Diversified Bacteria Causing Potato Blackleg and Soft Rot Related to Their Management

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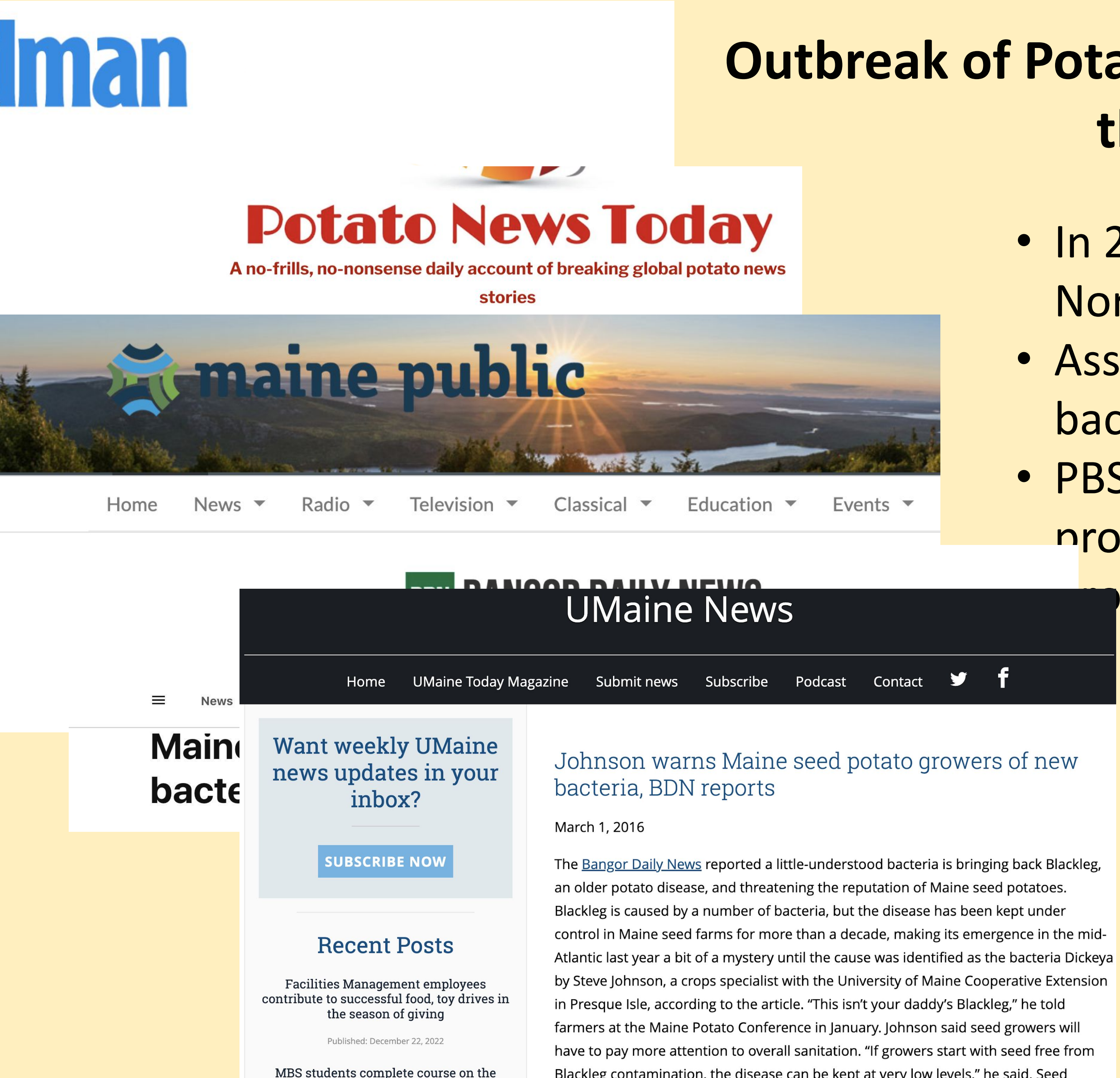
Outbreak of Potato Blackleg and Soft Rot (PBSR) in the Northeaster US



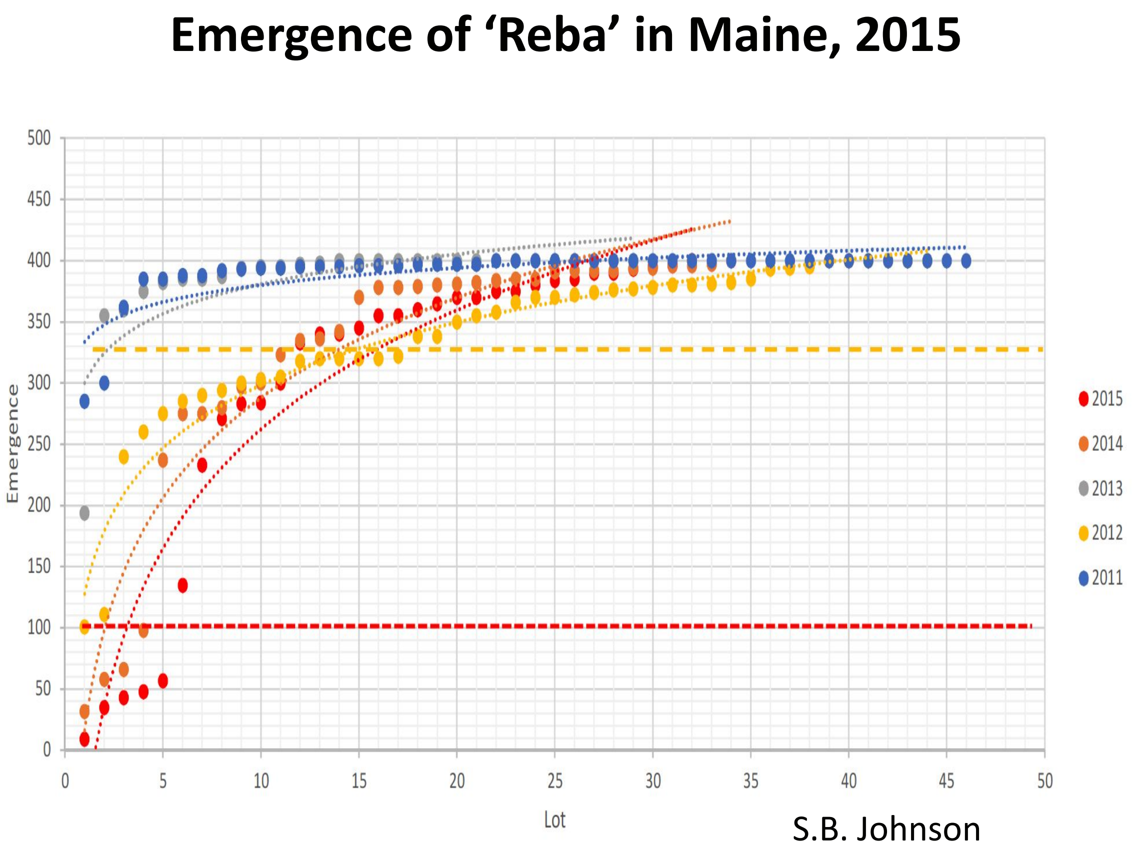
Johnson warns Maine seed potato growers of new bacteria, BDN reports

March 1, 2016

The Bangor Daily News reported a little-understood bacteria is bringing back Blackleg, an older potato disease, and threatening the reputation of Maine seed potatoes. Blackleg is caused by a number of bacteria, but the disease has been kept under control in Maine seed farms for more than a decade, making its emergence in the mid-Atlantic last year a bit of a mystery until the cause was identified as the bacteria Dickeya by Steve Johnson, a crops specialist with the University of Maine Cooperative Extension in Presque Isle, according to the article. "This isn't your daddy's Blackleg," he told farmers at the Maine Potato Conference in January. Johnson said seed growers will have to pay more attention to overall sanitation. "If growers start with seed free from Blackleg contamination, the disease can be kept at very low levels," he said. Seed

- In 2015, PBSR was observed in the Northeastern region
- Associated with seed transmission of a bacteria
- PBSR threatened Maine's potato production, which is a major seed producer

Low emergence of potatoes due to blackleg



S.B. Johnson

Symptoms of potato blackleg and soft rot

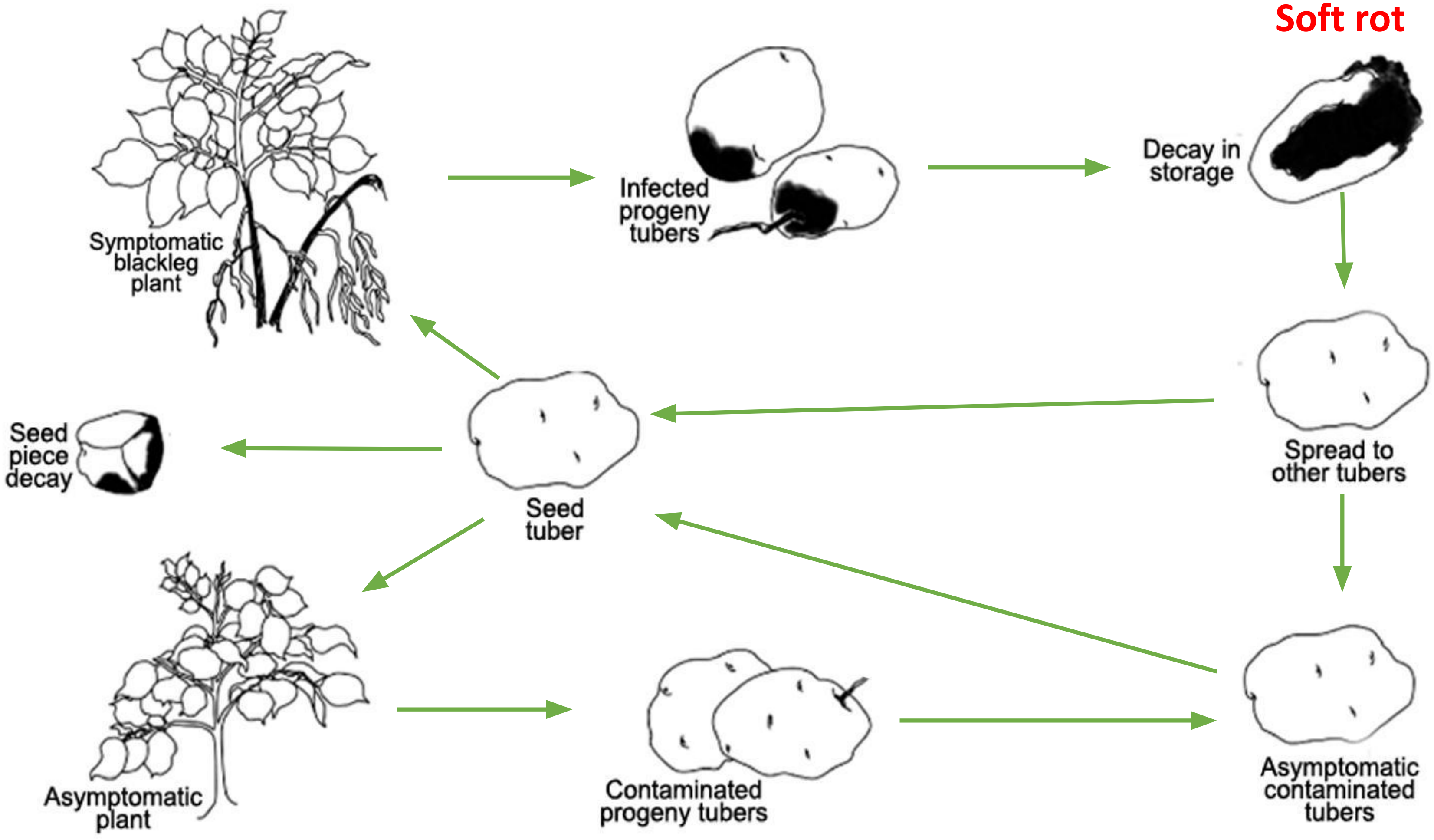
Stand loss, wilting and yellowing leaves, brown, black, and decayed stems, storage rot

