

SESSION 3

BACTERIAL DISEASES

O9 Characterization and diversity of *Pectobacterium* and *Dickeya* species in the Netherlands

Michiel Pel ((NIVIP, NVWA), WUR, The Netherlands)

O11 Glycoalkaloids from *Solanum spp* leaves modify virulence factors in *Dickeya solani* and *Pectobacterium brasiliense sp. nov.*

Anna Grupa-Urbańska (IHAR-PIB, Poland)

O12 Increase of glycoalkaloid content in potato tubers by greening as a method to reduce the spread of *Pectobacterium* and *Dickeya spp.* in seed production systems

Brice Dupuis (Agroscope, Switzerland)

P13 RNAseq expression analysis of resistant and susceptible potato tubers at early stage of infection with *Dickeya solani*

Renata Lebecka (IHAR-PIB, Poland)

P15 Evaluation of the phenotypic and genotypic diversity of *Ralstonia solanacearum* in metropolitan France and the risks for emergence of other species of the *Ralstonia spp.* complex

Antinéa Sallen (ANSES/inov3PT, INRAE-IGEPP, France)

P16 Is there any risk for potato crops to be infected by Apiaceae haplotypes of '*Candidatus Liberibacter solanacearum*'?

Laure Berton (FN3PT, France)

P17 Purple top complex disease a threat for the Ecuadorian and South America potato production and diversity

Xavier Cuesta (INIAP, Ecuador)

P18 Virulence of novel *Ralstonia pseudosolanacearum* (phylotype I) strains from rose, blueberry and mandevilla on seed potato

Bo van Doorn ((NIVIP, NVWA), WUR, The Netherlands)



Netherlands Food and Consumer
Product Safety Authority
*Ministry of Agriculture,
Nature and Food Quality*

Characterization and diversity of *Pectobacterium* and *Dickeya* species in the Netherlands

Chiel Pel

*National Institute for Vectors, Invasive plants and Plant health (NIVIP)
Netherlands Food and Consumer Product Safety Authority (NVWA)*

National Plant Protection Organisation (NPPO-NL)



Wageningen ≠ WUR

- > Not everyone from Wageningen is part of Wageningen University and Research (WUR)





NVWA



Vleesketen en voedselveiligheid



Diergeneesmiddelen



Horeca en ambachtelijke productie



Export



Alcohol en tabak



Productveiligheid



Levende dieren en diergezondheid



Natuur en milieu



Fytosanitair



Visketen



Dierproeven



Meststoffen



**Industriële productie
Dierlijke bijproducten
Microbiologie**



Gewasbescherming



Dierenwelzijn

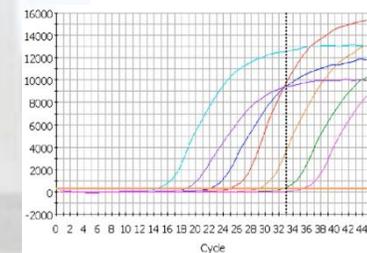


**Diervoeder
Europese subsidieregelingen
Cross compliance
Grondgebonden subsidies**



Facts and Organizational Structure of NIVIP

- › National Institute for Vectors, Invasive plants and Plant health
- › Head: dr. ir. Mieke Reyniers (+ 5 Managers)
- › +/- 110 colleagues
- › Divided over 8 departments:
 - Bacteriology
 - Virology
 - Mycology
 - Entomology
 - Nematology
 - Molecular Biology
 - Invasive Plants
 - Centre for Monitoring of Vectors
 - + Admin, Advisors, greenhouse staff



COMMISSION IMPLEMENTING REGULATION (EU) 2019/2072

of 28 November 2019

establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019

PART A

PESTS NOT KNOWN TO OCCUR IN THE UNION TERRITORY	
Quarantine Pests and their codes assigned by EPPO	
1. Bacteria	
1.	<i>Candidatus Liberibacter africanus</i> [LIBEAF]
2.	<i>Candidatus Liberibacter americanus</i> [LIBEAM]
3.	<i>Candidatus Liberibacter asiaticus</i> [LIBEAS]
4.	<i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i> (Hedges) Collins and Jones [CORBFL]
5.	<i>Pantoea stewartii</i> subsp. <i>stewartii</i> (Smith) Mergaert, Verdonck & Kersters [ERWIST]
6.	<i>Ralstonia pseudosolanacearum</i> Safni <i>et al.</i> [RALSPS]
7.	<i>Ralstonia syzygii</i> subsp. <i>celebesensis</i> Safni <i>et al.</i> [RALSSC]
8.	<i>Ralstonia syzygii</i> subsp. <i>indonesiensis</i> Safni <i>et al.</i> [RALSSI]
9.	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> (Ishiyama) Swings <i>et al.</i> [XANTOR]
10.	<i>Xanthomonas oryzae</i> pv. <i>oryzicola</i> (Fang <i>et al.</i>) Swings <i>et al.</i> [XANTTO]
11.	<i>Xanthomonas citri</i> pv. <i>aurantifolii</i> (Schaad <i>et al.</i>) Constantin <i>et al.</i> [XANTAU]
12.	<i>Xanthomonas citri</i> pv. <i>citri</i> (Hasse) Constantin <i>et al.</i> [XANTCI]

defined by EPPO

PART B

PESTS KNOWN TO OCCUR IN THE UNION TERRITORY	
Quarantine Pests and their codes assigned by EPPO	
1. Bacteria	
1.	<i>Clavibacter sepedonicus</i> (Spieckermann and Kottho) Nouioui <i>et al.</i> [CORBSE]
2.	<i>Ralstonia solanacearum</i> (Smith) Yabuuchi <i>et al.</i> Emend. Safni <i>et al.</i> [RALSSL]
3.	<i>Xylella fastidiosa</i> (Wells <i>et al.</i>) [XYLEFA]

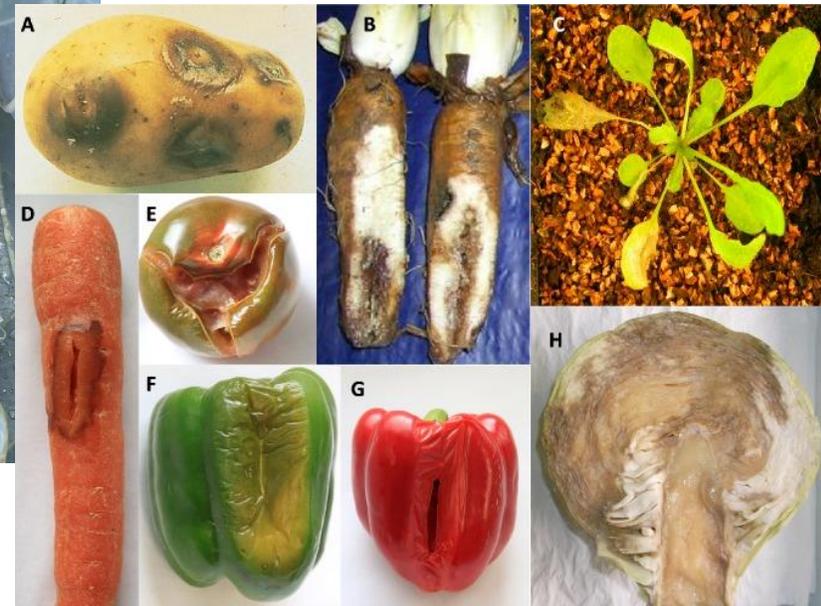


Research activities

- › Development of diagnostic tools/protocols
- › Phylogenetic research
- › Epidemiologic research
 - Risk assesment
 - Advisory tasks



Bacterial soft rot





Bacterial soft rot

- › Causes more crop loss than any other bacterial disease
 - Plants in field/greenhouse
 - Food in storage
- › Affects succulent plant parts (fruits, tubers, stems and bulbs)
- › Plants in nearly every plant family
 - Potato, carrot, tomato, cucumbers, melons, squash, pumpkins, cabbage, cauliflower.



Bacterial soft rot

- › Wide range of temperatures (20°C - 25°C)
- › Wet conditions
- › Symptoms
 - Water soaked spots -> sunken soft spots
 - Tissue becomes discolored and mushy
 - Seepage of moisture
 - Strong smell





Bacterial soft rot

- > *Pseudomonas*
- > *Bacillus*
- > *Burkholderia*
- > *Pantoea*
- > *Enterobacter*
- > *Klebsiella*
- > *Leuconostoc*
- > *Clostridium*
- > *Pectobacterium*
- > *Dickeya*



Bacterial soft rot

> Soft Rot *Pectobacteriaceae* (SRP)

– *Dickeya*

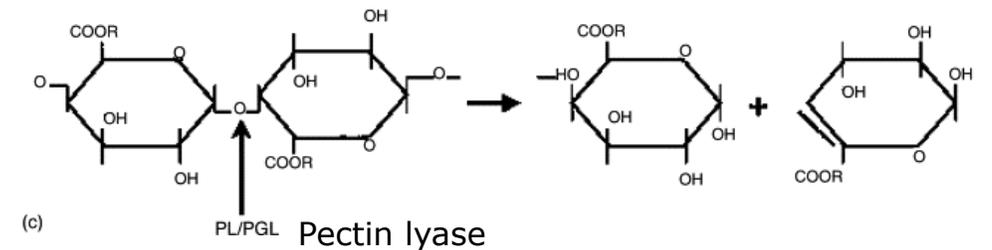
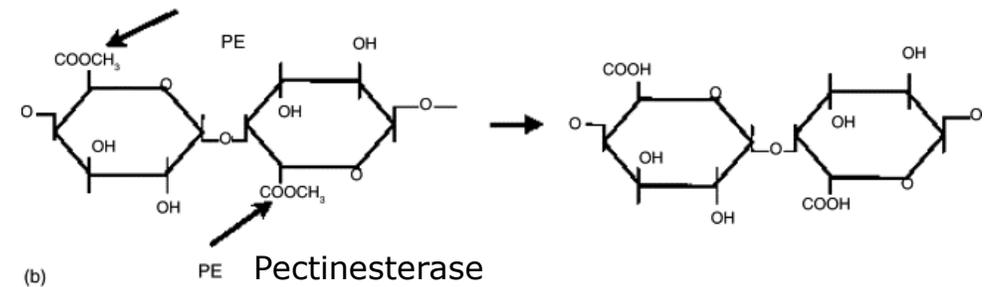
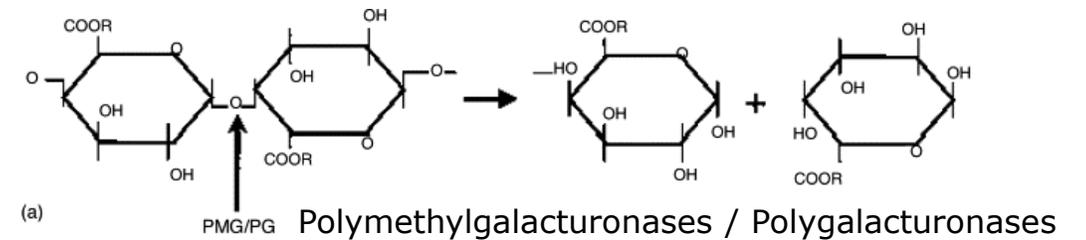
- After the American phytopathologist Robert S. Dickey, for his contribution to research on the *Erwinia chrysantemi* complex

– *Pectobacterium*

- A pectolytic/pectinolytic bacterium

> Produce different Plant Cell Wall degrading enzymes (PCWDE)

- Pectinases, cellulases, proteases





Blackleg of potato

- › Severe losses in potato industry
 - Europe
 - *Dickeya solani*
 - *Pectobacterium brasiliense*
 - USA
 - *Dickeya dianthicola*



- › In NL average annual losses of €12 million in seed potato industry





Current taxonomy

Family *Pectobacteriaceae*

⤴ parent ⤵ siblings ⤵ children

① **Name:** *Pectobacteriaceae* Adeolu *et al.* 2016

① **Category:** Family

① **Proposed as:** fam. nov.

① **Etymology:** Pec.to.bac.te.ri.a.ce'ae. N.L. neut. n. *Pectobacterium*, type genus of the family; L. fem. pl. suff. *-aceae*, ending to denote a family; N.L. fem. pl. n. *Pectobacteriaceae*, the family whose nomenclatural type is the genus *Pectobacterium*

① **Gender:** feminine

① **Type genus:** *Pectobacterium* Waldee 1945 (Approved Lists 1980)

① **Valid publication:** Adeolu M, Alnajar S, Naushad S, S Gupta R. Genome-based phylogeny *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* f.

① **IJSEM list:** Oren A, Garrity GM. Notification list. Notification that new names and new comb

① ▼ Child taxa:

Name	Nomenclatural status	Taxonomic status ▼
<i>Acerihabitans</i> Lee <i>et al.</i> 2021	validly published under the ICNP	correct name
<i>Biostraticola</i> Verburg <i>et al.</i> 2008	validly published under the ICNP	correct name
<i>Brenneria</i> Hauben <i>et al.</i> 1999	validly published under the ICNP	correct name
<i>Dickeya</i> Samson <i>et al.</i> 2005	validly published under the ICNP	correct name
<i>Lonsdalea</i> Brady <i>et al.</i> 2012	validly published under the ICNP	correct name
<i>Musicola</i> Hugouvieux-Cotte-Pattat <i>et al.</i> 2021	validly published under the ICNP	correct name
<i>Pectobacterium</i> Waldee 1945 (Approved Lists 1980)	validly published under the ICNP	correct name
<i>Sodalis</i> Dale and Maudlin 1999	validly published under the ICNP	correct name
" <i>Affinibrenneria</i> " Bian <i>et al.</i> 2021	not validly published	
" <i>Bruguierivorax</i> " Li <i>et al.</i> 2021	not validly published	
" <i>Prodigiosinella</i> " Duprey <i>et al.</i> 2019	not validly published	

Current taxa

> 20 species

▼ Child taxa:

Name	Nomenclatural status	Taxonomic status ▼
 <i>Pectobacterium actinidiae</i> Portier <i>et al.</i> 2019	validly published under the ICNP	correct name
 <i>Pectobacterium aquaticum</i> Pédrón <i>et al.</i> 2019	validly published under the ICNP	correct name
 <i>Pectobacterium aroidearum</i> Nabhan <i>et al.</i> 2013	validly published under the ICNP	correct name
 <i>Pectobacterium atrosepticum</i> (van Hall 1902) Gardan <i>et al.</i> 2003	validly published under the ICNP	correct name
 <i>Pectobacterium betavascularum</i> (Thomson <i>et al.</i> 1984) Gardan <i>et al.</i> 2003	validly published under the ICNP	correct name
 <i>Pectobacterium brasiliense</i> Portier <i>et al.</i> 2019	validly published under the ICNP	correct name
 <i>Pectobacterium cacticida</i> corrig. (Alcorn <i>et al.</i> 1991) Hauben <i>et al.</i> 1999	validly published under the ICNP	correct name
 <i>Pectobacterium carnegieana</i> (Standring 1942) Brenner <i>et al.</i> 1973 (Approved Lists 1980)	validly published under the ICNP	correct name
 <i>Pectobacterium carotovorum</i> (Jones 1901) Waldee 1945 (Approved Lists 1980)	validly published under the ICNP	correct name
 <i>Pectobacterium fontis</i> Oulghazi <i>et al.</i> 2019	validly published under the ICNP	correct name
 <i>Pectobacterium odoriferum</i> (Gallois <i>et al.</i> 1992) Portier <i>et al.</i> 2019	validly published under the ICNP	correct name
 <i>Pectobacterium parmentieri</i> Khayi <i>et al.</i> 2016	validly published under the ICNP	correct name
 <i>Pectobacterium parvum</i> Pasanen <i>et al.</i> 2020	validly published under the ICNP	correct name
 <i>Pectobacterium peruvienne</i> Waleron <i>et al.</i> 2022	validly published under the ICNP	correct name
 <i>Pectobacterium polaris</i> Dees <i>et al.</i> 2017	validly published under the ICNP	correct name
 <i>Pectobacterium polonicum</i> Waleron <i>et al.</i> 2019	validly published under the ICNP	correct name
 <i>Pectobacterium punjabense</i> Sarfraz <i>et al.</i> 2018	validly published under the ICNP	correct name
 <i>Pectobacterium quasiaquaticum</i> Ben Moussa <i>et al.</i> 2021	validly published under the ICNP	correct name
 <i>Pectobacterium versatile</i> Portier <i>et al.</i> 2019	validly published under the ICNP	correct name
 <i>Pectobacterium wasabiae</i> (Goto and Matsumoto 1987) Gardan <i>et al.</i> 2003	validly published under the ICNP	correct name
 <i>Pectobacterium cacticidum</i> (Alcorn <i>et al.</i> 1991) Hauben <i>et al.</i> 1999	orthographic variant	misspelling
 <i>Pectobacterium chrysanthemi</i> (Burkholder <i>et al.</i> 1953) Brenner <i>et al.</i> 1973 (Approved Lists 1980)	validly published under the ICNP	synonym
 <i>Pectobacterium cyripedii</i> (Hori 1911) Brenner <i>et al.</i> 1973 (Approved Lists 1980)	validly published under the ICNP	synonym
 <i>Pectobacterium rhapontici</i> (Millard 1924) Patel and Kulkarni 1951 (Approved Lists 1980)	validly published under the ICNP	synonym
 "<i>Pectobacterium delphinii</i>" Waldee 1945	not validly published	
 "<i>Candidatus Pectobacterium macerans</i>" corrig. Shirshikov <i>et al.</i> 2018	not validly published	
 "<i>Candidatus Pectobacterium maceratum</i>" Shirshikov <i>et al.</i> 2018	orthographic variant	
 "<i>Pectobacterium melonis</i>" (Giddings 1910) Waldee 1945	not validly published	
 "<i>Pectobacterium zantedeschiae</i>" Waleron <i>et al.</i> 2019	not validly published	

