Crazy roots with its crazy biological solutions

Lien Bosmans*, Wendy Vanlommel*, Céline Gilli, Matthias Lutz, Pablo Vargas, Hans Rediers, Bart Lievens

15 January 2019

*Research Centre Hoogstraten
Voort 71, B-2328 Hoogstraten (Meerle)
Tel.: +32 (0) 3 315 70 52
Lien.bosmans@proefcentrum.be
www.proefcentrum.be

C-IPM Coordinated Integrated Pest Management in Europe

Introduction

- Hairy root disease (HRD): rhizogenic agrobacteria
- First symptoms: 1970, UK
- Hydroponics: tomatoes, cucumber, eggplants

- Europe: Austria, Belgium, Denmark, France, Greece, the Netherlands, Poland, Switzerland, United Kingdom,…
- Other countries: Russia, Japan, New Zealand, USA,…

→ **Severe losses** in marketable yield

C-IPM Coordinated Integrated Pest Management in Europe

Introduction

- Symptoms of Hairy Root Disease (HRD)

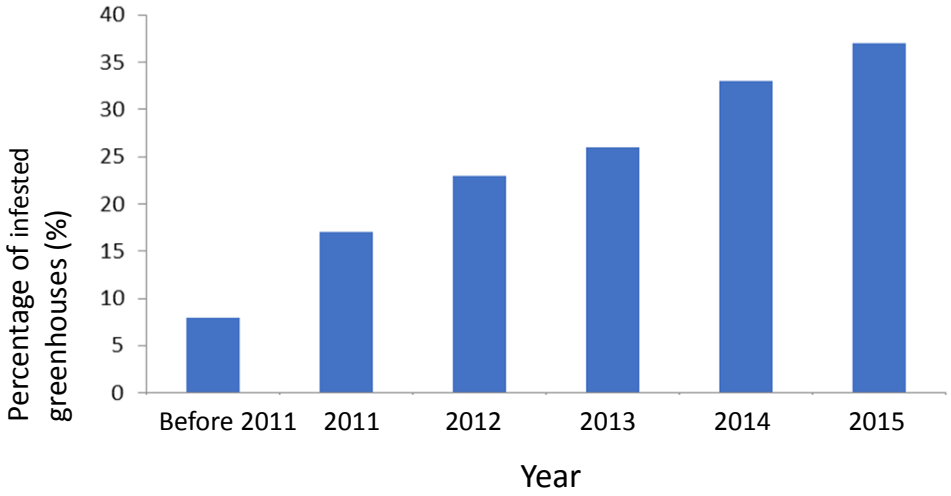
Healthy

HRD

Bosmans et al. (2017). Rhizogenic agrobacteria in hydroponic crops: epidemics, diagnostics and control. Plant Pathology. Doi: 10.1111/ppa.12687.

Introduction

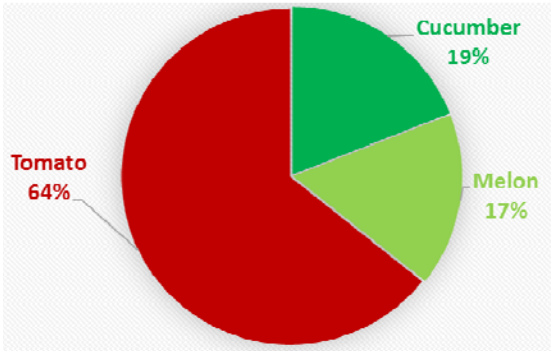
- Occurrence of HRD in Flanders

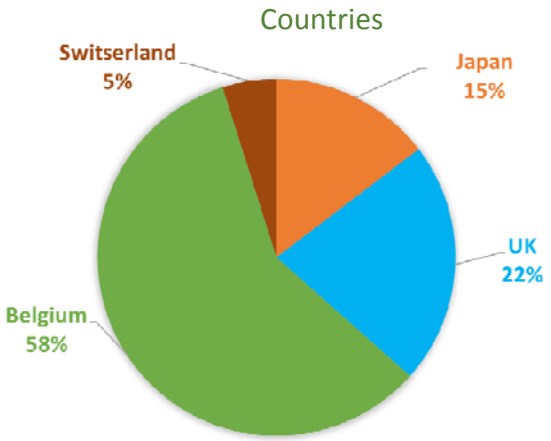


C-IPM Coordinated Integrated Pest Management in Europe

Introduction

- Collection of 41 isolates (different host species/ countries/ years of isolation)