Source: Steven B. Johnson

Disease Cycle – PBSR is a Seedborne Disease

Soft rot

Non-emerge
nce

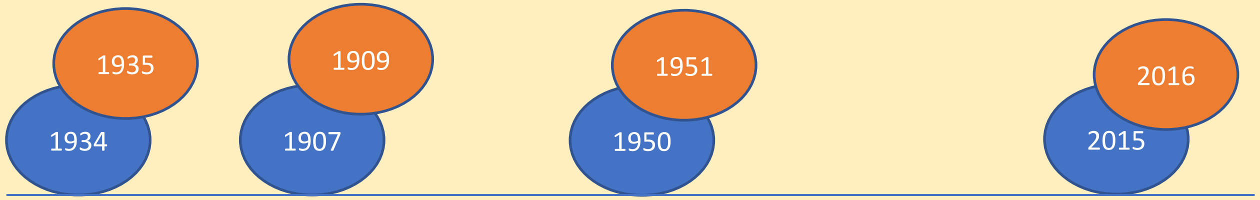


(modified from De Boer & Rubio, 2004)

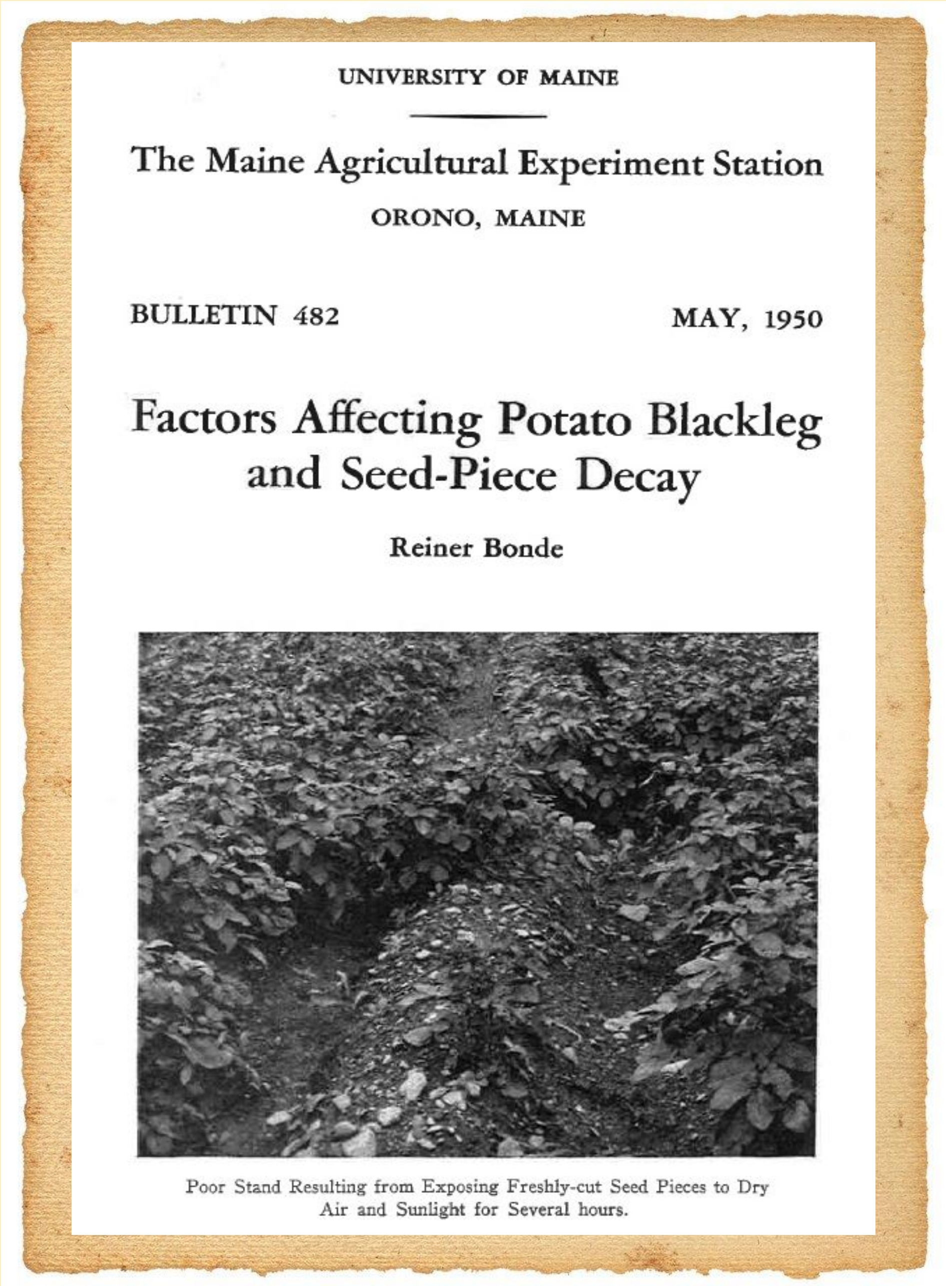
Questions on the PBSR outbreak

1. What are the origin and pathogens causing the outbreak?
3. How did the outbreak occur?
4. How do the pathogens survive?
5. How to manage the disease?

Outbreaks in history: old disease? New pathogen?



2015, Maine



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The Maine Agricultural Experiment Station

ORONO, MAINE

BULLETIN 482 — MAY, 1950

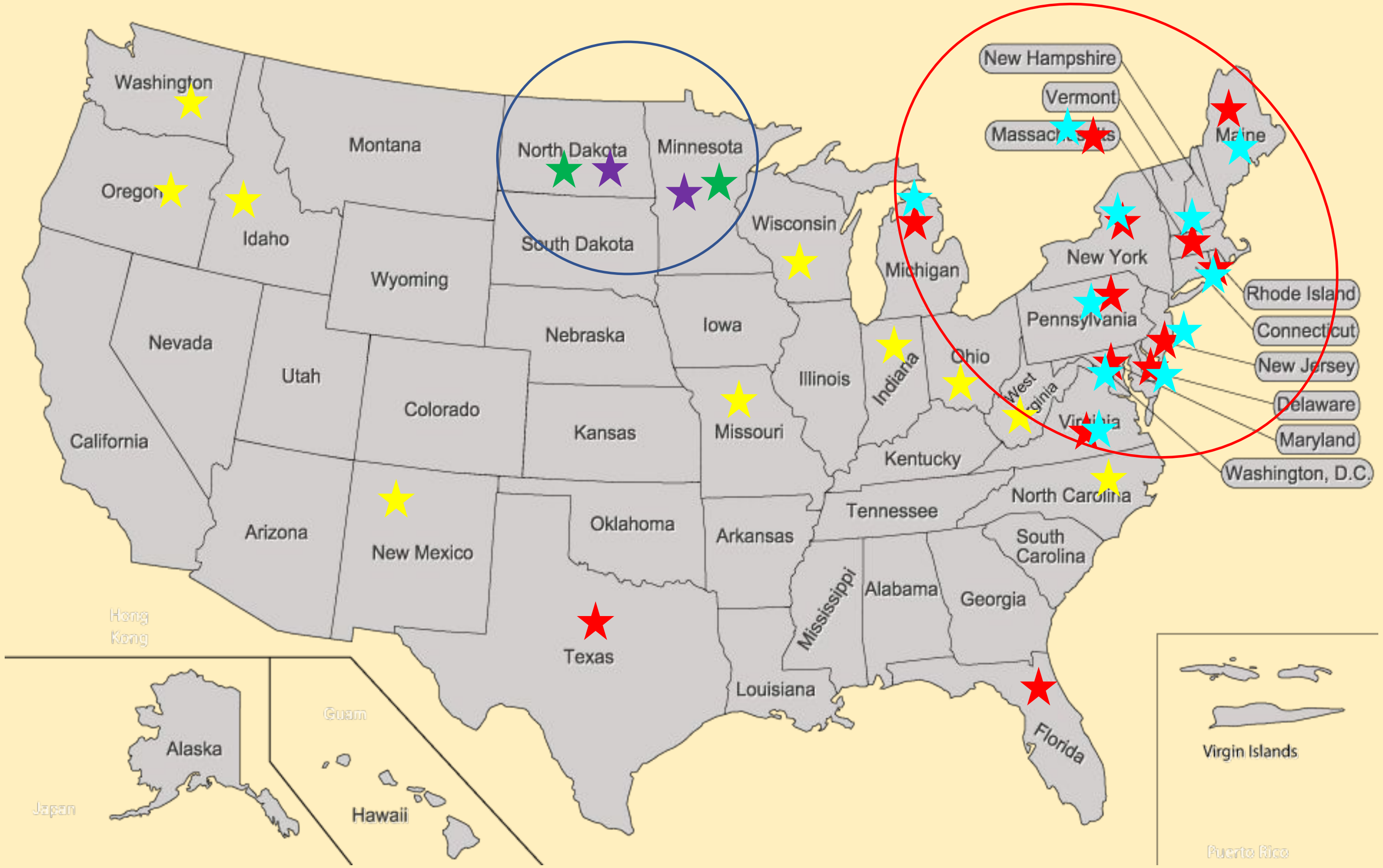
Factors Affecting Potato Blackleg and Seed-Piece Decay

Reiner Bonde

Poor Stand Resulting from Exposing Freshly-cut Seed Pieces to Dry Air and Sunlight for Several hours.

