



Instituut voor Landbouw- en Visserijonderzoek

Gezond pootgoed @ ILVO 2011

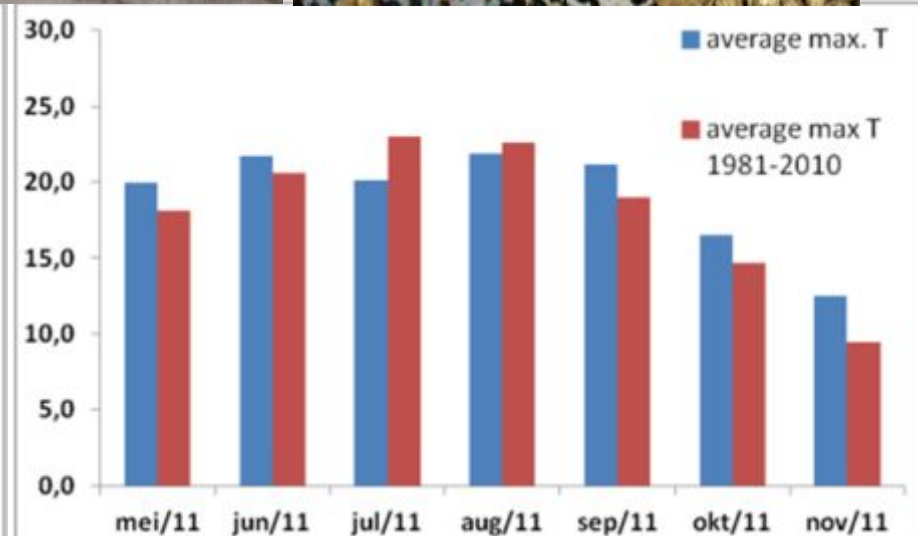
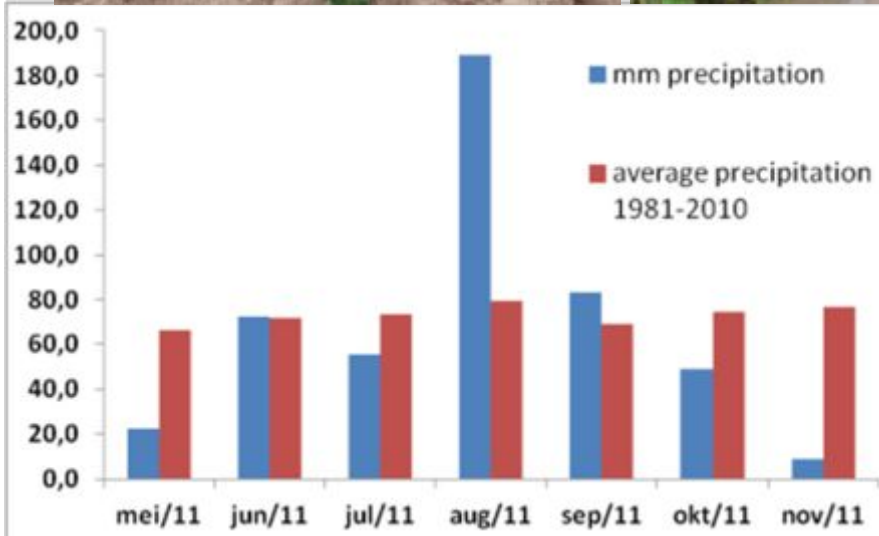
VVP – ALV
12 december 2011

Gezond pootgoed @ ILVO 2011



- Het aardappelseizoen 2011
- Top 5 ziekten in pootgoed
- Dickeya & Pectobacterium
- Projecten @ ILVO
- Survey pootgoed
- Identificatie & opsporing
- Virulentie in aardappelrassen
- Dickeya in waterlopen
- Contactbesmetting
- Akkerkwesties
- Bio Control

