Diversity and biology of potato blackleg causing Pectobacteriaceae

STØTTET AF

Kartoffelafgiftsfonden

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Aarhus, 7 December 2021

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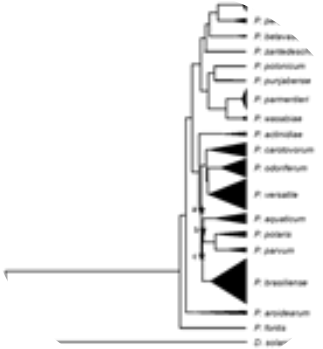
Outline presentation

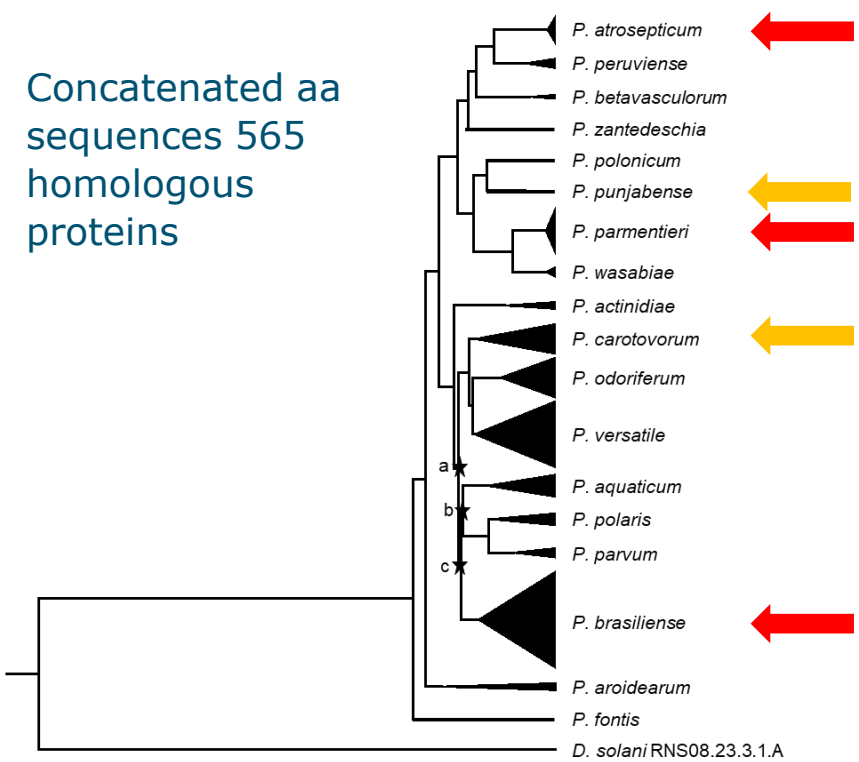
- Diversity
- Initial infection
- Colonization
- Spread
- Disease expression
- Management



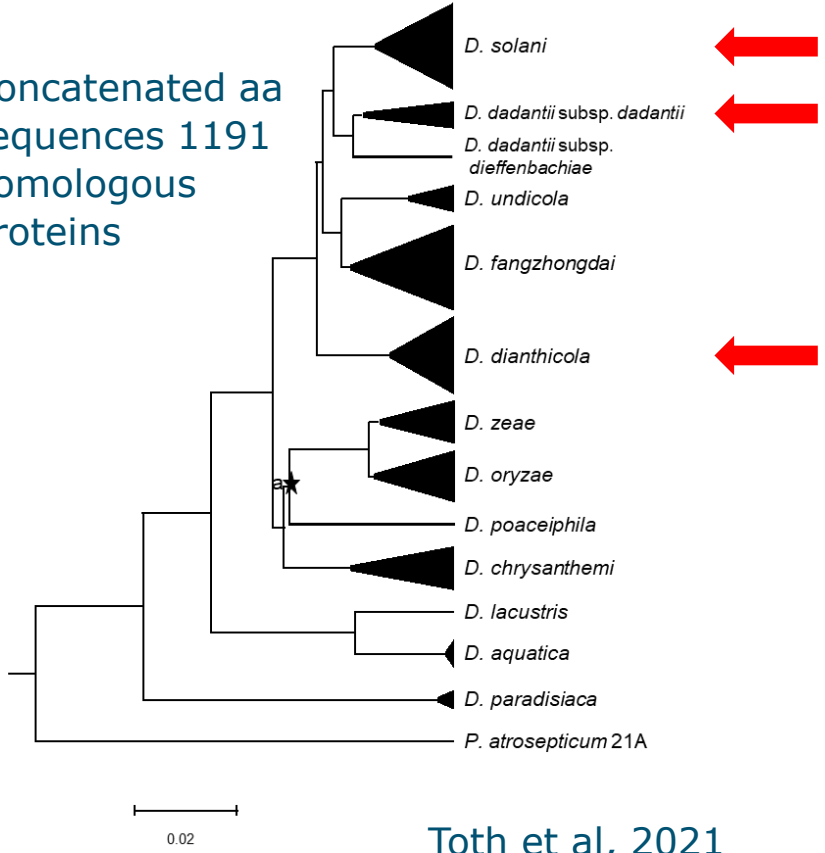
Diversity

Diversity blackleg causative agents

Concatenated aa sequences 565 homologous proteins



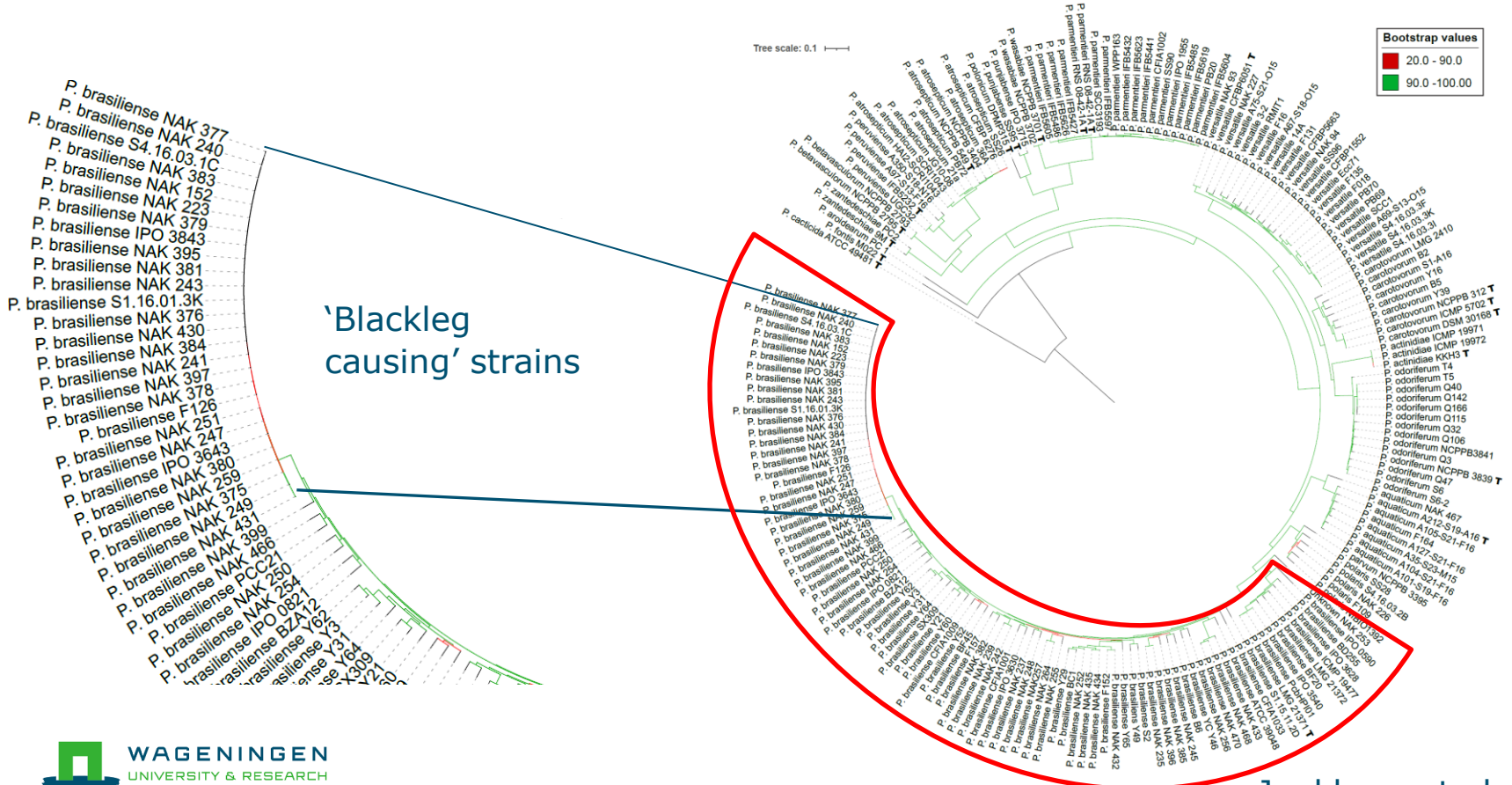
Concatenated aa sequences 1191 homologous proteins