Introduction

- Phenotypic diversity

	Catalase	pH					Temperature (°C)							Biofilm
		3	5	7	9	11	4	22	25	30	37	42	44	
ST15.13/043	■		■	■				■	■	■	■	■		■
ST15.13/045	■			■			■	■	■	■	■			
ST15.13/012	■		■	■	■			■	■	■	■	■		■
ST15.13/048			■	■				■	■	■	■	■	■	■
ST15.13/013			■	■	■		■	■	■	■	■			
ST15.13/040		■	■	■					■	■	■	■		
ST15.13/001			■	■					■	■	■	■		
ST15.13/046	■	■	■	■	■		■	■	■	■	■	■		■
ST15.13/042			■	■	■				■	■				
ST15.13/056			■	■	■		■	■	■	■	■	■		
ST15.13/059			■	■	■				■	■	■	■		■
ST15.13/064		■	■	■	■		■	■	■	■	■			
ST15.13/095			■	■					■	■	■	■	■	
ST15.13/091	■	■	■	■	■		■	■	■	■				
ST15.13/097		■	■	■	■			■	■	■	■			
ST15.13/098		■	■	■	■		■	■	■	■	■			■
ST15.13/060			■	■	■		■	■	■	■				■
ST15.13/004			■	■					■	■				
ST15.13/039			■	■	■				■	■	■			■
ST15.13/007		■	■	■	■				■	■				
ST15.13/054			■	■				■	■	■	■			
ST15.13/006			■	■				■	■	■	■	■		■
ST15.13/057			■	■			■	■	■	■	■			
ST15.13/077			■	■				■	■	■	■	■		
ST15.13/090	■	■	■	■	■			■	■	■	■			■
MAFF 106580			■	■					■	■	■			
MAFF 106587			■	■	■				■	■				
MAFF 106591		■	■	■				■	■	■	■	■		
MAFF 210265			■	■	■			■	■	■	■			
MAFF 210268			■	■				■	■	■	■	■	■	
MAFF 301724		■	■	■			■	■	■	■	■			
NCPPB 2655		■	■	■				■	■	■	■	■		
NCPPB 2656			■	■	■				■	■				■
NCPPB 2657				■	■		■	■	■	■	■	■		
NCPPB 2659			■	■					■	■	■	■		
NCPPB 2660			■	■				■	■	■	■	■		■
NCPPB 4042	■		■	■	■				■	■				■
NCPPB 4043			■	■				■	■	■	■	■	■	
NCPPB 4062			■	■				■	■	■	■	■		■
ST15.13/067			■	■					■	■	■			
ST15.13/073			■	■	■			■	■	■	■			■

Introduction

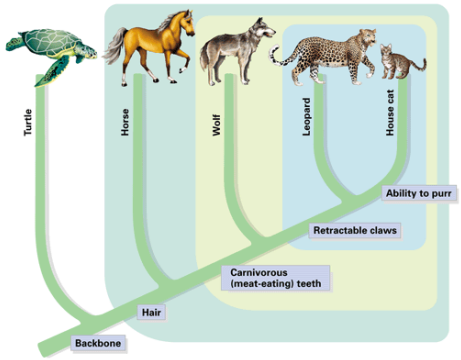
- Genetic diversity

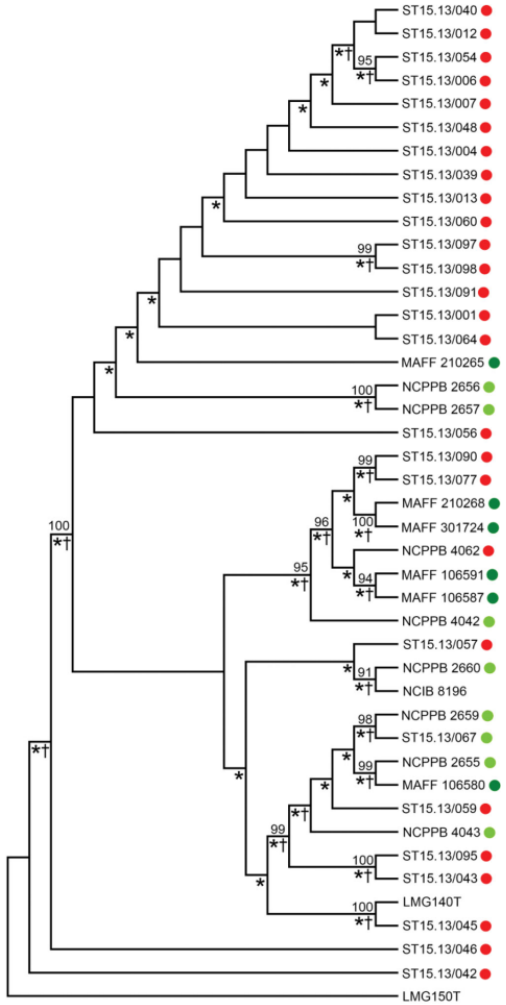
➢ Multilocus sequence analysis (MLSA)

4 core housekeeping genes (16S rRNA gene, *recA*, *rpoB* and *trpE)*

two regions located on the Ri plasmid (*rolB* and *virD2*)