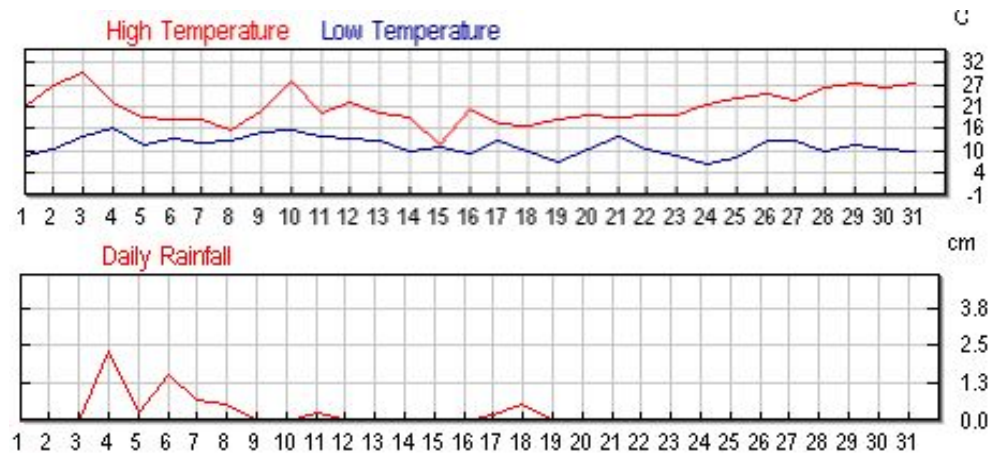
Het aardappelseizoen 2011



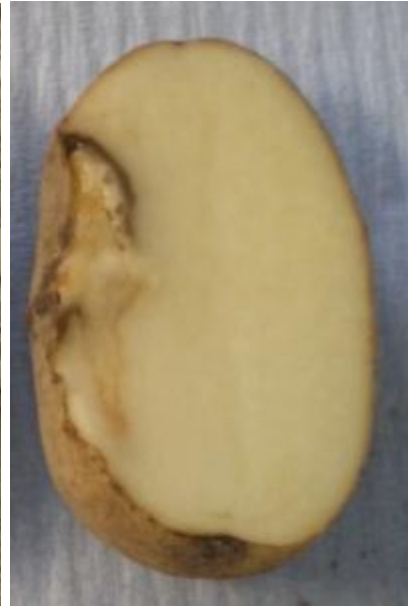
Het aardappelseizoen 2011



Als 1 rottende knol ~ 100 ml vocht lekt