New: *P. quasiaquaticum* and *D. parazeae* (water)

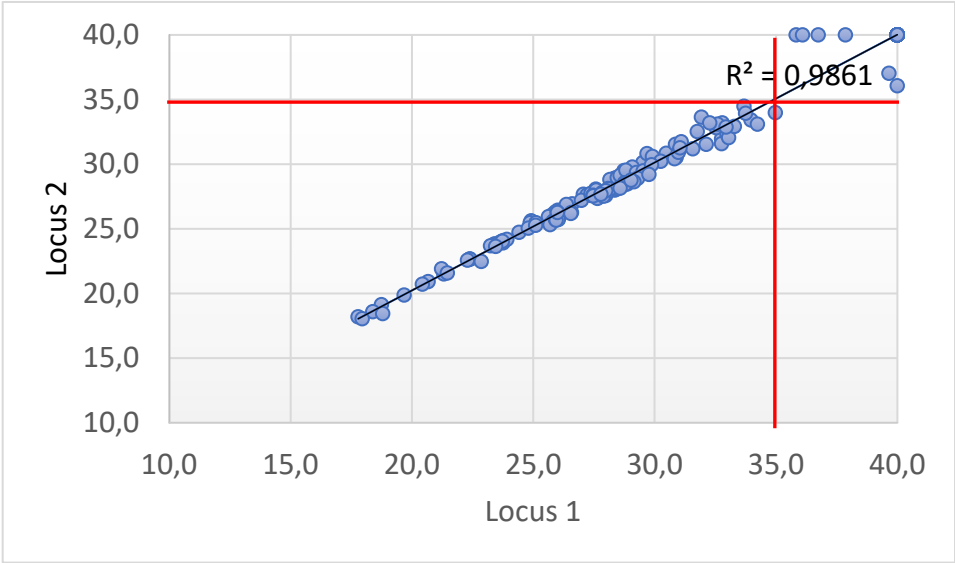
Toth et al, 2021

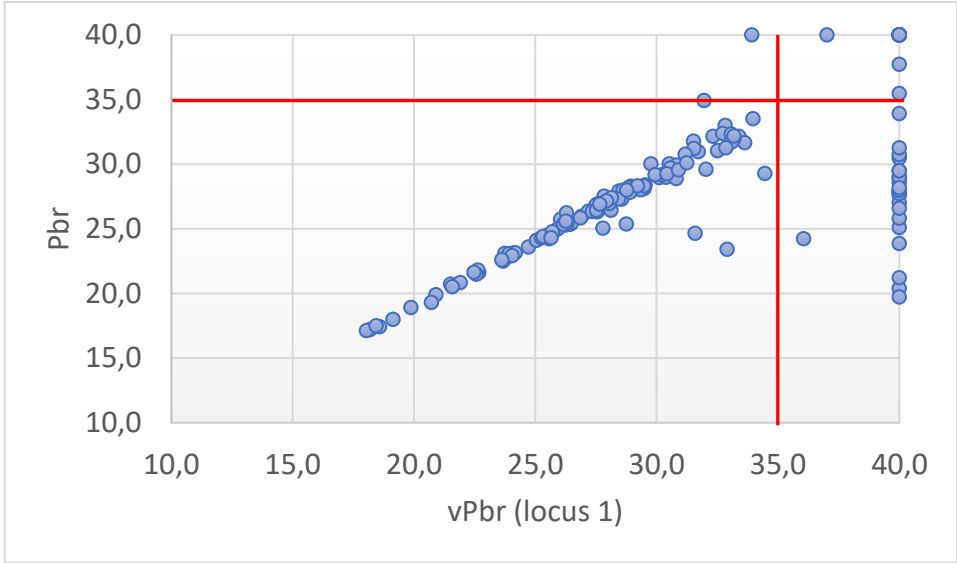
Blackleg causing capacity can be clade/strain specific



Jonkheer et al, 2021

TaqMan assays for blackleg causing Pbr strains





Comparison of vPbr assays based on two loci in a triplex setting (v. d. Lee & v. Gent)

TaqMan Pbr (Muzhinji et al., 2020) with vPbr assay 9

Comparison based on 172 field samples from 2 farms

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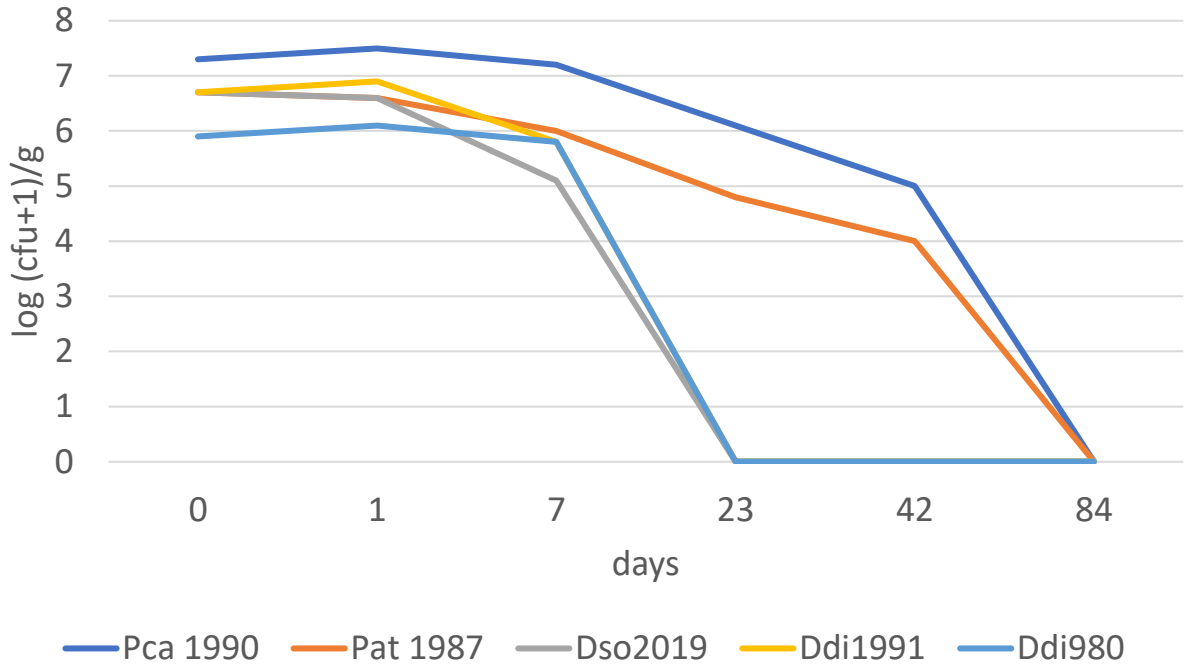
Initial infection

Soil as contamination source: survival planktonic cells (6-8 ^{o}C, 50% FC in peat soil)



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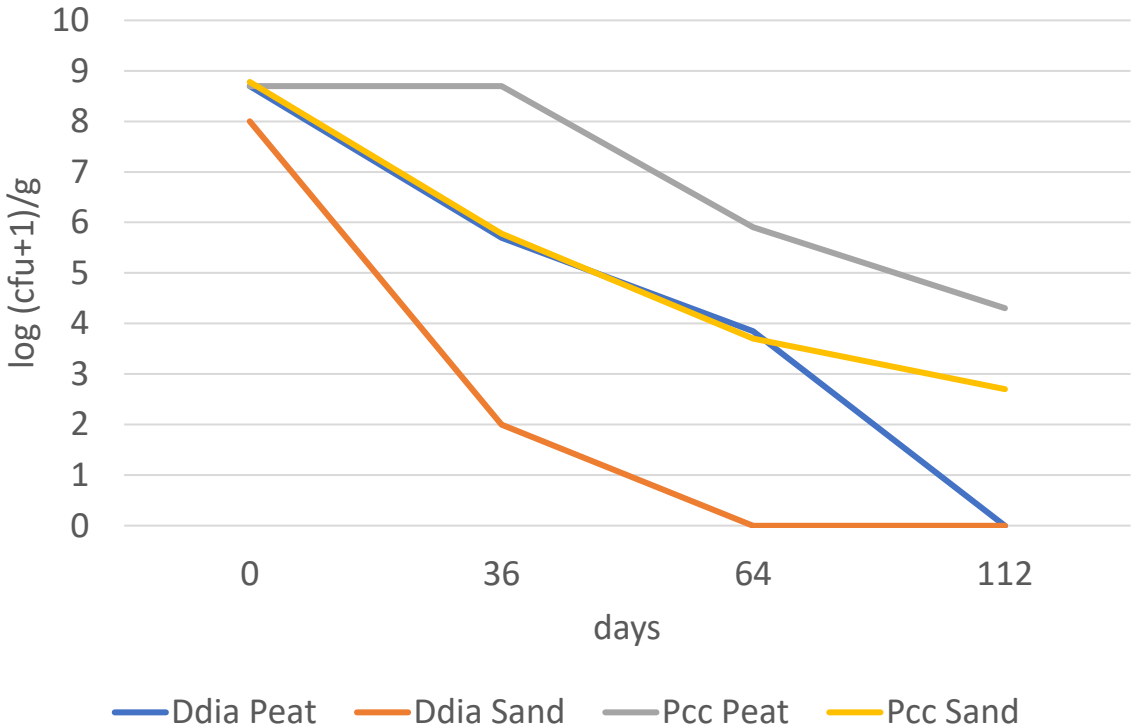
Soil as contamination source: Survival in rotten potato stems in peat soil (6-8 °C, 50% FC)