➢ Remarkable genotypic diversity





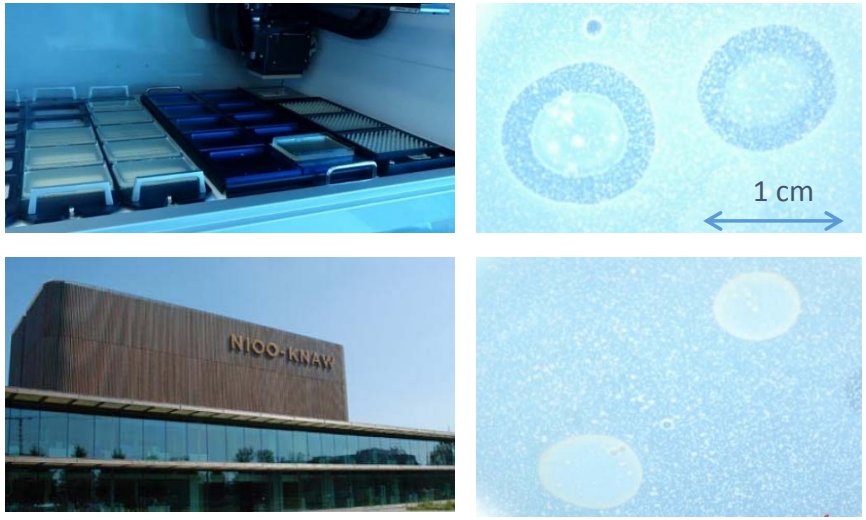
Bosmans et al. (2015). Assessment of the genetic and phenotypic diversity among rhizogenic Agrobacterium biovar 1 strains infecting solanaceous and cucurbit crops. FEMS Microbiology Ecology 91, 1-16.

C-IPM Coordinated Integrated Pest Management in Europe

Biological solution (Laboratorium)

- Screening

A collection of diverse bacterial isolates was screened for antagonistic activity against rhizogenic *Agrobacterium* biovar 1 using the agar overlay assay



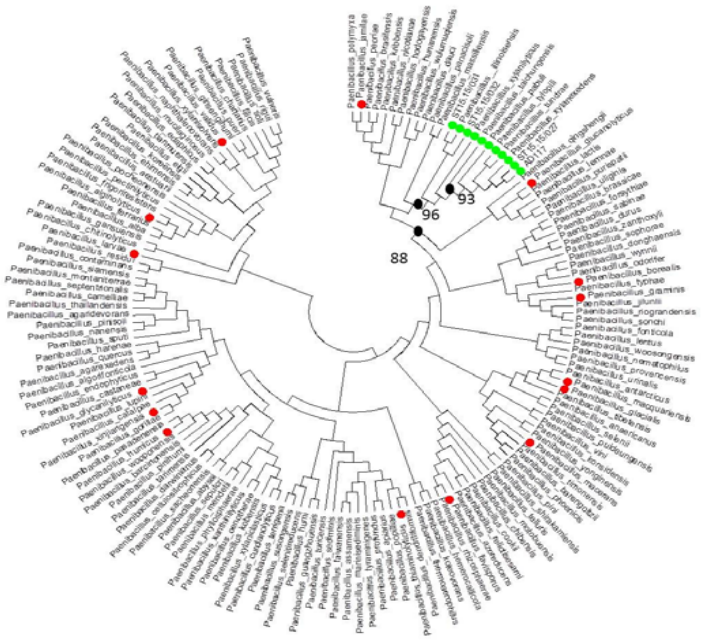
Bosmans et al. (2016). Agar composition affects in vitro screening of biocontrol activity of antagonistic microorganisms. Journal of Microbiological Methods 127, 7-9.

Biological solution (Laboratorium)

- Screening
  - ➢ Out of more than 130 strains tested a clade of phylogenetic

Paenibacillus clade:
P. illinoisensis
P. pabuli
P. taichungensis
P. tundrae
P. tylopili
P. xylanexedens
P. xylanilyticus