1% rotte tubers in 1000 T = ~ 5 m³ vocht



Top 5 ziekten in pootgoed in 2011: n°1



Top 5 ziekten in pootgoed in 2011: n°2



Top 5 ziekten in pootgoed in 2011: n°3



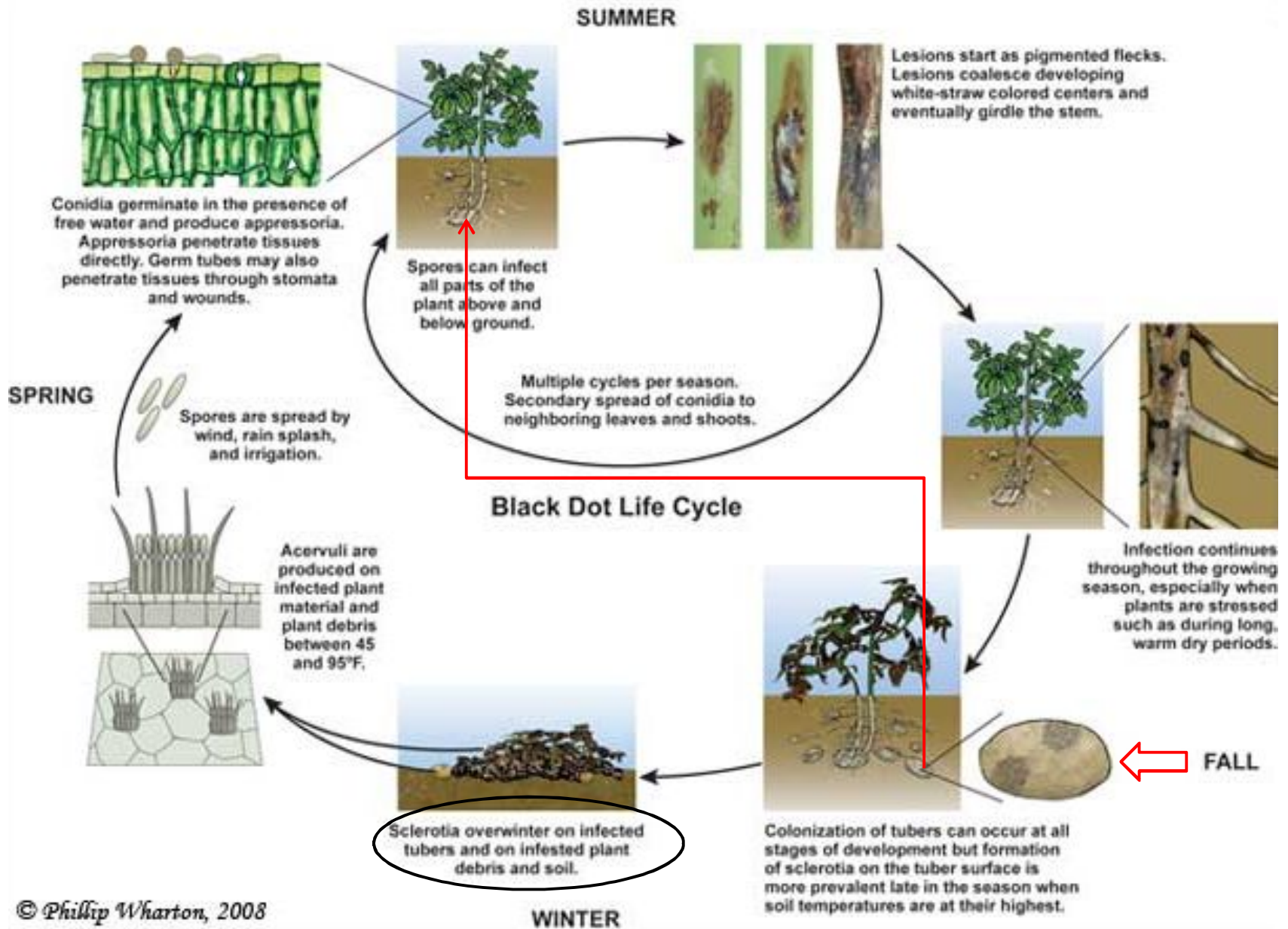