Dickeya dianthicola was associated with the PBRS outbreak

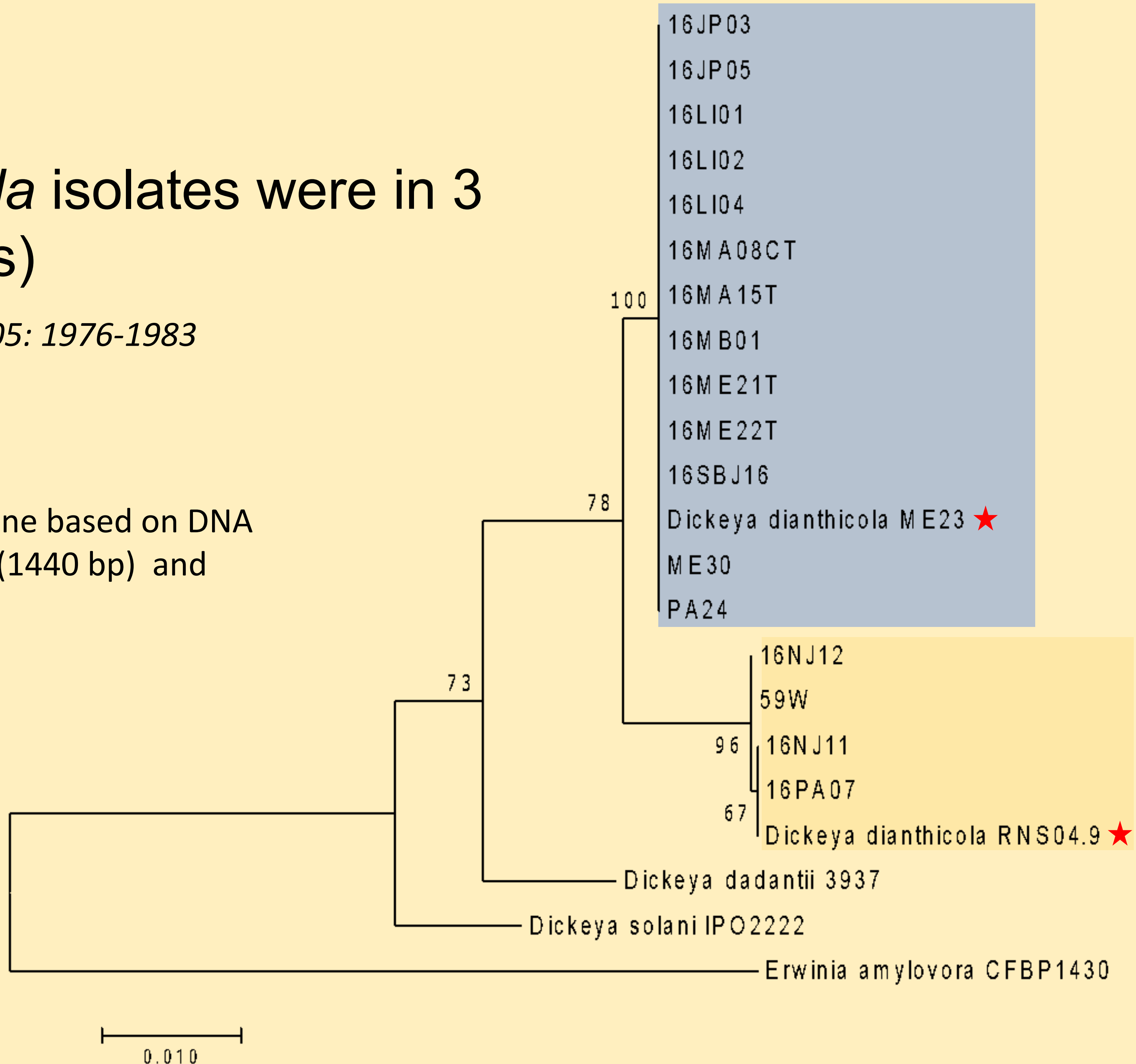
- **D. dianthicola**
- *D. chrysanthemi*
- *P. parmentieri*
- *P. brasiliense*
- Un-identified



Dickeya dianthicola isolates were in 3 groups (genotypes)

Ge et al. 2021. Plant Disease105: 1976-1983

Phylogenetic analysis was done based on DNA sequencing of the 16S rRNA (1440 bp) and gapA (850 bp) genes.



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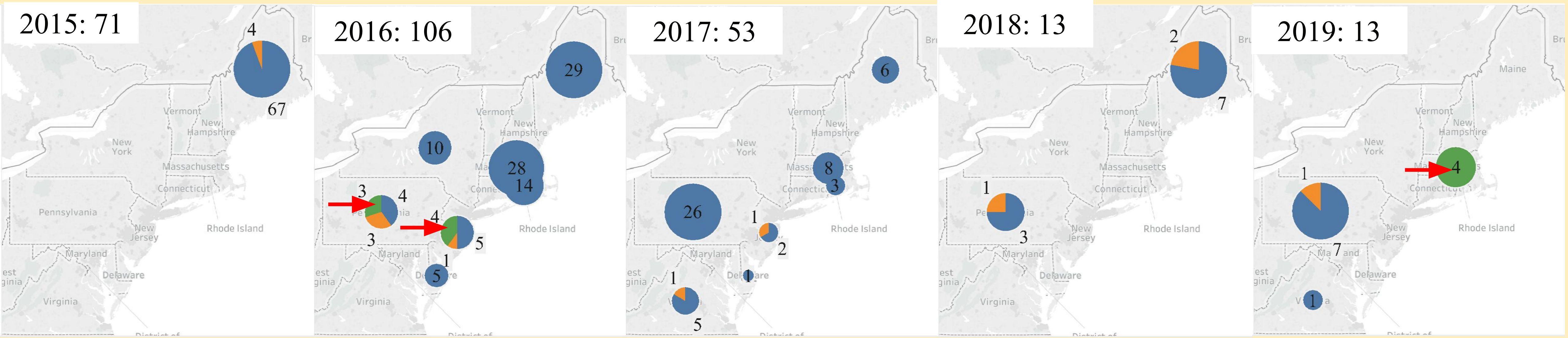
Genotypic distribution of *Dickeya dianthicola* in the NE US

Samples were collected from 2015 to 2019 (n = 256)

Ge et al. 2021. Plant Disease105: 1976-1983



Type I, predominant, comprising 95% in Maine, and 83% from all other states.

Type II, continuously present in at least one state every year at relatively lower percentages than Type I.

Type III, only present in 2016 in Pennsylvania and New Jersey, and 2019 in Massachusetts, and was not detected in Maine.

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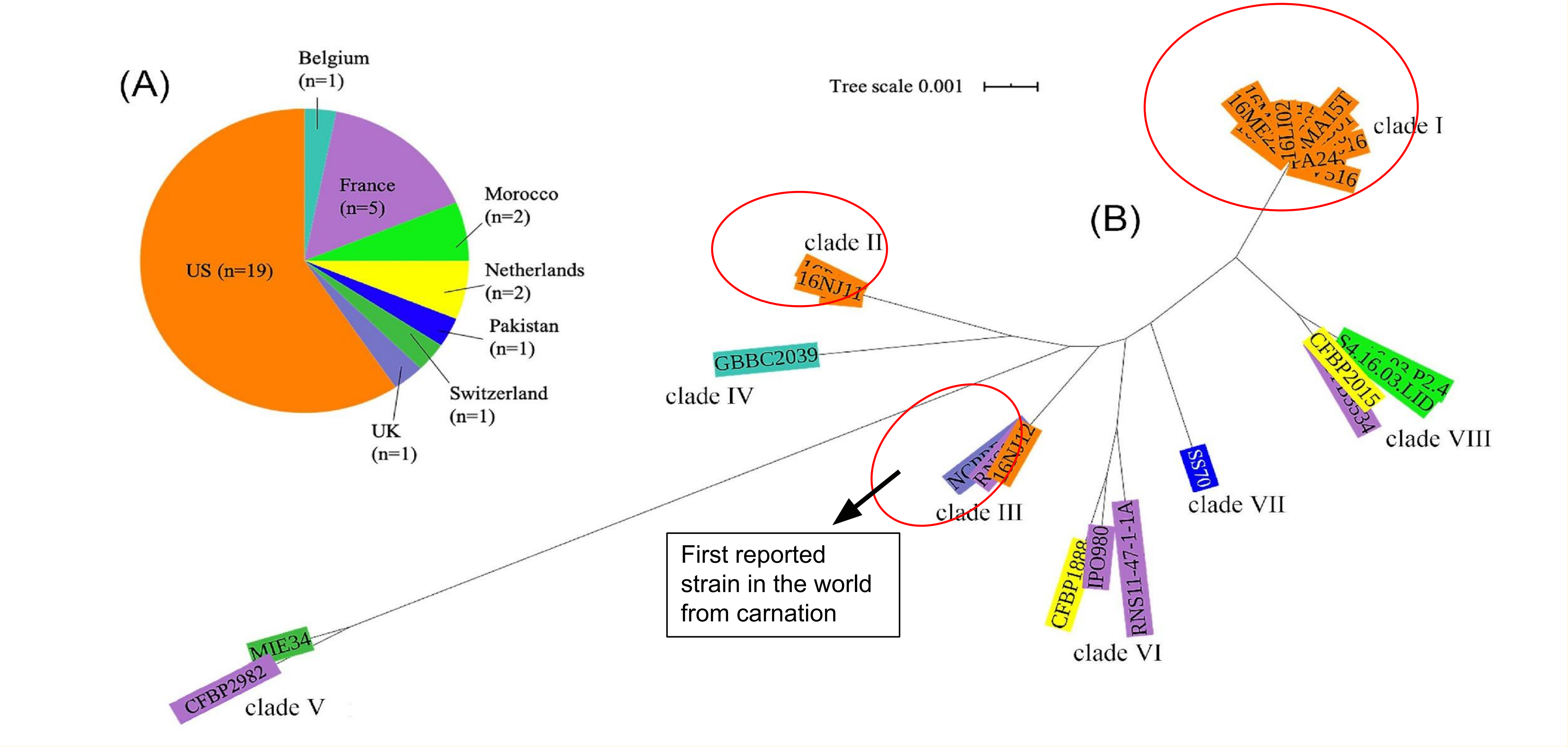
Phylogenetic tree based on SNPs in the core genome, N = 32

Ge et al. 2021. Plant Disease 105: 3946-3955



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Pennsylvania Flower Growers, Bulletin: 205

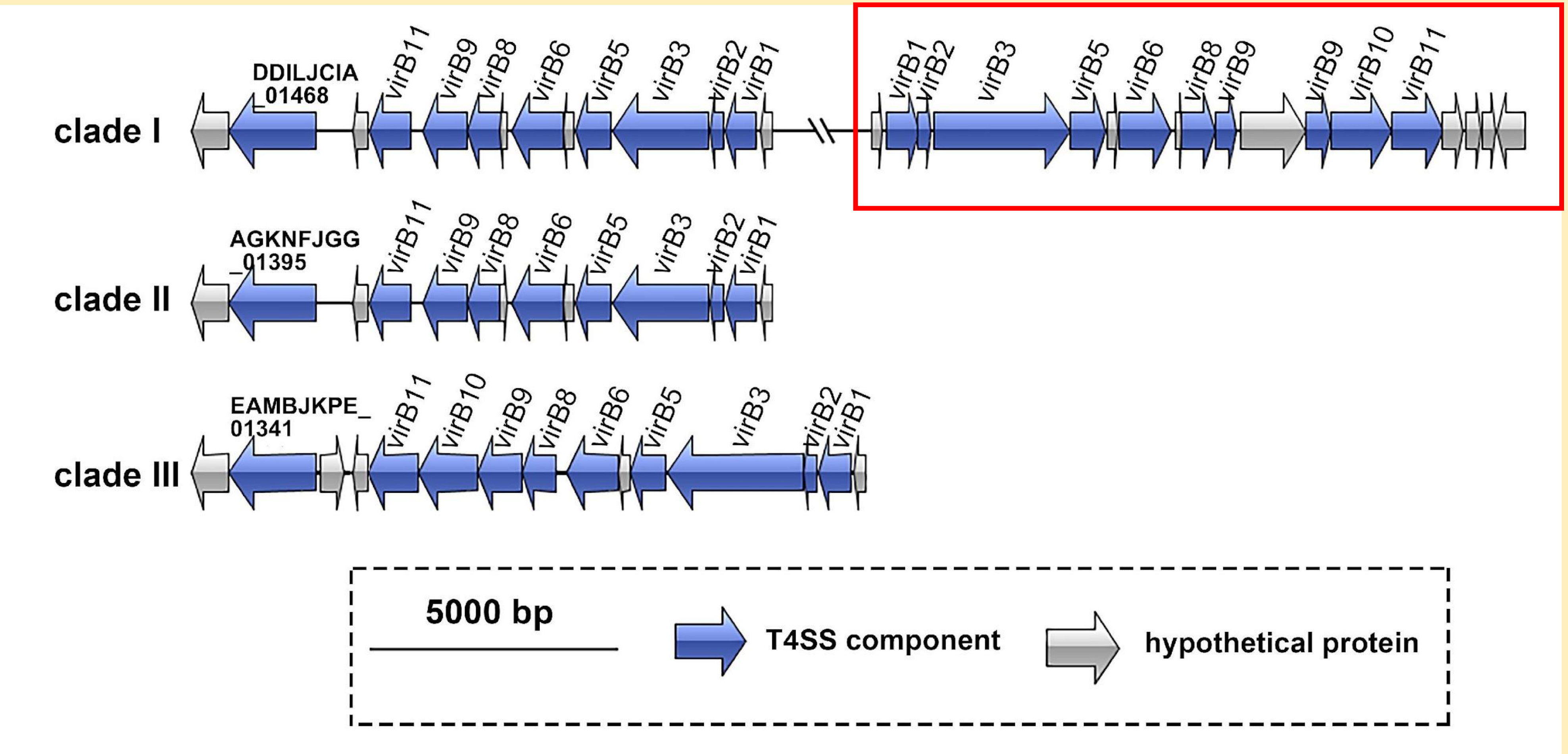
Bacterial Slow Wilt or Stunt of Carnations

Paul E. Nelson, Robert S. Dickey, and L. P. Nichols
Departments of Plant Pathology
Cornell University and The
Pennsylvania State University

Bacterial slow wilt or stunt, a serious disease of carnation in Europe, was first found in the United States in 1954-55, on carnation plants grown in western New York. In April 1962 it was found on carnations in southeastern Pennsylvania and again in November 1967 in the same range.

