Bern University
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Monitoring of imported and national seed lots in the control of pectinolytic bacteria in the Swiss potato branch

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swisssem

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BIOREBA

Schweizerische Eidgenossenschaft
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Station de recherche
Agroscope Changins-Wädenswil ACW

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Swiss Confederation

Federal Department of Economic Affairs FDEA
Federal Office for Professional Education and Technology OPET
Innovation Promotion Agency CTI

▸ School of Agricultural, Forest and Food Sciences HAFL

Introduction

Switzerland and potatoes:

- potatoes production: ~ **12'000 ha**
- Seed potatoes: ~ **1'500 ha**
- Each year **~50 ha rejected**

  mainly caused by pectinolytic bacteria

  Dsp *Dickeya* sp.

  Pa *Pectobacterium atrosepticum*

  Pc *Pectobacterium sp.*

- The pectinolytic bacteria situation in Switzerland

  (data based on plants samples from Agroscope ACW)

2012 42% fungi and 54% bacteria (n=150 plants)

(62%Dsp, 35% Pa, 2% Pc)

2013 60% fungi (?) and 40% bacteria (n=112 plants)

(53% Dsp, 2% Pa, 44% Pc)

The Problem!

the use of certified seed is no guarantee that the seed is free of these bacterial pathogens.

The Solution?

assess the post-harvest health status of seed tuber lots for *Dickeya* sp. or *Pectobacterium* sp.

Monitoring of imported and national seed lots (first implementation into praxis, financed by the seed potato companies)

25 imported seed lots (from France, Germany, Netherlands)

28 national seed lots (multiplication in Switzerland)

Agata, Agria, Alexandra, Amandine, Annabelle, Bintje, Challenger, Charlotte, Celtiane, Désirée, Ditta, Fontane, Gourmandine, Innovator, Jelly, Lady Christl, Lady Claire, Lady Felicia, Laura, Markies, Nicola, Panda, and Victoria

The Method

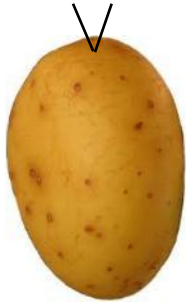
Analysis of the seed lots

- Analysis of 6 subsamples of 50 tubers (totally 300 tubers per seed lot)
- PCR analysis with enrichment (DPEM)
- Amplification with specific primers for

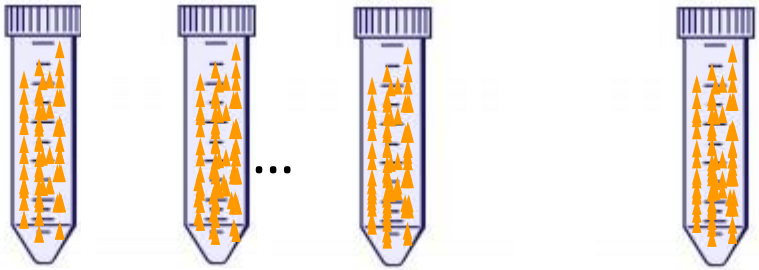
Dsp	*Dickeya* sp.	ADE1 / ADE2 (Nassar *et al*., 1996)
Pa	*Pectobacterium atrosepticum*	Y45 / Y46 (Frechon *et al*., 1998)
Pc	*Pectobacterium sp.*	Y1 / Y2 (Darrasse *et al*., 1994)
vPcc	virulent Pectobacterium	vPCC-F/vPCC-R (de Haan *et al*., 2008)

Material and methods I

Seed lot A:
300 tubers



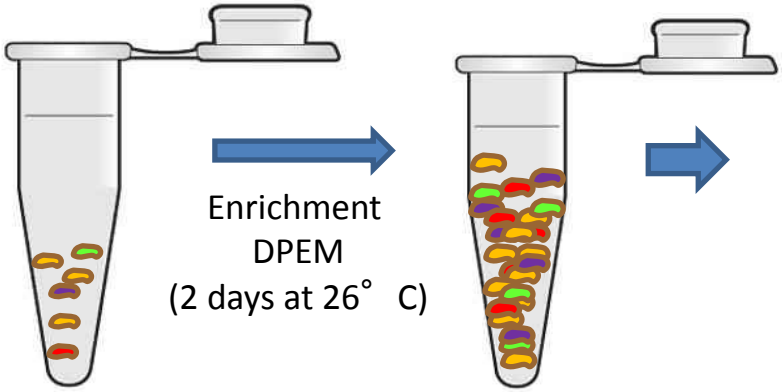
phosphate buffer, overnight at 100 rpm, 4 ° C



