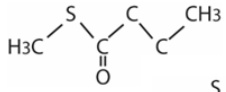
Number of detected proteins regulated by S-VOCs



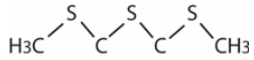
Number of proteins commonly regulated by S-VOCs



SM: S-methyl butanethioate



BMTMS: bis (methylthiomethyl) sulfide



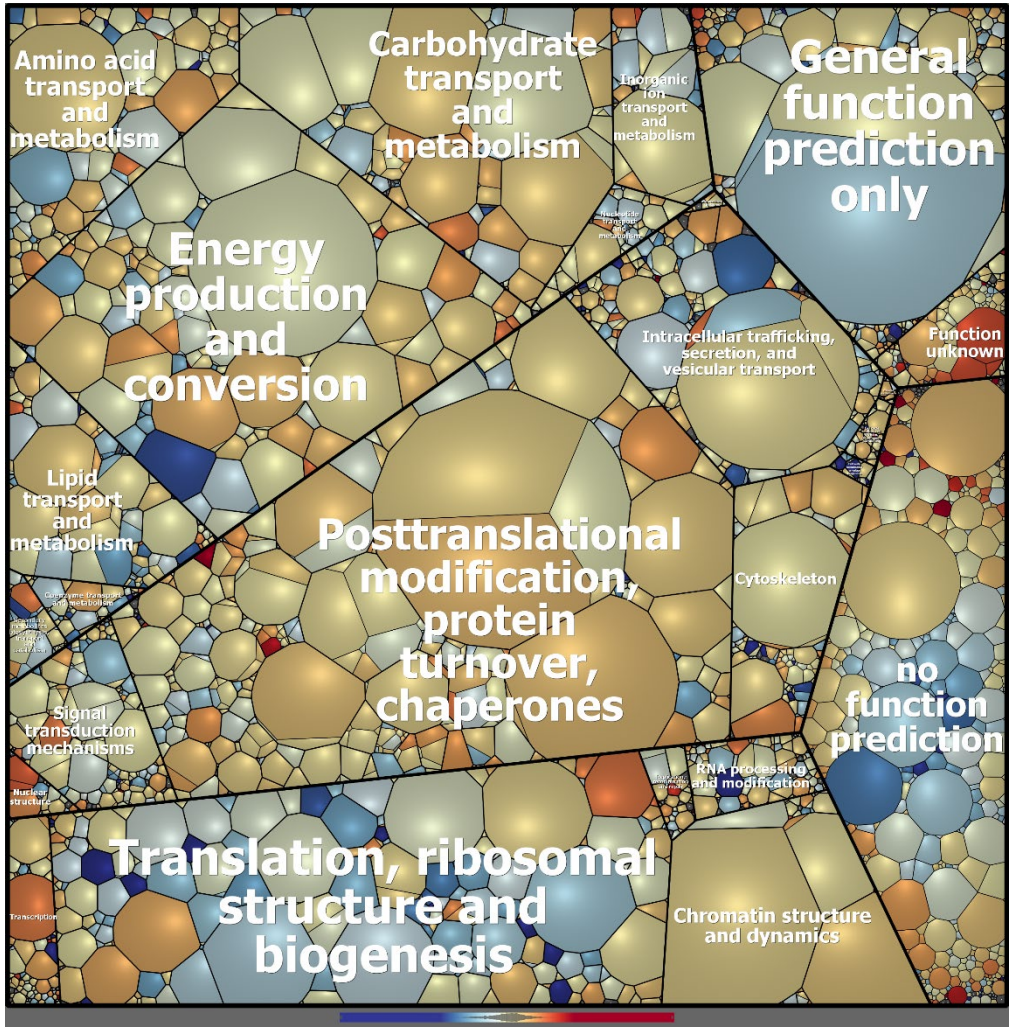
ERNST MORITZ ARNDT
UNIVERSITÄT GREIFSWALD

Wissen lockt. Seit 1456

Chinchilla et al., submitted

What are the mechanisms of action of S-VOCs on *P. infestans*?