Inoculum

Fragments still visible after 112 days

Weeds as contamination source

- In Scotland and the USA, in 71 of 130 weed species ‘Erwinia’ was found
  - Mainly ‘*P. carotovorum*’ (67 -98%)
  - Mainly during the growing season

- In Israel, *Cyperus rotundus* was found symptomless infected with *Dickeya solani* in 7-14% of the plants, but not 10 other plant species (Tsror et al., 2010)

Rain water as contamination source

- '*P. carotovorum*' recovered from 80% of ocean- and rain- water cells
- 5% of rocky mountain snow samples yielded '*P. carotovorum*'
- Cells are active as cloud condensation nuclei
- They are transported with cloud systems
- And deposited in precipitation at inland sites
- However, in 2021 in NL no indications were found for the presence of SRP in rainwater during growing season (in total 20 samples from 10 places

Franc et al., 1988, 1998

Aerosols as contamination source I

Nr of Pectobacterium cells released during flailing		
Experiment	*Pectobacterium*, "disease-free" crop	*Pectobacterium* in symptomatic crop
1	$5x10^8$/ha	10^8/ha
2	$7x10^7$/ha	$8x10^7$/ha

- Pectobacterium can survive in aerosols 2 h at 65% RH and 18 ^oC
- Estimated nrs Pectobacterium cells deposited per m^2 from aerosols:
  - Distance to source 50 m : 1000
  - Distance to source 100 m: 100
  - Distance to source 1000 m: 3

Perombelon et al. 1979

Aerosols as contamination source (II)

- Set up experiment
  - Seed potato field crops in NL with and without blackleg symptomatic plants
  - Experimental field Dsol infected plants (inoculated seed)
  - Air sampling: 20 min (plant growth) or 10 min (during haulm destruction)
  - During haulm destruction: sampling on machine
  - Enrichment TaqMan + metagenomics

300 L air/min

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Moments of sampling

Selection

Spraying pesticides

Chemical haulm destruction

Flailing

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TaqMan results

nr	Date	Crop	Action	Symptomatic plants in field	Ppar		Pbra		Dsol		Patr		Pode	Pbeta
					HZPC	WUR	HZPC	WUR	HZPC	WUR	HZPC	WUR	HZPC	HZPC
1	June	Exp. Field	Selection	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	June	Field Crop 1	Selection	N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	June	Exp. Field	Selection	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	June	Exp. Field	Selection	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	June	Field crop 2	Selection	N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	June	Field crop	Spraying	N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	June	Field crop	Spraying	N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	June	Exp. Field	Selection	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	July	Exp. Field	Selection	Y	ND	ND	nd	ND	ND	ND	ND	ND	ND	ND
10	July	Exp. Field	Selection	Y	ND	ND	30	ND	ND	ND	ND	ND	ND	ND
11	July	Exp. Field	Selection	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	July	Exp. Field	Selection	Y	34	ND	ND	ND	ND	ND	ND	ND	ND	ND
13	July	Field crop	Flailing	Y	25	22	25	23	ND	ND	ND	ND	27	ND
14	July	Field crop	Flailing	Y	25	21	26	22	ND	ND	ND	ND	31	ND
15	July	Field crop	Flailing	Y	29	28	34	26	ND	ND	ND	ND	33	ND
16	July	Field crop	Flailing	Y	24	21	25	21	ND	ND	ND	ND	33	ND
17	July	Field crop	Flailing	N	33	28	30	25	ND	ND	ND	ND	32	ND
18	July	Field crop	Flailing	N	ND	ND	27	22	ND	ND	ND	ND	ND	ND
19	July	Field crop	Flailing	N	ND	36	26	22	ND	ND	ND	ND	ND	ND
20	July	Field crop	Flailing	N	ND	34	29	25	ND	ND	ND	ND	ND	ND
21	August	Field crop	Chem./Flailing	N	28	23	27	20	ND	ND	ND	ND	26	ND
22	August	Field crop	Chem./Flailing	N	28	26	22	23	33	ND	ND	ND	27	ND
23	August	Field crop	Chem./Flailing	N	27	26	24	23	33	ND	ND	ND	28	ND
24	August	Field crop	Chem./Flailing	N	29	27	23	24	ND	ND	ND	ND	27	ND

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All generic Erwinia TaqMan results were positive

Insects can transmit Erwinia's

- Pectobacterium be transmitted by various species of flies
- Pectobacterium is found in densities up to 2 million bacterial cells per fly
- Pectobacterium can survive up to 72 h on flies
- Infected insects are found from May till September, but not in October
- Flies can be contaminated with *Pectobacterium* present in potato dump piles and carry them up to 200 m after which they can infect (damaged) plants
- *P. brasiliense* is detected on insects on sticky yellow traps (NAK, Vreeburg, pers. comm.)

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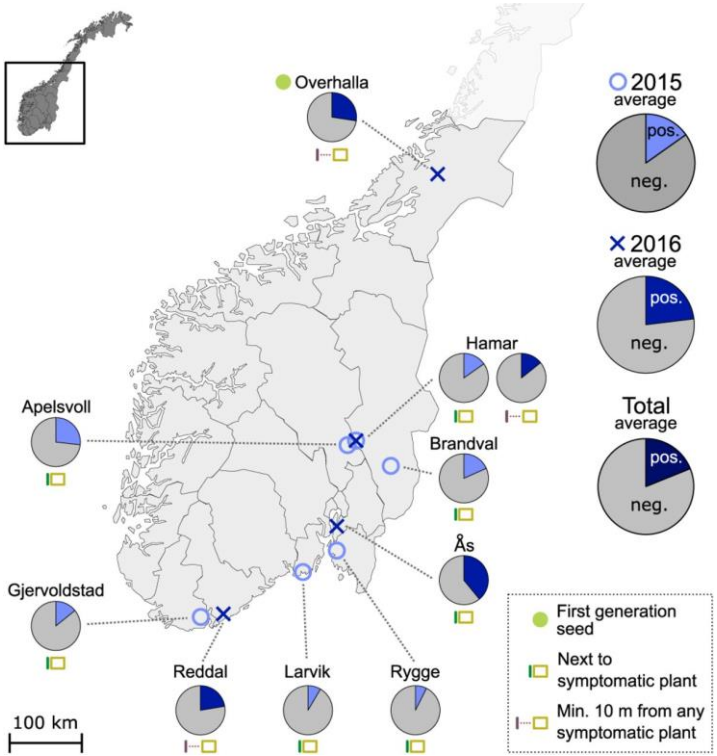
SRP on insects in Norway