1 2 3 ... 6

6 x 50 tubers

centrifugation – pellet suspension



Enrichment
DPEM
(2 days at 26° C)

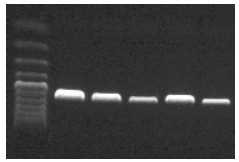
6 tubes (1 to 6)

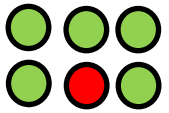
multiplication

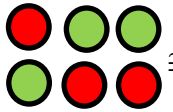
DNA extraction

PCR or Q-PCR

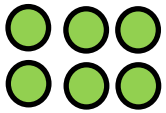
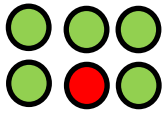
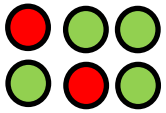
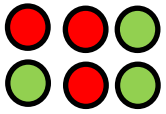
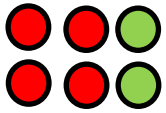
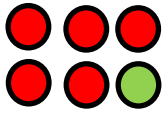
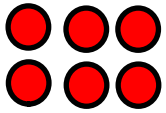
gel



Dsp  ≅ **0.4 %**

Pc  ≅ **1.4 %**

Estimation of incidence «*I*»

1)		p= 0	$I \cong 0\%$	-
2)		p= 1	$I \cong 0.4\%$	+
3)		p= 2	$I \cong 0.8\%$	+
4)		p= 3	$I \cong 1.4\%$	++
5)		p= 4	$I \cong 2.2\%$	++
6)		p= 5	$I \cong 3.5\%$	+++
7)		p= 6	$3.5\% < I < 100\%$	+++

$$I = \left(1 - \left(\frac{N - p}{N}\right)^{\frac{1}{n}}\right) * 100$$

p = number of positive composite samples
N = total number of composite samples
n = number of potato tubers combined together

(De Boer S.H., 2002, Plant Disease / Vol. 86 N° 9)
Geng et al., 1983; Maury et al., 1985; ISTA 2013)

In our study (300 tubers):
N= 6
n= 50

The health status assessment

assess the health status of seed tuber lots for *Dickeya* sp. or *Pectobacterium* sp.

25 imported seed lots (from France, Germany, Netherlands)

28 national seed lots (multiplication in Switzerland)

Agata, Agria, Alexandra, Amandine, Annabelle, Bintje, Challenger, Charlotte, Celtiane, Désirée, Ditta, Fontane, Gourmandine, Innovator, Jelly, Lady Christl, Lady Claire, Lady Felicia, Laura, Markies, Nicola, Panda, and Victoria

sample	variety	seeds lot n°	**Dsp** Q-PCR	**Pa** Q-PCR	**Pc** PCR	**vPcc** PCR	decision
n_1	X	123456-789	0%	0%	0%	0%	

sample	variety	seeds lot n°	**Dsp** Q-PCR	**Pa** Q-PCR	**Pc** PCR	**vPcc** PCR	decision
n_2	Y	123456-789	**1.4%**	0%	0.4%	0%	

sample	variety	seeds lot n°	**Dsp** Q-PCR	**Pa** Q-PCR	**Pc** PCR	**vPcc** PCR	decision
n_3	Z	123456-789	**0.4%**	0%	**3.5%**	0%	

Dsp *Dickeya* sp.
Pa *Pectobacterium atrosepticum*
Pc *Pectobacterium sp.*
vPcc virulent Pectobacterium

official field controller inform us if black leg symptoms are observed and we go on the field to collect plants samples.

Results: health assessment

assess the health status of seed tuber lots for *Dickeya* or *Pectobacterium*

25 Import seed tuber lots (from France, Germany, Netherlands)

28 multiplication seed tubers lots (multiplication in Switzerland)