Top 5 ziekten in pootgoed in 2011: n°4



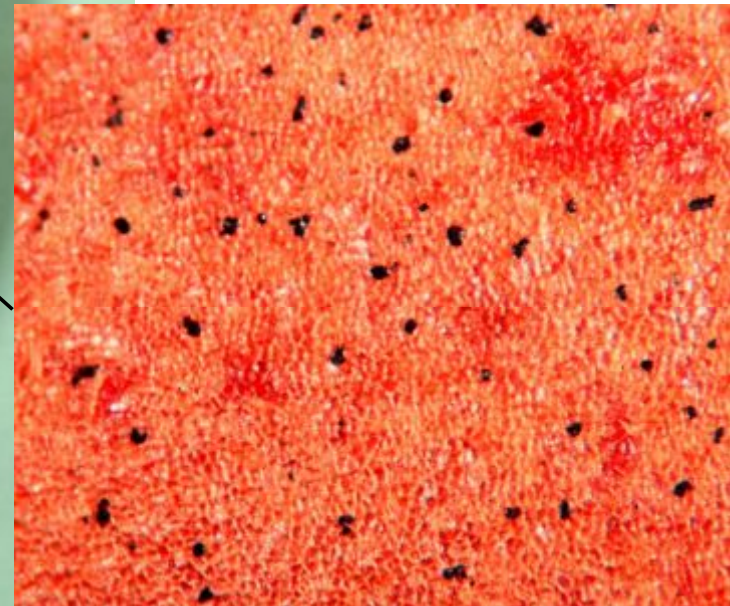
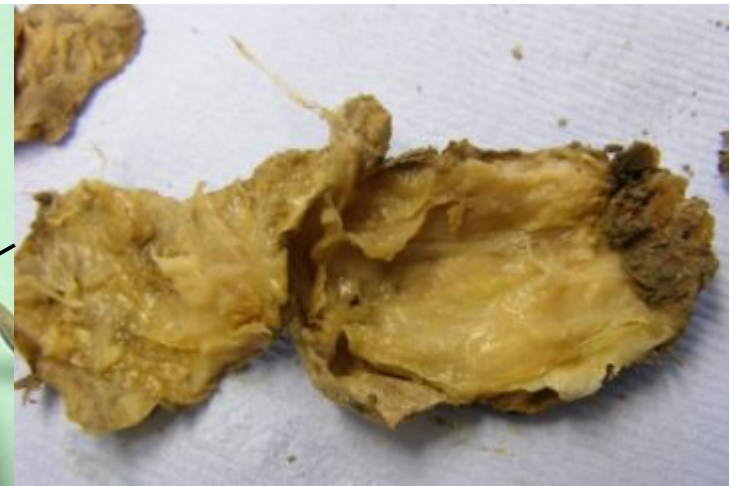
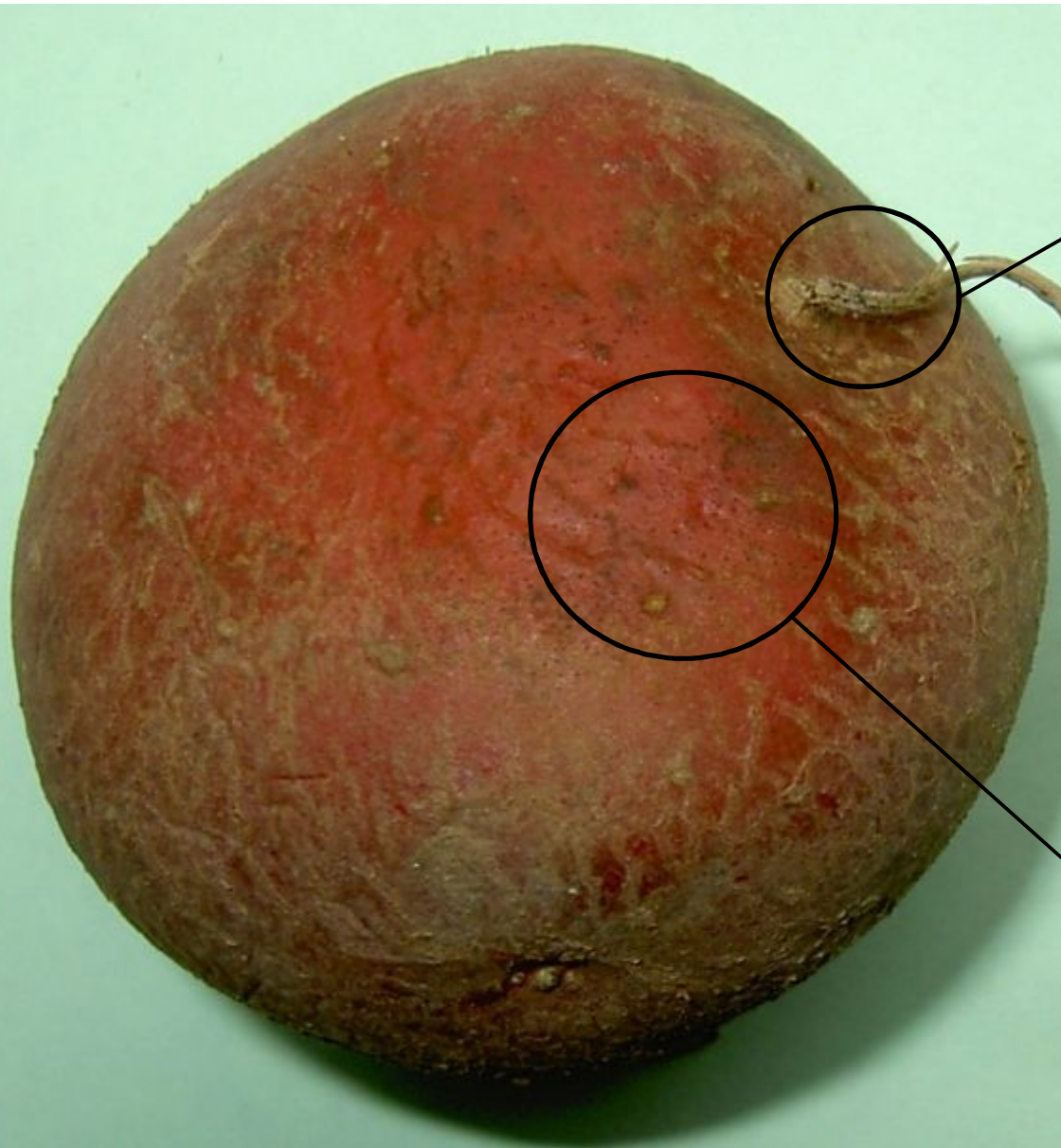
Top 5 ziekten in pootgoed in 2011: n°5