Patent application

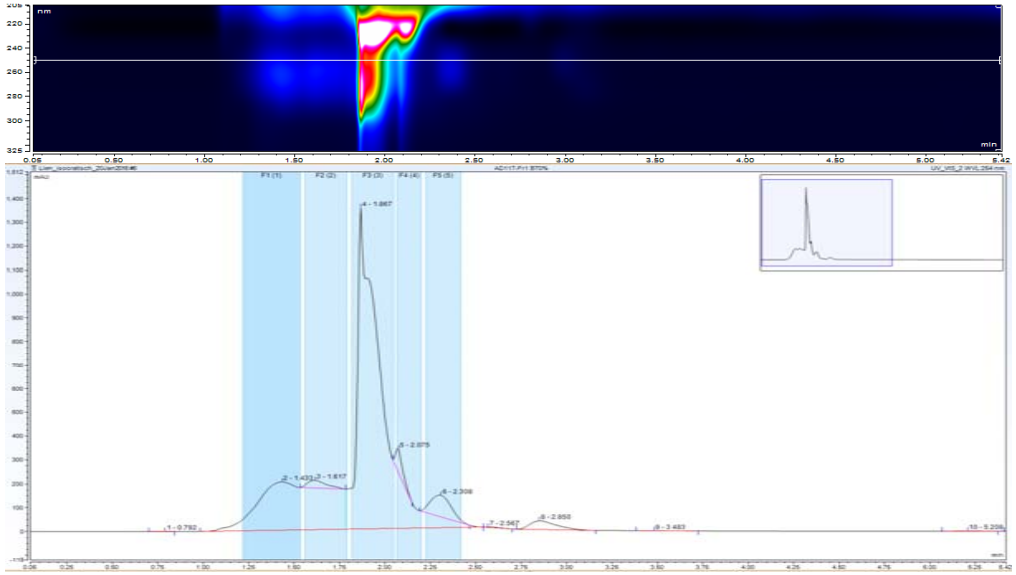


Bosmans et al. (2017). Potential for biocontrol of hairy root disease by a Paenibacillus clade exhibiting antagonistic activity against rhizogenic Agrobacterium biovar 1 strains. Frontiers in Microbiology 8, 243031.

C-IPM Coordinated Integrated Pest Management in Europe

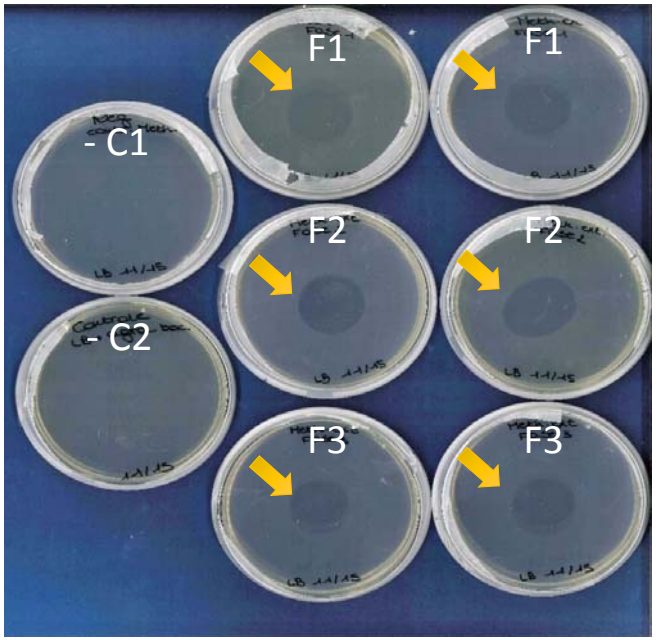
Biological solution (Laboratorium)

- Preliminary characterization of the compound(s)



Biological solution (Laboratorium)

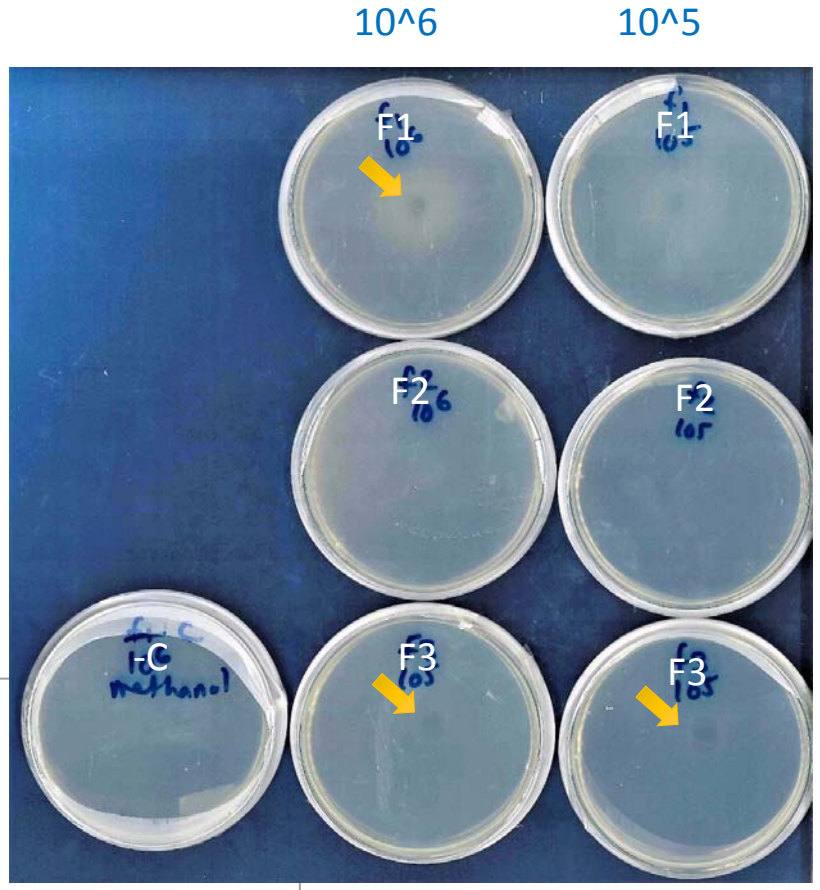
- Preliminary characterization of the compound(s)
  - Compound extraction was done using 65% methanol + 0.1% formic acid
  - Spotting of 10 ul on LB agar spiked with 10^6 CFU/ml Agrobacterium
  - F1: phase 1 of crude extract (supernatant)
  - F2: phase 2 of crude extract (supernatant after centrifugation)
  - F3: phase 3 of crude extract (resuspended pellet after centrifugation)
  - - C1 & C2: control