Type IV secretion system (T4SS) involved in genetic exchange or effector translocation

Ge et al. 2021. Plant Disease 105: 3946-3955



Conclusions

1. *Dickeya dianthicola* genotype I was the predominant strain associated with the outbreak of 2015

Bacterial communities in PBSR potato tissues examined using metagenomics

***Dickeya* detected**

Group of samples / # of Reads	2207	308	983	851	1128	28788	11000	17029	17013	25405	12585	4901	24841	6369	16508	24727	24902	9241	13219	11389	597	2186
D. aquatica	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. chrysanthemi	3	5	4	6	5	1	1	1	1	24	4	18	23	5	1	1	1	2	1	2	1	20
D. dadantii	92	73	87	82	81	99	98	98	97	96	95	95	95	94	99	99	99	99	99	98	97	96
D. dianthicola	91	72	86	82	81	98	98	97	97	95	95	94	94	94	99	99	99	99	99	97	97	96
D. fangzhongdai	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
D. paradisiaca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. zeae	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. aroidearum	0	1	1	1	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. atrosepticum	1	6	1	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1
P. betavasculorum	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. cacticida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. c. subsp. actinidiae	0	3	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. c. subsp. brasiliense	1	10	1	3	1	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	1
P. c. subsp. carotovorum	2	8	4	9	2	0	0	1	0	1	1	2	0	0	0	0	0	0	0	0	0	1
P. c. subsp. odoriferum	2	11	4	9	3	0	1	1	1	1	1	2	0	1	0	0	0	0	0	1	1	1
P. parmentieri	3	14	4	4	4	0	1	1	1	1	1	1	2	1	0	0	0	0	0	1	2	2
P. polaris	3	8	4	2	4	0	1	1	1	1	1	1	2	1	0	0	0	0	0	1	2	1

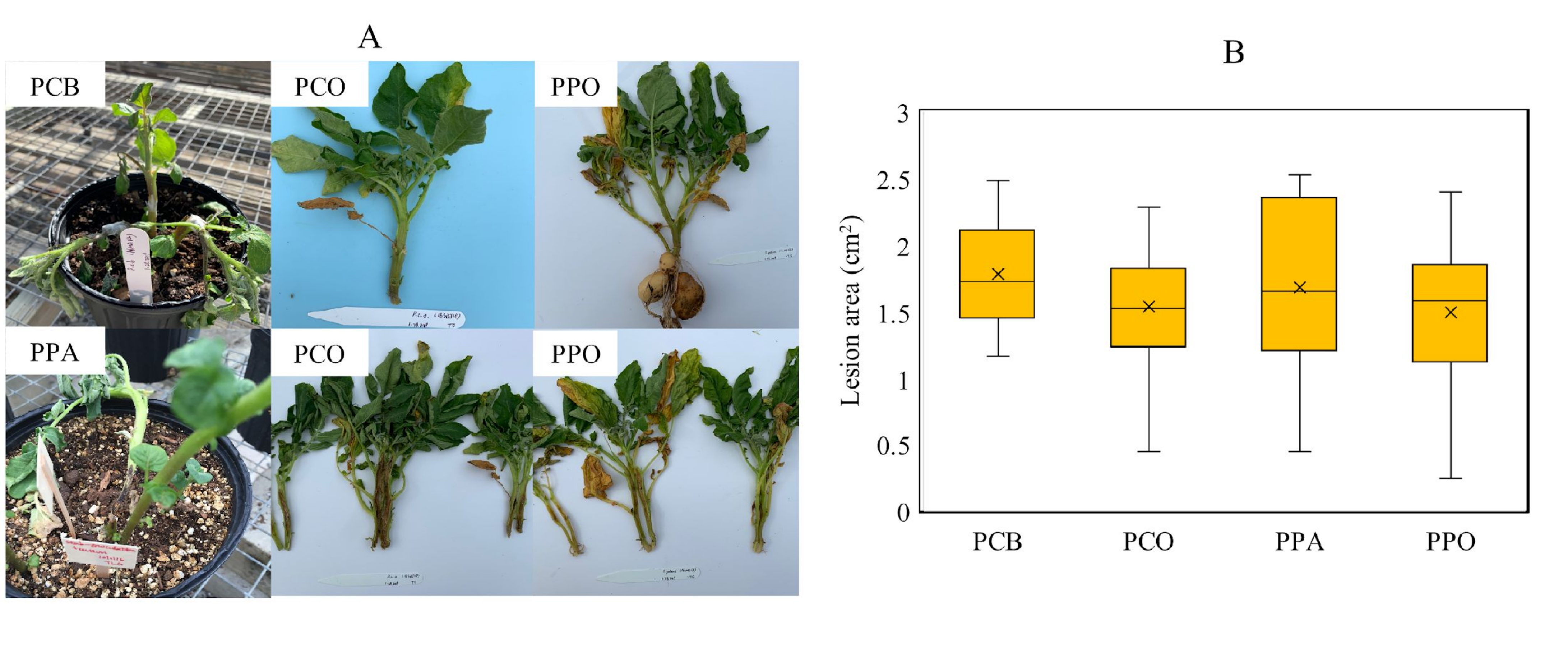
***Dickeya* detected**

Group of samples / # of Reads	16892	20840	4256	5627	2449	4331	4880	4286	16452	1611	22873	9098	1554	2429	1323	844	824	1988	4537	802	1702
D. aquatica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. chrysanthemi	2	3	4	2	6	2	5	8	9	5	16	3	9	7	5	2	1	1	6	8	3
D. dadantii	96	94	89	88	80	79	72	59	56	55	48	42	40	40	38	33	26	22	20	20	17
D. dianthicola	95	94	88	88	80	78	71	59	56	55	47	42	40	40	38	32	26	22	20	20	16
D. fangzhongdai	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
D. paradisiaca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. zeae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. aroidearum	0	0	1	0	0	0	0	0	0	0	0	1	0	0	9	1	1	1	1	2	1
P. atrosepticum	0	0	1	0	0	0	1	1	0	1	1	1	0	1	1	1	0	0	1	2	1
P. betavasculorum	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
P. cacticida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. c. subsp. actinidiae	1	1	0	0	0	1	0	0	0	1	0	1	0	1	25	1	71	1	1	1	1
P. c. subsp. brasiliense	0	0	1	0	0	0	1	0	0	1	0	1	0	1	1	2	0	0	1	2	2
P. c. subsp. carotovorum	2	0	3	5	0	1	23	10	0	19	0	54	0	1	16	43	0	29	17	4	77
P. c. subsp. odoriferum	3	0	6	9	1	3	24	10	0	38	0	54	0	1	16	44	0	62	39	10	78
P. parmentieri	0	3	3	2	17	8	3	26	38	4	42	1	54	45	9	19	2	14	37	63	3
P. polaris	0	3	2	2	17	17	3	26	38	4	43	1	55	53	9	19	2	15	37	61	3

***Pectobacterium* detected**

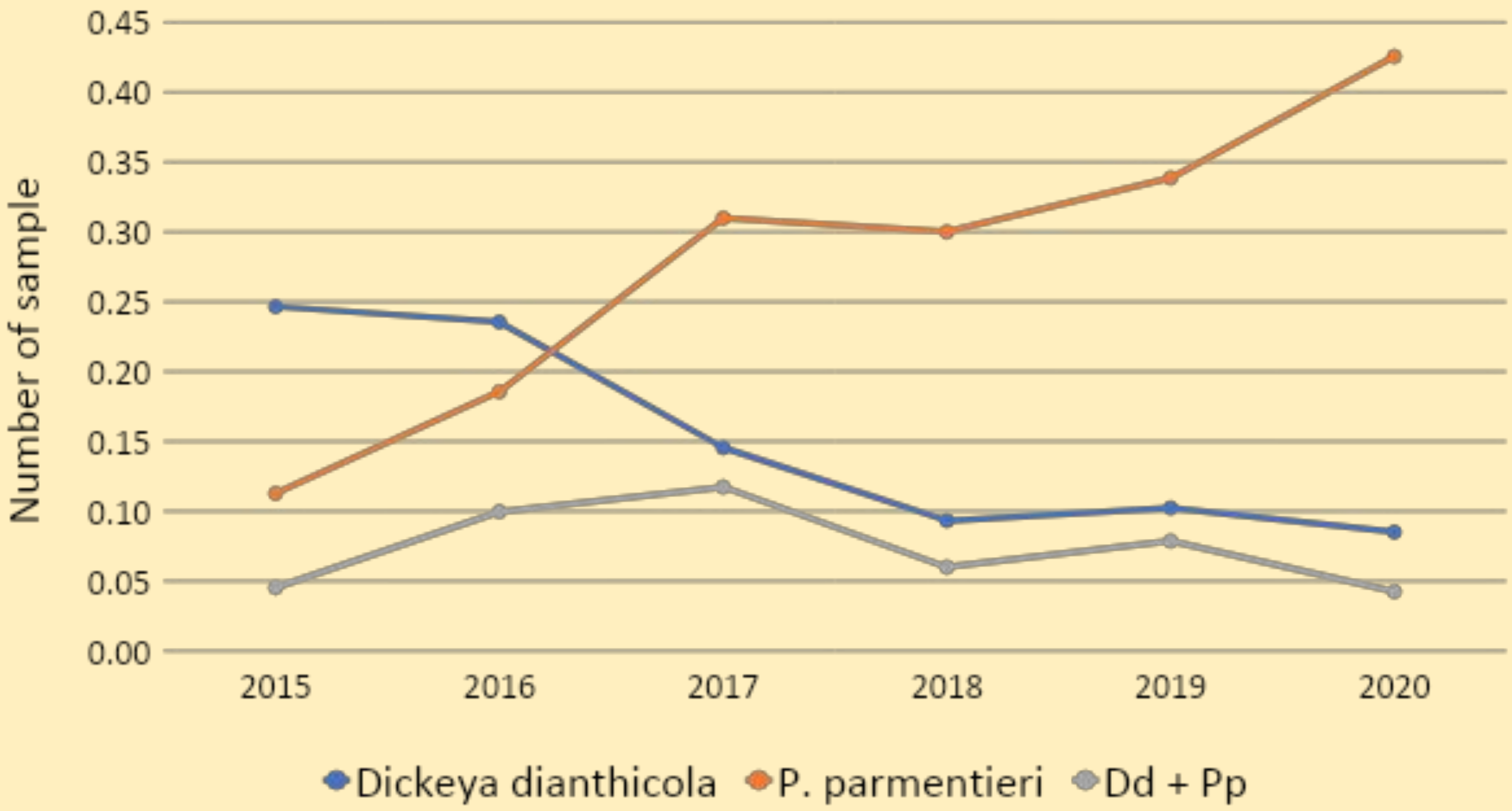
Group of samples / # of Reads	834	705	9368	1957	3374	3928	3833	3305	7954	7583	12674	673	3568	3999	14622	1489	4202	4501	3706	21015	23215	17039
D. aquatica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. chrysanthemi	1	4	7	1	1	2	2	2	0	0	0	5	0	4	3	0	3	4	0	5	1	3
D. dadantii	30	15	4	4	4	2	2	2	1	1	0	4	2	1	1	1	1	1	1	1	0	0
D. dianthicola	30	14	4	4	4	2	2	1	1	0	0	4	2	1	1	1	1	1	1	0	0	0
D. fangzhongdai	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. paradisiaca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. zeae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. aroidearum	1	1	2	1	3	0	18	12	1	1	1	0	2	0	1	51	0	1	1	0	0	0
P. atrosepticum	2	1	3	1	0	1	1	0	0	0	0	1	1	1	1	1	0	2	32	0	1	0
P. betavasculorum	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. cacticida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. c. subsp. actinidiae	1	1	1	3	13	0	6	16	0	0	0	0	70	2	0	43	0	1	2	0	0	0
P. c. subsp. brasiliense	3	1	2	2	6	0	3	1	1	1	1	0	1	0	1	1	0	5	32	0	1	0
P. c. subsp. carotovorum	31	8	2	82	71	25	60	36	94	91	97	3	25	1	6	2	0	35	63	0	57	0
P. c. subsp. odoriferum	60	9	2	84	72	26	61	38	95	93	98	4	26	2	16	2	0	37	64	0	63	0
P. parmentieri	8	72	88	9	3	71	11	35	3	7	1	90	2	93	69	3	97	60	33	98	41	98
P. polaris	6	72	86	9	3	71	12	36	3	7	1	91	2	94	82	3	97	59	3	98	41	99