A clear pattern for BMTMS

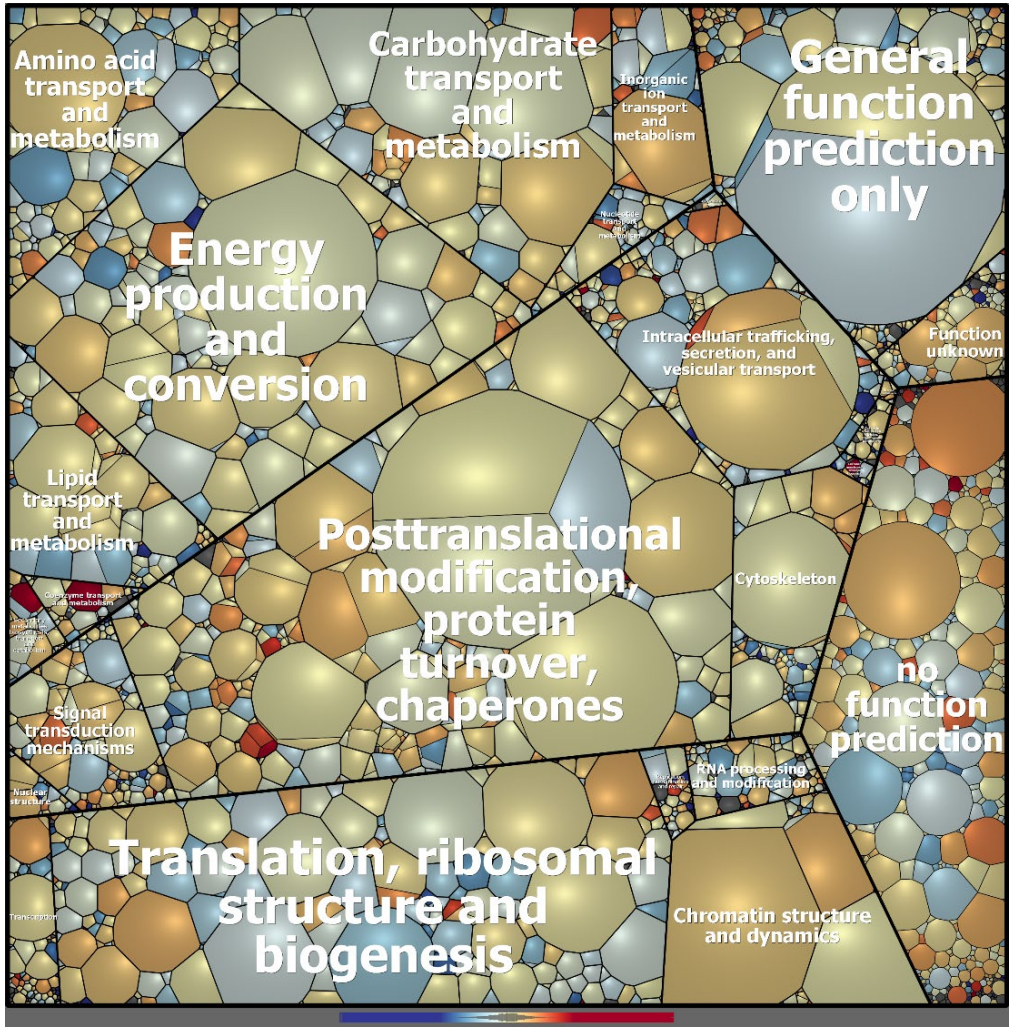


ID	*Fold change*	*Molecular function*	*Biological process*
D0NR65 PITG_15407	0.074	60S ribosomal protein L34	Large subunit 60S[(1)]
D0NY29 PITG_18052	0.114	60S ribosomal protein L3 and related proteins	Large subunit 60S[(1)]
D0N4E3 PITG_06237	0.183	60s ribosomal protein L2/L8	Large subunit 60S[(1)]
D0NG62 PITG_11099	0.21	60s ribosomal protein L18	Large subunit 60S[(1)]
D0MU72 PITG_01833	0.223	60S ribosomal protein L13a	Large subunit 60S[(1)]
D0NFC2 PITG_10450	OFF	putative ribosomal S6 kinase	Regulation of the small subunit 40S
D0NLP0 PITG_13312	2.06	40S ribosomal protein S25	Small subunit 40S [(1)]
D0P0D1 PITG_19608	2.05	Box H/ACA small nucleolar RNP component (NHP2)	Pseudouridylation of ribosomal RNA [(2)]
D0N4G5 PITG_06263	OFF	H/ACA ribonucleoprotein complex subunit 4/ pseudo-uridine synthase (DKC1)	Pseudouridylation of ribosomal RNA [(2)]
D0NS88 PITG_15652	OFF	H/ACA small nucleolar RNP component (GAR1)	Pseudouridylation of ribosomal RNA [(2)]

[(1)] KEGG pif03010: Ribosome ; [(2)] KEGG pif 03008: Ribosome biogenesis

What are the mechanisms of action of S-VOCs on *P. infestans*?