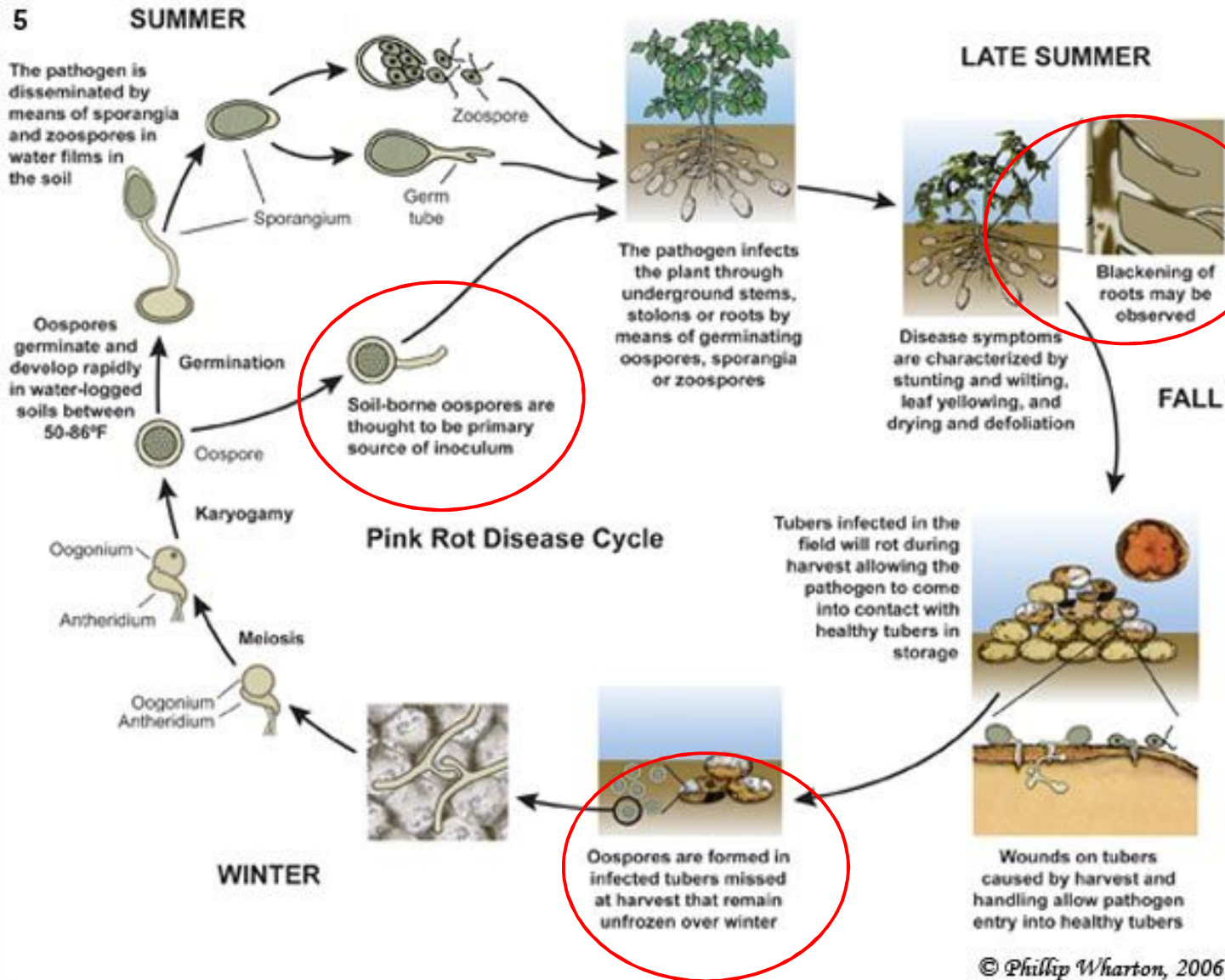
Colletotrichum coccodes (zwarte spikkel)