Pathogenicity of *Pectobacterium* spp.



PCO: *P. carotovora* subsp. *odoriferum*, PCB: *P. brasiliense,* PPO: *P. polaris*, PPA: *P. parmentieri*

Pathogen detection of potato samples indicating a progressive change of PBSR epidemic



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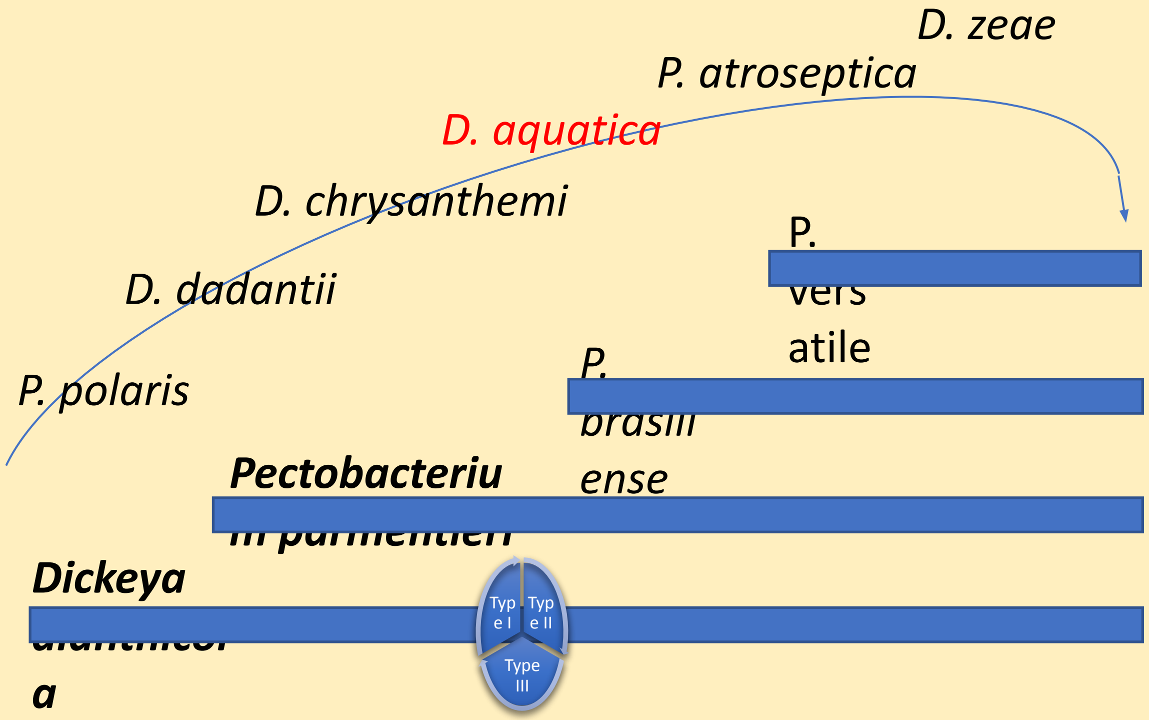
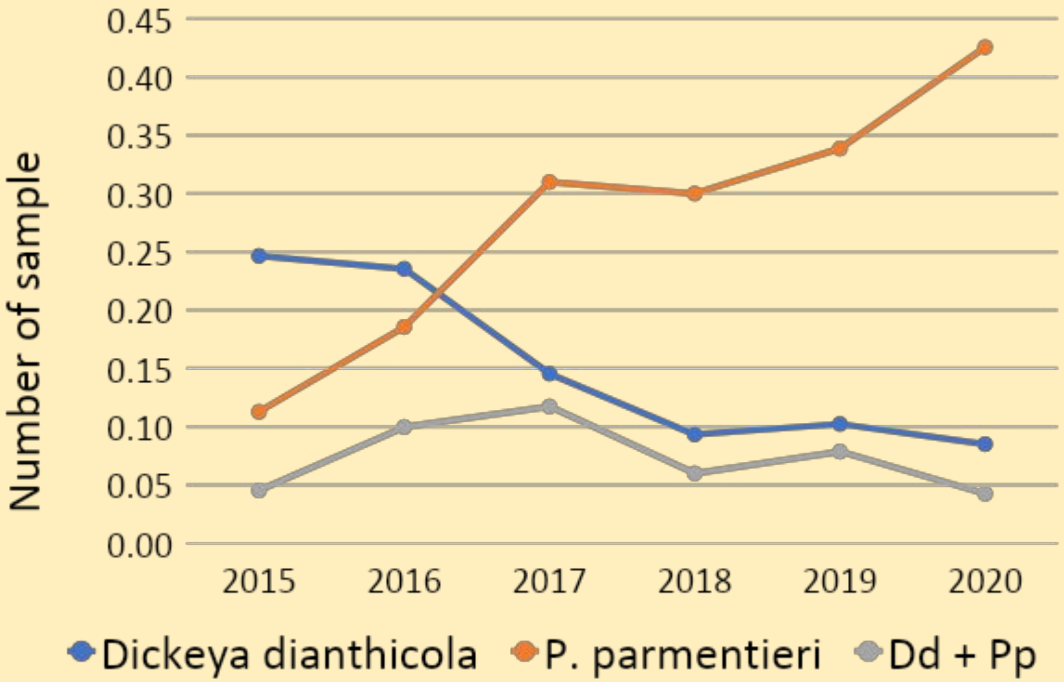
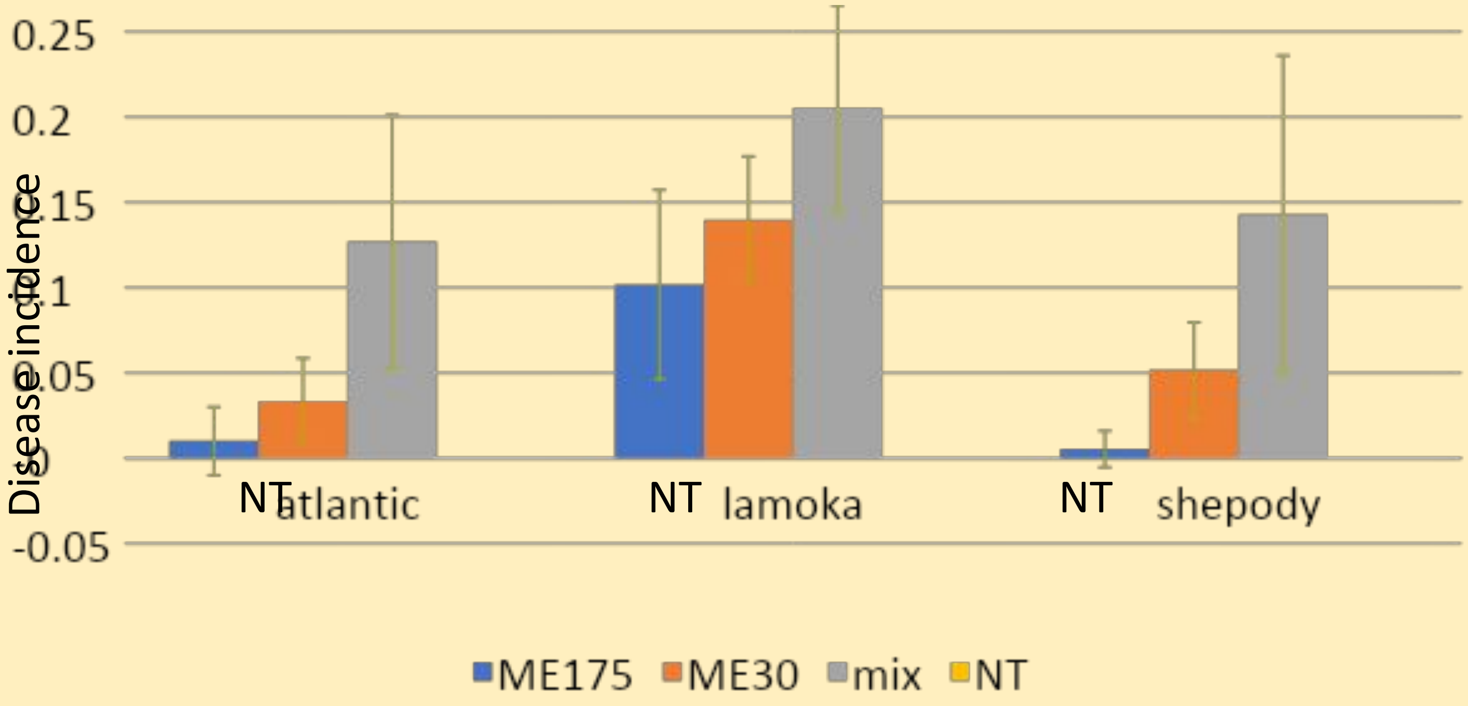
Taxonomy of PBRS pathogens





2018 field trial –
Blackleg of potato inoculated with *D. dianthicola* OR/AND *P. parmentieri*

Ge et al. 2021. Microorganisms 9: 316.