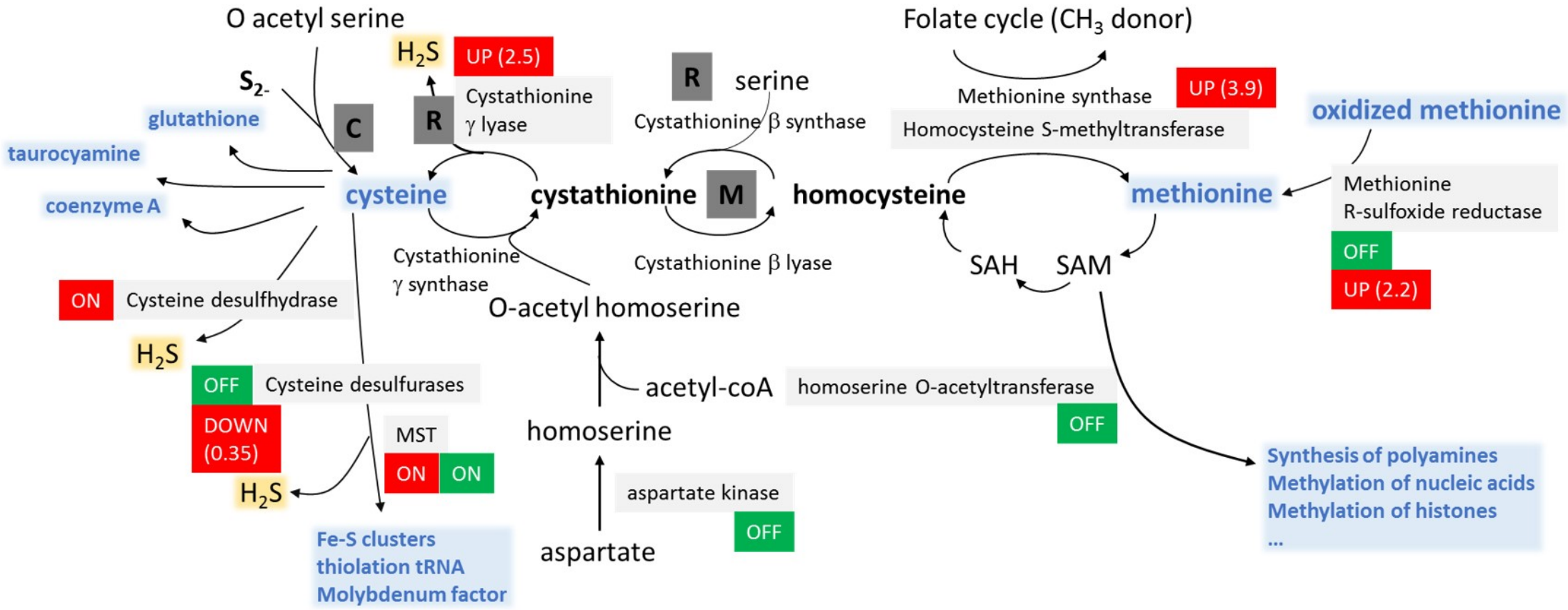
No clear pattern for MMTS (nor DMTS)



sVOC	*ID*	*Fold change*	*Molecular function*	*Biological process*
DMTS	D0MUU0 PITG_01172	2.2	Methionine-R-sulfoxide reductase *(EC : 1.8.4.14 ?)*	Reduction of oxidized methionine
	D0NST2 PITG_16069	4.3	Chaperone HSP104	Refolding of disaggregated proteins
	D0NJX9 PITG_12948	1.969	Thioredoxin/protein disulfide isomerase	Cell redox homeostasis
	D0MRE5 PITG_00674	2.42	Carbonic anhydrase	Protection against oxidative stress
	D0NKS8 PITG_12541	ON	Selenoprotein T	Anti-oxidant
MMTS	D0MUU0 PITG_01172	OFF	Methionine-R-sulfoxide reductase *(EC : 1.8.4.14 ?)*	Reduction of oxidized methionine
	D0NRD5 PITG_15492	2.034	Alkyl hydroperoxide reductase, thiol specific antioxidant = Peroxiredoxin 2 = thioredoxin peroxidase (uses thioredoxin)	Cell redox homeostasis
	D0N359* PITG_05579	2.957	Catalase	ROS detoxification
	D0NVL8 PITG_17249	0.40	Glutaredoxin (if reduced, higher ROS effect)	Redox regulation