Colletotrichum coccodes (zwarte spikkel)



Phytophthora erythroseptica (roodrot)

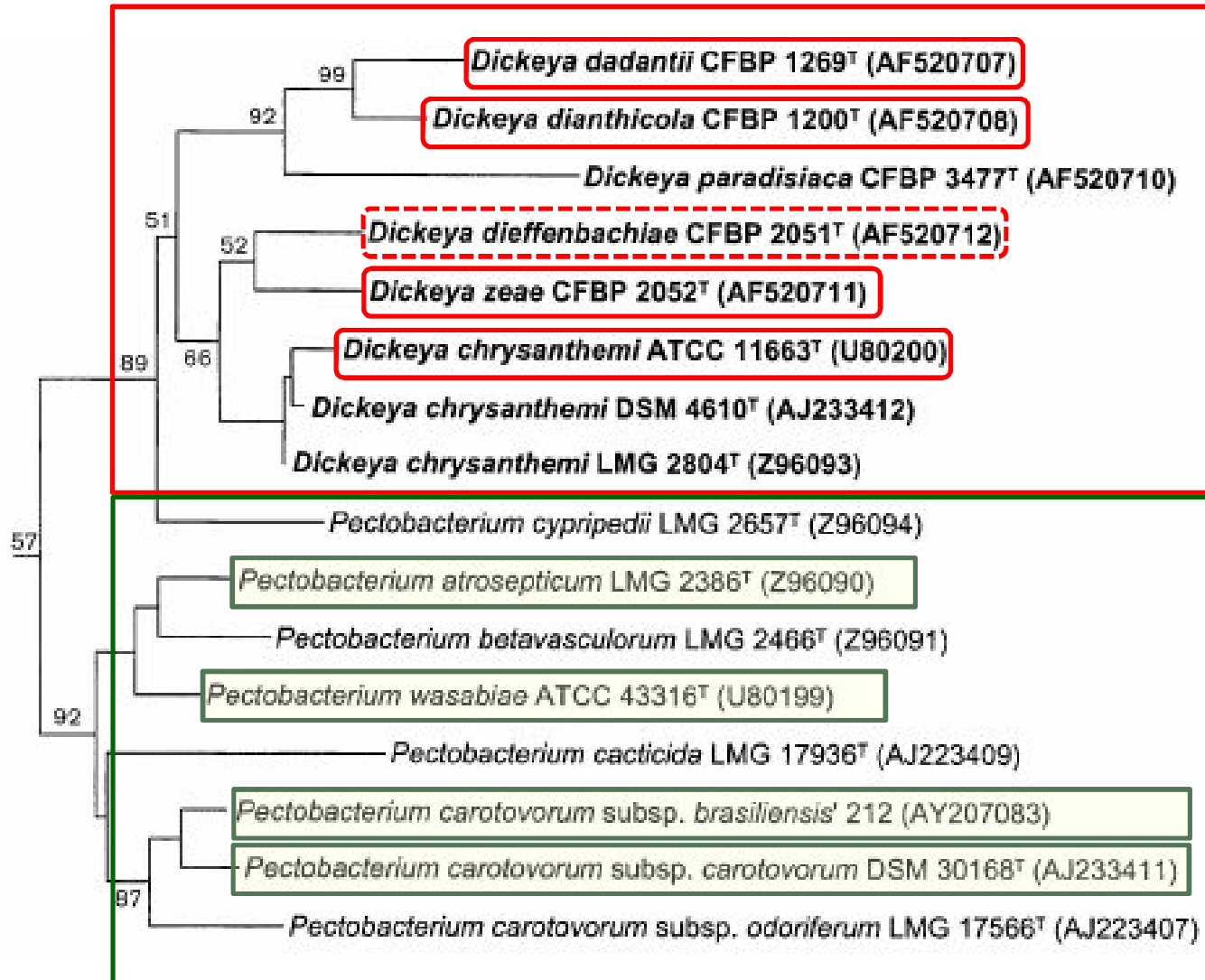