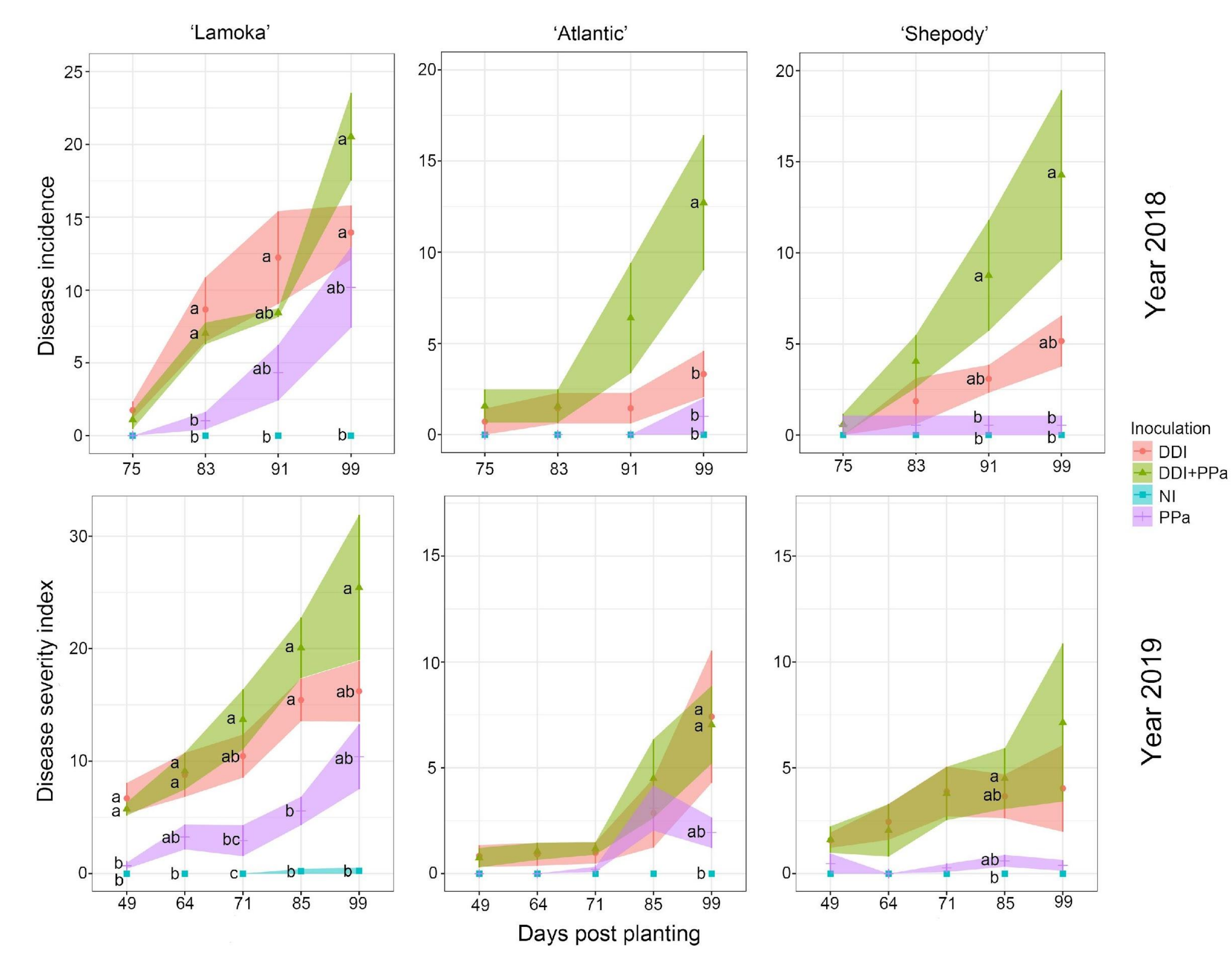


- *D. dianthicola* is more aggressive than *P. parmentieri*
- Co-infection of *D. dianthicola* and *P. parmentieri* aggravate disease severity

- ***ME175**: Pectobacterium parmentieri;* ***ME30**: Dickeya dianthicola; mix: combined;* **NT**: non-inoculated

Synergism effect from co-inoculation of *Dickeya dianthicola* (DDI) and *Pectobacterium parmentieri* (PPA) in the field

Ge et al. 2021. Microorganisms 9: 316.



Conclusions

2. Multiple species of *Dickeya* and *Pectobacterium* cause potato blackleg and soft rot

3. Co-infection with multiple species of bacteria may have a synergistic effect making the disease even worse

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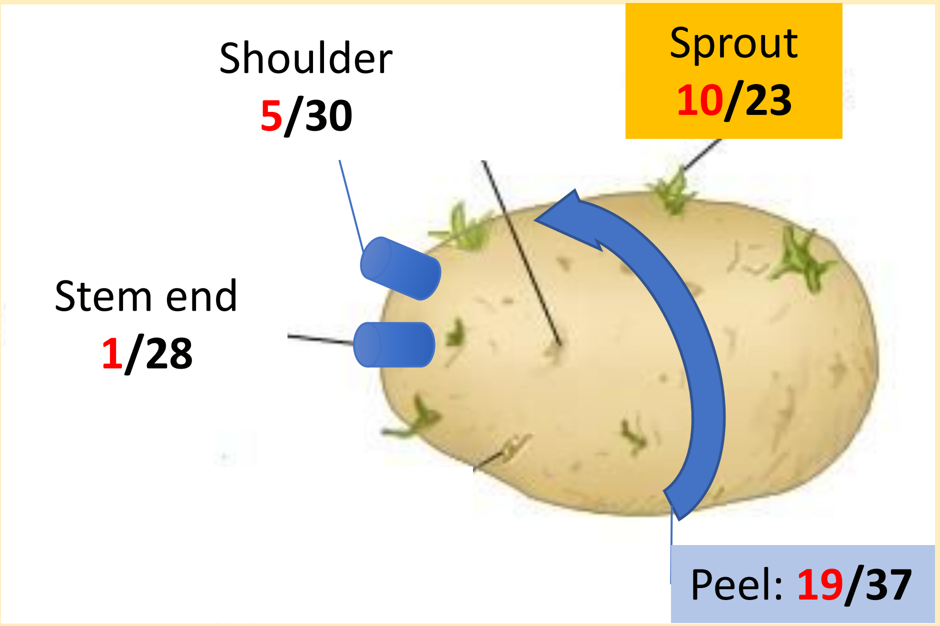
Bacterial distribution on potato tubers

- Potato tubers usually harbor low numbers of the bacteria in a **quiescent** form, often for a long time (Helias et al., 2000).
  - stolon end (Czajkowski et al., 2009)
  - lenticels and suberized wounds (Pérombelon, 2002).
- Bacteria concentrated in potato stems in the first 15–20 cm above ground level (Hélias et al., 2000)
- Bacteria reside in lenticels in dormant status

Hao/Secor tests (% *Dickeya* positive)



Hao tested 5 months later than Secor

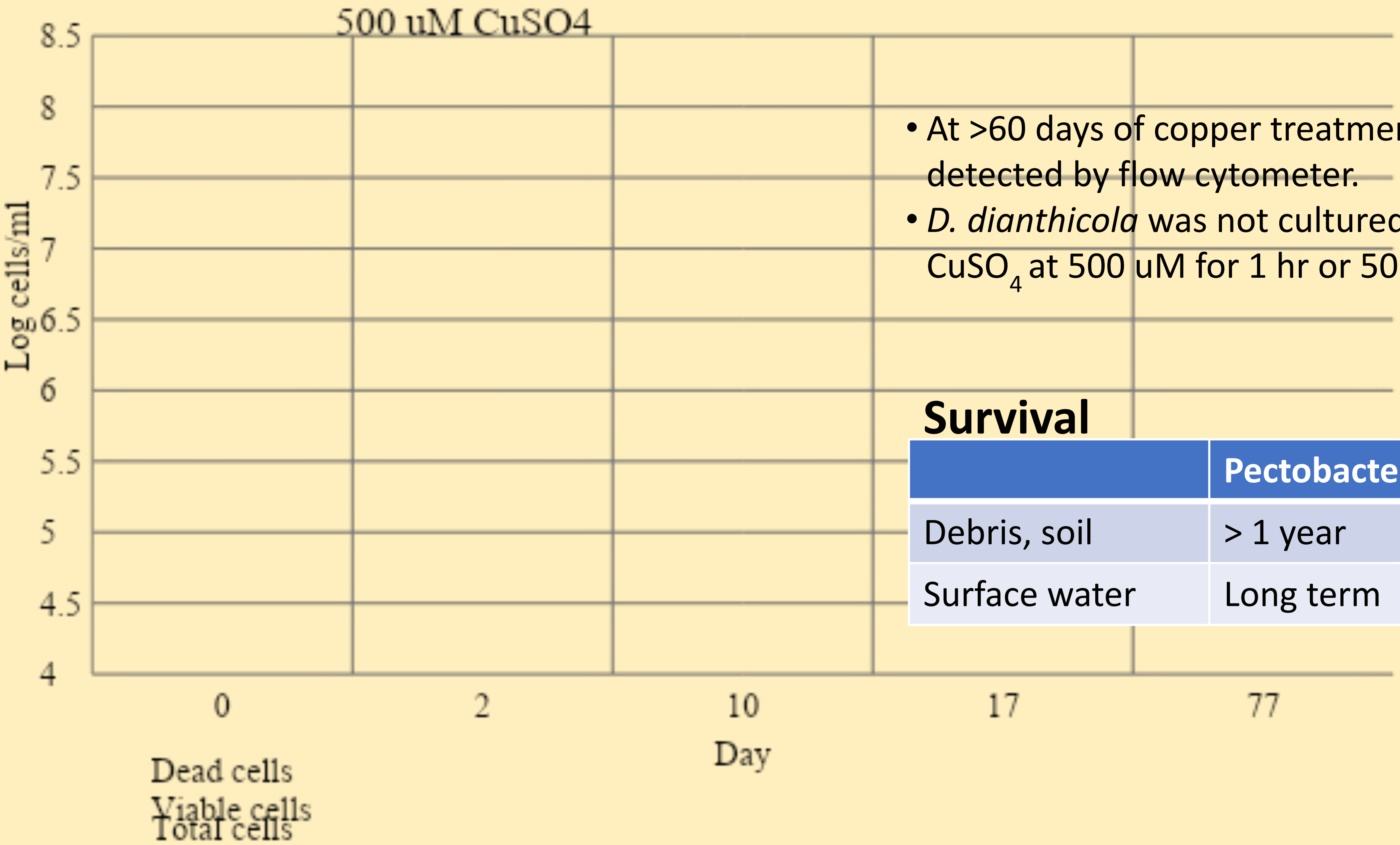
Chi-square test on tissue distribution of *Dickeya dianthicola* (ME30) and *Pectobacterium parmentieri* (ME175) on potatoes

Category	Frequency			Chi-Sq	df	P value
	Stem	Tuber (observed)	Tuber (expected)[a]			
ME30 positive	307 (40.3)	29 (23.8)	49 (40.3)	13.64	1	<0.001
ME30 negative	454 (59.7)	93 (76.2)	73 (59.7)			
Total	761	122	122			
ME175 positive	394 (51.8)	77 (63.1)	63 (51.8)	6.43	1	<0.01
ME175 negative	367 (48.2)	45 (36.9)	59 (48.2)			
Total	761	122	122			

Values are presented as number (percentage) of sample.

[a] the percentage of pathogens on stems were viewed as the expected percentage of pathogens on tubers.