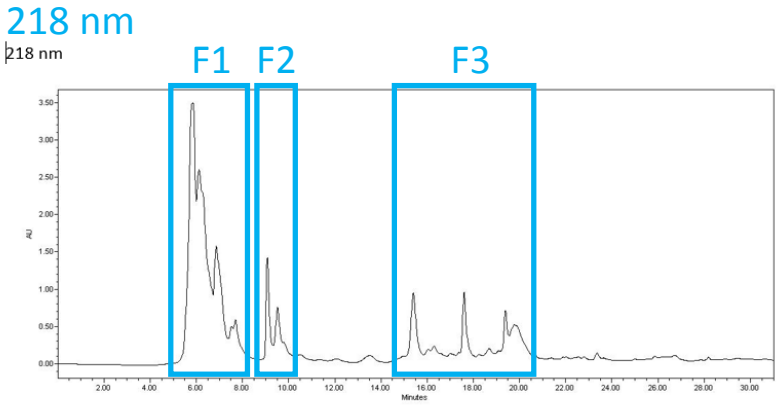


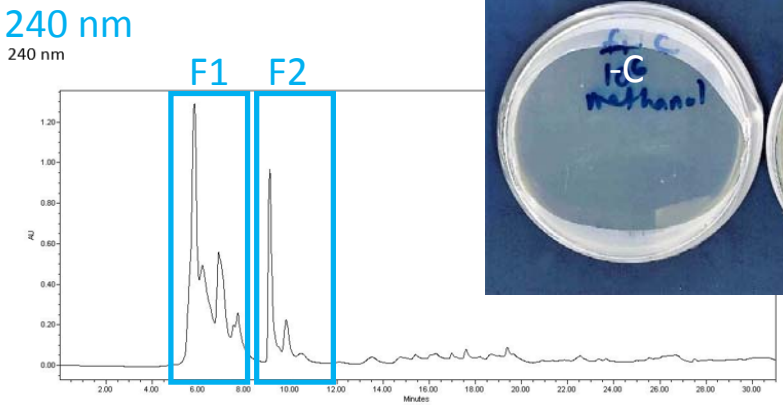
C-IPM Coordinated Integrated Pest Management in Europe

Biological solution (I)

- Preliminary characterization of the compound(s)
  - 2 ml extracted compound was run on an HPLC column with water/acetonitrile mobile phase
  - 3 fractions of 7 runs were pooled
  - F3 seems to contain the compound responsible for the antagonistic activity against rhizogenic Agrobacterium, and slight activity in F1 (to be confirmed)

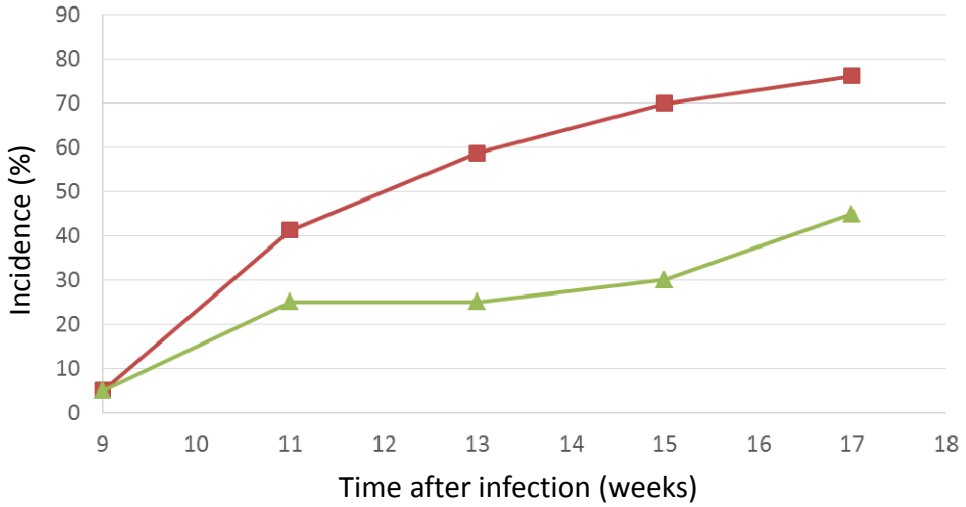






Biological solution (Greenhouse)