MMTS and DMTS perturb sulphur metabolism in *P. infestans*

Changes induced by DMTS and MMTS



Chinchilla et al., submitted

Effects of bacterial S-VOCs on late blight development

- ✓ Potato-associated bacteria emit S-VOCs with strong anti-oomycete activity
- ✓ They do not induce plant defences but act on the pathogen
- ✓ They are efficient *in planta* (on leaf discs and in pots)
- ✓ Different S-VOCs induce different changes in *P. infestans* proteome
- ✓ MMTS and DMTS, both toxic to *P. infestans,* perturb redox balance and S metabolism

⬇

Are these molecules interesting for late blight control?

⬇

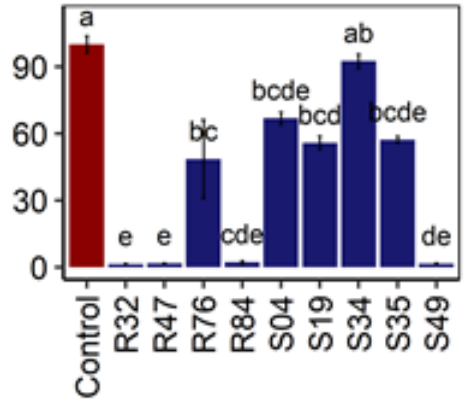
Outlook: testing MMTS effects on non-target organisms

Charlotte Joller

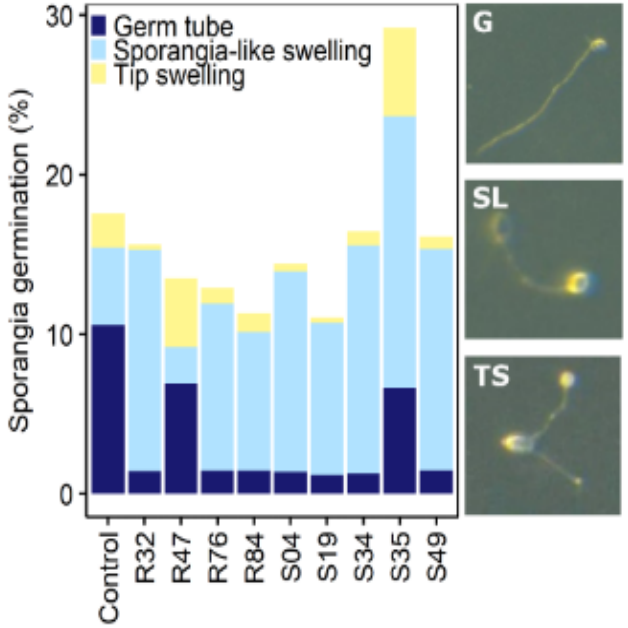
Using bacterial strains rather than pure metabolites: *in vitro* tests