Current taxonomy

① ▼ Child taxa:

Name	Nomenclatural status	Taxonomic status ▼
 <i>Dickeya aquatica</i> Parkinson <i>et al.</i> 2014	validly published under the ICNP	correct name
 <i>Dickeya chrysanthemi</i> (Burkholder <i>et al.</i> 1953) Samson <i>et al.</i> 2005	validly published under the ICNP	correct name
 <i>Dickeya dadantii</i> Samson <i>et al.</i> 2005	validly published under the ICNP	correct name
 <i>Dickeya dianthicola</i> Samson <i>et al.</i> 2005	validly published under the ICNP	correct name
 <i>Dickeya fangzhongdai</i> Tian <i>et al.</i> 2016	validly published under the ICNP	correct name
 <i>Dickeya lacustris</i> Hugouvieux-Cotte-Pattat <i>et al.</i> 2019	validly published under the ICNP	correct name
 <i>Dickeya oryzae</i> Wang <i>et al.</i> 2020	validly published under the ICNP	correct name
 <i>Dickeya parazeae</i> Hugouvieux-Cotte-Pattat and Van Gijsegem 2021	validly published under the ICNP	correct name
 <i>Dickeya poaceiphila</i> Hugouvieux-Cotte-Pattat <i>et al.</i> 2020	validly published under the ICNP	correct name
 <i>Dickeya solani</i> van der Wolf <i>et al.</i> 2014	validly published under the ICNP	correct name
 <i>Dickeya undicola</i> Oulghazi <i>et al.</i> 2019	validly published under the ICNP	correct name
 <i>Dickeya zeeae</i> Samson <i>et al.</i> 2005	validly published under the ICNP	correct name
 <i>Dickeya dieffenbachiae</i> Samson <i>et al.</i> 2005	validly published under the ICNP	synonym
 <i>Dickeya paradisiaca</i> (Fernandez-Borrero and Lopez-Duque 1970) Samson <i>et al.</i> 2005	validly published under the ICNP	synonym

> *12 species*

① ▼ Child taxa:

Name ▼	Nomenclatural status	Taxonomic status
 <i>Dickeya dadantii</i> subsp. <i>dadantii</i> (Samson <i>et al.</i> 2005) Brady <i>et al.</i> 2012	validly published under the ICNP	correct name
 <i>Dickeya dadantii</i> subsp. <i>dieffenbachiae</i> (Samson <i>et al.</i> 2005) Brady <i>et al.</i> 2012	validly published under the ICNP	correct name

Current taxonomy

▼ Child taxa:

Name ▼	Nomenclatural status	Taxonomic status
Musicola keenii Hugouvieux-Cotte-Pattat <i>et al.</i> 2021	validly published under the ICNP	correct name
Musicola paradisiaca (Fernandez-Borrero and Lopez-Duque 1970) Hugouvieux-Cotte-Pattat <i>et al.</i> 2021	validly published under the ICNP	correct name

> 2 species

INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

Volume 71, Issue 10

Research Article

Proposal for the creation of a new genus *Musicola* gen. nov., reclassification of *Dickeya paradisiaca* (Samson *et al.* 2005) as *Musicola paradisiaca* comb. nov. and description of a new species *Musicola keenii* sp. nov.

Nicole Hugouvieux-Cotte-Pattat¹ , Cécile Jacot des-Combes², Jérôme Briolay² , Leighton Pritchard³

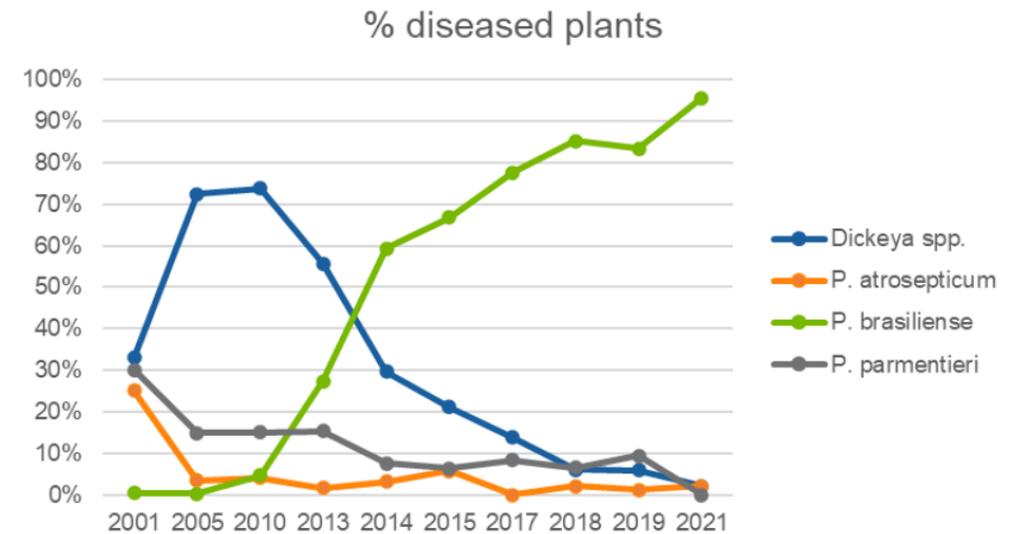
View Affiliations

Published: 07 October 2021 | <https://doi.org/10.1099/ijsem.0.005037>



Blackleg of potato

- > Most prevalent species appears to be dynamic
- > Many different species are present in NL



Source Inge van Duivenbode, NAK Emmeloord



Blackleg of potato

- > Most prevalent species appears to be dynamic
- > Many different species are present in NL

Species	# isolates
<i>P. actinidiae</i>	1
<i>P. aquaticum</i>	1
<i>P. atrosepticum</i>	1
<i>P. brasiliense</i>	151
<i>P. parmentieri</i>	5
<i>P. polaris</i>	4
<i>P. punjabense</i>	6
<i>P. versatile</i>	1
<i>Pectobacterium</i> spp.	139
<i>D. solani</i>	17
<i>D. chrysanthemi</i>	5
<i>D. dadantii</i>	3
<i>D. zeae</i>	35

Source Inge van Duivenbode, NAK Emmeloord



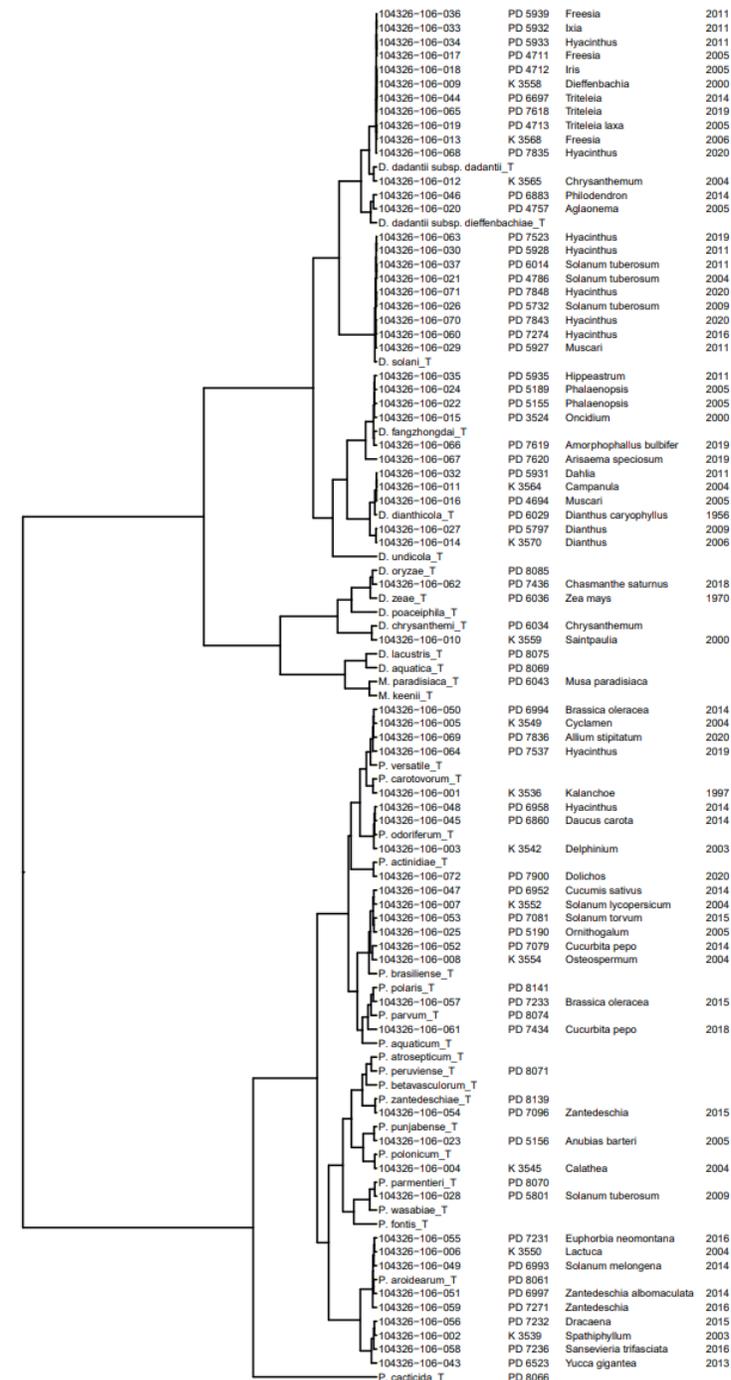
Collection at NIVIP

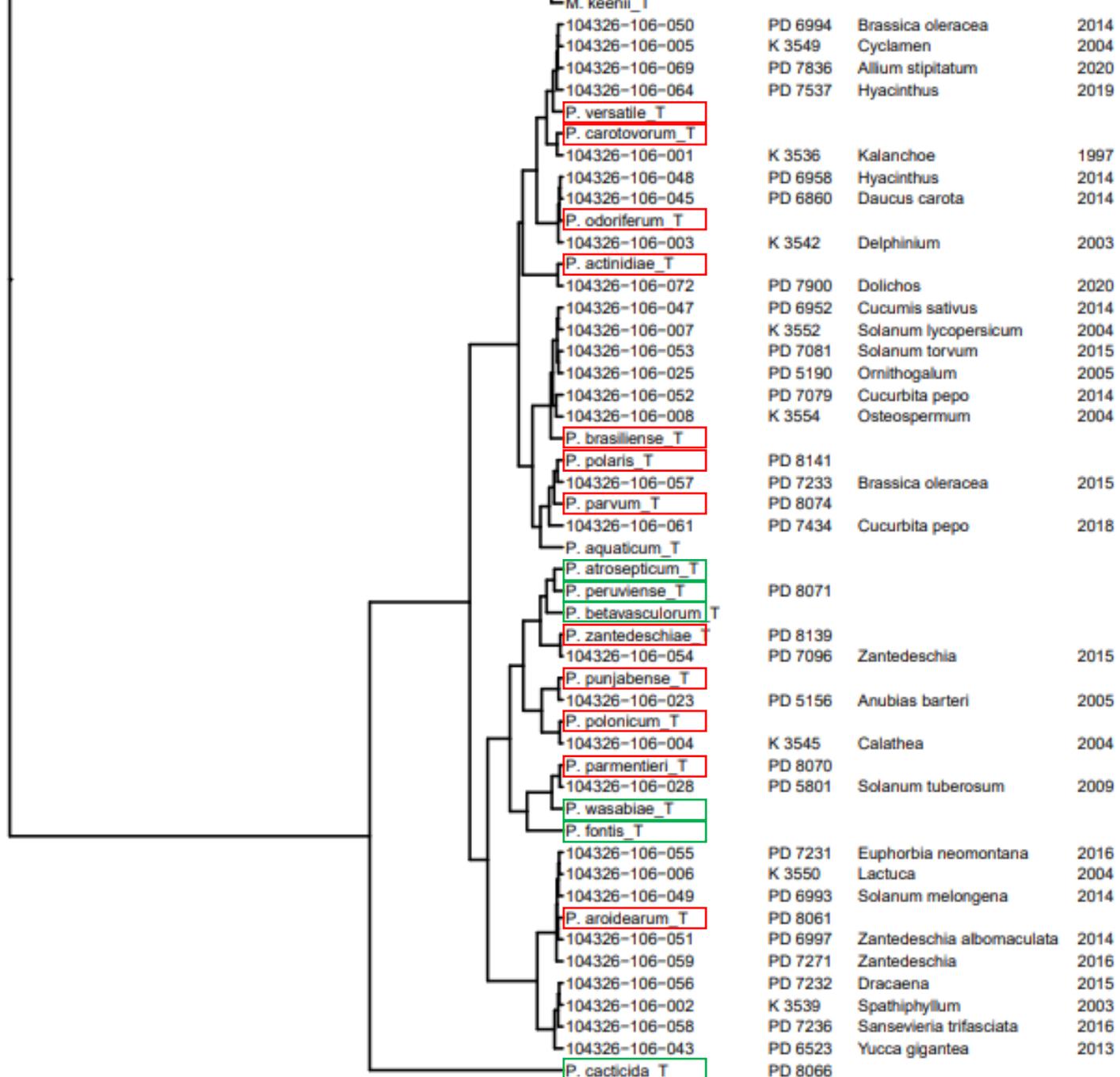
- › Which species are found in the Netherlands?
 - We get questions from third countries
 - Identify potential dangers

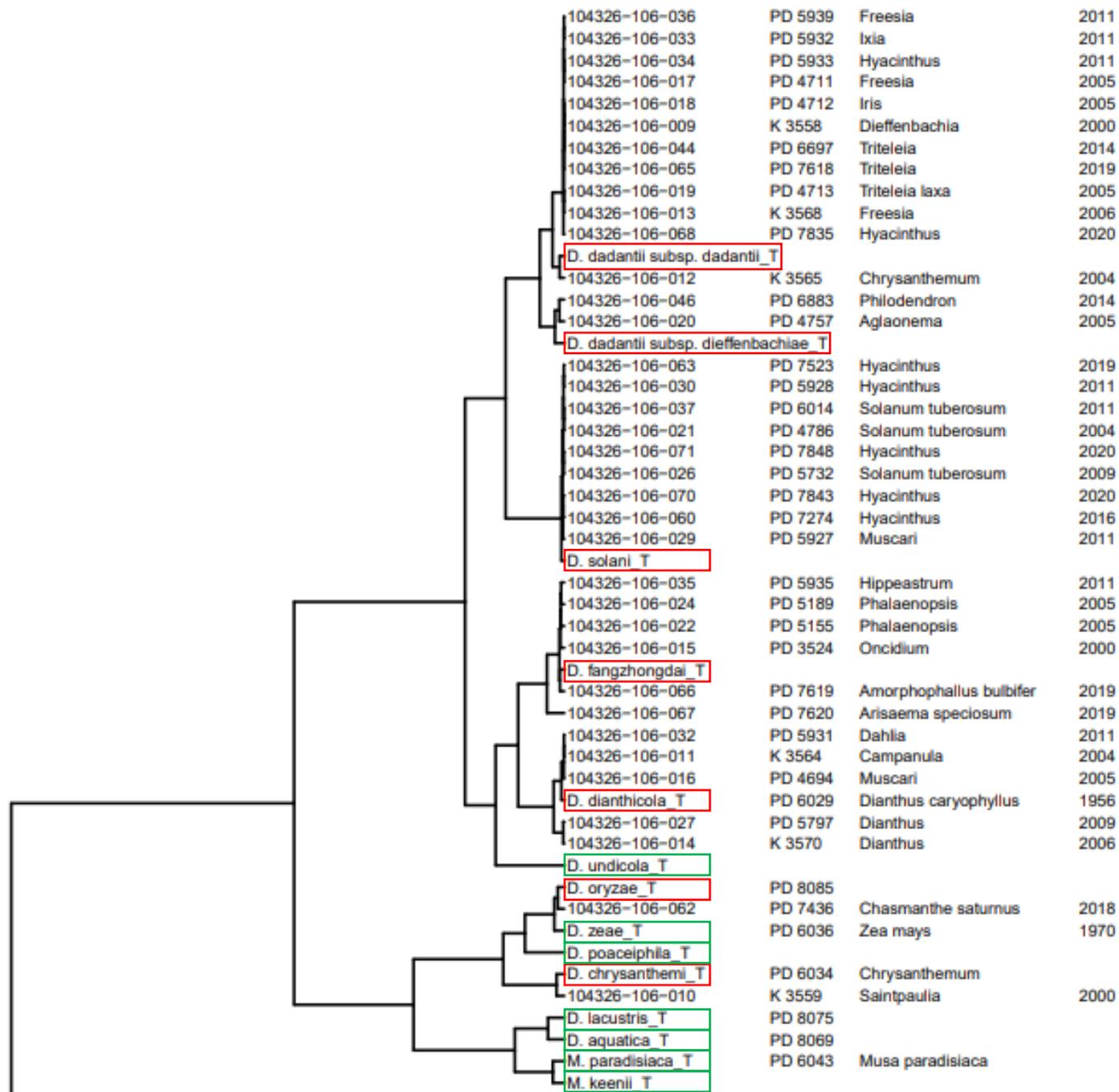
- › ~400 isolates in collection (1980's until now)
 - *E. chrysanthemi* or *E. carotovora*