Delia floralis = turnip root fly = bloemvlieg

Plutella xylostella = diamondback moth = koolmot

Chrysoperla carnea = lacewings = gaasvlieg

D. solani: +-
P. parmentieri: +-
P. atrosepticum: ++

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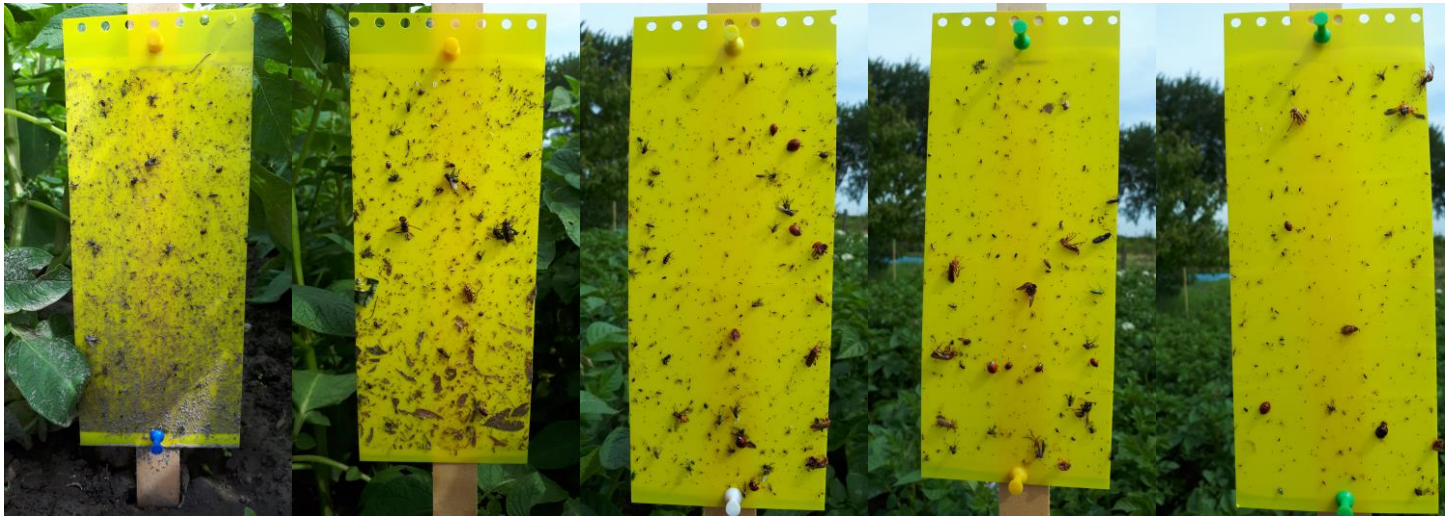
Total: 2000 insects

Rossmann et al. (2018)

In NL, insects can be present in high numbers



30 cm 60 cm 90 cm 120 cm 150 cm

Data Tjalling Douma, Agrico, 19 June 2021, NL, collection of one week

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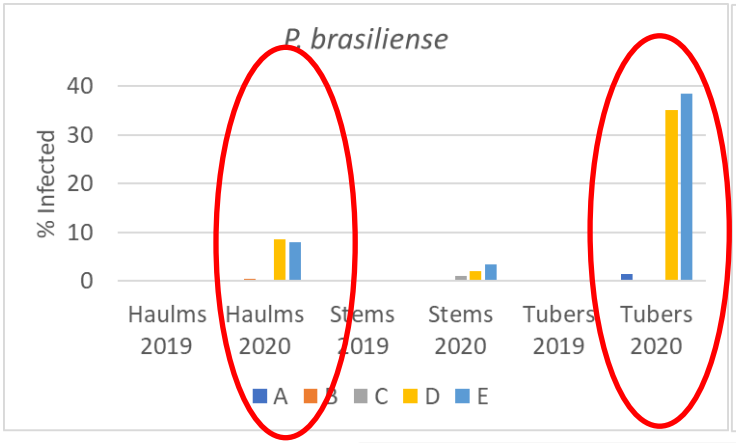
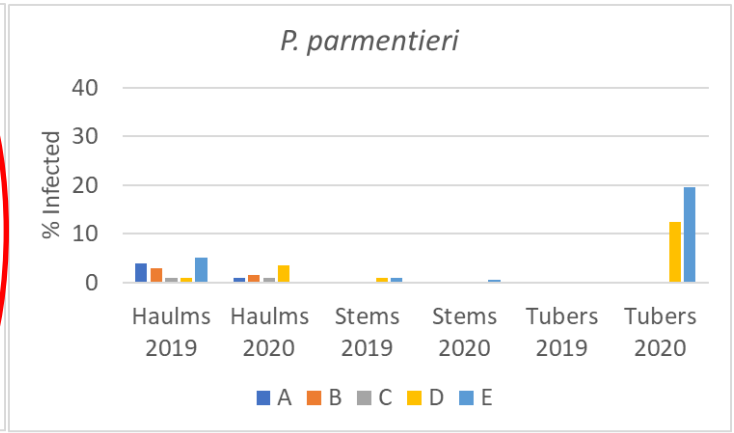
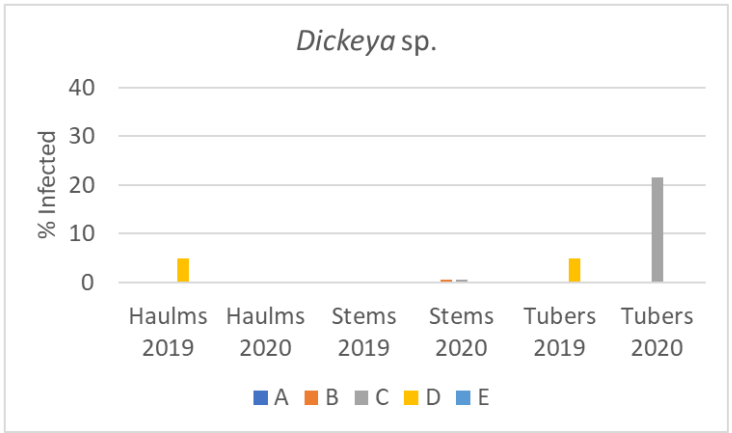
Survey minituber crops

- 5 growers in the Netherlands
- cv. Agria
- 2019 (100 plants/grower) and 2020 (200 plants per grower)
- Haulms (top), stems (basis) and tubers (composite sample all tubers) of individual plants were analysed with enrichment TaqMan assays

Infections minituber crop (before haulm destruction)





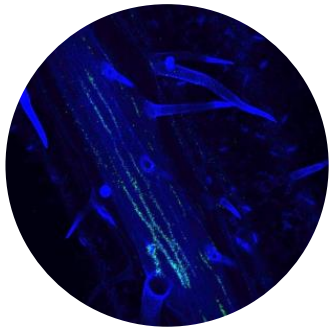


Van der Wolf et al., 2021

Discussion results 2020

- For Pbr and Ppar (growers D and E),
    - Haulm infections did not coincide with infected tubers:
        - Infections came from air-borne inoculum
    - Tuber infections did not coincide with haulm infections:
        - infections came from soil or from inoculum leaking downward → populations grown in (ageing) leaves are responsible?
- For Dickeya sp. (grower C)
    - Not *D. solani* or *D. dianthicola*
    - Only tubers were infected → Soil-borne inoculum