Mout De Vrieze

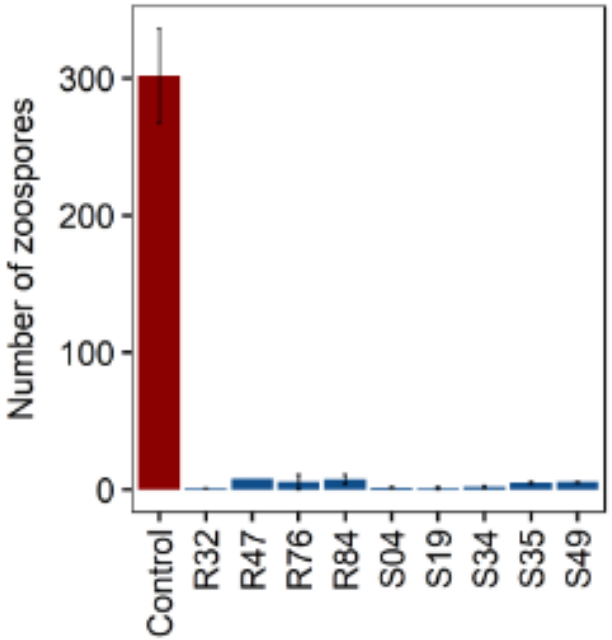
Mycelial growth



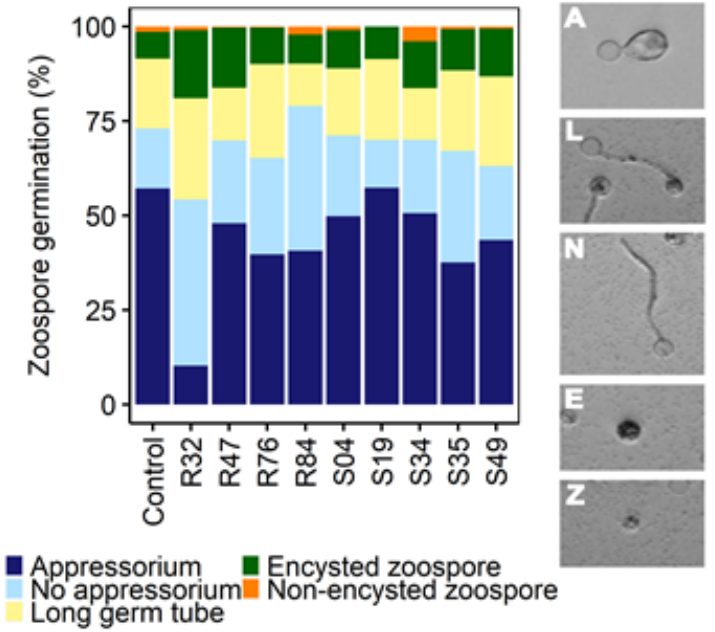
Sporangia germination



Zoospore release

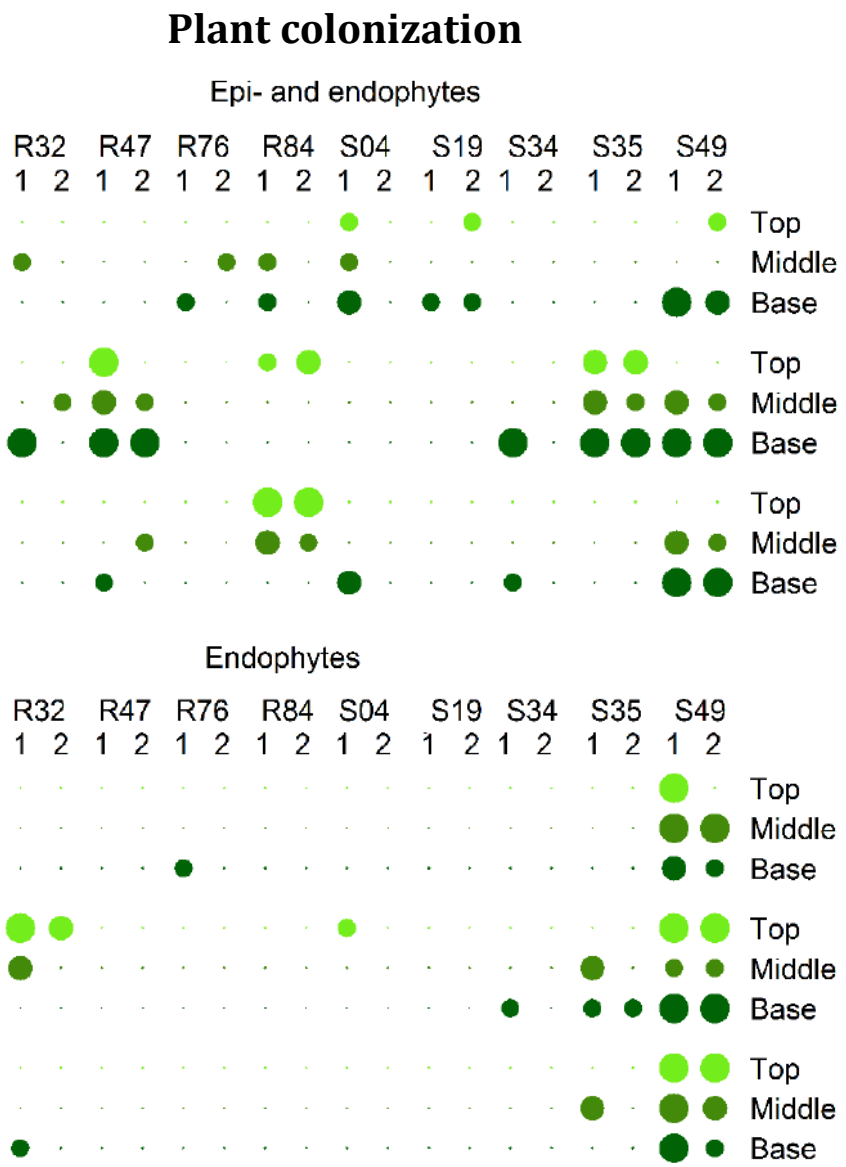


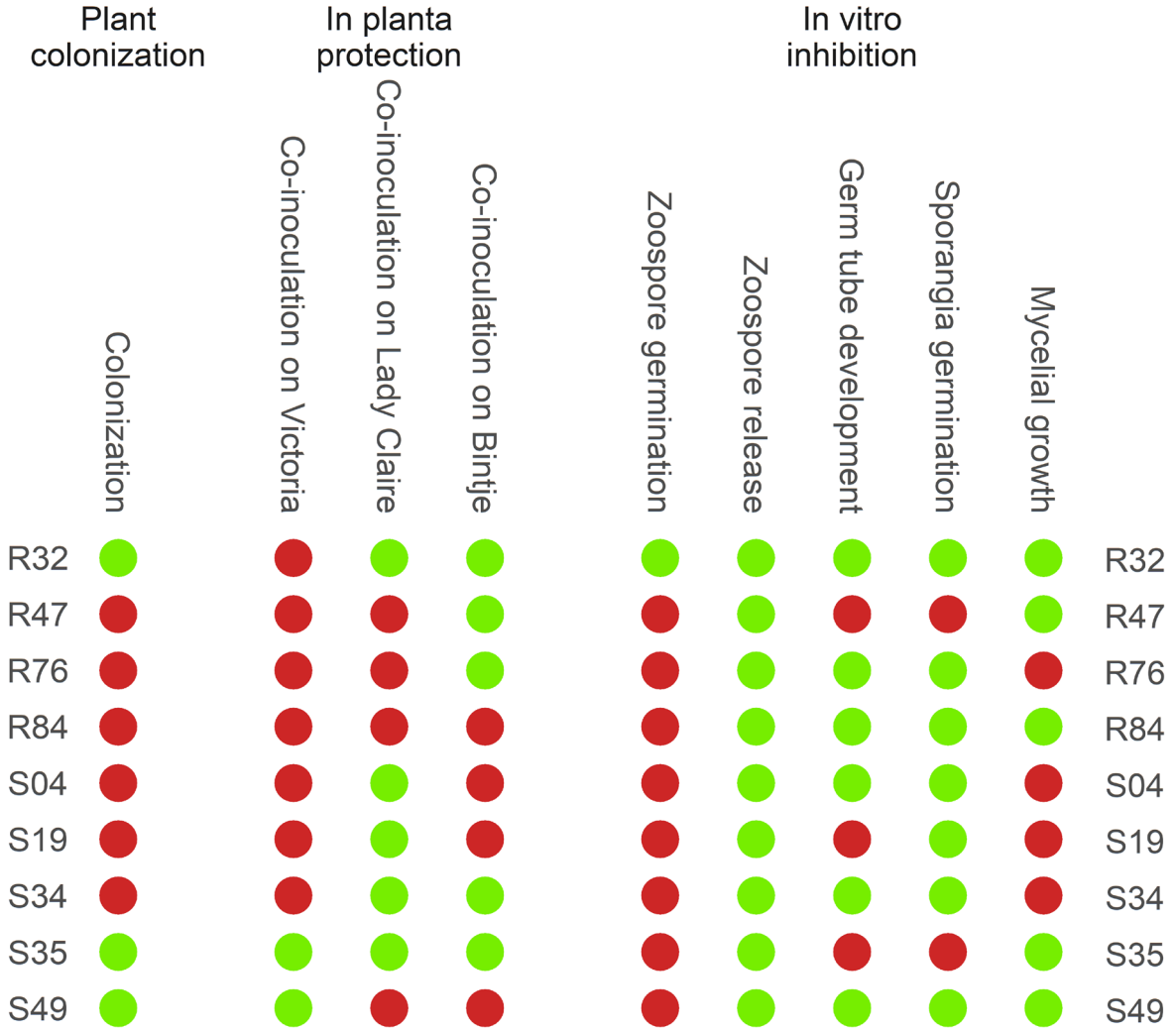
Zoospore germination



Ravikumar, De Vrieze et al., in prep

Using bacterial strains rather than pure metabolites: *in planta*

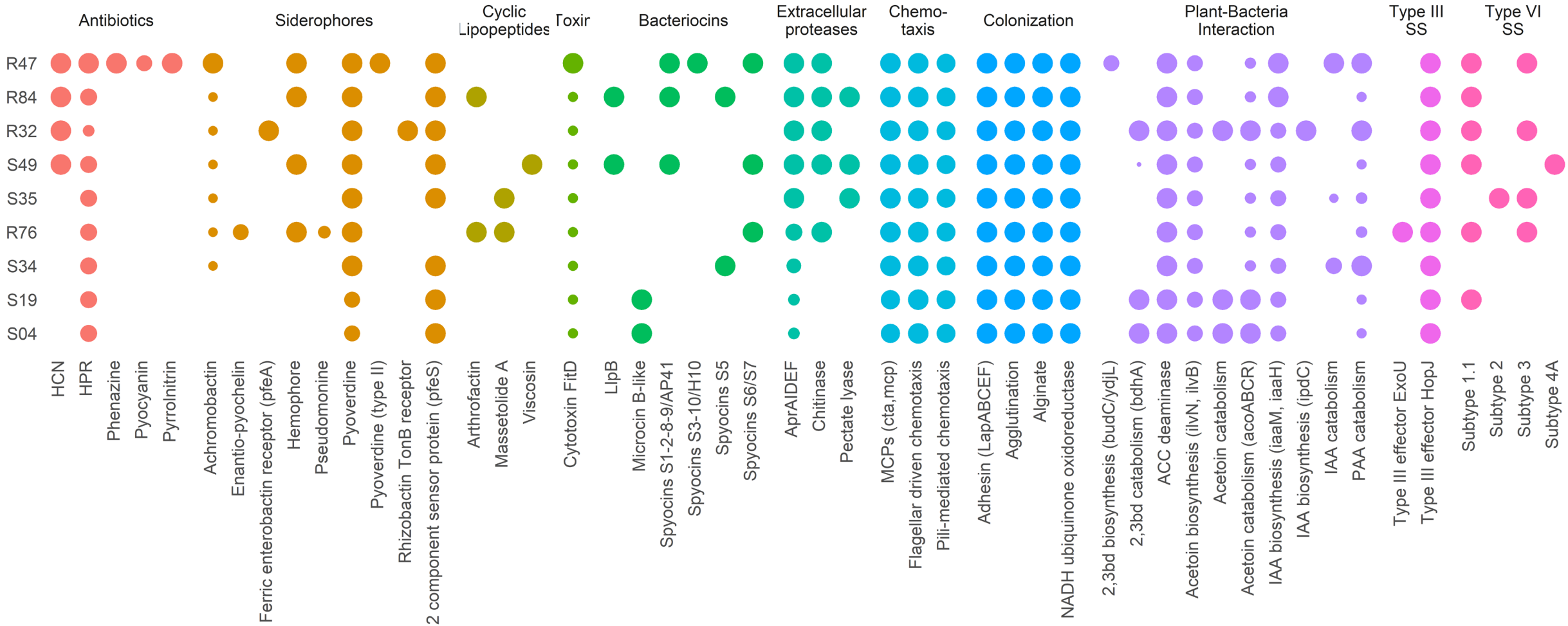




Ravikumar, De Vrieze et al., in prep