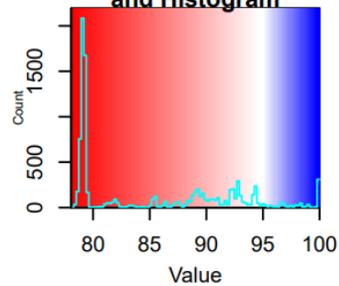
- > Selected ~ 70 isolates
- > Sampled in the Netherlands
 - But not necessarily from the Netherlands!
- > Collected all type-strains
- > Illumina sequencing
 - ~ 70 isolates
 - Type-strain of which no WGS data was available at NCBI
- > *de novo* assembly (RAPT at NCBI)
- > Fast-ANI



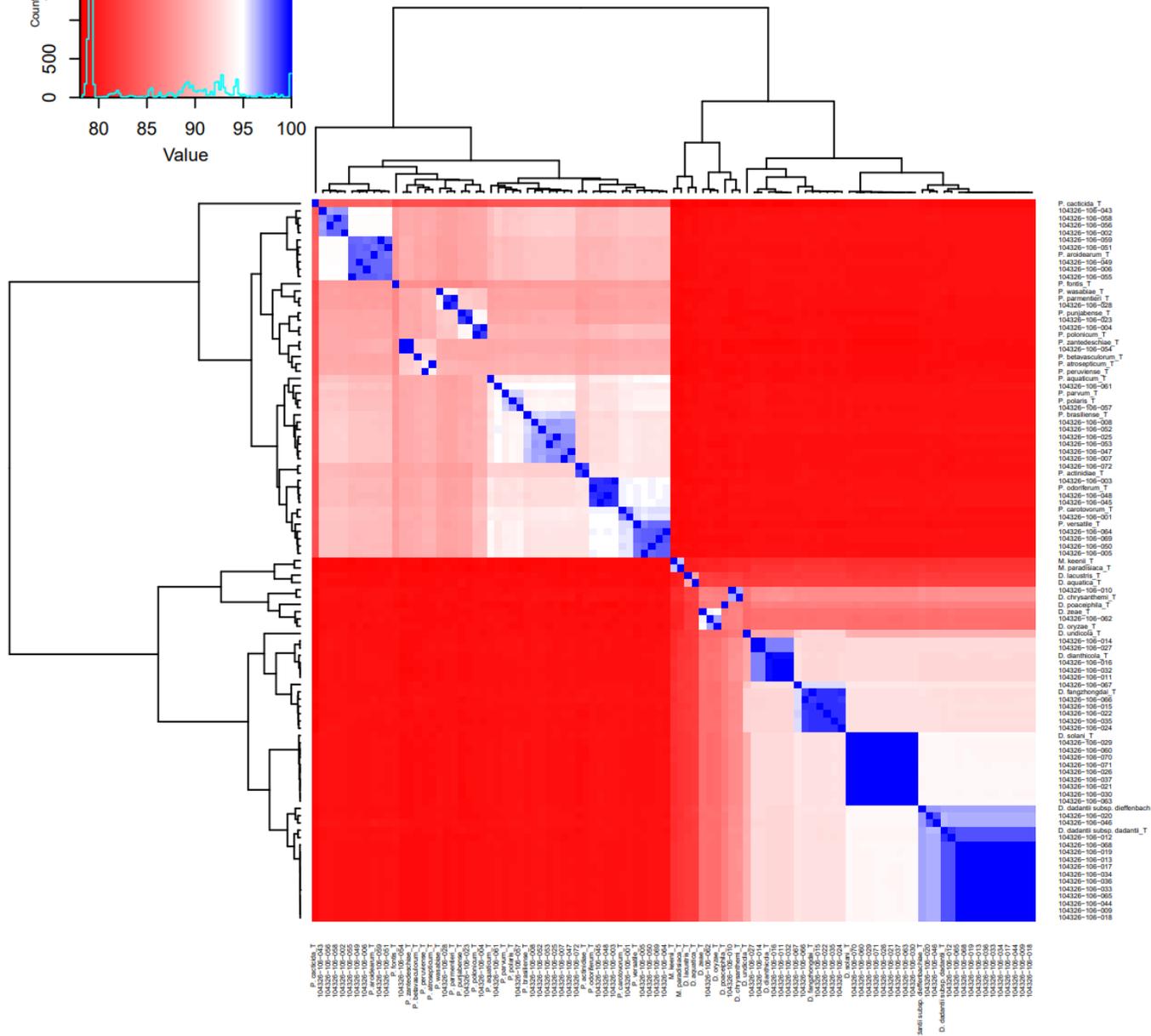




Color Key
and Histogram

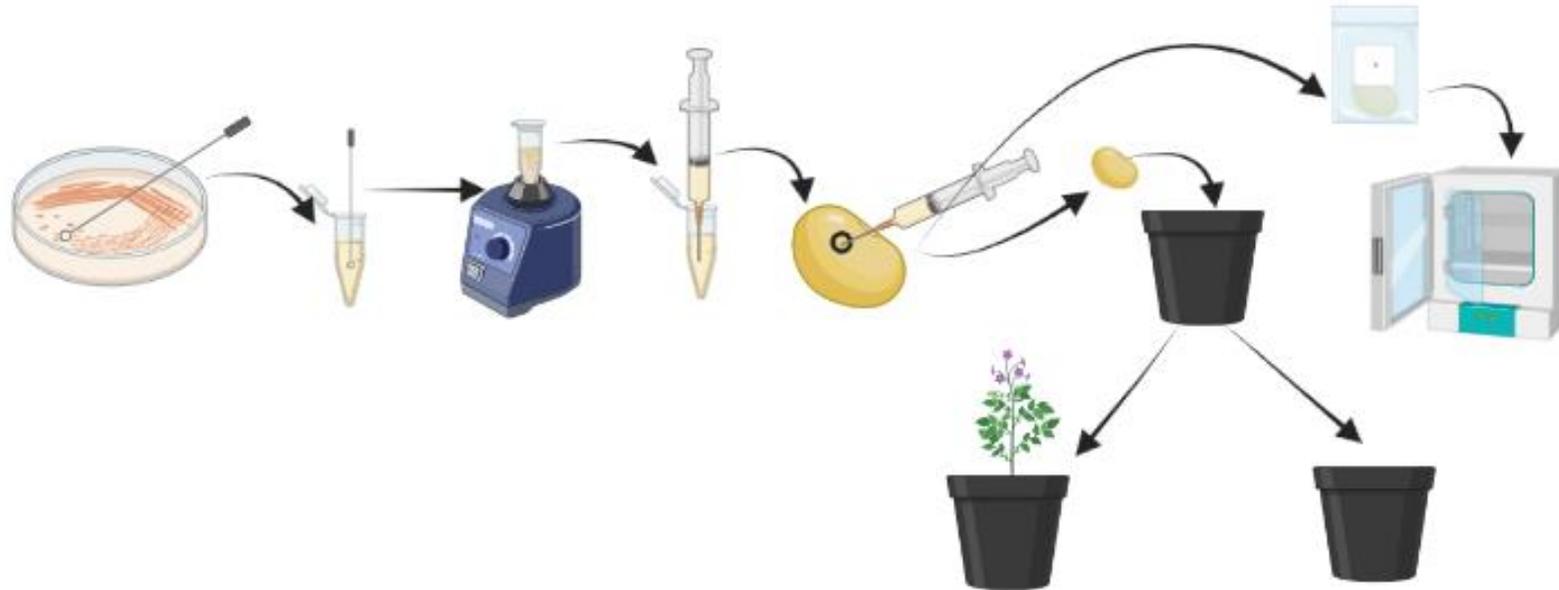


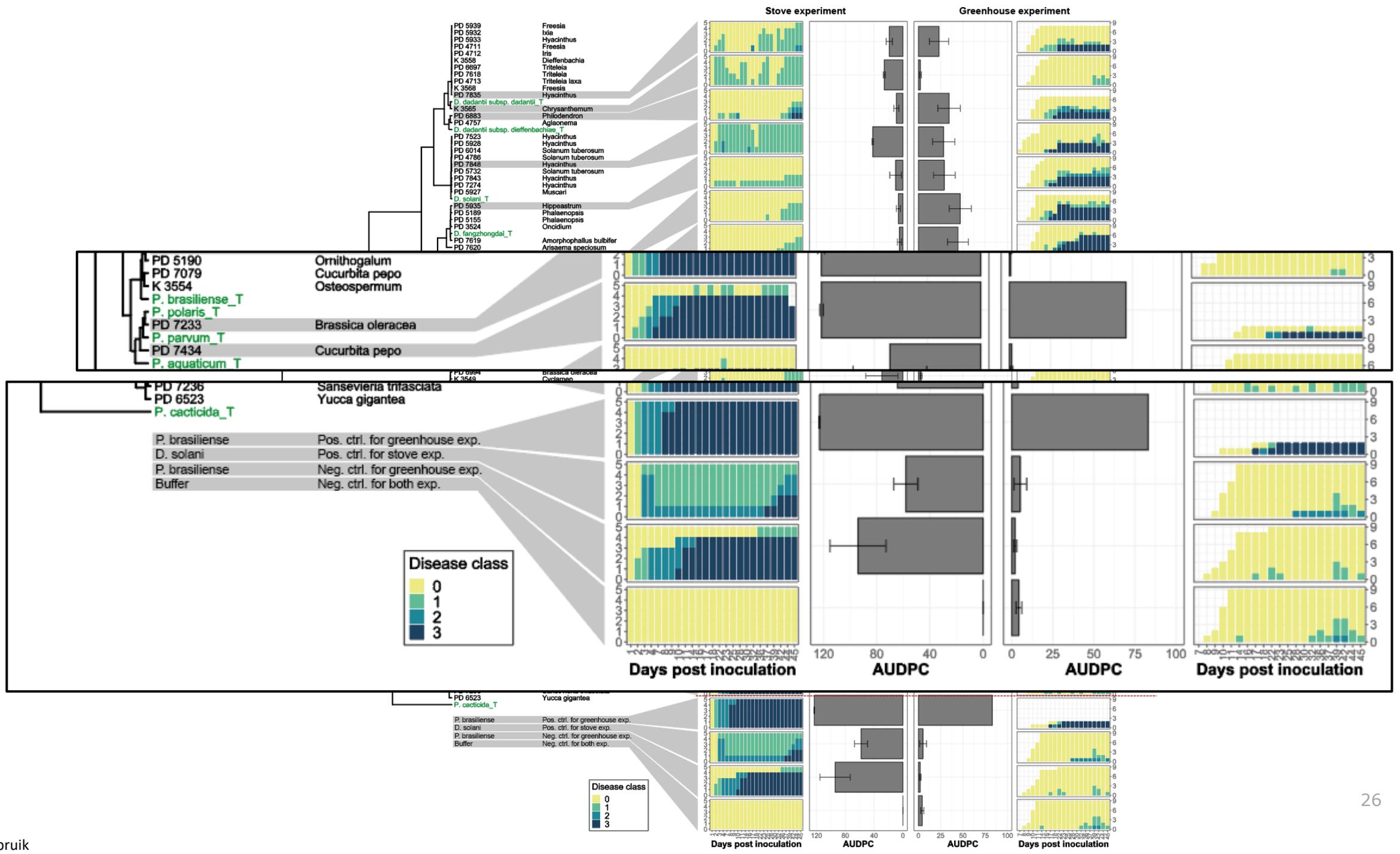
fastANI heatmap





- > Is the presence of these isolates a problem?







Conclusions

- › Large diversity of *Pectobacterium* and *Dickeya* isolates was found
- › Only a limited number appear to be highly virulent on potato
- › It is hard to link virulence to the phylogenetic position



Acknowledgements

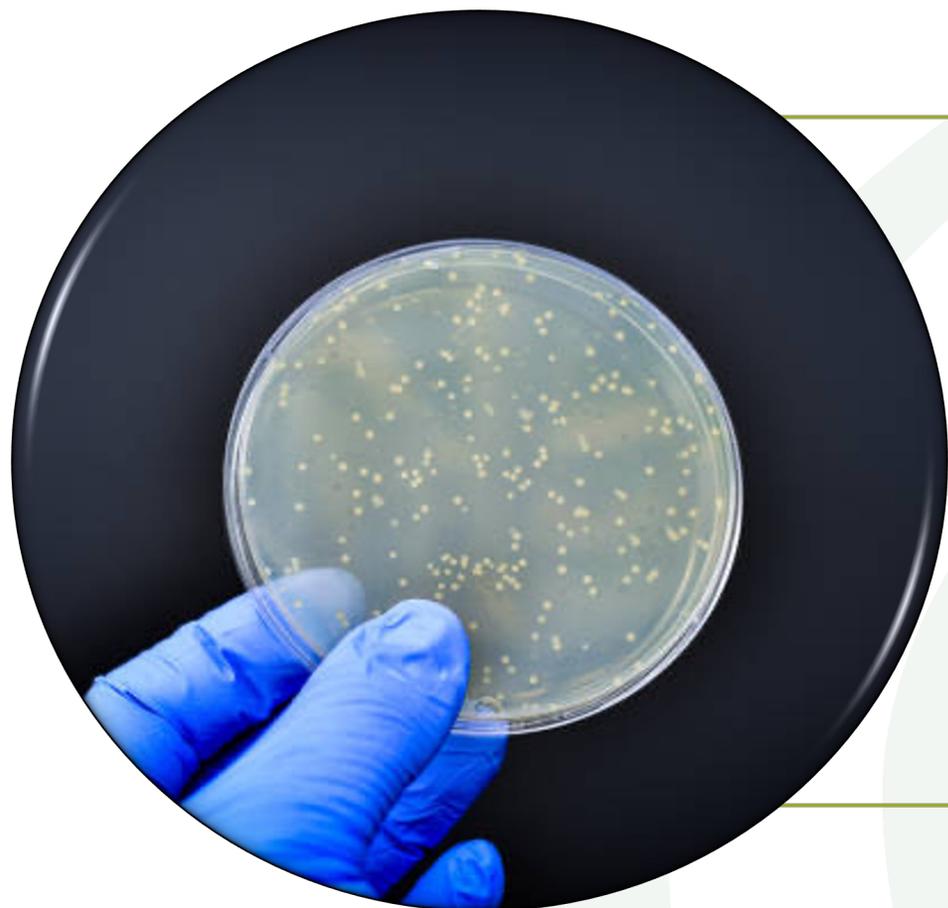
- > Bacteriology team
 - Marisja Boksebeld
- > Molecular biology team
 - Tom Raaymakers
 - Michael Visser
- > NAK
 - Inge van Duivenbode



**Glycoalkaloids from *Solanum spp* leaves modify
virulence factors in *Dickeya solani* and
Pectobacterium brasiliense sp. nov.**

Grupa-Urbańska A, Sołtys-Kalina D, Lebecka R

The Plant Breeding and Acclimatization Institute (IHAR) - National Research Institute



Pectobacterium brasiliense (Pcb)

Strain: Pcb3M16

Reference: Lebecka & Michalak, 2020



Dickeya solani (Ds)

Strain: IFB0099

Reference: Golanowska et al., 2015

Introduction to bacterial pathogens and their impact



Classification:
Gram-negative
bacteria
family
Pectobacteriaceae.



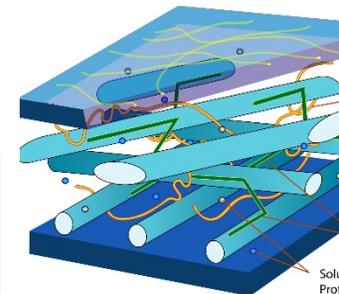
Major threats to
potato crops,
causing big
yearly losses.



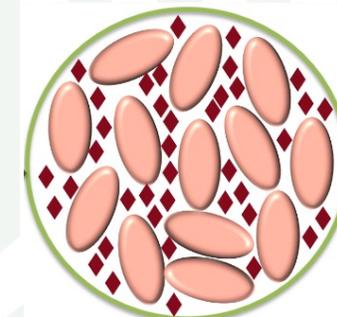
Ds and Pcb
cause **soft rot**
and **blackleg**,
among top **10**
destructive plant
pathogens.



Chemical
protection
against bacterial
diseases is **not**
practiced.



Ds and Pcb
virulence is
mainly due to
plant cell wall
degrading
enzymes
(**PCWDEs**).