Every seed lot is planted on an average of 6 field (1 to 2 ha)

samples	Class	Dsp Q-PCR	Pa Q-PCR	Pc PCR	vPcc PCR	decision
Imp_1	S	0%	0%	0%	0%	
Imp_2	SE	0%	**0.4%**	**0.4%**	0%	
Imp_3	S	0%	0%	**0.4%**	0%	
Imp_4	SE	0%	0%	**>3.5%**	0%	
Imp_5	S	0%	0%	**0.4%**	0%	
Imp_6	S	0%	0%	**>3.5%**	0%	
Imp_7	S	0%	0%	0%	0%	
Imp_8	S	0%	0%	0%	0%	
Imp_9	S	0%	0%	0%	0%	
Imp_{10}	S	0%	0%	**0.8%**	0%	
Imp_{11}	S	0%	0%	**0.4%**	0%	
Imp_{12}	SE	0%	0%	0%	0%	
Imp_{13}	SE	0%	0%	0%	0%	
Imp_{14}	SE	0%	0%	0%	0%	
Imp_{15}	SE	0%	0%	**0.8%**	0%	
Imp_{16}	SE	0%	0%	**0.4%**	0%	
Imp_{17}	SE	0%	0%	0%	0%	
Imp_{18}	SE	0%	0%	**1.4%**	0%	
Imp_{19}	SE	0%	0%	**1.4%**	0%	
Imp_{20}	SE	0%	**1.4%**	**3.5%**	0%	
Imp_{21}	SE	0%	0%	**>3.5%**	**0.4%**	
Imp_{22}	S	**0.4%**	**1.4%**	**3.5%**	**0.4%**	
Imp_{23}	S	0%	0%	0%	0%	
Imp_{24}	SE	0%	0%	**1.4%**	**0.4%**	
Imp_{25}	S	0%	**0.4%**	**>3.5%**	**0.4%**	

Results: health assessment

assess the health status of seed tuber lots for *Dickeya* or *Pectobacterium*

25 Import seed tuber lots (from France, Germany, Netherlands)

<u>28 multiplication seed tubers lots (multiplication in Switzerland)</u>

Every seed lot is planted on an average of 6 field (1 to 2 ha)

samples	Class	Dsp Q-PCR	Pa Q-PCR	Pc PCR	vPcc PCR	decision
$Mult_{1}$	SE2	0%	0%	**1.4%**	0%	
$Mult_{2}$	SE2	0%	0%	**1.4%**	**0.4%**	
$Mult_{3}$	SE2	0%	0%	**3.5%**	**0.4%**	
$Mult_{4}$	SE2	0%	0%	**2.2%**	0%	
$Mult_{5}$	S	0%	0%	**0.8%**	0%	
$Mult_{6}$	SE1	0%	0%	**0.4%**	0%	
$Mult_{7}$	S	0%	**0.4%**	**0.4%**	0%	
$Mult_{8}$	SE2	0%	0%	**0.4%**	0%	
$Mult_{9}$	SE2	**0.4%**	0%	**0.4%**	0%	
$Mult_{10}$	SE1	0%	0%	**0.4%**	0%	
$Mult_{11}$	SE3	0%	0%	**0.8%**	**0.4%**	
$Mult_{12}$	SE2	0%	0%	**0.8%**	0%	
$Mult_{13}$	F 4	**0.4%**	0%	**0.4%**	0%	
$Mult_{14}$	S	0%	0%	**0.8%**	**0.4%**	
$Mult_{15}$	S	0%	0%	**2.2%**	0%	
$Mult_{16}$	F 3	0%	0%	**0.4%**	**0.8%**	
$Mult_{17}$	F 4	0%	0%	**0.4%**	0%	
$Mult_{18}$	S	0%	0%	**0.4%**	0%	
$Mult_{19}$	S	0%	0%	**0.8%**	0%	
$Mult_{20}$	F 3	0%	0%	0%	**0.4%**	
$Mult_{21}$	F 4	0%	0%	**>3.5%**	**0.8%**	
$Mult_{22}$	F 4	0%	0%	**3.5%**	**0.4%**	
$Mult_{23}$	S	0%	0%	**0.8%**	0%	
$Mult_{24}$	F 3	0%	0%	**0.8%**	**0.4%**	
$Mult_{25}$	SE2	0%	0%	**2.2%**	**1.4%**	
$Mult_{26}$	SE1	0%	0%	**2.2%**	0%	
$Mult_{27}$	SE1	0%	0%	**0.4%**	0%	
$Mult_{28}$	SE2	0%	0%	**2.2%**	0%	