- Evaluation strains under greenhouse conditions
  - Application of a combination of two of these strains in greenhouse conditions resulted in a significant reduction of HRD

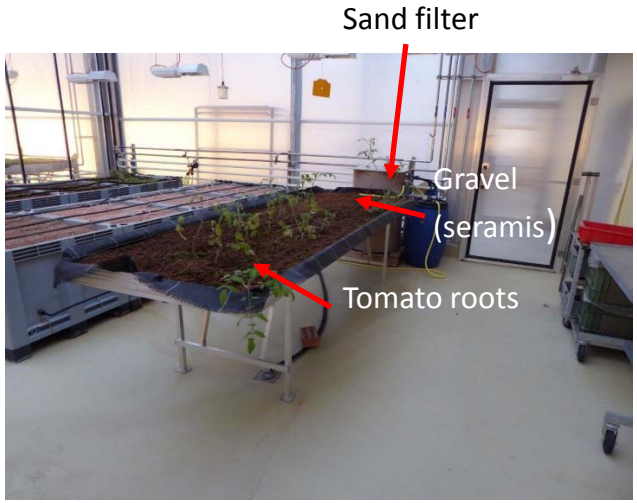


Bosmans et al. (2016). Development of a qPCR assay for detection and quantification of rhizogenic agrobacterium biovar 1 strains. European Journal of Plant Pathology 145 , 719-730.

C-IPM Coordinated Integrated Pest Management in Europe

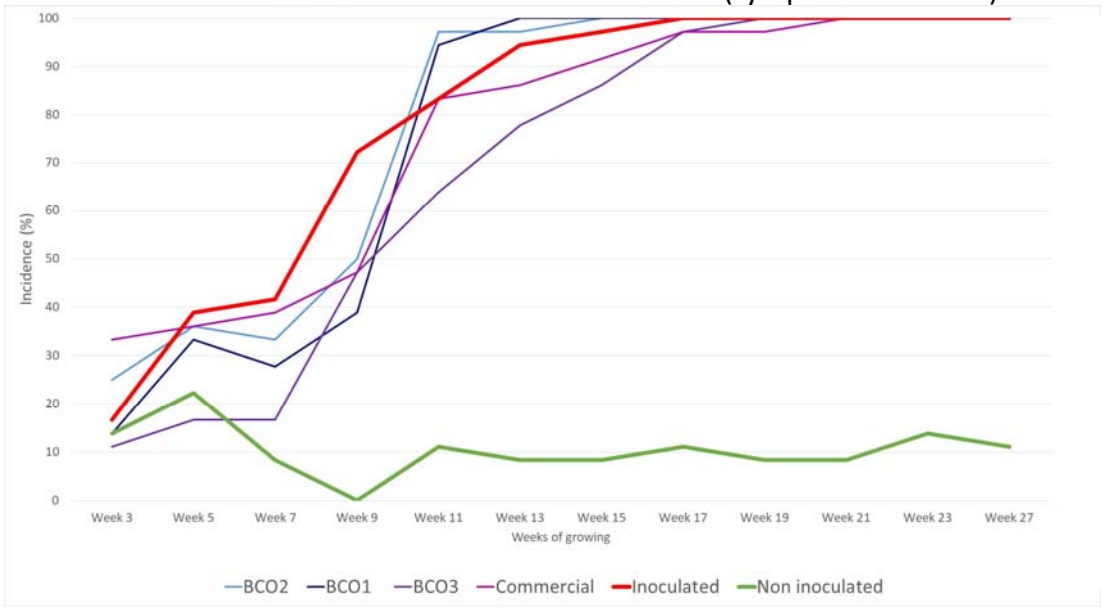
Biological solution (Laboratorium)