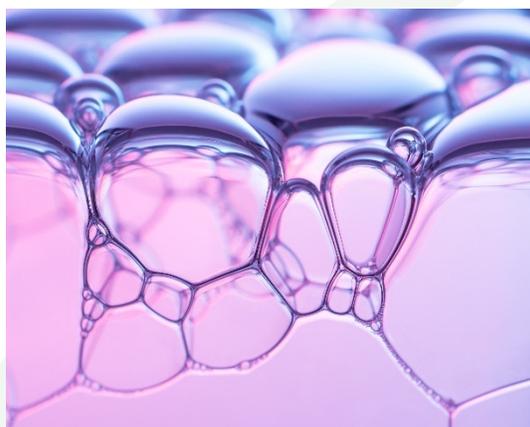


The expression
of these
enzymes is
controlled by
**quorum
sensing (QS)**
systems.

Glycoalkaloids (GAs) in Potato Plants



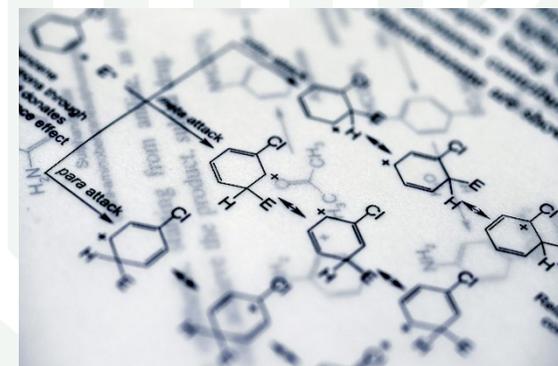
Potato plants contain **protective metabolites** that defend against threats like insects, herbivores, and pathogens.



Potato plants produce **glycoalkaloids (GAs)**, toxins that defend against bacteria, fungi, viruses, and insects.



GAs Composition:
 α -chaconine and α -solanine: 95% of total GAs.



Other GAs:
solasonine,
solamargine, leptinine I,
& leptinine II.

Materials & Methods

GAs sources:

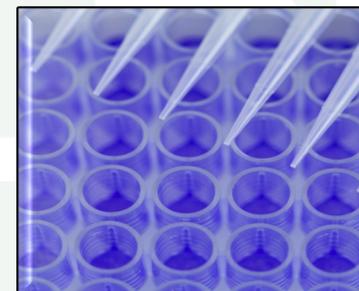
- ✓ 3 potato cultivars (Mieszko, Owacja, Tajfun)
- ✓ 3 wild species (*S. chacoense*, *S. maglia*, *S. garsiae*)
- ✓ 2 interspecific *Solanum* spp. hybrids (DG 00-683; DG 08-305)

Analytical technique:

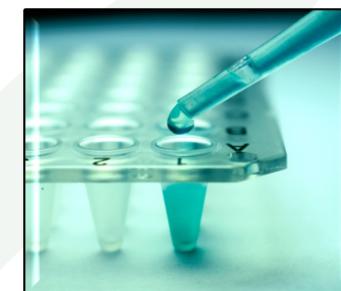
High-Performance Liquid Chromatography-Mass Spectrometry (**HPLC-MS**)



1. GAs & Pectinolytic Activity - **Crystal Violet Pectate (CVP) medium**

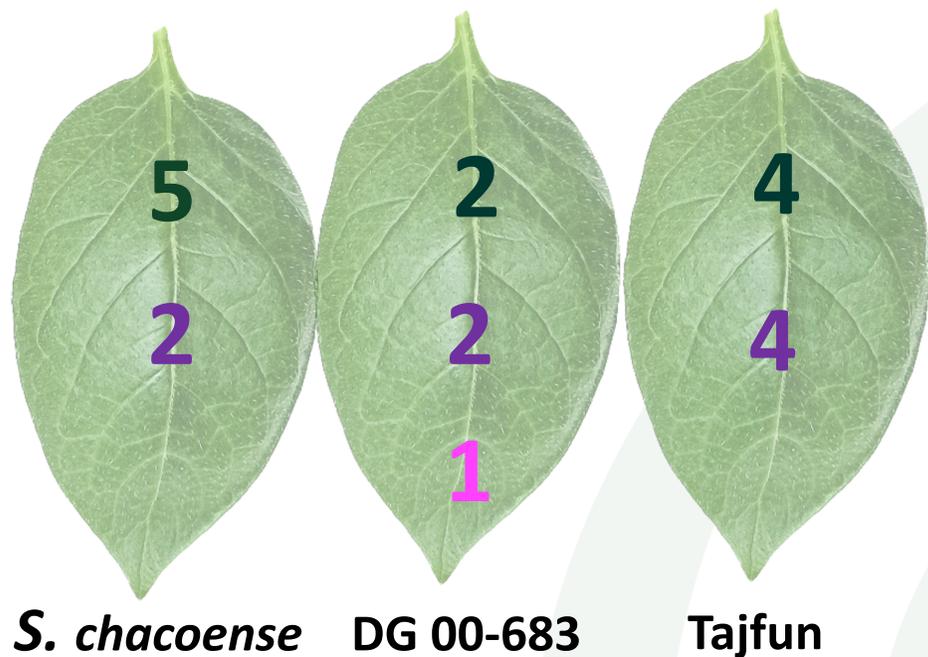


2. GAs & Biofilm Formation- **Microtiter plate assay stained with Crystal Violet**



3. GAs & QS **Gene Expression-quantitative PCR**

Selected GAs and their composition



α -chaconine



α -solanine



leptinine I



peak area on HPLC-MS histograms:

C = 0;

1 = $0 < C < 25,000$;

2 = $25,000 < C < 50,000$;

3 = $50,000 < C < 75,000$;

4 = $75,000 < C < 100,000$;

5 = $100,000 < C < 125,000$

Objective: To explore the potential of GAs, particularly from *Solanum* spp. leaves, as inhibitors against *Pectobacterium* and *Dickeya*.

Hypothesis: GAs can inhibit the growth, QS, enzymatic activity, and biofilm formation of these bacteria.

GAs impact on pectinolytic activity of bacterial isolates

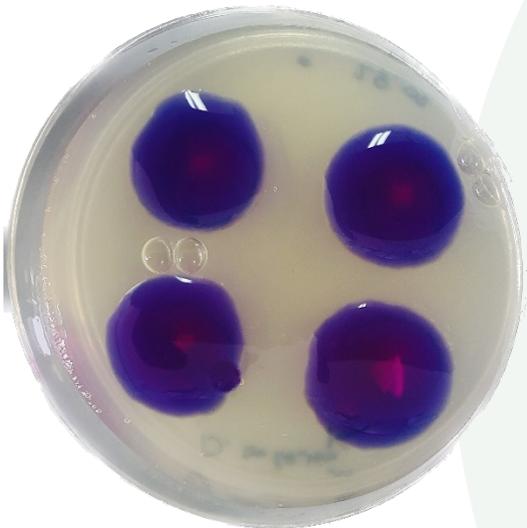
Assay Medium:
Crystal Violet
Pectate (CVP)
medium

**GAs
Supplementation:**
0.8 mg/ml of CVP

Assessment:
incubated at T 31°C
for 48 h

Inoculation:
Suspension:
10⁹ CFU mL⁻¹
Method: toothpick

Replicates:
3 biological
4 technical



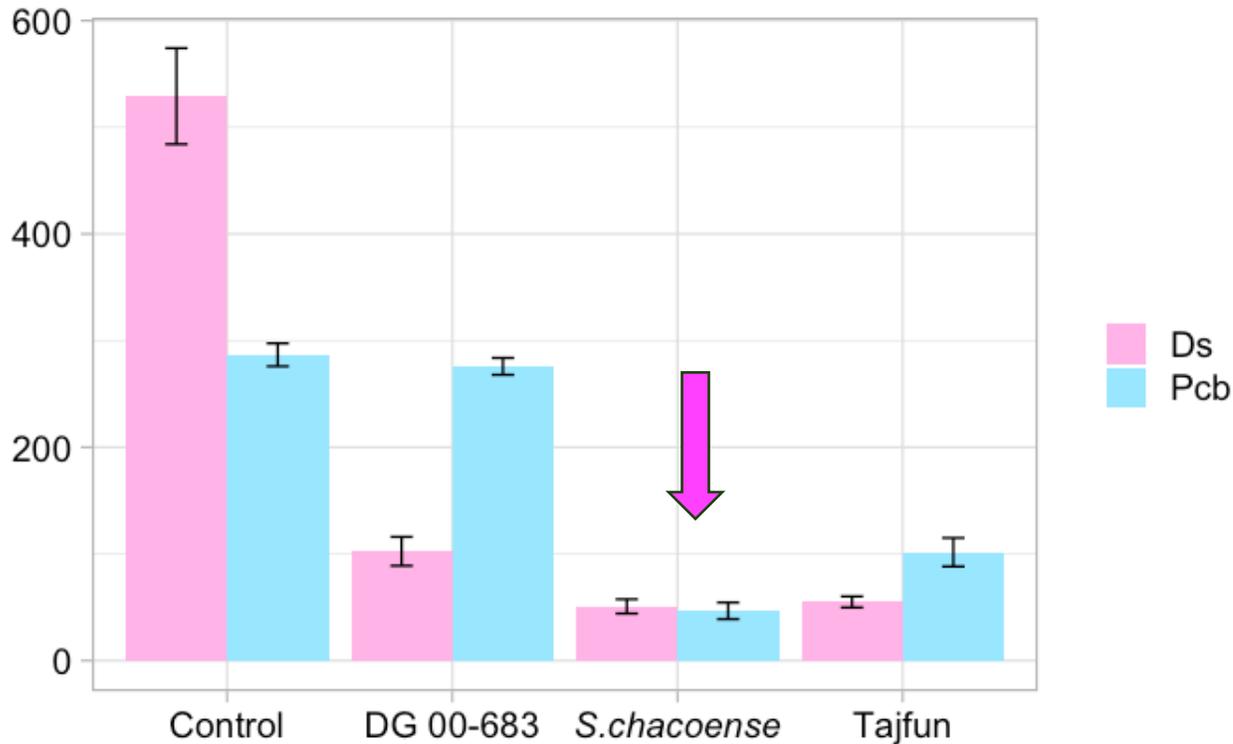
Control without GAs



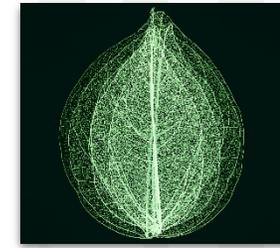
GAs from the cultivar Tajfun

Measurement of cavity volumes formed by Ds in CVP medium with and without GAs.

Effect of GAs on pectinolytic activity of bacterial isolates



GAs, from *S. chacoense*, significantly inhibited the pectinolytic activity of both bacterial strains.



Ds showed similar responses to GAs from **DG 00-683** and **Tajfun**, but both were weaker than the response to GAs from *S. chacoense*.



Pcb exhibited no change in activity with GAs from DG 00-683 compared to the control.

Biofilm Formation

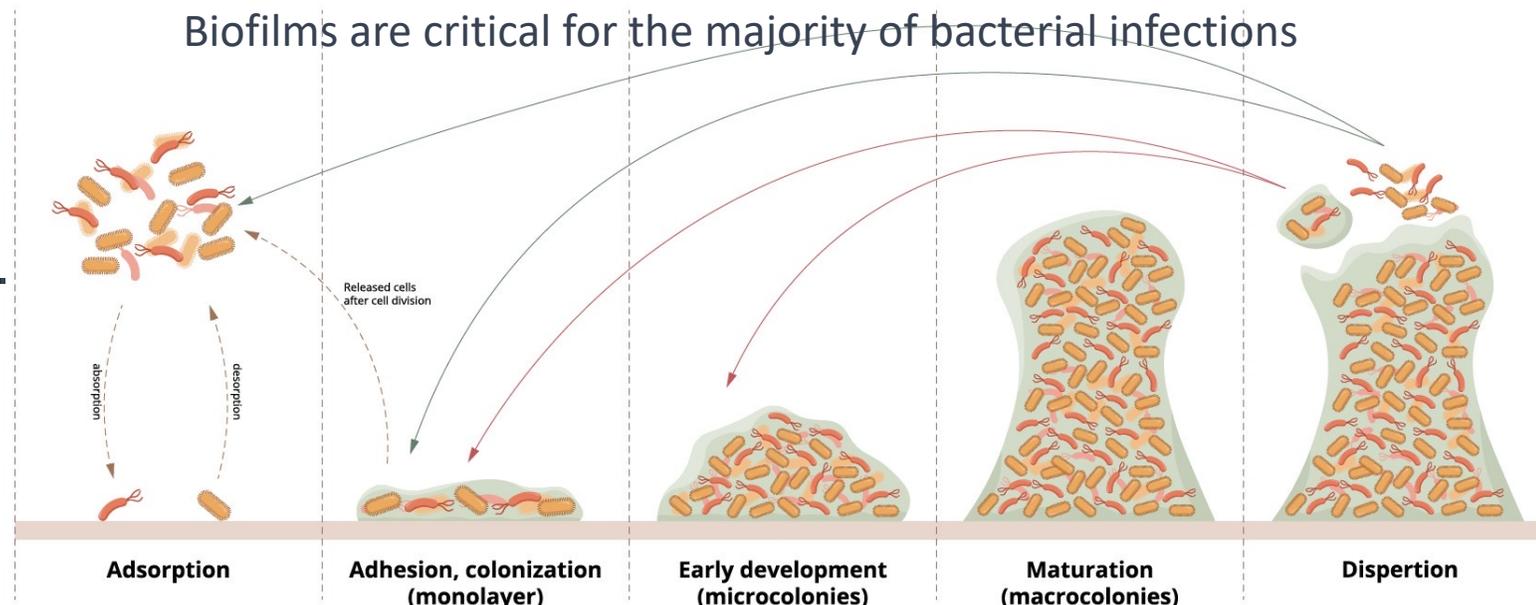
Structured community of microbial cells enclosed in a self-produced polymeric matrix adherent to a surface.

Components:

Microbial Cells: "Bacteria or microorganisms forming layers."

Extracellular Polymeric Substances (EPS):

"Mixture of polysaccharides, proteins, nucleic acids, and lipids."



Biofilm lifecycle

Ma et al., 2022

Our research focused on observing the **early stages of biofilm formation**.

We specifically analyzed the biofilm after **6** hours of bacterial growth.

GAs role in bacterial biofilm formation

Biofilm Assessment

Microtiter plate assay
Stained with Crystal Violet

Bacterial suspension

10^6 CFU

Incubation

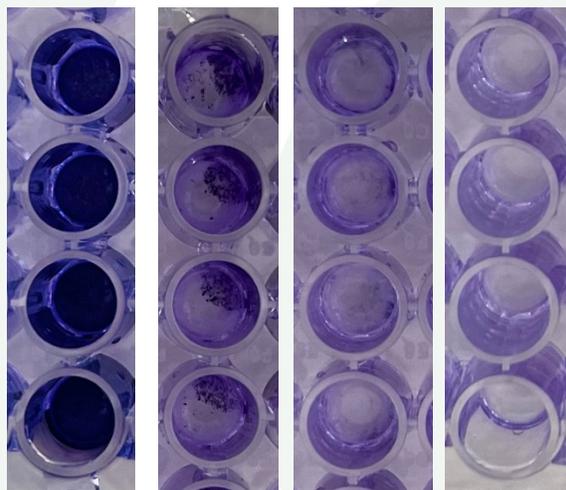
6 h at 30°C

Biofilm Quantification

560 nm OD

Replicates

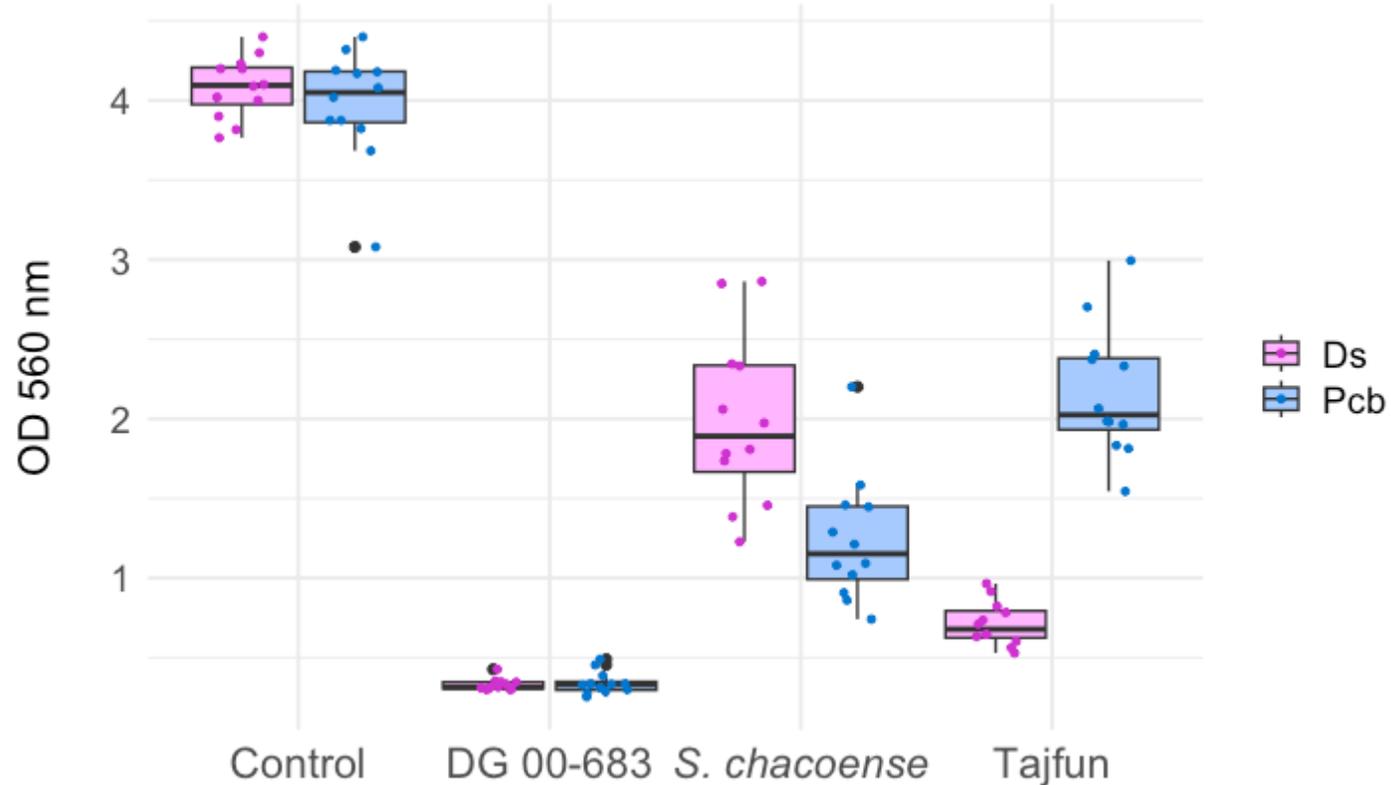
3 biological
4 technical



1 2 3 4

1. Control
2. Tajfun
3. *S. chacoense*
4. DG 00-683

Biofilm formation inhibition by GAs in *Ds* and *Pcb*



DG 00-683 GAs: Most effective for both *Ds* and *Pcb*

***S. chacoense* GAs:** Noticeable reduction, but less than DG 00-683

Tajfun GAs: Highly effective against *Ds*

Quorum Sensing in *Dickeya* & *Pectobacterium*: A Key player in plant pathogenicity

QS plays an important role in bacterial growth, virulence, motility and biofilm formation.