Results: field observations

25 imported seed lots

% Blackleg

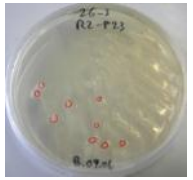
samples	Class	Dsp Q-PCR	Pa Q-PCR	Pc PCR	vPcc PCR	decision	field report
Imp_1	S	0%	0%	0%	0%		< 0.1%
Imp_2	SE	0%	**0.4%**	**0.4%**	0%		< 0.1%
Imp_3	S	0%	0%	**0.4%**	0%		< 0.1%
Imp_4	SE	0%	0%	**>3.5%**	0%		< 0.1%
Imp_5	S	0%	0%	**0.4%**	0%		< 0.1%
Imp_6	S	0%	0%	**>3.5%**	0%		< 0.1%
Imp_7	S	0%	0%	0%	0%		< 0.1%
Imp_8	S	0%	0%	0%	0%		< 0.1%
Imp_9	S	0%	0%	0%	0%		< 0.1%
Imp_{10}	S	0%	0%	**0.8%**	0%		< 0.1%
Imp_{11}	S	0%	0%	**0.4%**	0%		< 0.1%
Imp_{12}	SE	0%	0%	0%	0%		< 0.1%
Imp_{13}	SE	0%	0%	0%	0%		< 0.1%
Imp_{14}	SE	0%	0%	0%	0%		< 0.1%
Imp_{15}	SE	0%	0%	**0.8%**	0%		< 0.1%
Imp_{16}	SE	0%	0%	**0.4%**	0%		< 0.1%
Imp_{17}	SE	0%	0%	0%	0%		< 0.1%
Imp_{18}	SE	0%	0%	**1.4%**	0%		< 0.1%
Imp_{19}	SE	0%	0%	**1.4%**	0%		< 0.1%
Imp_{20}	SE	0%	**1.4%**	**3.5%**	0%		< 0.1%
Imp_{21}	SE	0%	0%	**>3.5%**	**0.4%**		< 0.1%
Imp_{22}	S	**0.4%**	**1.4%**	**3.5%**	**0.4%**		0.1-0.2%
Imp_{23}	S	0%	0%	0%	0%		< 0.1%
Imp_{24}	SE	0%	0%	**1.4%**	**0.4%**		< 0.1%
Imp_{25}	S	0%	**0.4%**	**>3.5%**	**0.4%**		< 0.1%

28 multiplication seed lots

% Blackleg

samples	Class	Dsp Q-PCR	Pa Q-PCR	Pc PCR	vPcc PCR	decision	field report
$Mult_1$	SE2	0%	0%	**1.4%**	0%		0.1%
$Mult_2$	SE2	0%	0%	**1.4%**	**0.4%**		< 0.1%
$Mult_3$	SE2	0%	0%	**3.5%**	**0.4%**		< 0.1%
$Mult_4$	SE2	0%	0%	**2.2%**	0%		< 0.1%
$Mult_5$	S	0%	0%	**0.8%**	0%		< 0.1%
$Mult_6$	SE1	0%	0%	**0.4%**	0%		< 0.1%
$Mult_7$	S	0%	**0.4%**	**0.4%**	0%		0.2%
$Mult_8$	SE2	0%	0%	**0.4%**	0%		< 0.1%
$Mult_9$	SE2	**0.4%**	0%	**0.4%**	0%		< 0.1%
$Mult_{10}$	SE1	0%	0%	**0.4%**	0%		< 0.1%
$Mult_{11}$	SE3	0%	0%	**0.8%**	**0.4%**		0.5%
$Mult_{12}$	SE2	0%	0%	**0.8%**	0%		1%
$Mult_{13}$	F 4	**0.4%**	0%	**0.4%**	0%		0.1%
$Mult_{14}$	S	0%	0%	**0.8%**	**0.4%**		< 0.1%
$Mult_{15}$	S	0%	0%	**2.2%**	0%		0.1-1.5%
$Mult_{16}$	F 3	0%	0%	**0.4%**	**0.8%**		< 0.1%
$Mult_{17}$	F 4	0%	0%	**0.4%**	0%		< 0.1%
$Mult_{18}$	S	0%	0%	**0.4%**	0%		< 0.1%
$Mult_{19}$	S	0%	0%	**0.8%**	0%		< 0.1%
$Mult_{20}$	F 3	0%	0%	0%	**0.4%**		< 0.1%
$Mult_{21}$	F 4	0%	0%	**>3.5%**	**0.8%**		0.1%
$Mult_{22}$	F 4	0%	0%	**3.5%**	**0.4%**		0.1%
$Mult_{23}$	S	0%	0%	**0.8%**	0%		0.1%
$Mult_{24}$	F 3	0%	0%	**0.8%**	**0.4%**		< 0.1%
$Mult_{25}$	SE2	0%	0%	**2.2%**	**1.4%**		< 0.1%
$Mult_{26}$	SE1	0%	0%	**2.2%**	0%		< 0.1%
$Mult_{27}$	SE1	0%	0%	**0.4%**	0%		< 0.1%
$Mult_{28}$	SE2	0%	0%	**2.2%**	0%		< 0.1%