Viable but not culturable (VBNC) status used for dormancy



- At >60 days of copper treatment, viable cells can also be detected by flow cytometer.
- *D. dianthicola* was not cultured when treated with $CuSO_4$ at 500 uM for 1 hr or 50 uM for 2 hr

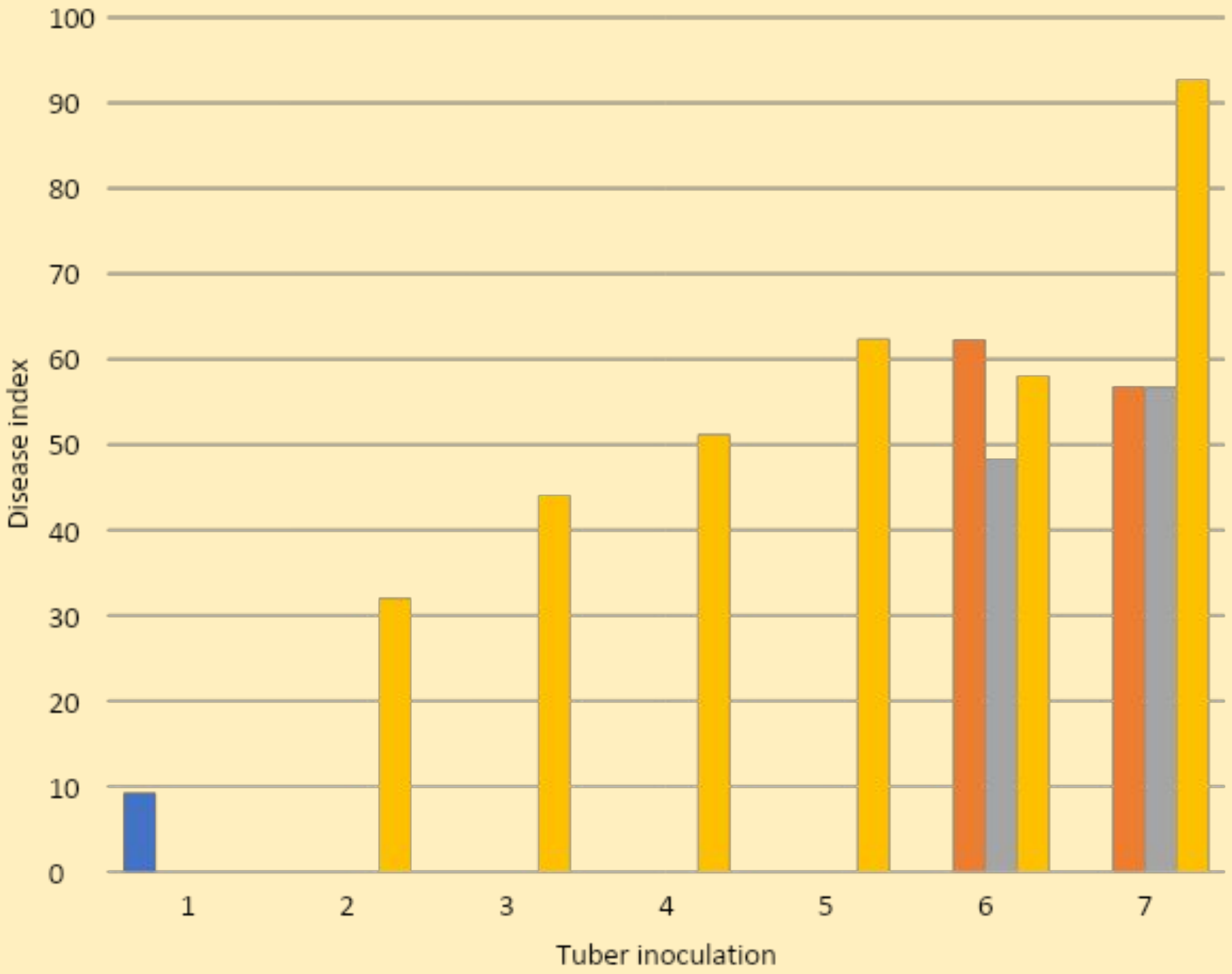
Survival

	Pectobacterium	Dickeya
Debris, soil	> 1 year	< ½ years
Surface water	Long term	Long term

Storage period is the key for tuber cross-infection, 2022

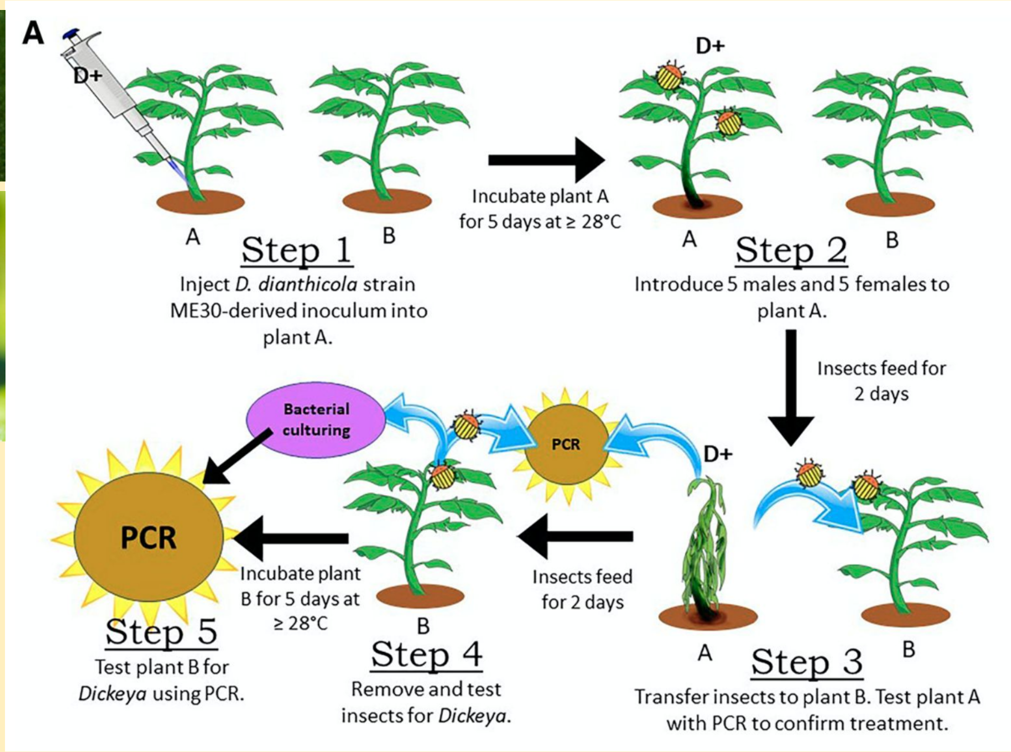
- Obs. 1. Cutting. Epidemics occurs in a long-stored CUT tubers under unfavorable conditions, but not if planted immediately after cutting
- Obs. 2. Non-cutting. Disease incidence is higher for longer storage period

Inoculation method: whole potato tubers were inoculated with *Dickeya dianthicola* strains: Types I, II, III using vacuum infiltration and mixed with non-inoculated tubers. NI: non-inoculated.



Dickeya dianthicola is not vectored by green peach aphids (*Myzus persicae*) and Colorado potato beetles (*Leptinotarsa decemlineata*)

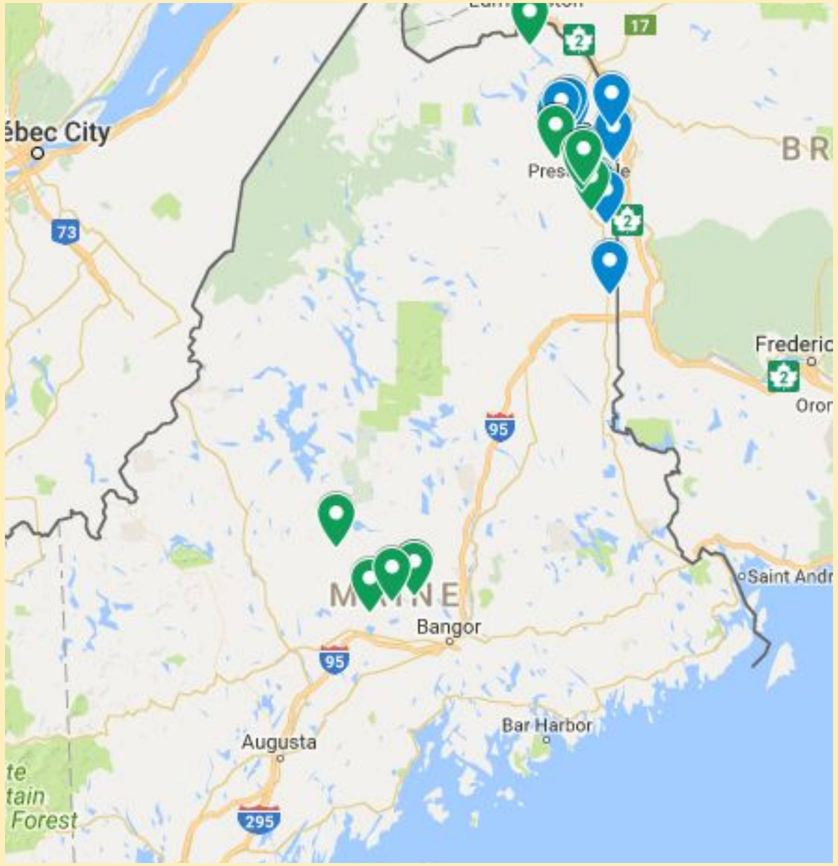
Insinga et al. 2021. PhytoFrontiers 1:160-172



- But *Pectobacterium* is transmitted by flies

Can water be a source of inoculum?
-Dickeya dianthicola found in water courses

- Australia: there is a direct link from waterways to infected potatoes via irrigation (Cother & Gilbert, 1990), **but no direct evidence of infection**
- The bacteria found in **Sweden** (Olsson, 1985), **The Netherlands** (Roozen, 1990; van Vuurde and de Vries, 1992), **Scotland** (Cahill et al., 2010), **England** (Toth et al., 2012), **USA** (Florida, Norman et al., 2003)
- **South Africa**, van der Waals et al. identified **pathogenic species** which included *Dickeya* spp., *Pectobacterium* spp.
- **Finland**: *D. dianthicola* showed **pathogenic** on potato plants and tubers (Laurila et al., 2006, 2008, 2010).