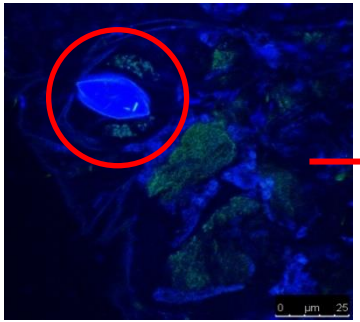
Twinkl.com



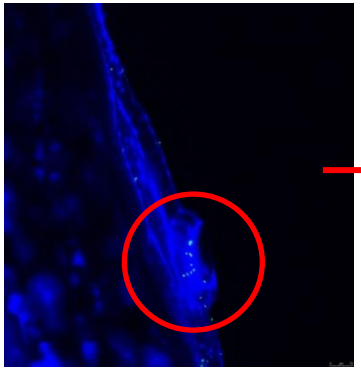
Colonization

Leaf infections: colonization pathway

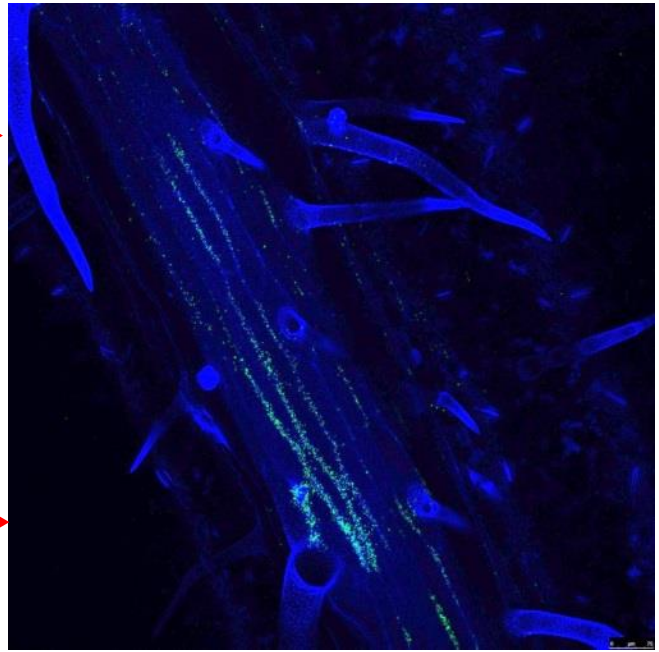
Abaxial side



Stomata day 1



Hydathodes day 1



Vascular infection leaves

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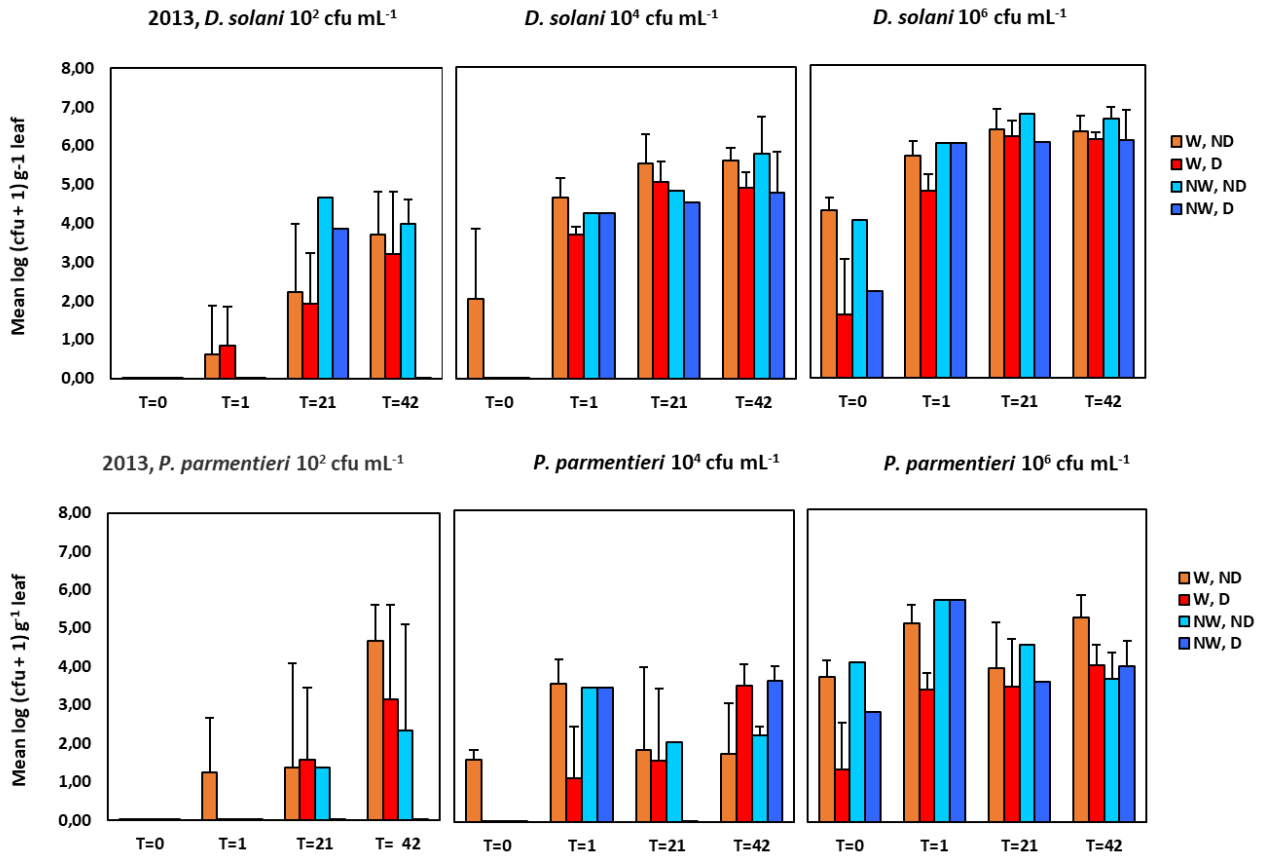
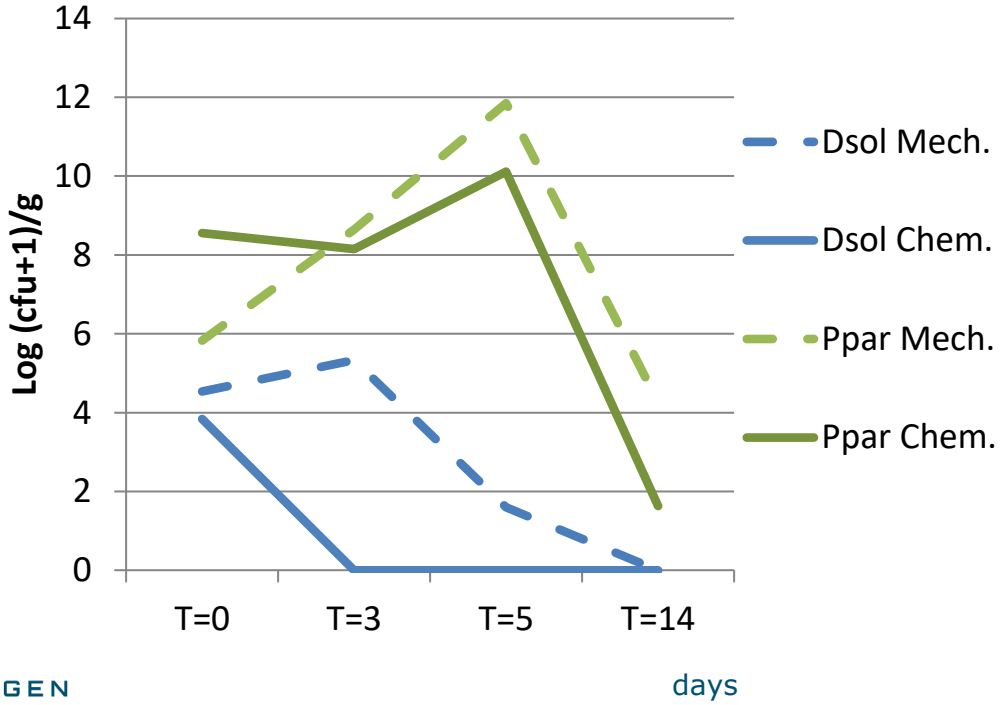
Risks of haulm infections