Results: diseased plants diagnostic



Bacterial isolation

DNA extract

samples	Dsp Q-PCR	Pa Q-PCR	Pc PCR	vPcc PCR	decision	field report
Imp_{22}	**0.4%**	**1.4%**	**3.5%**	**0.4%**		0.1-0.2%
$Mult_{11}$	0%	0%	**0.8%**	**0.4%**		0.5%
$Mult_{12}$	0%	0%	**0.8%**	0%		1%
$Mult_{13}$	**0.4%**	0%	**0.4%**	0%		0.1%
$Mult_{15}$	0%	0%	**2.2%**	0%		0.1-1.5%
$Mult_{23}$	0%	0%	**0.8%**	0%		0.1%

	Field information			diseased plant diagnostic		
samples	number of field	+ black leg	plantes sampeled	Dsp Q-PCR	Pa Q-PCR	Pc PCR
Imp_{22}	8	2	6	0	0	**3**
$Mult_{11}$	3	3	12	0	0	**11**
$Mult_{12}$	1	1	4	0	0	**4**
$Mult_{13}$	3	1	1	0	0	**1**
$Mult_{15}$	6	6	10	0	0	**10**
$Mult_{23}$	4	1	5	**1**	0	**4**

- # Monitoring 2013

Pectobacterium sp. cause black-leg symptoms in field

Pectobacterium…

P. atrosepticum (Pa)

P. wasabiae (Pwas)

P. carotovorum (Pc)

P. carotovorum subsp. *carotovorum* (Pcc)

P. carotovorum subsp. *brasiliensis* (Pbra)

Results: diseased plants diagnostic

	Field information			diseased plant diagnostic					
samples	number of field	+ black leg	plantes sampeled	**Dsp** Q-PCR	**Pa** Q-PCR	**Pc** PCR	**Pcc** PCR	**Pbra** PCR	**Pwas** PCR
Imp_{22}	8	2	6	0	0	**3**	**6**	0	**5**
$Mult_{11}$	3	3	12	0	0	**11**	0	**7**	0
$Mult_{12}$	1	1	4	0	0	**4**	0	**2**	0
$Mult_{13}$	3	1	1	0	0	**1**	**1**	0	0
$Mult_{15}$	6	6	10	0	0	**10**	0	**6**	0
$Mult_{23}$	4	1	5	**1**	0	**4**	0	**2**	0

Dsp *Dickeya* sp.

Pa *Pectobacterium atrosepticum*

Pc *Pectobacterium sp.*

Pcc *Pectobacterium carotovorum* subsp. *carotovorum*

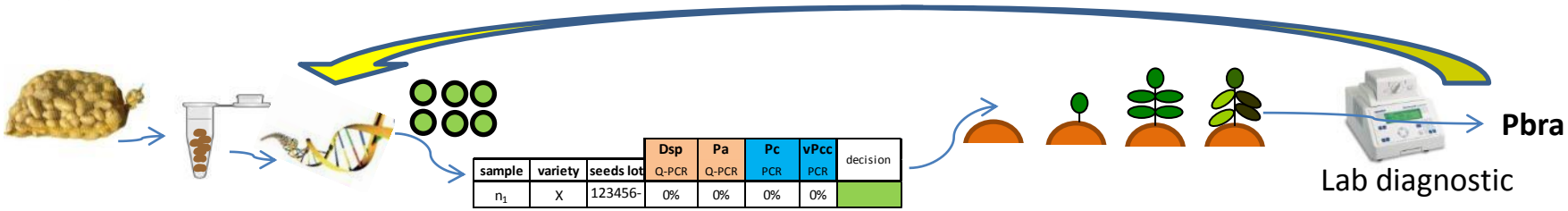
Pbra *Pectobacterium carotovorum* subsp. *brasiliensis*