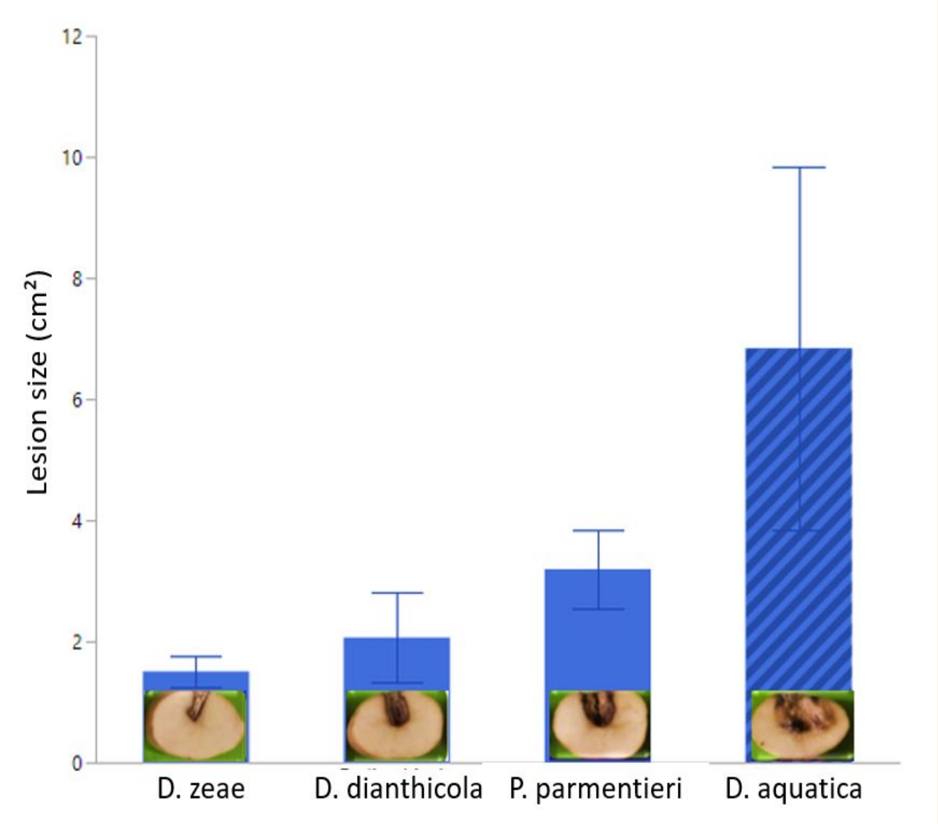


Dickeya isolated from water

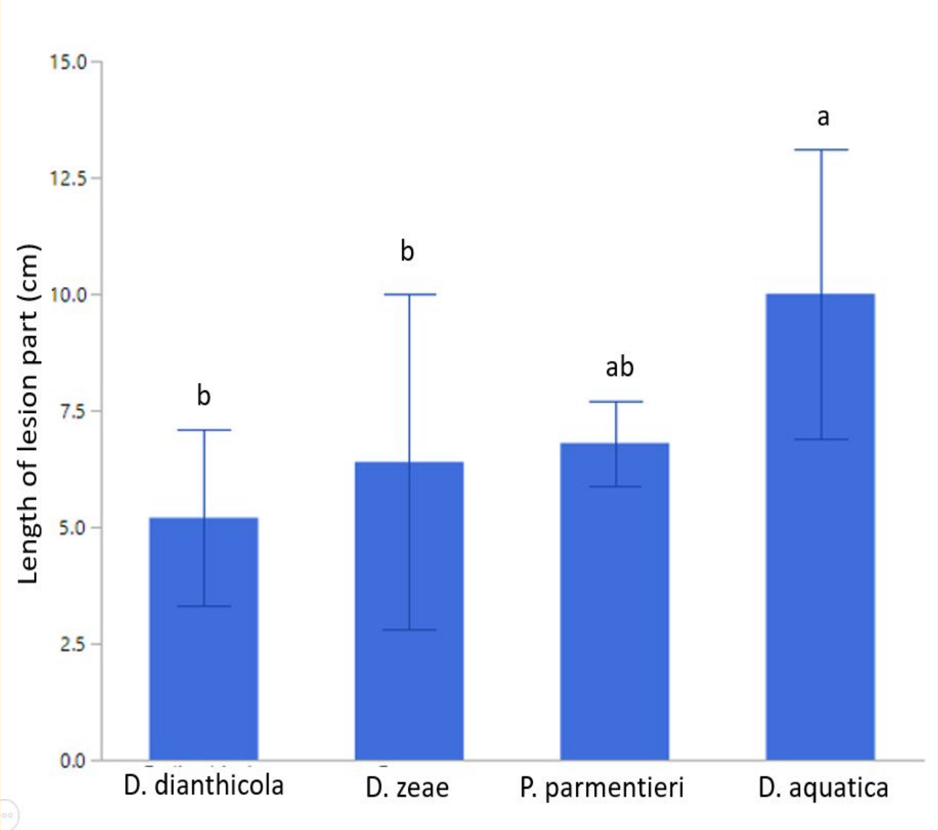
- Maine
  - 40.5% water samples were *Dickeya* spp. *(D. dadantii, D. zeae, D. dianthicola, D. aquatica)*
  - *Pectobacterium brasiliense*, *P. carotovorum*, *P. atrosepticum* and *P. parmentieri*
  - Water samples from one pond treated with chlorine were negative for both *Dickeya* spp., and *Pectobacterium* spp.,
- MA: one water sample showed *Dickeya* positive
- Canadian results supported this finding

Pathogenicity assay

- Poking Method



- Stem injection



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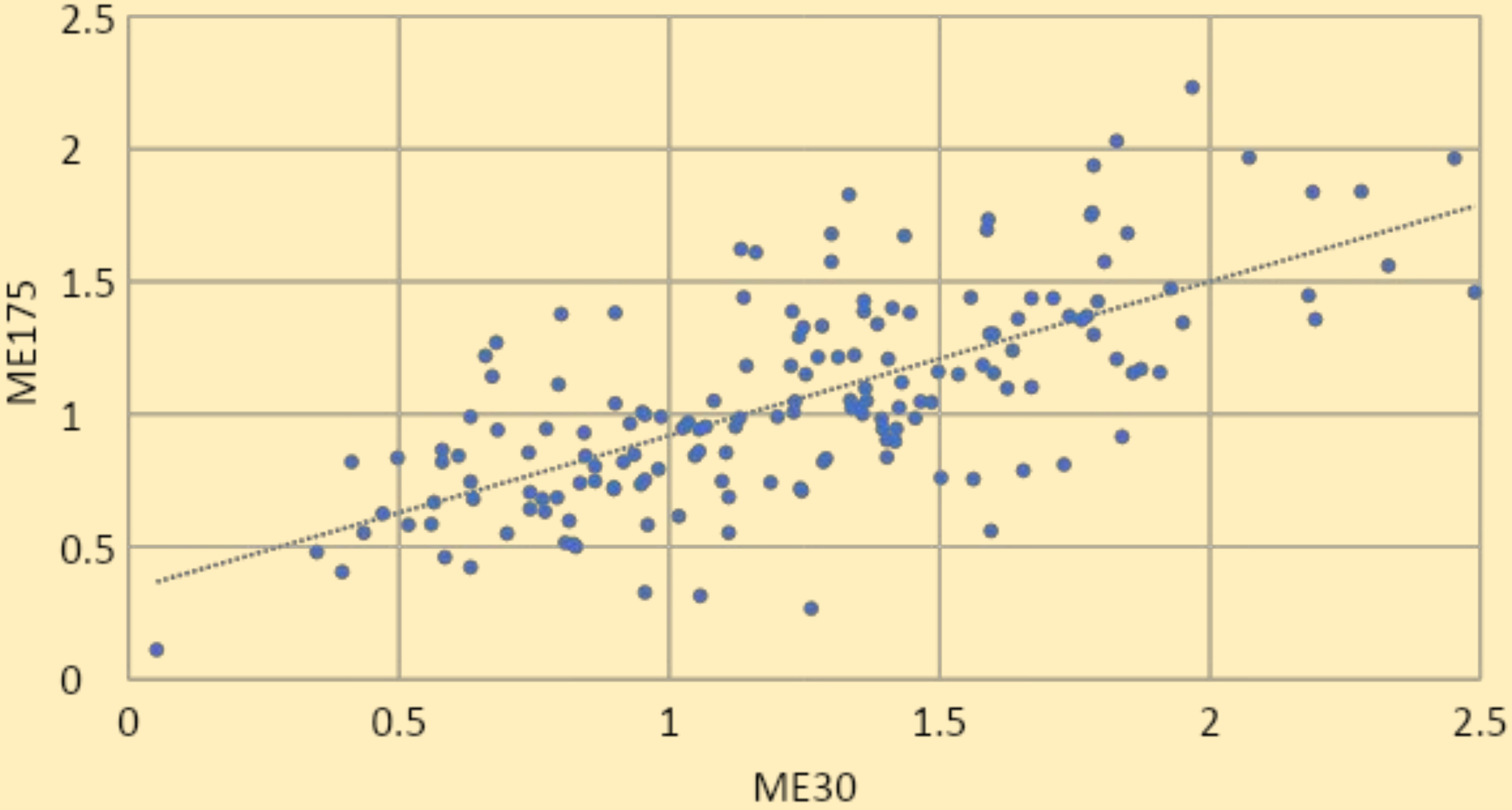
Conclusions

4. *Dickeya dianthicola* is more associated with stem infection and *Pectobacterium parmentieri* is for tuber infection
5. PBSR pathogens survive on potato tubers and may stay alive in dormant status
6. *D. dianthicola* is not likely transmitted by insects
7. Water can be an important source of inoculum of PBSR

Responses of 204 potato diploid clones to *Dickeya dianthicola* ME30 and *Pectobacterium parmentieri* ME175, 2017



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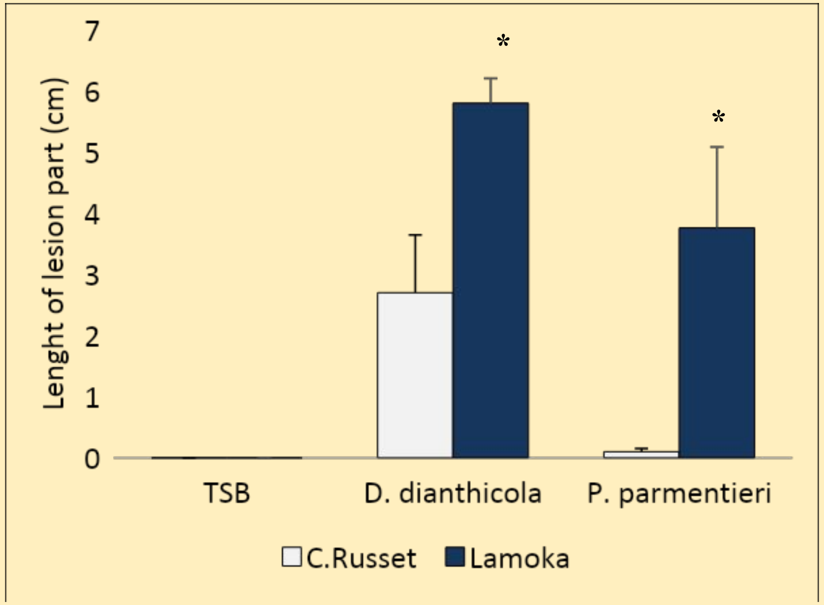
Potato varieties have various responses to bacterial infection

a) *D. dianthicola* b) *P. parmentieri*

'Lamoka'

'Caribou Russet'



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Conclusions

8. Some potato varieties are tolerant to PBSR although not completely resistant.

9. Breeding for resistance is a possibility for managing PBSR

Summary

- Multiple species of *Dickeya* and *Pectobacterium* cause PBSR, and the prevalent species tend to be switched in the past years
- The predominant Type I strain was indigenous, homogeneous, endemic to the US, and distinct from previously reported *D. dianthicola* strains, suggesting a recent incursion of this strain into potato production
- Management of PBSR is needed

PBSR management based on disease epidemiology

Nature of the pathogens	Management strategies
Seed-borne: soil dwellers, groundwater	Seed testing and certification; Mini tubers free of infection Seed treatment
Accumulation of infection via seed generations	Crop rotation
Broad host range, alternative hosts	Remove weeds and volunteer potatoes
Plant responds to PBSR at different levels	Selecting tolerant varieties
Survive better in water but soil	Clean irrigation water
Spread via: • free water in the soil • machinery • aerosols: up to 3,000 feet	• Drainage, harvest under drying conditions • Sanitation, disinfection of equipment, rouging, cultural practices
Infection via wounds (cutting) and natural openings, lenticels	Avoid bruising and damaging of tubers
Temperature regulates bacterial activities	• Temperatures < 40 F • Avoid harvest at T > 65 F and rapid dry tubers
Rot in storage	Adjust ventilation, temperature & moisture in storage

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Thank you