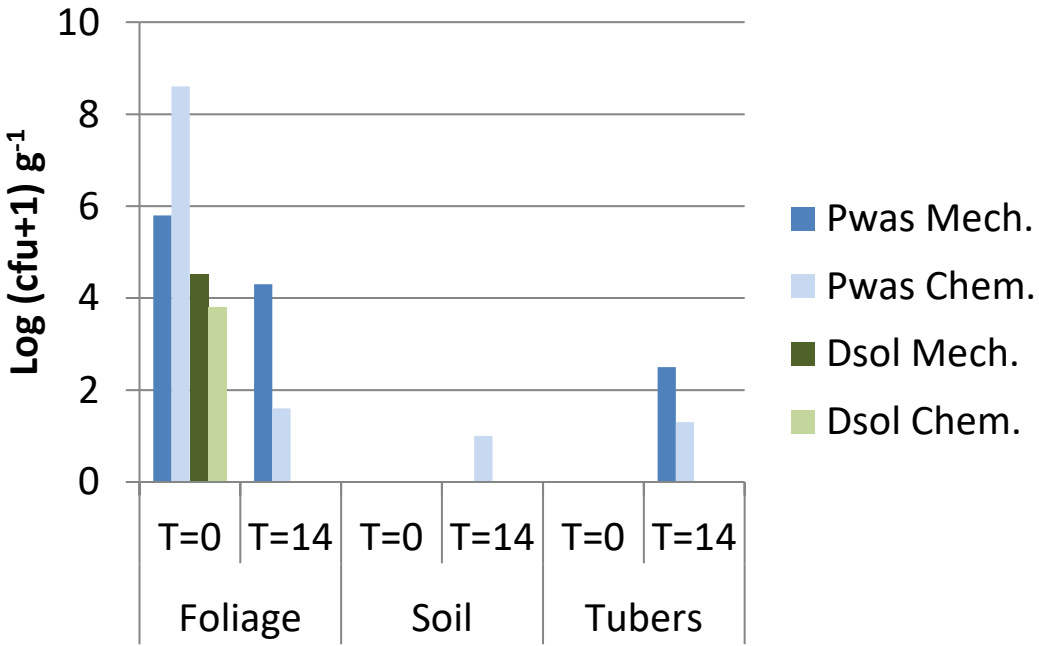


Kastelein et al., 2020

Population dynamics in destructed haulms

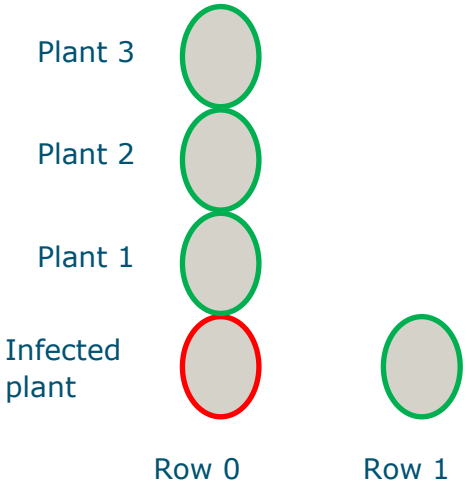


From destructed haulms to soil and tubers

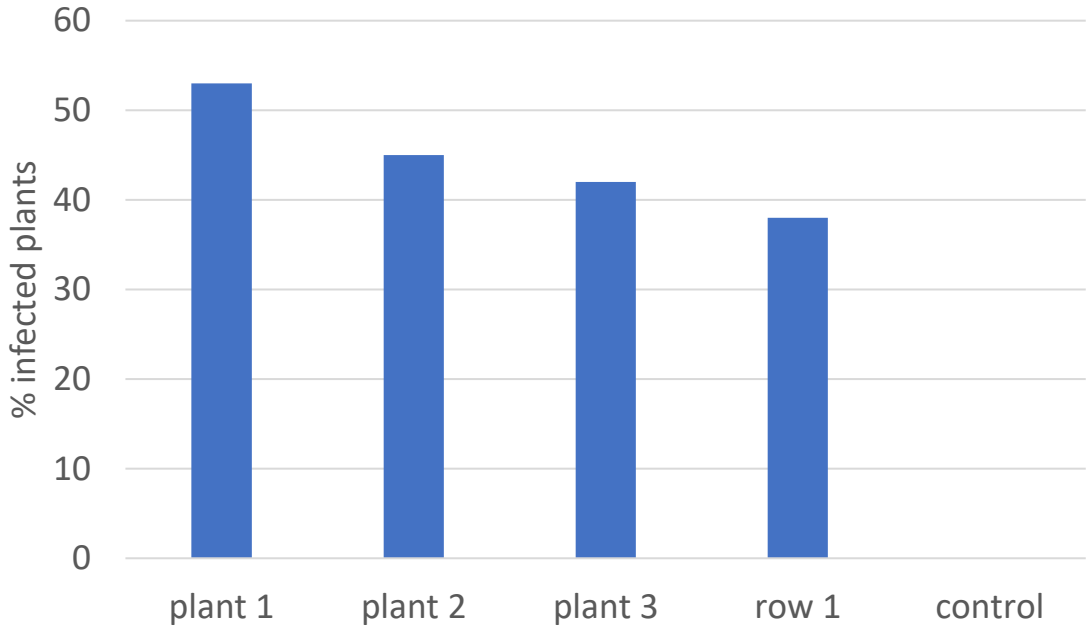


Spread

During cultivation: spread in the field by a release from rotten tubers

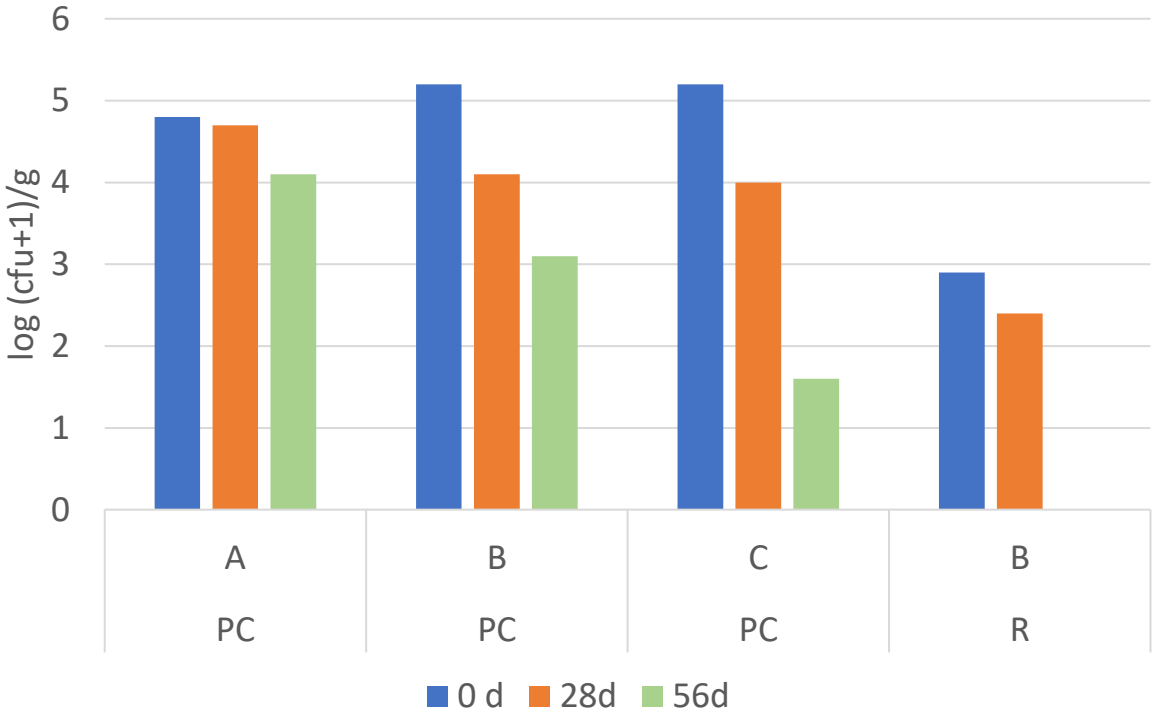


D. solani/Kondor



Boomsma et al., ...

Survival of *P. carotovorum* on tubers contaminated at grading during storage at 4 °C

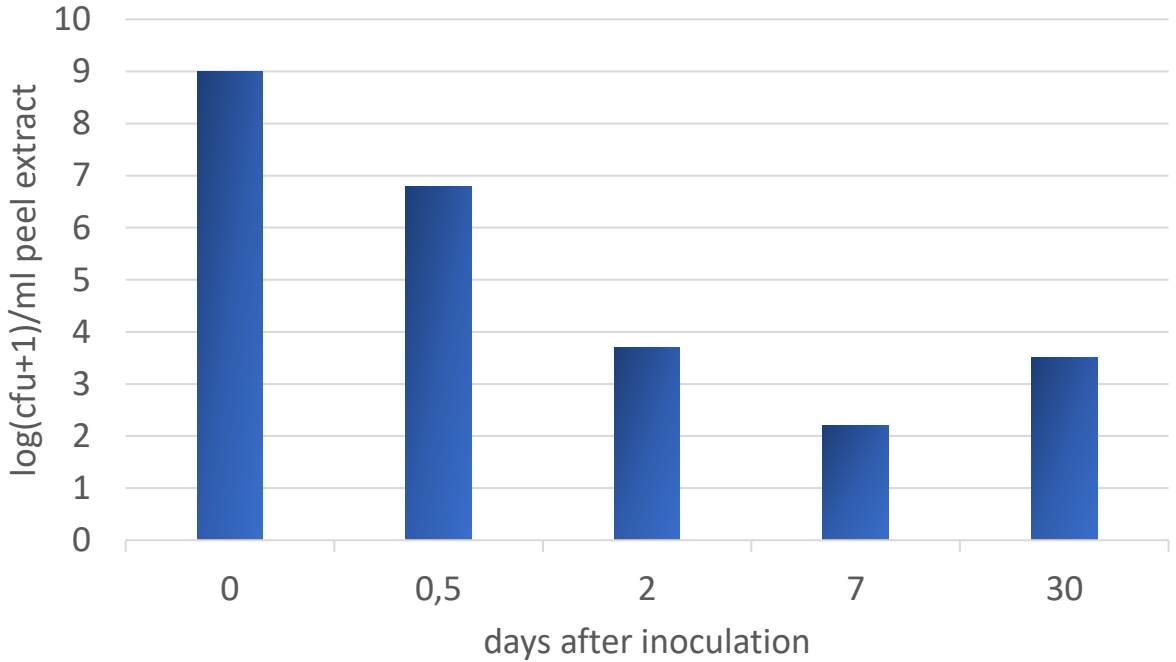


Grader

Cultivar
(Pentland Crown, Record)

Elphinstone & Perombelon, 1986

Long term survival of Dickeya in tuber decay on tubers



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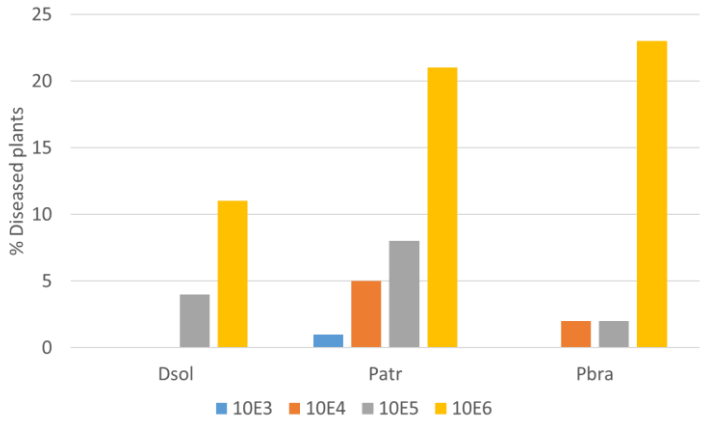
Factors determining expression of potato blackleg