Pwas *Pectobacterium wasabiae*

samples	**Dsp** Q-PCR	**Pa** Q-PCR	**Pc** PCR	**vPcc** PCR	decision	field report	mostly founded bacteria
Imp_{22}	**0.4%**	**1.4%**	**3.5%**	**0.4%**		0.1-0.2%	*P. wasabiae*
$Mult_{11}$	0%	0%	**0.8%**	**0.4%**		0.5%	*P.c.* subsp. *brasiliensis*
$Mult_{12}$	0%	0%	**0.8%**	0%		1%	*P.c.* subsp. *brasiliensis*
$Mult_{13}$	**0.4%**	0%	**0.4%**	0%		0.1%	*P.c.* subsp. *carotovorum*
$Mult_{15}$	0%	0%	**2.2%**	0%		0.1-1.5%	*P.c.* subsp. *brasiliensis*
$Mult_{23}$	0%	0%	**0.8%**	0%		0.1%	*P.c.* subsp. *brasiliensis*

Back to the Method

DNA extract from the original seed lots were used again to analyze *Pectobacterium* sp. which might cause balck leg.



- Analysis of 6 subsamples of 50 tubers (totally 300 tubers per seed lot)
- PCR analysis with enrichment (DPEM)
- Amplification with specific primers for

Dsp	*Dickeya* sp.	ADE1 / ADE2 (Nassar *et al*., 1996)
Pa	*Pectobacterium atrosepticum*	Y45 / Y46 (Frechon *et al*., 1998)
Pc	*Pectobacterium sp.*	Y1 / Y2 (Darrasse *et al*., 1994)
vPcc	virulent Pectobacterium	vPCC-F/vPCC-R (de Haan *et al*., 2008)

→	**Pbra**	*Pectobacterium carotovorum* subsp. *brasiliensis*	BR1f/L1r (Duarte *et al*., 2004)
	Pwas	*Pectobacterium wasabiae*	PhF/PhR (De Boer *et al*., 2012)