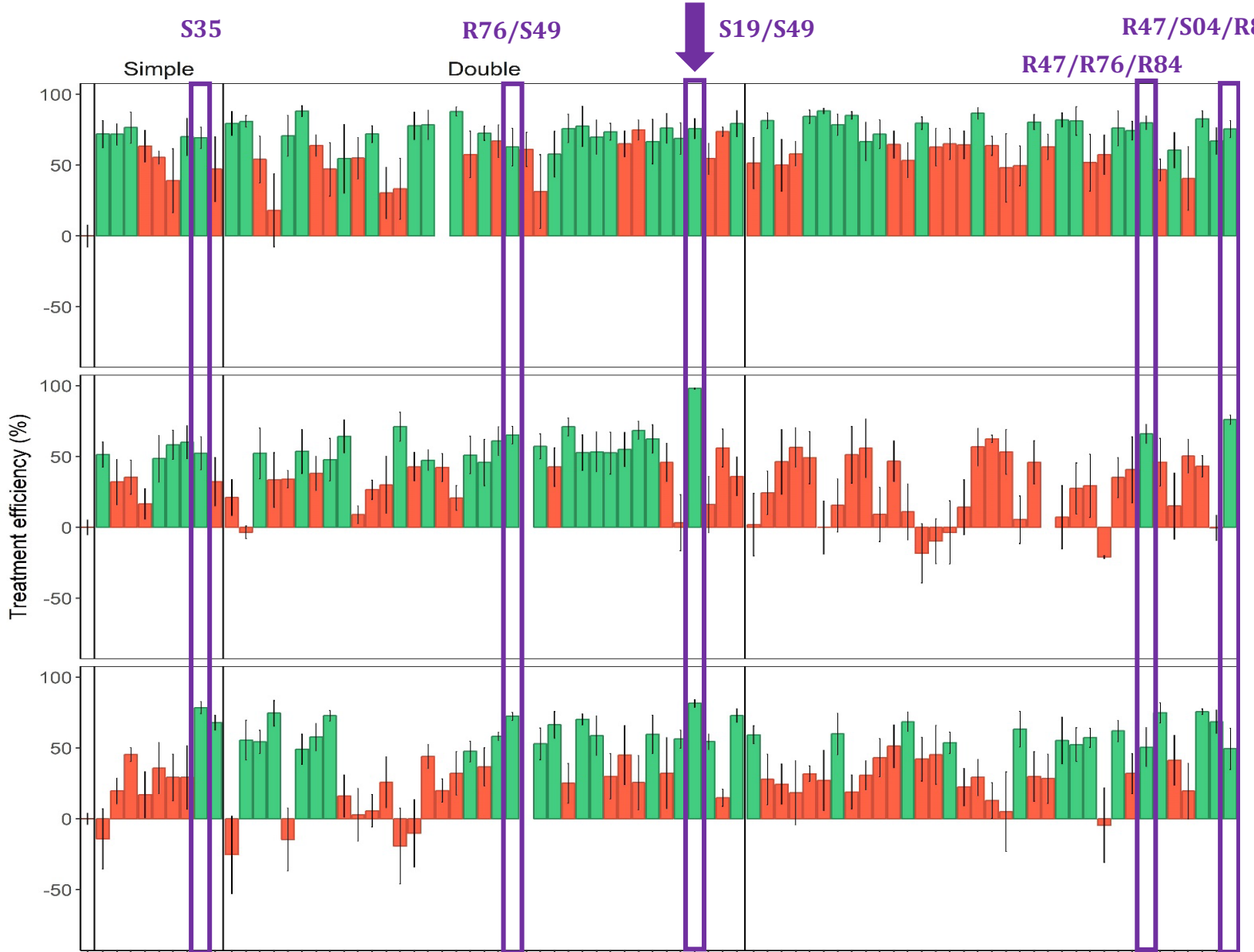
Potato-associated *Pseudomonas* differ in genomic capabilities

In collaboration with
Adithi Ravikumar
and **Christian Ahrens**



Ravikumar, De Vrieze et al., in prep

Could we increase the protective efficacy by combining different strains?



Yes, we can increase efficacy by combining strains…

…. with **different modes of action** on the pathogen

… which **do not interfere with each other's growth**

De Vrieze et al., 2019, Front Microb

Effects of bacterial strains on late blight development

- ✓ Potato-associated bacteria strongly inhibit *P. infestans* mycelium, sporangia and zoospores
- ✓ They are able to colonize potato plants epiphytically, some of them endophytically
- ✓ Some show very good protection in leaf discs assays, yet this is cultivar-dependent
- ✓ Strain consortia lead to more consistent protection across different cultivars

Outlook 1:

Testing efficacy in detached leaves and whole plant infection assays using pot and microplot experiments

Outlook 2:

Letting the plant select the most helpful members of its microbiome by a «vaccination» approach

WISSENSCHAFT. BEWEGEN
GEBERT RÜF STIFTUNG

Collaboration with Brice Dupuis

Agroscope

Vivien Pichon

Thanks to all contributors !

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