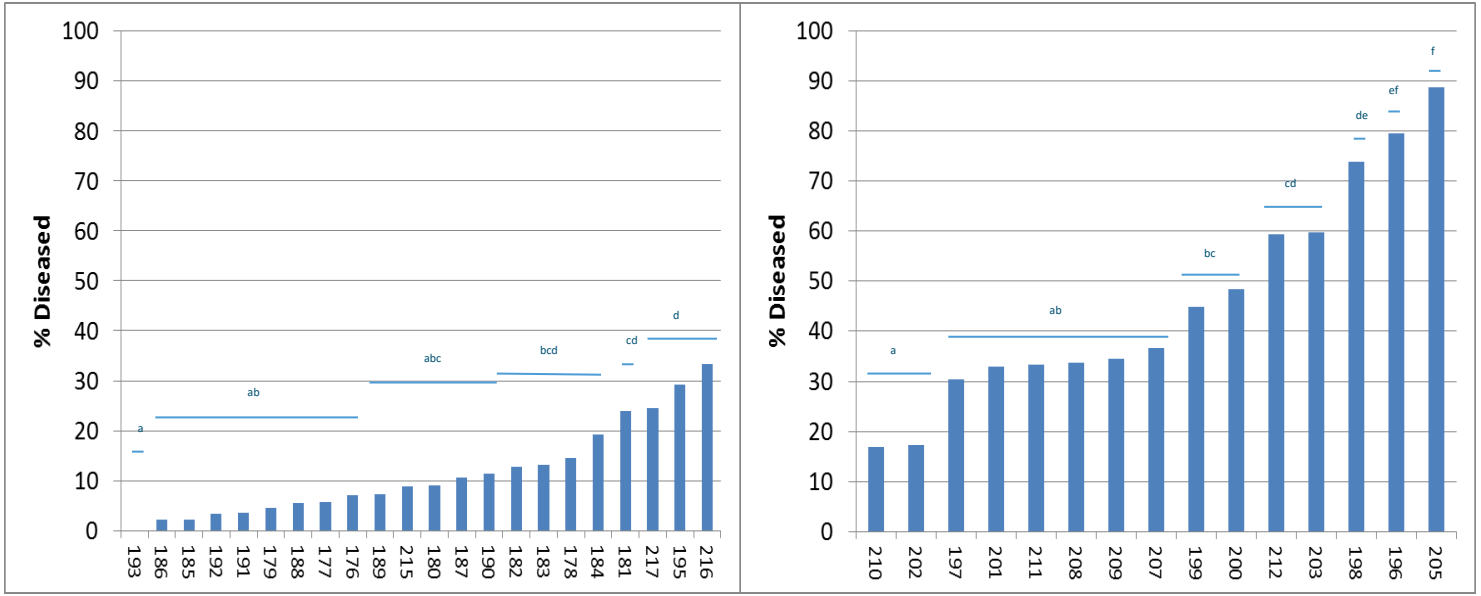
- Potato cultivar
- Pathogen density
- Pathogen variant
- Environmental conditions (temperature, humidity)
- Suppressiveness seed tubers??

  (Ability to prevent disease expression)

Suppressivess seed lot: set up experiment

- Seed lots of two cultivars (ca. 20 per cultivar per year) collected from different locations and growers in the NL
    - Tested for a low level of SRP's
    - Analysed for dry weight content and minerals
    - Inoculated with 10^6 cfu/ml of *D. solani* or not inoculated
    - 100 tuber per treatment were planted in a (randomized block design) in a sandy soil in the North of the NL

Increasing suppressiveness: a new management tool?



Spunta

Kondor

(P=0.05)

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Management

Management tools

- Selection of pathogen-free seed lots → Testing!
- Hygiene
- Cultivation measures?
- Resistance?
- Use of suppressiveness seed lots?
- Physical treatments of tubers?
- Biological control?

Cultivation measures

- Avoid water logging of soil → drainage
- Avoid damage of your crop → air-borne inoculum
- *No surface water for irrigation → can be contaminated*
- Selection and roguing: only useful early in season?!
- Plant a high grade seed lot at a distance of at least 100 meter from a low grade seed lot → splash dispersal, spread by soil water
- Flailing should be done preferably during dry weather conditions, sunshine and little wind
- Full field spraying followed by flailing (after 5 days) is preferred above flailing followed by spraying
- Avoid the presence of haulm debris/volunteers on soil above tubers

Cultivation measures

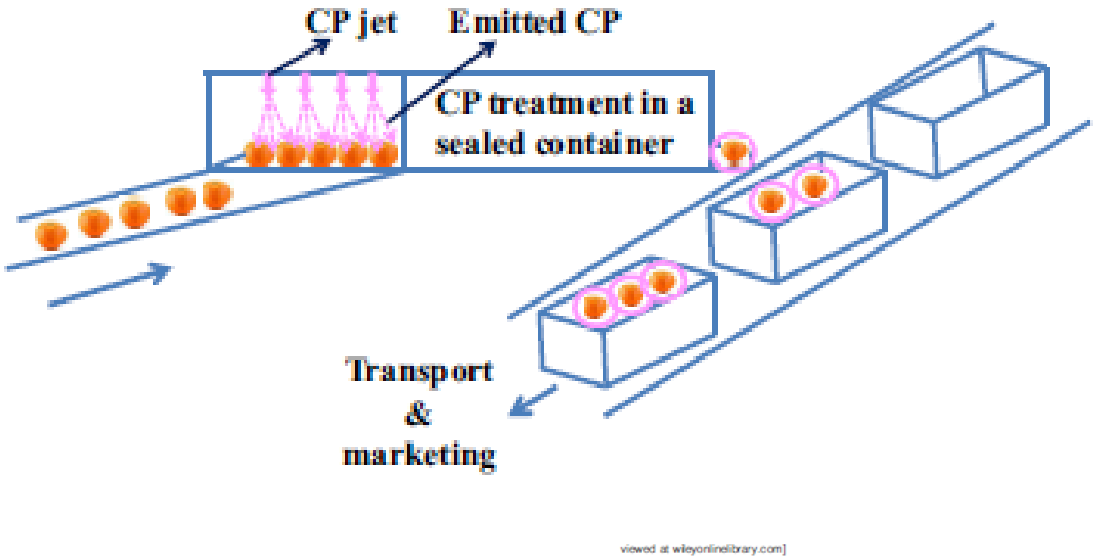
- Harvest manually as long as possible
- Harvest under dry conditions
- Postpone harvest of tubers till rotten mother tubers are gone (beware of rainy season!)
- Remove rotten tubers from harvesters and graders
- Avoid wounding of tubers
- After harvest: Dry, dry, dry .....
- Store tubers in well-ventilated rooms at low temperatures

Potential management tools

- Selection of pathogen-free seed lots → Testing
- Hygiene
- Cultivation measures?
- Resistance?
- Use of suppressiveness seed lots?
- Physical treatments of tubers?
- Biological control?

Physical treatments

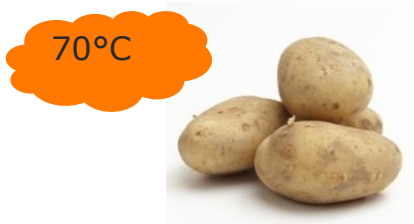
- Thermotherapy
  - Warm water
  - Steam
  - Hot air
- ‘Cold plasm’
- UV-light



(Siddique et al, 2018)

Steam treatment

5 s steam (1.2a)



Maintenance of seed vitality

Destruction of pathogen on surface

Blowing away inoculum surface

Production of antimicrobial compounds

Afek & Orenstein 2002

Steam treatment

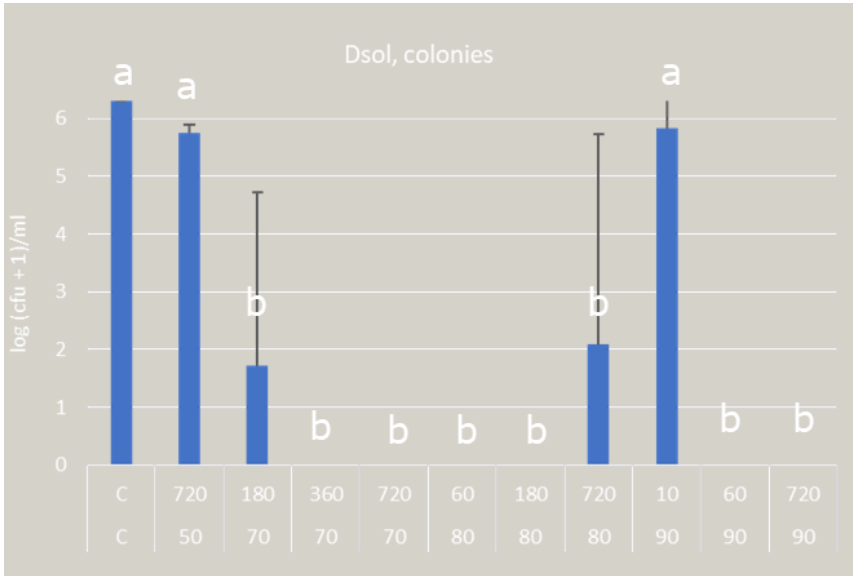
	Pecto-bacterium	*H. solani*	*R. solani*	*C. coccodes*	*Fusarium* spp.	*S. scabies*	*S. subterranae*
Pootgoed							
Controle	47	46	40	52	35	42	41
Stoom	2	3	2	3	2	2	2
Naoogst							
Controle	26	32	31	44	20	27	26
Stoom	3	4	5	8	4	5	5