It operates through auto-inducers (AIs), which give an idea of bacterial density.

These auto-inducers are chemical signals, such as acyl-homoserine lactones (AHL).

Ds

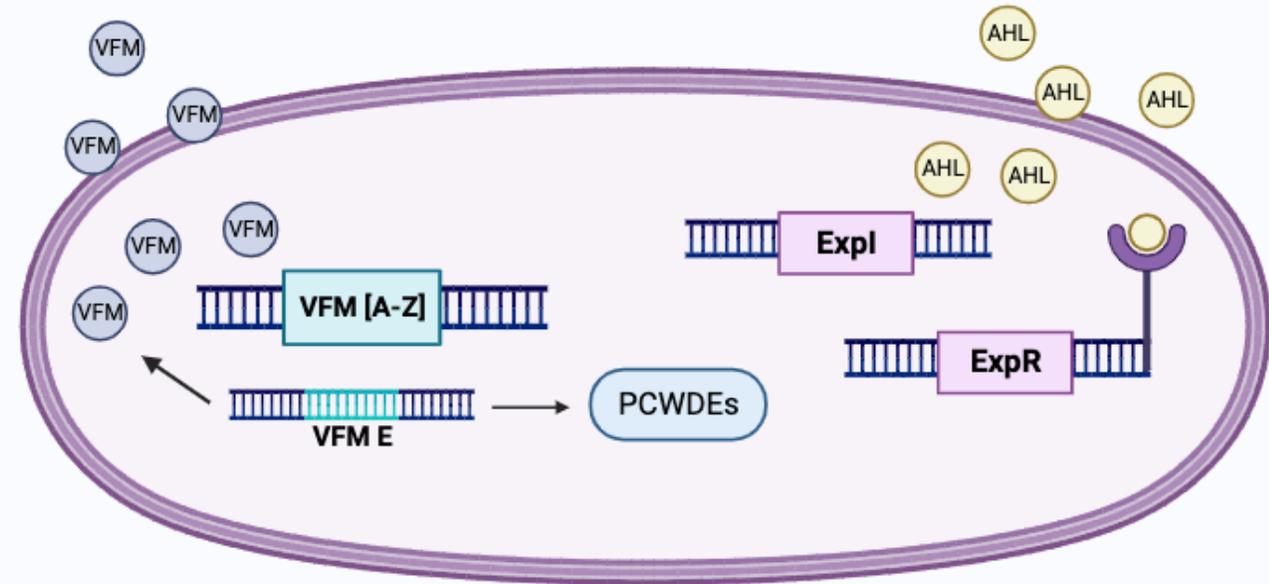
Uses two QS systems:

- AHL-based (synthase *ExpI* and sensory protein *ExpR*)
- *Vfm* system which has 26 genes (*VFM A-Z*)

Notably, ***VFM E* plays a crucial role in PCWDEs production.**

Pcb

QS is focused on AHL production, detection, and response.



As bacteria grow, they produce AHLs via the enzyme *ExpI*. When AHL levels are high, they bind to *ExpR* and activate QS-related genes.

Methodology for Quorum Sensing genes expression analysis

**GAs
Supplementation**
0.8 mg/ml

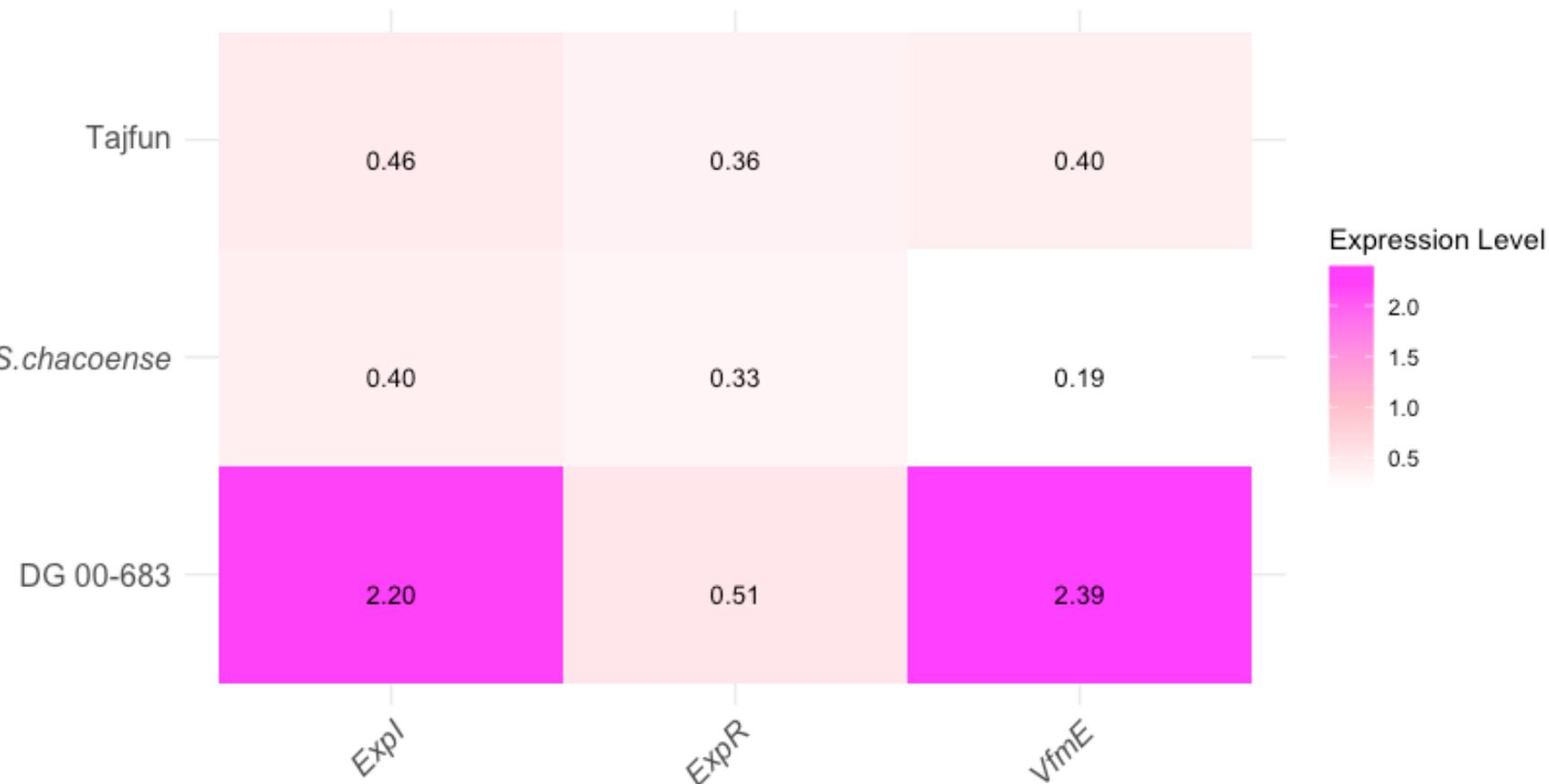
Incubation:
t 30°C for 8 h
180 rpm

RNA Isolation

qPCR to analyze
the expression of
**ExpI, ExpR, &
VfmE**

Relative gene expression of ExpI, ExpR, and VfmE was calculated using $2^{-\Delta\Delta C_t}$ method.

Impact of GAs on QS gene expression in *Dickeya solani*



DG 00-683: highest expression of Expl & VfmE.

***S. chacoense*:** strongly suppresses all genes. White cell (0.19) for **VfmE** indicates minimal expression.

Tajfun: moderate expression levels.

Key Findings:

- GAs, particularly from *S. chacoense*, significantly **inhibited pectinolytic activity** of Ds and Pcb.
- GAs from DG 00-683 most effectively **inhibited biofilm formation** in both Ds and Pcb.
- Varying impacts on QS gene expression: DG 00-683 highest for ExlI & VfmE; *S. chacoense* suppressed all tested genes; Tajfun – at moderate levels.

Conclusion:

Glycoalkaloids show potential as natural inhibitors against key virulence factors of Ds and Pcb, suggesting a possible eco-friendly alternative for controlling potato bacterial diseases. Further studies are needed.



Thank you for your attention.

The Plant Breeding and Acclimatization Institute (IHAR) - National Research Institute

Radzików
05-870 Błonie, Poland
tel. 22 733 45 00
NIP-PL: 5290007029
REGON: 000079480
e-mail: postbox@ihar.edu.pl
www.ihar.edu.pl

Anna Grupa-Urbańska

Platanowa 19
05-831 Młochów

e-mail: a.grupa@ihar.edu.pl



Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Federal Department of Economic Affairs,
Education and Research EAER

Agroscope



Instytut Hodowli i Aklimatyzacji Roślin - Państwowy Instytut Badawczy
Plant Breeding and Acclimatization Institute - National Research Institute
Radzików, 05-870 Błonie, Poland

Increase of glycoalkaloid content in potato tubers by greening as a method to reduce the spread of *Pectobacterium* and *Dickeya* spp. in seed production systems

Dorota Sołtys-Kalina, Anna Grupa-Urbańska, Renata Lebecka, Maud Tallant, Isabelle Kellenberger, Brice Dupuis

04.09.2023



Context

- Diseases caused by *Dickeya* and *Pectobacterium* species are responsible for losses of about EURO 46 M annually for the potato sector of the European Union
- There are no commercial products available to control the spread of these bacteria in seed potato production systems either in the field or during storage



Hypothesis

The glycoalkaloids (GAs) naturally produced by the tuber after greening will allow the control of *Pectobacterium* and *Dickeya* in seed potatoes



How to proceed practically?

The seed potatoes are harvested in two steps:

1. The potato harvester digs up the potatoes and leaves them on the soil exposed to sunlight for about ten days. The seed tubers will become green and increase their glycoalkaloid content
2. The seed tubers are then harvested and stored at low temperature waiting to be planted the next season



Digging out the potatoes
Source: Hardox



The tubers are exposed to sunlight
Source: Gardening know how



The tubers become green
Source: ABC rural



Harvest performed after greening
Source: Standen



How did we verify our hypothesis

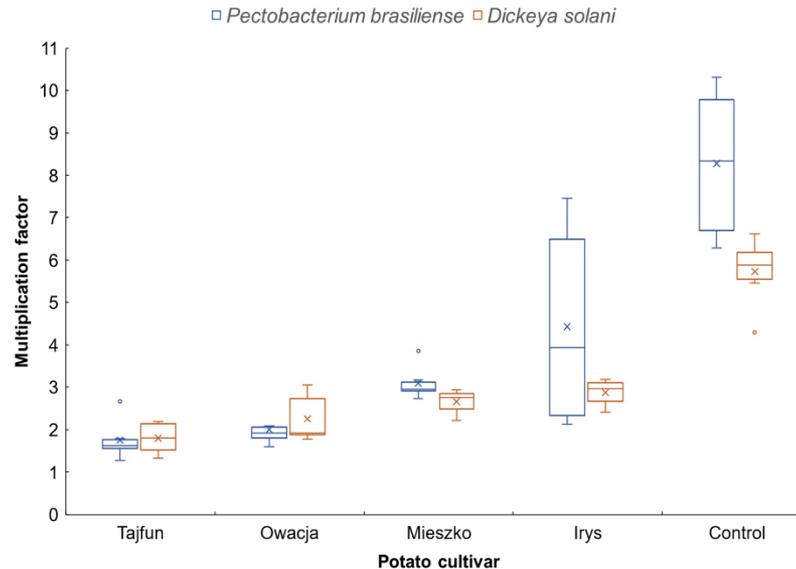
1. Effect of GAs on bacteria growth in vitro
2. Effect of GAs on bacteria viability in vitro
3. Effect of artificial greening in the field
4. Effect of natural greening in the field
5. Effect of greening on potato yield



Effect of GAs on bacteria growth in vitro

- Materials and methods
 - GAs were extracted from the leaves of four potato varieties (Tajfun, Mieszko, Irys, and Owacja)
 - Two bacterial strains (*Pectobacterium brasiliense* Pcb3M16 and *Dickeya solani* IFB0099) were grown in broth medium with the four different GAs extracts, and the OD was measured.

- Results

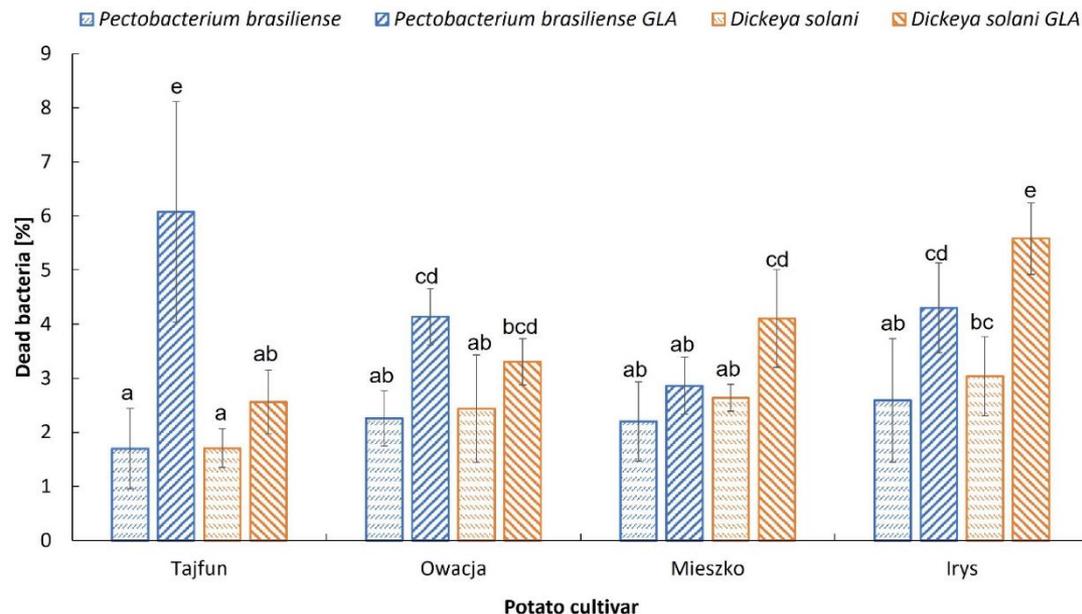




Effect of GAs on bacteria viability in vitro

- Materials and methods
 - GAs were extracted for the leaves of the same four potato varieties.
 - The same two bacterial strains were grown in broth medium with the four different GAs extracts, and the viability was measured with a CyFlow Space flow cytometer equipped with a blue laser.