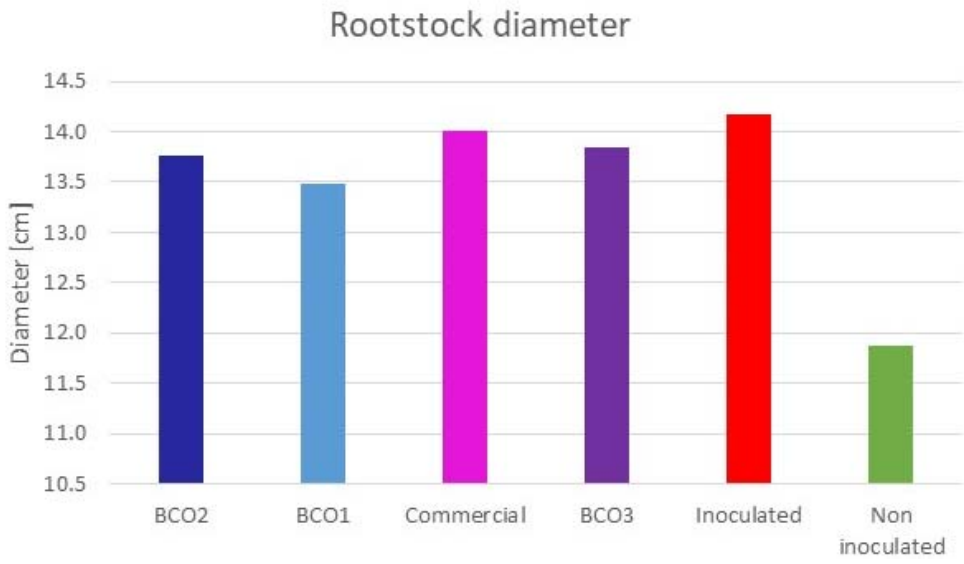
- Screening (Agroscope)
  - About 400 isolates extracted from the system
  - About 4% of the isolates showed high activity against *A. rhizogenes* in laboratory trials
  - 12 isolates were selected for more tests



Biological solution (Greenhouse)

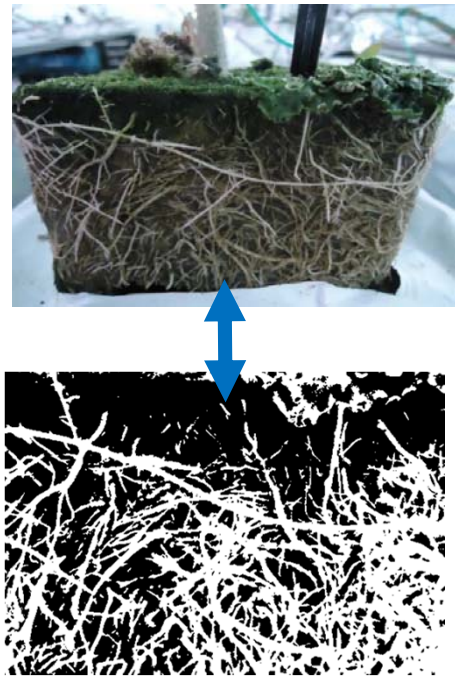
- Evaluation strains under greenhouse conditions (eggplants)
  - Artificial inoculation of *A. rhizogenes*
  - BCO: 2 from Belgium (BCO1 and BCO2), 1 isolated from the screening (BCO3), 1 commercial *Bacillus amyloliquefaciens*, RhyzoVital® 42.
  - Inoculation of BCOs in 6 times (one before plantation and then five times each week)
  - Application of three different strains in greenhouse conditions resulted in no significant reduction of HRD : infection rate (symptoms on roots)

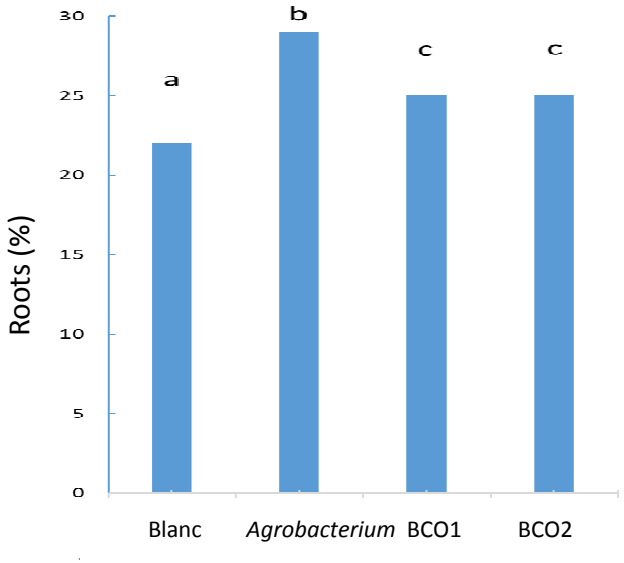




Biological solution (Greenhouse)

- Tunnelexperiment (PSKW): no significant reduction of HRD ?
- Greenhouse experiment (PCH): no significant reduction of HRD ?
- Small greenhouse experiment (12 weeks) (PCH): significant reduction of HRD