- Nozzle 20 cm above conveyer belt
- 10 s, 70 oC
- Air drying
- Progeny tested 120 days after planting

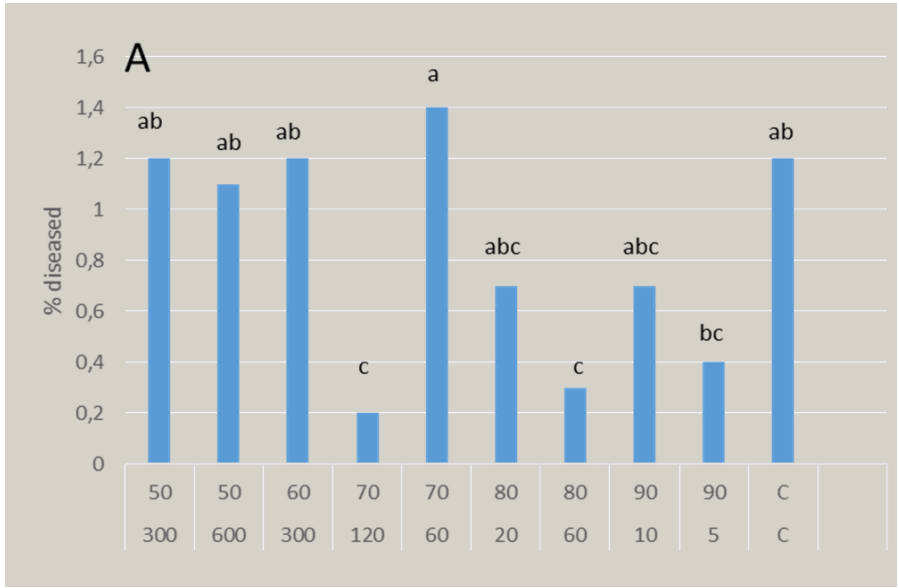
Afek & Orenstein, 2001

Steam treatment

Effect colonies on plates



Field: effect on Dsol infected tubers



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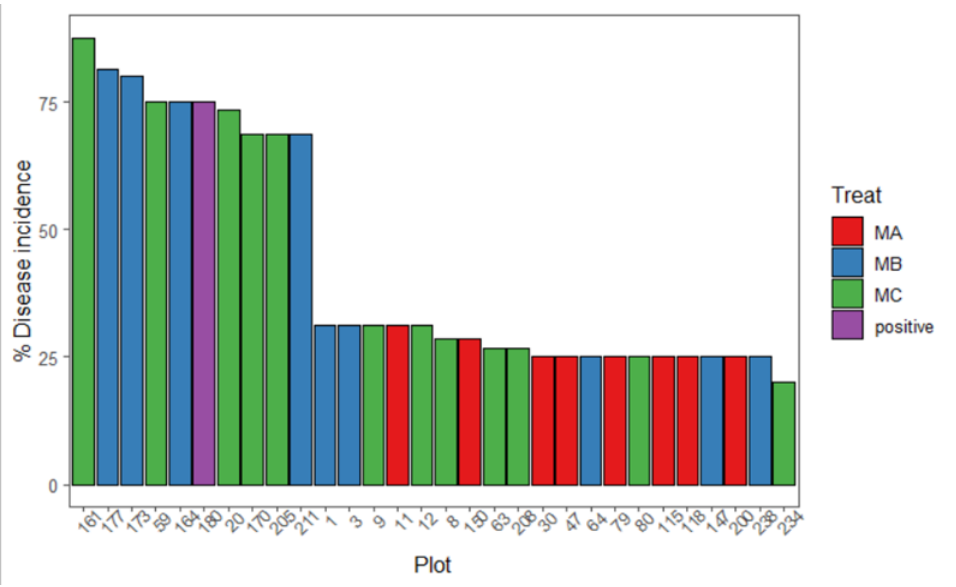
Results not reproducible!

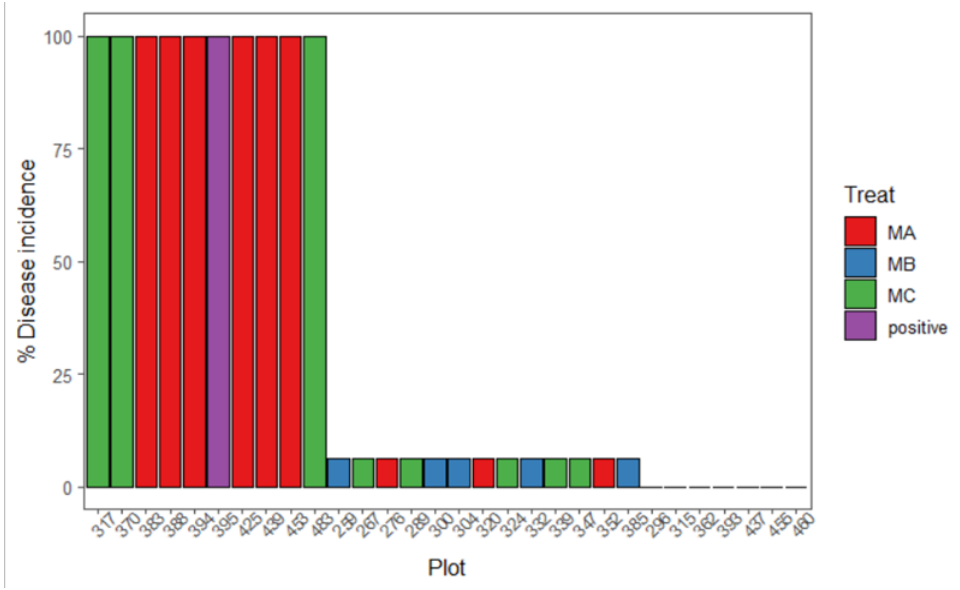
Potential management tools

- Selection of pathogen-free seed lots → Testing
- Hygiene
- Cultivation measures?
- Resistance?
- Use of suppressiveness seed lots?
- Physical treatments of tubers?
- Biological control?

BCA treatments: results 2019



Dickeya solani



Pectobacterium brasiliense

Experiments 2020, 2021: results not reproducible!

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Biological control Impression 26 June 2019

Pcb

Pcb +BCA MC 69

Water

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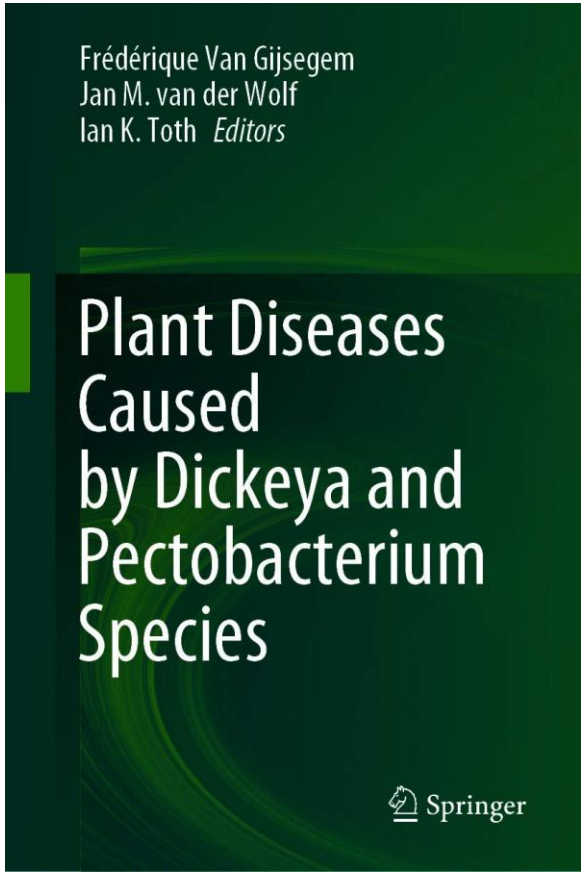
Not reproducible

Nederlandse Aardappel Organisatie

For further reading



2021

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- Mirjam Kooman (NAK
- Inge van Duivenbode (NAK)
- Jack Gros (Agrico)
- Jan Gottschall (NAO)

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Anna Paulowna B.A.

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Ray Spence, Guardian, July 2020