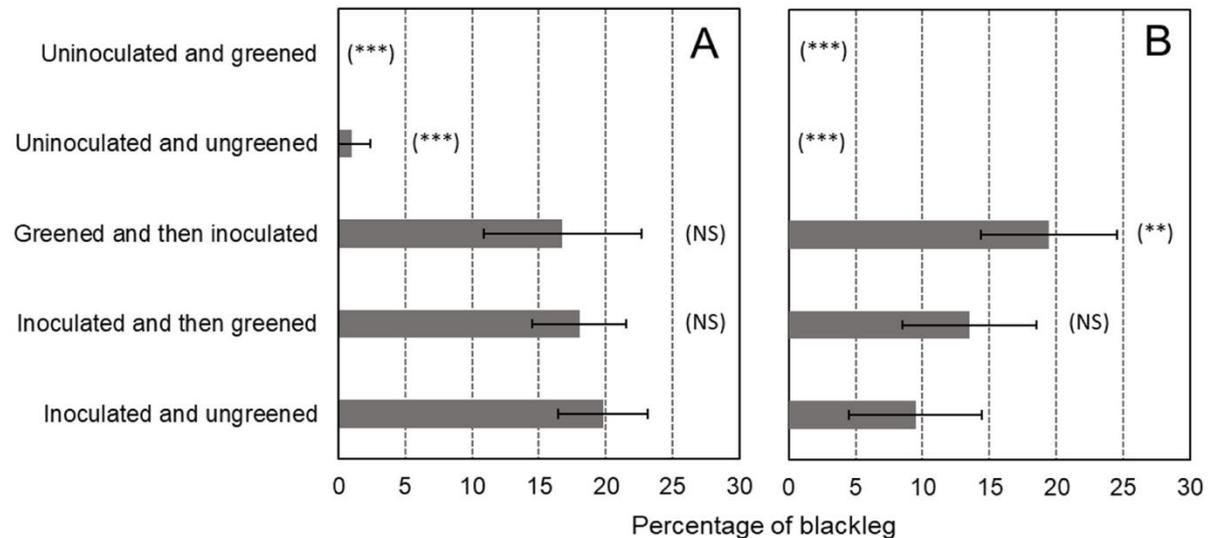
- Results



Effect of artificial greening in the field

- Materials and Methods
 - Tubers of cv. Agria were exposed to artificial light for 10 days before and after inoculation with *D. dianthicola* 8823.
 - They were then planted in the field and the development of blackleg symptoms was assessed. This trial has been repeated two consecutive years.

Results

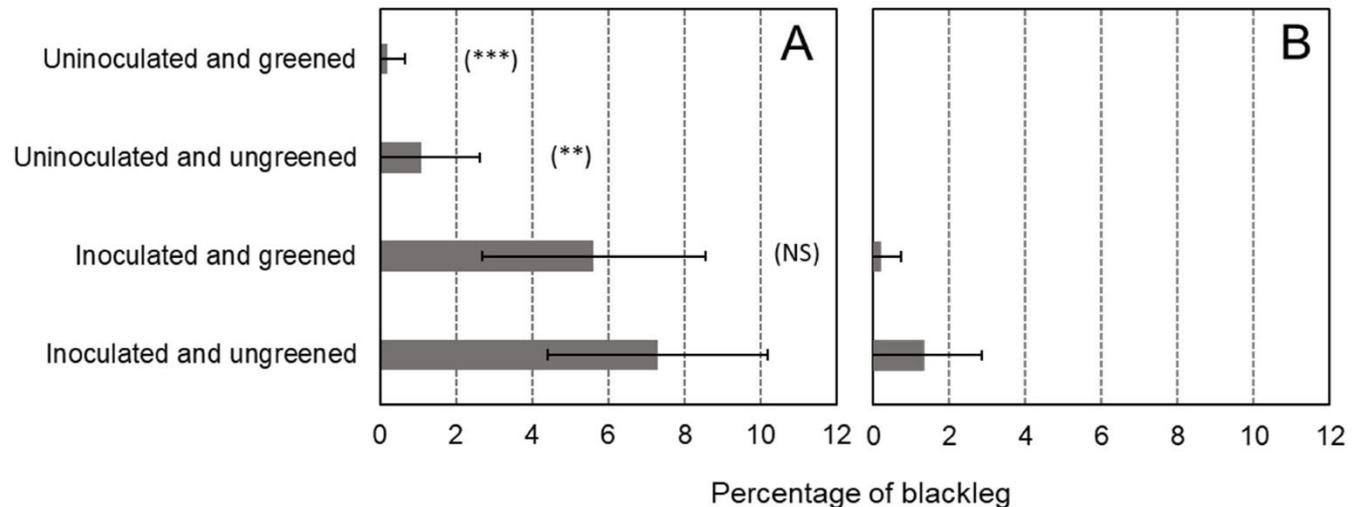


Effect of natural greening in the field

■ Materials and Methods

- Tubers of cv. Agria were inoculated with *D. dianthicola* 8823 and planted in the field.
- At harvest, they were exposed to sunlight for 10 days.
- The harvested tubers were planted the following year for blackleg assessment. This trial has been repeated two consecutive years.

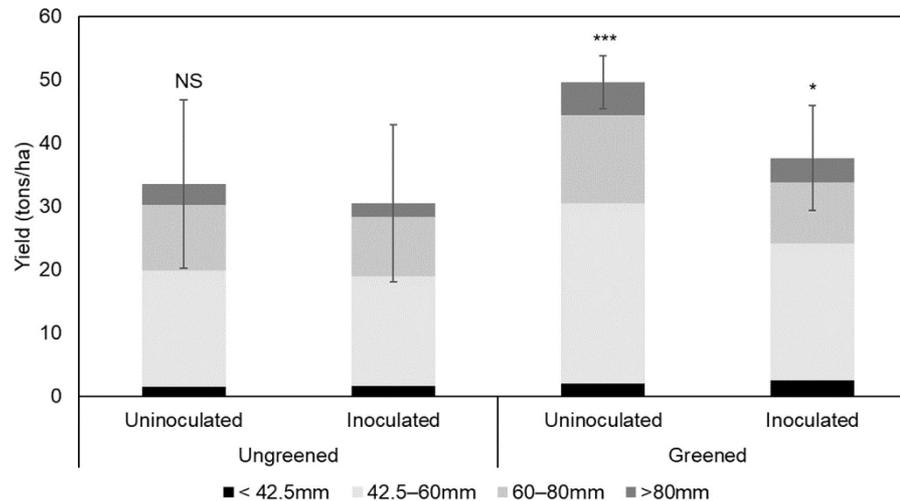
■ Results





Effect of greening on potato yield

- Materials and Methods
 - The yield was measured in both field trials
- Results
 - Artificial greening trials



- Natural greening trials → no significant effect



Conclusions

- **In the growth media:** all GAs isolated from the four cultivars appeared to be bacteriostatic and bactericidal against both bacteria strains. The inhibitory effect varied among GAs from the different cultivars
- **In the field:** Except for a one-year field trial, the blackleg incidence was lower in plants grown from green seed tubers without the yield being affected. The blackleg control was marginal, probably due to the low production of GAs by the tubers of cv. Agria after greening



Perspectives

New field trials are currently performed with different varieties presenting different GAs content after greening (high and low content)



For more informations



microorganisms



Article

Increase of Glycoalkaloid Content in Potato Tubers by Greening as a Method to Reduce the Spread of *Pectobacterium* and *Dickeya* spp. in Seed Production Systems

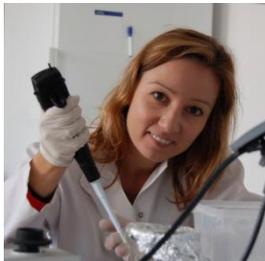
Dorota Sołtys-Kalina ¹, Anna Grupa-Urbańska ¹, Renata Lebecka ¹, Maud Tallant ², Isabelle Kellenberger ³ and Brice Dupuis ^{2,*}

¹ Plant Breeding and Acclimatization Institute–National Research Institute, Platanowa 19, 05-831 Młochów, Poland

² Agroscope, Plant Production Systems, Route de Duillier 50, 1260 Nyon, Switzerland

³ Agroscope, Plant Protection, Route de Duillier 50, 1260 Nyon, Switzerland

* Correspondence: brice.dupuis@agroscope.admin.ch





Thank you for your attention

Brice Dupuis

brice.dupuis@agroscope.admin.ch

Agroscope good food, healthy environment



Institut Hodowli i Aklimatyzacji Roślin - Państwowy Instytut Badawczy
Plant Breeding and Acclimatization Institute - National Research Institute
Radzików, 05-870 Błonie, Poland



RNAseq expression analysis of resistant and susceptible potato tubers at an early stage of infection with *Dickeya solani*



Lebecka R., Grupa-Urbańska A., Sołtys-Kalina D., Szajko K.

Department of Potato Genetics and Parental Lines, Plant Breeding and Acclimatization Institute, Radzików, E-mail: r.lebecka@ihar.edu.pl

Introduction

Soft rot of potato tubers is caused by pectinolytic bacteria of many different species, including *Dickeya solani*. It causes severe losses of potato yield. Chemical control is seldom used for this bacterial disease. The use of genetic resistance from wild potato relatives, found in the diploid clone DG 00-270, may be the genetic solution for improving of medium and low resistance levels to pectinolytic bacteria in cultivated potatoes. The mapping population (F1) was derived from the cross between two diploid potato clones, DG 00-270 (the resistant maternal parent, ♀) and DG 08-305 (the susceptible pollen parent, ♂). We discovered two strong and reproducible QTLs for resistance to *D. solani* on potato chromosomes IV and II. The objective of this study was to identify genes related to the complex trait of potato tuber soft rot resistance, in the early stage of infection, 8 hours post-inoculation. This moment corresponds to the transition from the early latent phase of infection to the symptomatic phase.

Materials

Tubers of the five most resistant and five most susceptible to soft rot diploid individuals from the F1 population (Fig. 1.).

Methods

Potato tubers wound-inoculated with bacteria, mock-inoculated and not wounded were sprayed with water and kept in closed boxes at a temperature 27°C. Tubers were cut after 8 h and tissue was cut out along the wound and immediately frozen in liquid nitrogen. RNA was isolated from three tubers per genotype, resulting in six bulked samples (Fig.2.), prepared in three replicates.

Sequencing was performed on Illumina NovaSeq6000 sequencing platform. Reads were mapped to the reference genome *Solanum tuberosum* DM 1-3 516 R44 (NCBI RefSeq GCA_000226075.1). Differential gene expression analysis was performed with DESeq2 (Love et. al. 2014).

Results

Differences among treatments explained 77 % of the variance, between resistance level – 15% (Fig. 3.).

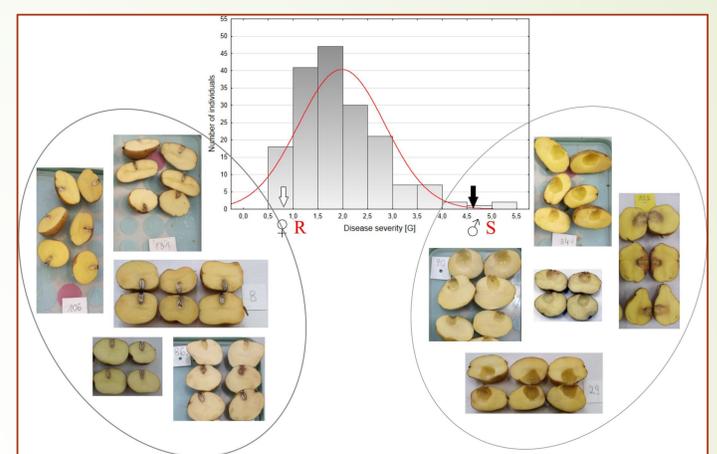


Fig. 1. Symptoms of infection three days post inoculation with *D. solani* in selected individuals from the mapping population.

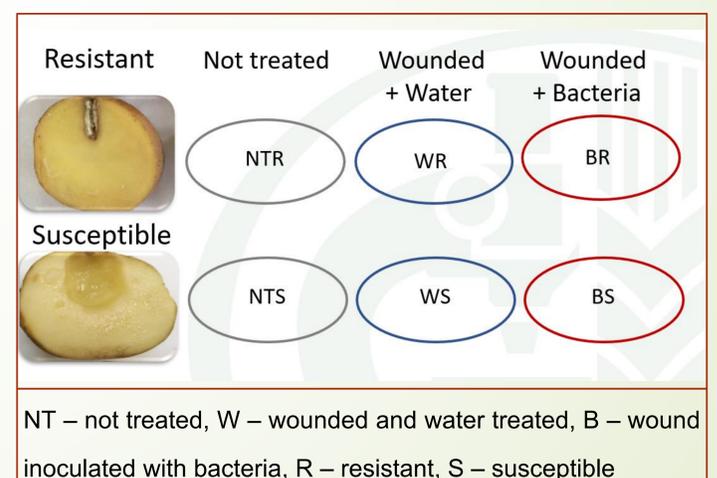


Fig. 2. Experiment layout.

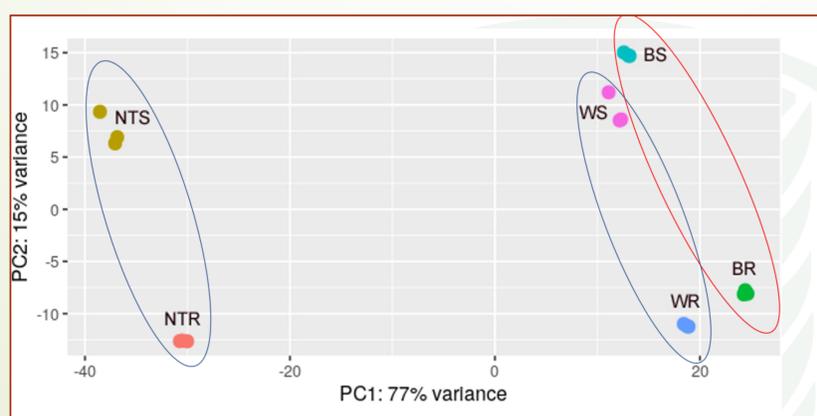


Fig. 3. Principal Component Analysis of sequenced samples.

Out of 408 significantly differentially expressed genes in BS vs BR samples (351 genes only in BR and 57 genes in both BR and WR bulks, shown in Fig. 4), twenty-five were selected that were associated with: stress response, resistance to bacteria, pathogenesis, resistance genes, defense mechanisms, pathogen recognition, wound healing, suberization (peroxidases, 5 of which were found only in BR samples, feruloyl Co-A, and cytochrome P450). The expression of selected genes will be further evaluated by qPCR in individual samples of RNA from resistant and susceptible individuals.

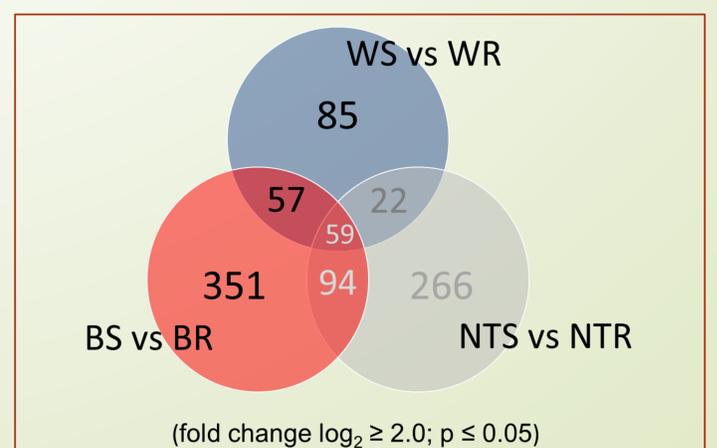


Fig. 4. The number of significantly differentially expressed genes in the bulk of RNA from „resistant” individuals in comparison with the „susceptible” one.