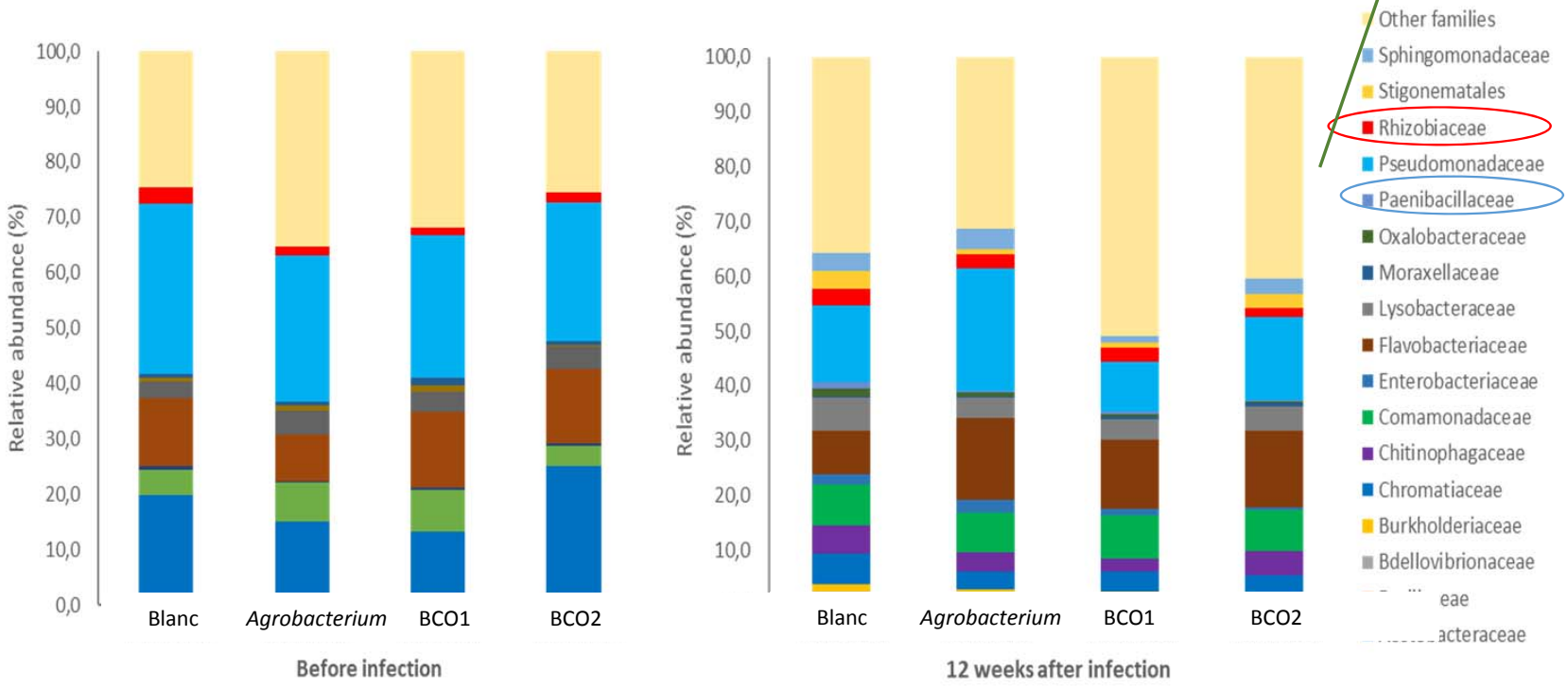




• Biological solution (Greenhouse)

- Small greenhouse experiment (PCH):
  - ➢ Illumina sequencing → microbial community

BCO concentration to low?!



Bosmans et al. (2019). Effect of biocontrol agent Paenibacillus sp. ST15.15/027 on hairy root disease and associated tomato rhizosphere bacterial community composition in greenhouse conditions. *In prep.*

General Conclusions

- *Agrobacterium* biovar 1 strains encompass a genetically and phenotypically diverse group of agrobacteria
- *Paenibacillus* holds great potential for biocontrol of HRD
- Further research required in BARATom :VLAIO(01/11/2018–30/10/2022) Kuleuven-PCH-PSKW-Scientia Terrae
  - Antibiofilm molecules
  - BCO: concentration, commercial BCO's, frequency,…

C-IPM Coordinated Integrated Pest Management in Europe

KU LEUVEN

- Pablo Vargas
- Bart Lievens
- Hans Steenackers
- Hans Rediers
- More info: hans.rediers@kuleuven.be

Vegenov BBV

- Céline Hamon
- Charlotte Roby
- Daniel Le Corre

caté

- Alain Guillou
- Lucie Drogou
- Marine Guerret

PROEFCENTRUM HOOGSTRATEN

- Wendy Vanlommel
- Lien Bosmans

PROEFSTATION VOOR DE GROENTETEELT

- Bart Van Calenberge
- Lieve Wittemans

Agroscope

- Céline Gilli
- Matthias Lutz
- Sandrine Eberlé
- Timea Szikora

Scientia Terrae Research Institute

- Barbora Jostiakova
- Stefan Van Kerckhove

C-IPM Coordinated Integrated Pest Management in Europe

AGENTSCHAP INNOVEREN & ONDERNEMEN

C-IPM Coordinated Integrated Pest Management in Europe