Analyses of the latent infection of seed tubers for Pbra and Pwas

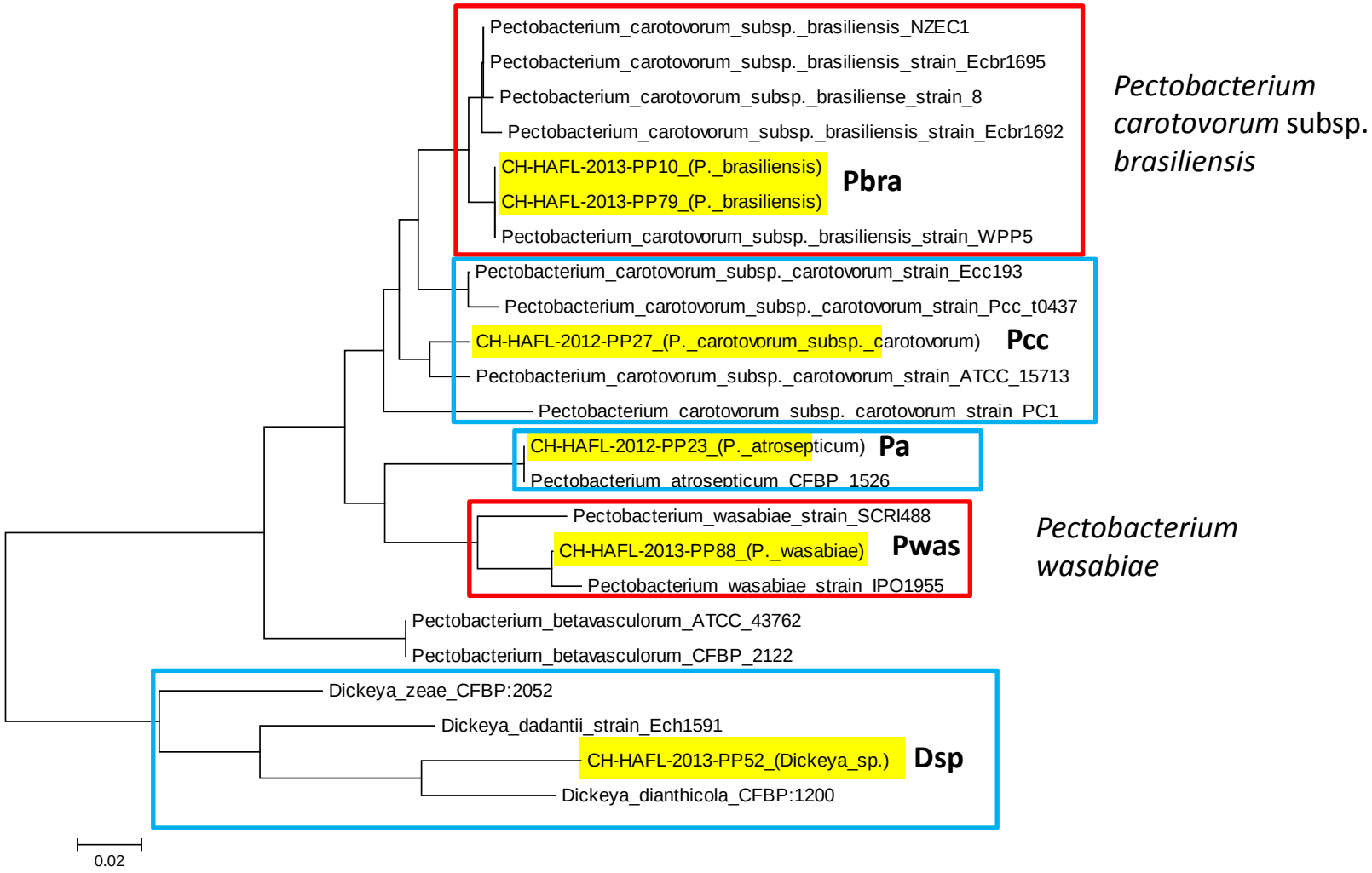
samples	Class	Dsp Q-PCR	Pa Q-PCR	Pc PCR	vPcc PCR	decision	field report	field diagnostic	*P. wasabiae*	*P. c.* subsp. *brasiliensis*
Imp_1	S	0%	0%	0%	0%		< 0.1%	-	0%	0%
Imp_2	SE	0%	**0.4%**	**0.4%**	0%		< 0.1%	-	0%	0%
Imp_3	S	0%	0%	**0.4%**	0%		< 0.1%	-	0%	0%
Imp_4	SE	0%	0%	**>3.5%**	0%		< 0.1%	-	0%	0%
Imp_5	S	0%	0%	**0.4%**	0%		< 0.1%	-	0%	0%
Imp_6	S	0%	0%	**>3.5%**	0%		< 0.1%	-	0%	0%
Imp_7	S	0%	0%	0%	0%		< 0.1%	-	0%	0%
Imp_8	S	0%	0%	0%	0%		< 0.1%	-	0%	0%
Imp_9	S	0%	0%	0%	0%		< 0.1%	-	0%	0%
Imp_{10}	S	0%	0%	**0.8%**	0%		< 0.1%	-	0%	0%
Imp_{11}	S	0%	0%	**0.4%**	0%		< 0.1%	-	0%	0%
Imp_{12}	SE	0%	0%	0%	0%		< 0.1%	-	0%	0%
Imp_{13}	SE	0%	0%	0%	0%		< 0.1%	-	0%	0%
Imp_{14}	SE	0%	0%	0%	0%		< 0.1%	-	0%	0%
Imp_{15}	SE	0%	0%	**0.8%**	0%		< 0.1%	-	0%	0%
Imp_{16}	SE	0%	0%	**0.4%**	0%		< 0.1%	-	0%	0%
Imp_{17}	SE	0%	0%	0%	0%		< 0.1%	-	0%	0%
Imp_{18}	SE	0%	0%	**1.4%**	0%		< 0.1%	-	0%	0%
Imp_{19}	SE	0%	0%	**1.4%**	0%		< 0.1%	-	0%	0%
Imp_{20}	SE	0%	**1.4%**	**3.5%**	0%		< 0.1%	-	0%	0%
Imp_{21}	SE	0%	0%	**>3.5%**	**0.4%**		< 0.1%	-	**1.4%**	0%
Imp_{22}	S	**0.4%**	**1.4%**	**3.5%**	**0.4%**		0.1-0.2%	*P. wasabiae*	0%	0%
Imp_{23}	S	0%	0%	0%	0%		< 0.1%	-	0%	0%
Imp_{24}	SE	0%	0%	**1.4%**	**0.4%**		< 0.1%	-	0%	0%
Imp_{25}	S	0%	**0.4%**	**>3.5%**	**0.4%**		< 0.1%	-	0%	0%