Evaluation of the phenotypic and genotypic diversity of *Ralstonia solanacearum* in metropolitan France and the risks for emergence of other species of the *Ralstonia* spp. complex

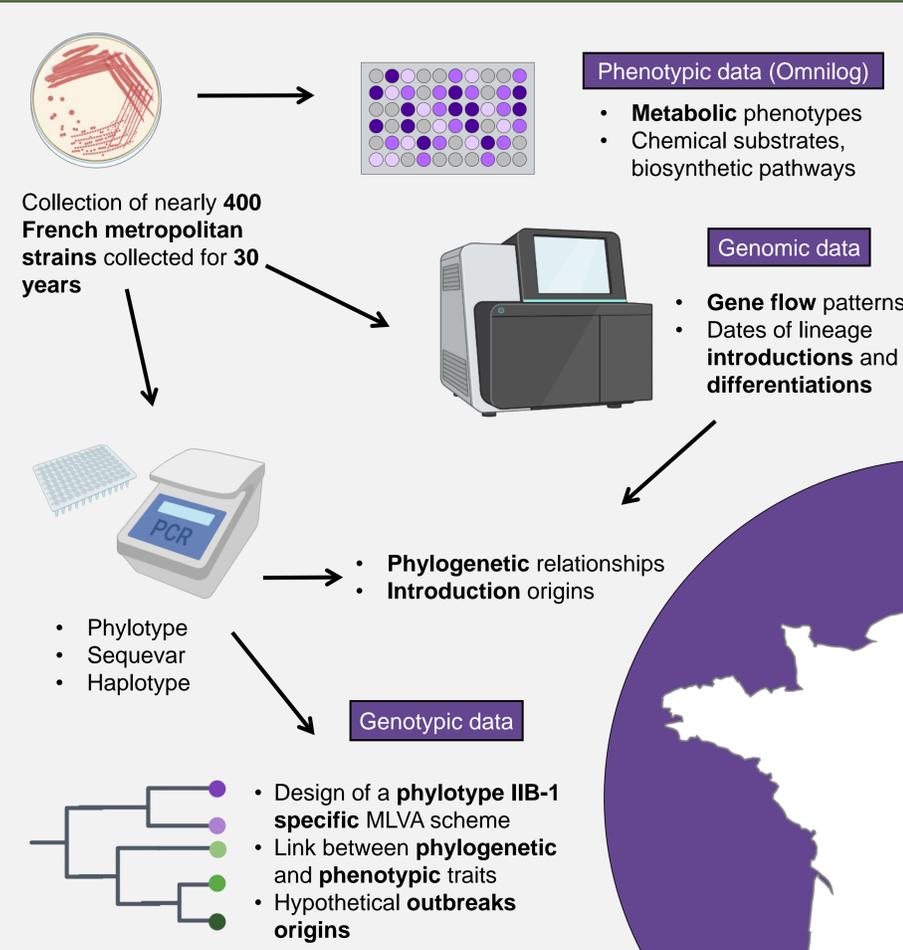


Antinea Sallen^{1,2}, Aurélie Leclerc², Sandrine Paillard¹, Philippe Reignault¹, Amandine Cunty^{1*} and Anne-Claire Le Roux^{2*}

¹Plant Health Laboratory, ANSES, Angers, France ; ²FN3PT/Inov3PT, INRAE-IGEPP, Le Rheu, France. *These authors contributed equally to this work. antinea.sallen@anses.fr or antinea.sallen@inov3pt.fr

<h2 style="margin: 0;">Background</h2>	<ul style="list-style-type: none"> The <i>Ralstonia solanacearum</i> species complex (RSSC) includes distinct species (<i>R. solanacearum</i>, <i>R. pseudosolanacearum</i> and 3 ssp. of <i>R. syzygii</i>) and is considered one of the most damaging plant pathogens worldwide; as such, the European Union considers the whole complex as quarantine organisms. Recent findings of <i>R. pseudosolanacearum</i> in Europe are of great concern, and highlight strong survival and adaptation capabilities There is a genuine risk for emergence of other species of the RSSC in France, especially in the context of climate change 	<h2 style="margin: 0;">Goals</h2>	 <ul style="list-style-type: none"> Determining the phenotypic and genotypic diversity of the French metropolitan RSSC strains Assessing the risk for emergence and establishment of RSSC strains (other than IIB-1) in Europe
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1 Epidemiological approach



Phenotypic data (Omnilog)

- Metabolic phenotypes
- Chemical substrates, biosynthetic pathways

Genomic data

- Gene flow patterns
- Dates of lineage introductions and differentiations

Genotypic data

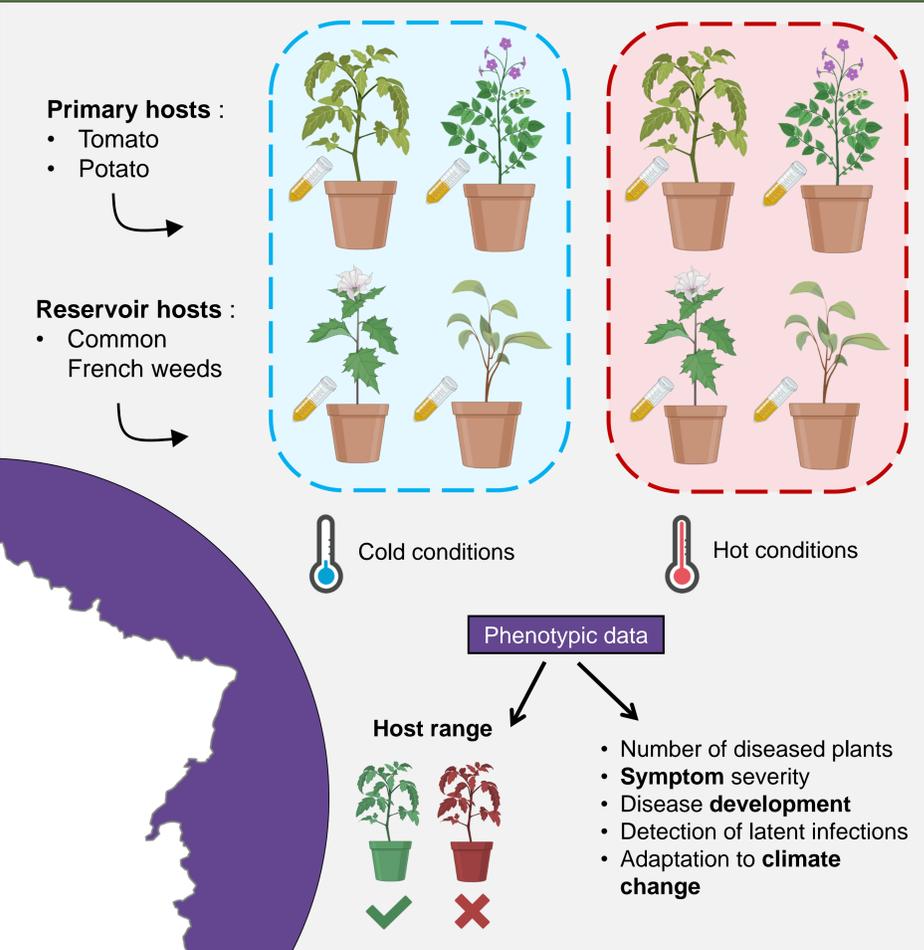
- Phylogenetic relationships
- Introduction origins

Phylotype

- Sequevar
- Haplotype

- Design of a **phylotype IIB-1 specific MLVA scheme**
- Link between **phylogenetic** and **phenotypic** traits
- Hypothetical **outbreak origins**

2 Host range and pathogenicity



Primary hosts :

- Tomato
- Potato

Reservoir hosts :

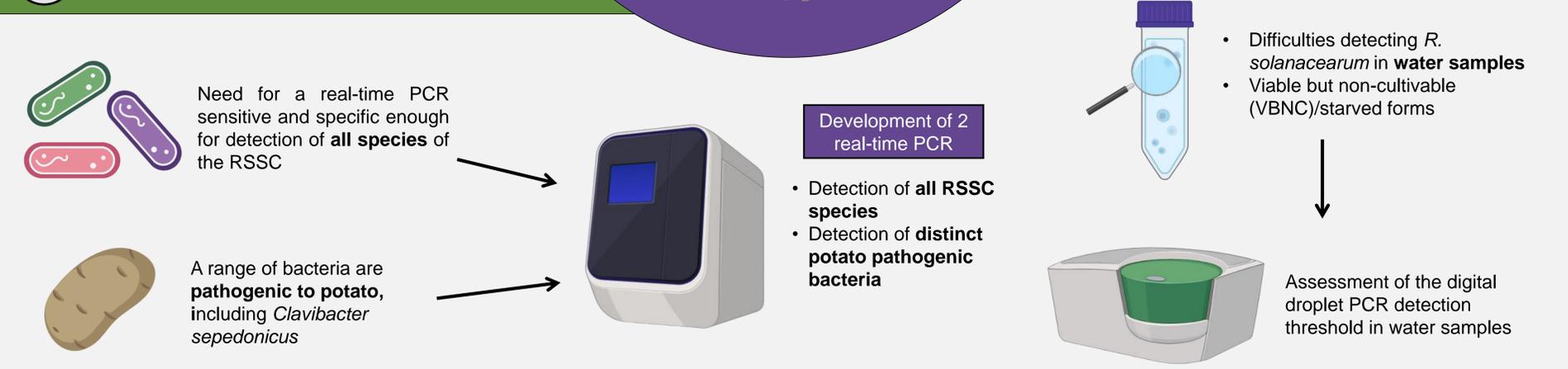
- Common French weeds

Phenotypic data

Host range

- Number of diseased plants
- Symptom** severity
- Disease **development**
- Detection of latent infections
- Adaptation to **climate change**

3 Detection and identification tools



Need for a real-time PCR sensitive and specific enough for detection of all species of the RSSC

A range of bacteria are pathogenic to potato, including *Clavibacter sepeadonicus*

Development of 2 real-time PCR

- Detection of **all RSSC species**
- Detection of **distinct potato pathogenic bacteria**

Difficulties detecting *R. solanacearum* in water samples

- Viable but non-cultivable (VBNC)/starved forms

Assessment of the digital droplet PCR detection threshold in water samples

<p>Spatial and temporal evolution of the :</p> <ul style="list-style-type: none"> Phenotypic diversity Genotypic diversity <p>of French metropolitan RSSC strains</p>	<ul style="list-style-type: none"> Interaction with their environment Pathogenicity Host range Phylogenetic relationships Introduction origins Invasion routes 	<ul style="list-style-type: none"> Assessment of the risk for emergence of other RSSC species in France (and Europe) Basis for the development of detection tools and epidemiological monitoring on host crops Support to the stakeholders likely to be impacted : seed potato and food potato, but also solanaceous and ornamental plant production industries 	<h2 style="margin: 0;">Conclusion</h2>
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EAPR Pathology and Pests Section Meeting, 3-6 September 2023, Arras (France)

Is there any risk for potato crops to be infected by Apiaceae haplotypes of ‘*Candidatus Liberibacter solanacearum*’?

BERTON L.¹, NEVEUX M-S.¹, HUCHET E.¹, MOREE C.³, ZEAITER H.³, LATY P.³, LE HINGRAT Y.¹, GOBERT V.², LE ROUX A-C.¹

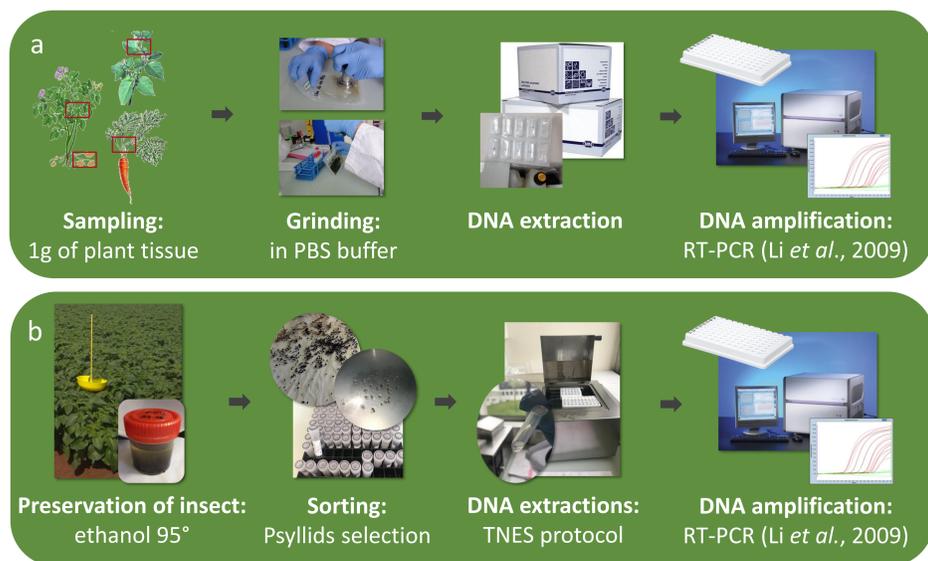
INTRODUCTION

Candidatus Liberibacter solanacearum (Lso) is a phloem-limited bacterium transmitted by psyllids to Solanaceous and Apiaceous plants. Haplotypes A and B of Lso are spread by *Bactericera cockerelli* and cause Zebra Chip disease of potato, mainly in the Americas and New-Zealand. These haplotypes and their vector have not been reported in Europe. The recent detection in Europe of Lso haplotypes C, D and E on Apiaceae crops led the potato industry to wonder about the risk of transmission of Apiaceae haplotypes to potato by their vectors *Bactericera trigonica* or *Trioza apicalis*. The objectives of this study are i) to estimate the occurrence of the disease and its vectors in potato fields and the close environment and ii) to assess the ability of psyllids to infect potato plants in a place where the Apiaceae haplotypes of Lso occurred.

CROP'S MONITORING Material & Methods

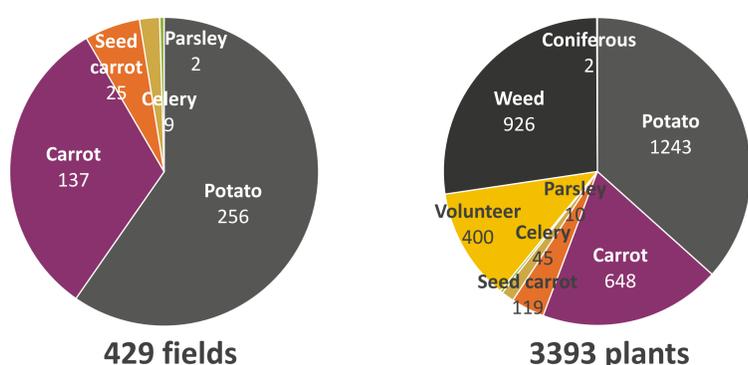
- Plant were collected in potato fields close to Apiaceae fields (< 1 km) during 6 summers (2016 - 2021)
- Sampling per plot:
 - 5 potato plants
 - 5 Apiaceae plants (carrot, celery, parsley)
 - 3 to 5 weeds (majority black nightshade - *Solanum nigrum*)
 - 4 potato volunteers
- Psyllids were collected (yellow traps) twice a week in some locations
- Analyses were carried out as described below (figure 1)

Figure 1: Plant (a) and insect (b) analysis methods



Results

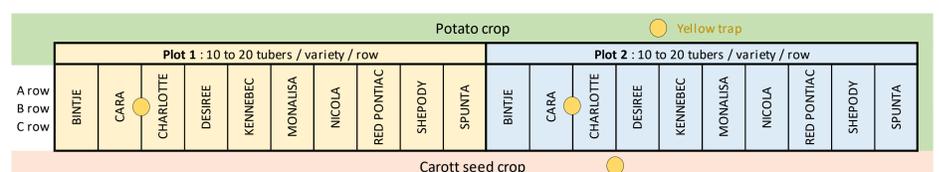
Figure 2: Number of fields and plants sampled and analysed



- ✓ Lso was not detected in any of the potato plants analysed
- ✓ Lso was detected into 49% of the seed carrot plants tested
- ✓ Lso was detected into 1 nettle - *Urtica dioica* (0,1% of the weed analysed)
- ✓ Lso was detected into 18% of the 112 psyllids analyzed

TRANSMISSION ASSESSMENT Experimental set-up

Figure 3: Plot plan

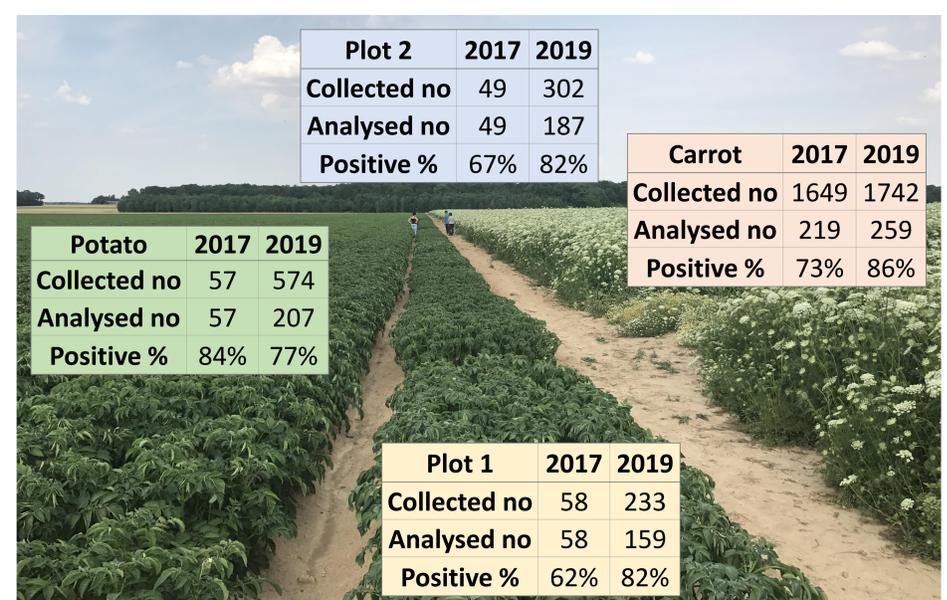


- 3 years of experiments (2017 - 2019)
- 10 potato cultivars were tested
- Plants were sampled 3 times during the growing period:
 - 9 potato plants per cultivar and plot
 - 5 carrot plants
- Analyses were carried out as described previously (figure 1)

Results

- ✓ Lso was not detected in any of the 508 potato plants analysed
- ✓ Lso was detected in 70% of the 40 seed carrot plants tested
- ✓ Lso was detected in 79% of the 1195 psyllids analysed

Figure 4: Number of psyllids collected in each crop



CONCLUSIONS

- ✓ These results suggest that Lso transmission to potato plants by Apiaceae psyllids is unlikely
- ✓ The highest danger for the potato industry in Europe is the risk of introduction of *B. cockerelli* and Lso haplotypes A and B through trade exchanges

¹ FN3PT/inov3PT (Fédération Nationale des Producteurs de Plants de Pomme de Terre), INRAE, Le Rheu, France; ² FN3PT/inov3PT, Achicourt, France; ³ Comité Centre et Sud, Laurière, France

Purple top complex disease a threat for the Ecuadorian and South America potato production and diversity

Cuesta X¹, Racines M¹, Rivadeneira J¹, Castillo C¹

Introduction

Potato purple top complex (PPT) caused by *Candidatus* Phytoplasma spp. and *Candidatus* Liberibacter solanacearum (CaLso) is an emerging disease in Ecuador, which has caused significant crop losses, the disease is reported in other solanaceous crops such as tomato, eggplant, and pepper^[1]. Management is based on the periodic application of insecticides for vector control *Bactericera cockerelli* (Bc), PPT has become one of the main constraints. Large diversity of potato is found in South America, it is estimated in about 100 wild species, >5000 native potatoes, that could be in danger. The objective of this research was to reduce the negative effects of the disease through the development of an integrated disease management strategy.