Analyses of the latent infection of seed tubers for Pbra and Pwas

samples	Class	Dsp Q-PCR	Pa Q-PCR	Pc PCR	vPcc PCR	decision	field report	field diagnostic	*P. wasabiae*	*P. c.* subsp. *brasiliensis*	
$Mult_{1}$	SE2	0%	0%	**1.4%**	0%		0.1%	-	0%	0%	
$Mult_{2}$	SE2	0%	0%	**1.4%**	**0.4%**		< 0.1%	-	0%	0%	
$Mult_{3}$	SE2	0%	0%	**3.5%**	**0.4%**		< 0.1%	-	0%	**0.4%**	
$Mult_{4}$	SE2	0%	0%	**2.2%**	0%		< 0.1%	-	0%	0%	
$Mult_{5}$	S	0%	0%	**0.8%**	0%		< 0.1%	-	0%	0%	
$Mult_{6}$	SE1	0%	0%	**0.4%**	0%		< 0.1%	-	0%	0%	
$Mult_{7}$	S	0%	**0.4%**	**0.4%**	0%		0.2%	-	0%	0%	
$Mult_{8}$	SE2	0%	0%	**0.4%**	0%		< 0.1%	-	0%	0%	
$Mult_{9}$	SE2	**0.4%**	0%	**0.4%**	0%		< 0.1%	-	0%	**0.4%**	
$Mult_{10}$	SE1	0%	0%	**0.4%**	0%		< 0.1%	-	0%	0%	
$Mult_{11}$	SE3	0%	0%	**0.8%**	**0.4%**		0.5%	*P.c.* sub. *brasiliensis*	0%	**0.8%**	☑
$Mult_{12}$	SE2	0%	0%	**0.8%**	0%		1%	*P.c.* sub. *brasiliensis*	0%	**0.4%**	☑
$Mult_{13}$	F 4	**0.4%**	0%	**0.4%**	0%		0.1%	*P.c.* sub. *carotovorum*	0%	0%	
$Mult_{14}$	S	0%	0%	**0.8%**	**0.4%**		< 0.1%	-	0%	**0.4%**	
$Mult_{15}$	S	0%	0%	**2.2%**	0%		0.1-1.5%	*P.c.* sub. *brasiliensis*	**0.4%**	**0.8%**	☑
$Mult_{16}$	F 3	0%	0%	**0.4%**	**0.8%**		< 0.1%	-	**1.4%**	**0.4%**	
$Mult_{17}$	F 4	0%	0%	**0.4%**	0%		< 0.1%	-	0%	0%	
$Mult_{18}$	S	0%	0%	**0.4%**	0%		< 0.1%	-	0%	0%	
$Mult_{19}$	S	0%	0%	**0.8%**	0%		< 0.1%	-	0%	0%	
$Mult_{20}$	F 3	0%	0%	0%	**0.4%**		< 0.1%	-	0%	0%	
$Mult_{21}$	F 4	0%	0%	**>3.5%**	**0.8%**		0.1%	-	**0.8%**	**0.4%**	
$Mult_{22}$	F 4	0%	0%	**3.5%**	**0.4%**		0.1%	-	0%	0%	
$Mult_{23}$	S	0%	0%	**0.8%**	0%		0.1%	*P.c.* sub. *brasiliensis*	0%	**0.4%**	☑
$Mult_{24}$	F 3	0%	0%	**0.8%**	**0.4%**		< 0.1%	-	0%	0%	
$Mult_{25}$	SE2	0%	0%	**2.2%**	**1.4%**		< 0.1%	-	**1.4%**	**0.4%**	
$Mult_{26}$	SE1	0%	0%	**2.2%**	0%		< 0.1%	-	0%	0%	
$Mult_{27}$	SE1	0%	0%	**0.4%**	0%		< 0.1%	-	0%	0%	
$Mult_{28}$	SE2	0%	0%	**2.2%**	0%		< 0.1%	-	**0.4%**	0%	

Results: sequencing of the malate dehydrogenase gene (*mdh*)

Maximum likelihood phylogeny analysis of *Pectobacterium* spp. and *Dickeya* spp. based on malate dehydrogenase (*mdh*) gene.



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Summary

- **Low latent infection** of imported and swiss seed lots 2013 (3 out of 53 positive for Dsp).
- **Low disease expression** in the field, although there were favorable climatic conditions for disease expression.
- **No Pa** and **almost no Dsp** could be identified in the fields with blackleg.
- **Unexpected** aggressive *Pectobacterium* sp.:

  Pbra (4 seed lots) and Pwas (1 seed lot)

  could be identified as causal agent of the black leg in field.

- **Pbra** was found as latent infection in the seed tubers of the 4 concerned seed lots. ☑
- **Pwas** could not be found as latent infection… ☒

- **First report** of Pbra and Pwas in Switzerland

- This monitoring will be repeated next year…