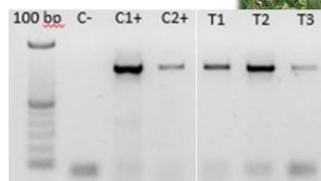
Management strategy

Monitoring



Diagnosis

Molecular analysis



Symptoms



Plant breeding



Seed transmission



IDM

- Chemical control
- Biorrational products
- Cultural practices
- Early varieties

Training/diffusion



References

- [1] Mora, V., et al. 2021. Frontiers in Microbiol. 12: 700663.
[2] Carrillo, C. et al., 2019. Bull. Insectol, 72, 85-91.
[3] Caicedo, J. et al., 2020. Australasian Plant Disease Notes, 15, 1-3.

Results

Diagnosis

In Ecuador, phytoplasmas were identified in 2018, Bc in 2019^[2] and CaLso in 2020^[3], as a result, up to 100% potato yield losses were reported whilst the cultivated area was reduced by 56%. Colombia reported Bc at the beginning of 2021, while Peru at the end of 2021, no reports of CaLso.

Transmission

The symptoms of PPT and its negative effects were transmitted through the seed tuber (Figure 1).

Breeding

The species *S. albicans*, *S. galapagense*, *S. colombianum* and *S. andreaeanum* showed antibiosis and antixenosis resistance response to Bc. Early potato varieties showed less incidence of PPT.

Biorrational products

Neem extract, potassium soap and kaolin had positive effect to reduce Bc population.

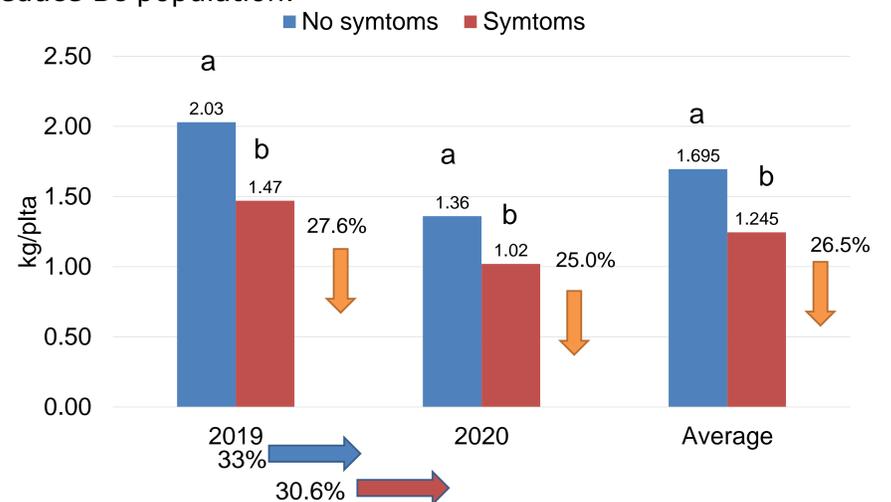


Figure 1. Effect of PPT symptoms on yield in two crop cycles

With the support of **PATAFEST** project a multi-actor consortium composed by 18 partners whose objective is to protect potato against emerging new pests, INIAP plans in the next four years to characterize wild, native, improved Ecuadorian varieties and European cultivars for Bc and CaLso resistance to identify at least 30 CaLso resistant varieties, a digital tool for diagnostic detection of CaLso and a pesticide free natural solution to control Bc will be validated under Ecuadorian conditions.

Conclusions

- Biodiversity of potato and other solanaceous crops from South America could be at risk.
- IDM strategy based on monitoring, high quality seed, natural products, early potato varieties, cultural practices, biorrational products and chemical control is the recommended technology to prevent PPT disease.
- High-quality seed production programs are essential.
- Breeding for resistance/tolerance to PPT is necessary.
- PATAFEST will contribute to the development of IDM technology to reduce the negative effects of CaLso

Acknowledgements

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Virulence of novel *Ralstonia pseudosolanacearum* (phylo type I) strains from rose, blueberry and mandevilla on seed potato

B.J.A. van Doorn¹, P.R. Overeem¹, M.J.C. Pel¹, N.N.A. Tjou-Tam-Sin¹, J.L.J. van de Bilt¹, P.P.M.A. Gorkink-Smits¹, N.M. Landman¹, A.M. Bocsanczy², D.J. Norman² and M. Bergsma¹. ¹Bacteriology, Netherlands Institute for Vectors, Invasive plants and Plant health (NIVIP), National Plant Protection Organization of the Netherlands (NPPO-NL), Wageningen, the Netherlands. ²University of Florida Department of Plant Pathology and Mid-Florida Research and Education Center-Apopka, USA

INTRODUCTION

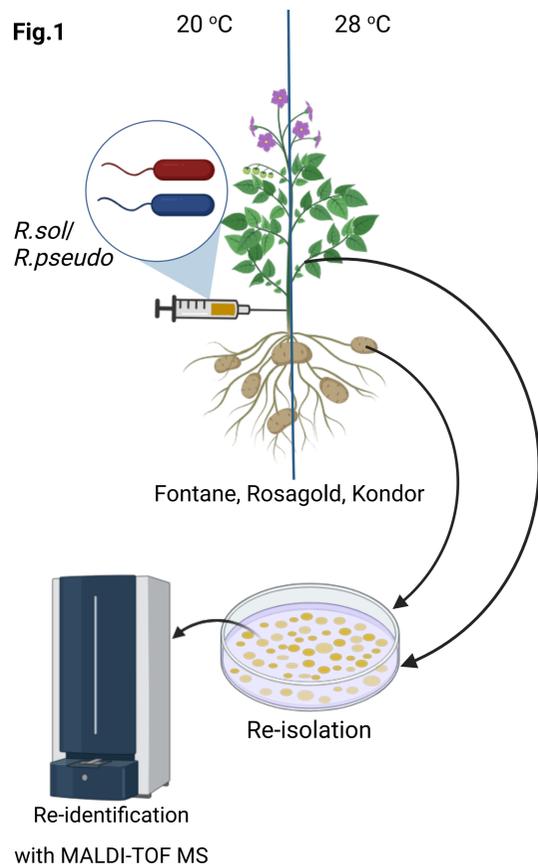
Ralstonia solanacearum (*R. sol*; phylotype (phy) IIB), the causal agent of brown rot disease on potato, causes great economical losses by affecting potato in temperate regions. It is thought that members of *Ralstonia pseudosolanacearum* (*R. pseudo*; phy I) are not pathogenic at low temperatures and usually are found in warmer climates. *R. pseudo* strain PD 7123 isolated from rose in the Netherlands, strain P824 isolated from blueberry in the USA and strain P781 from mandevilla in Florida are phylogenetically closely related and could therefore share a common host. The virulence and ability of these novel strains to multiply latently in potato in temperate regions is unknown.

Objective:

- Assess the virulence and presence of latent infections of the mentioned *R. pseudo* strains on three commercial seed potato cultivars (Fontane, Kondor, Rosagold) under warm (28°C) and temperate (20°C) temperatures.

METHODS

- Inoculation of cultivars Fontane, Rosagold and Kondor with the three *R. pseudo* isolates and the positive control *R. sol* (PD2762)
- Incubation at 20 or 28 °C at 80% RH
- Score symptom development during 5 weeks
- Re-isolation from plants on mSMSA medium
- Harvesting the daughter tubers to evaluate symptom development and to confirm the (latent) presence of *R. pseudo*
- MALDI-TOF MS to confirm the identity of re-isolated bacteria



CONCLUSION

- Differences in symptom development in distinctive potato cultivars inoculated with various *R. pseudo* strains demonstrate the specific interactions between strains and host under warm and temperate climatic conditions
- R. pseudo* poses a serious threat to potato cultivation, due to its ability to latently infect daughter tubers under temperate conditions

RESULTS

Fig. 2 Disease class distribution per time point, representing disease progress and comparison of the Area Under the Disease Progress Curve (AUDPC) per treatment combination. **A)** Disease progress of three potato varieties at 28°C (n=8). **B)** Disease progress for tomato at 28°C (n=10). **C)** Disease progress of three potato varieties at 20°C (n=8). **D)** Disease progress for tomato at 20°C (n=10). AUDPC per temperature treatment for each isolate and potato combination **E)** and tomato **F)**. Error bars indicate the standard error. Letters above the bars indicate significant differences (p<0.05).

Virulence on potato

- Differences in wilt symptom severity in potato cultivars and between strains
- At 28°C *R. pseudo* strains are more aggressive on tomato and potato than *R. sol*
- Wilt symptoms present in all plants 42 dpi at 28°C
- Disease severity much lower for *R. pseudo* than *R. sol* at 20°C, with less symptoms
- For *R. pseudo*, most potato plants do not develop symptoms at 20°C
- Nearly all symptomatic and asymptomatic plants positive for *R. sol*/*R. pseudo*

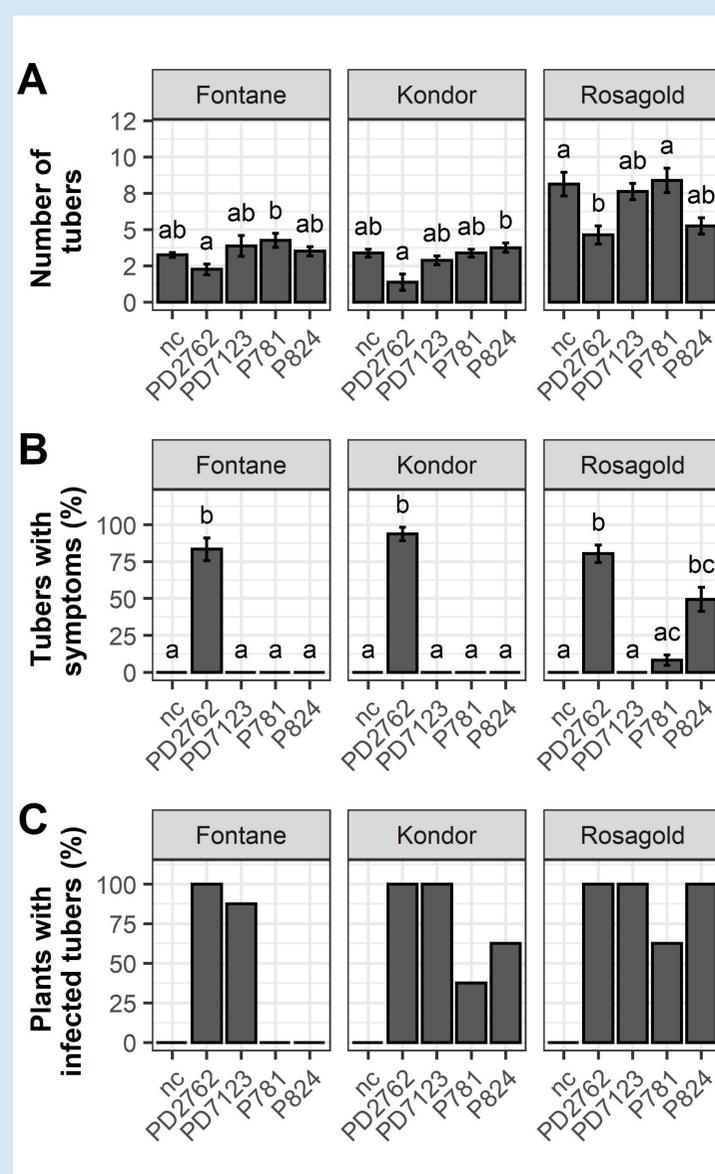
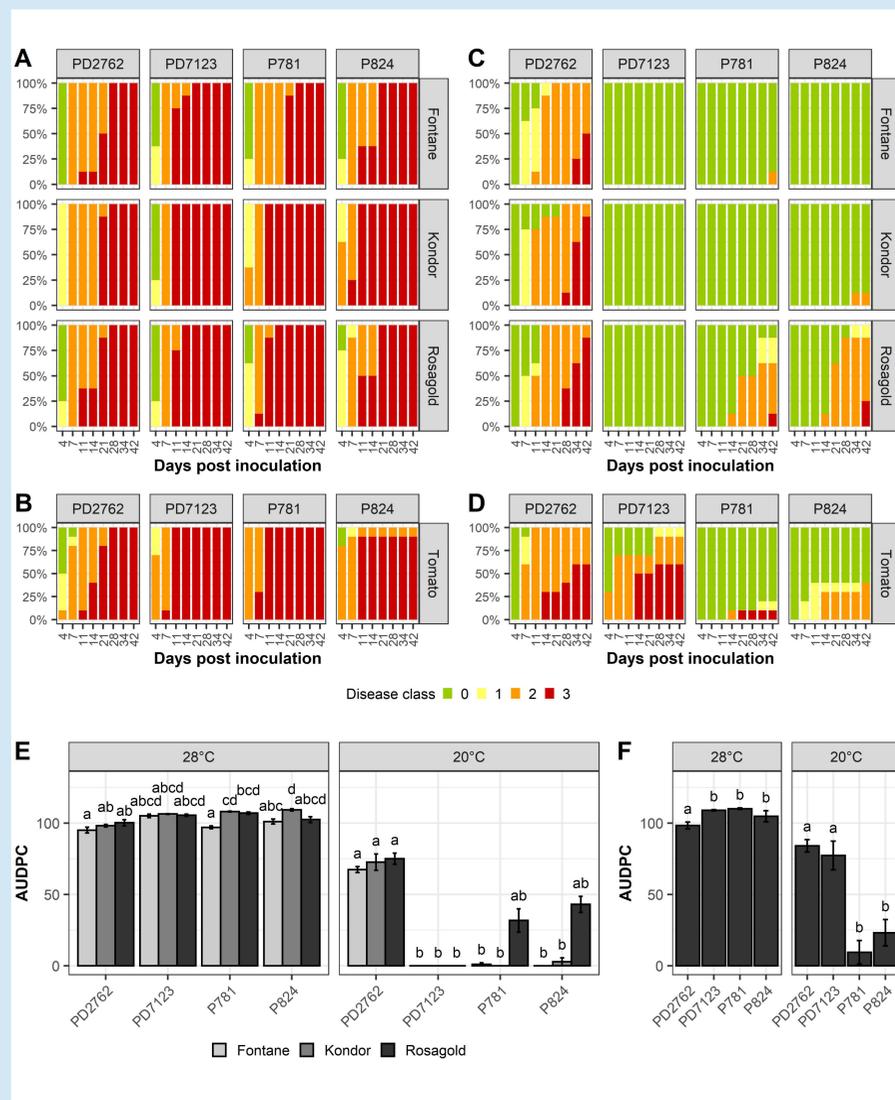


Fig. 3 Indicators of yield and latent infections in potato tubers at 20°C. **A)** Number of tubers per plant recovered. **B)** Percentage of tubers with symptoms. **C)** Percentage of plants from which at least one tuber was infected with *Ralstonia*. Error bars indicate the standard error. Letters above the bars indicate significant differences (p<0.05).

Systemic infections in the daughter tubers

- 42 dpi at 28°C none of the inoculated plants had tubers
- Most of the inoculated plants at 20°C did have daughter tubers 42 dpi
- R. sol* caused at least 80% symptomatic tubers, irrespective of the cultivar
- R. pseudo* strains did not cause any symptoms in Fontane and Kondor
- 0-49% symptoms on Rosagold, depending on the strain
- Successful infection strongly depends on strain and cultivar interaction

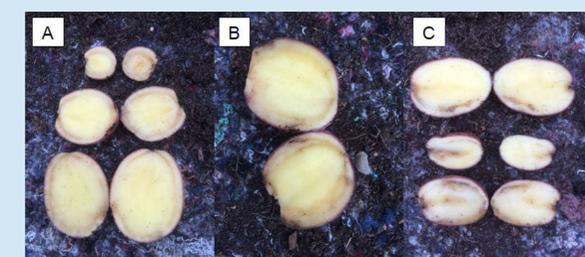


Fig. 4 Typical brown rot symptoms developed on daughter tubers of cv. Rosagold at 20°C, 42 dpi after inoculation with **(A)** phy IIB strain PD2762, **(B)** phy I strain P781, **(C)** phy I strain P824.

Contact

- Bo van Doorn
- b.j.a.vandoorn@nvwa.nl

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