Dickeya & *Pectobacterium*

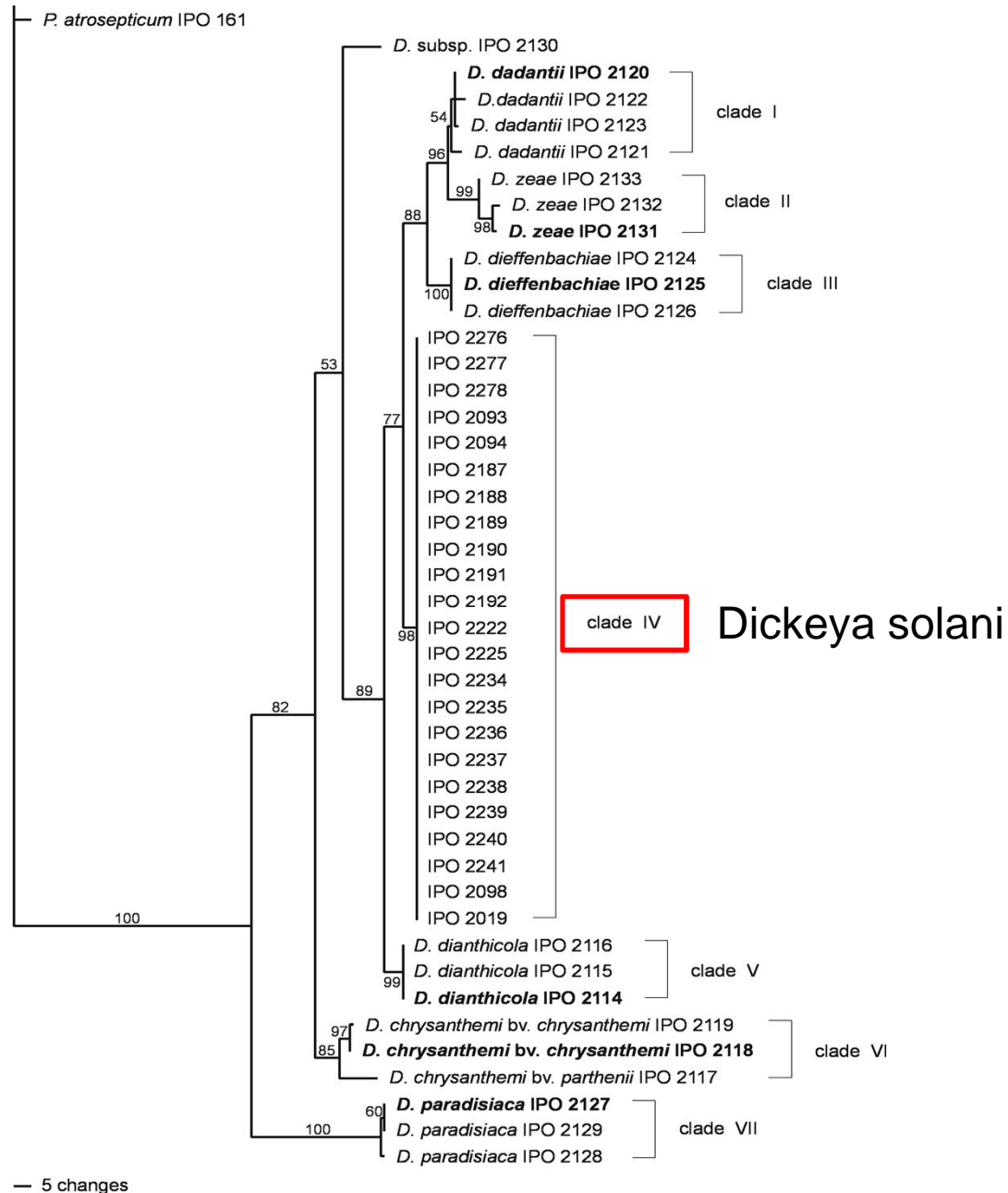


Robert S. Dickey
1921-1991

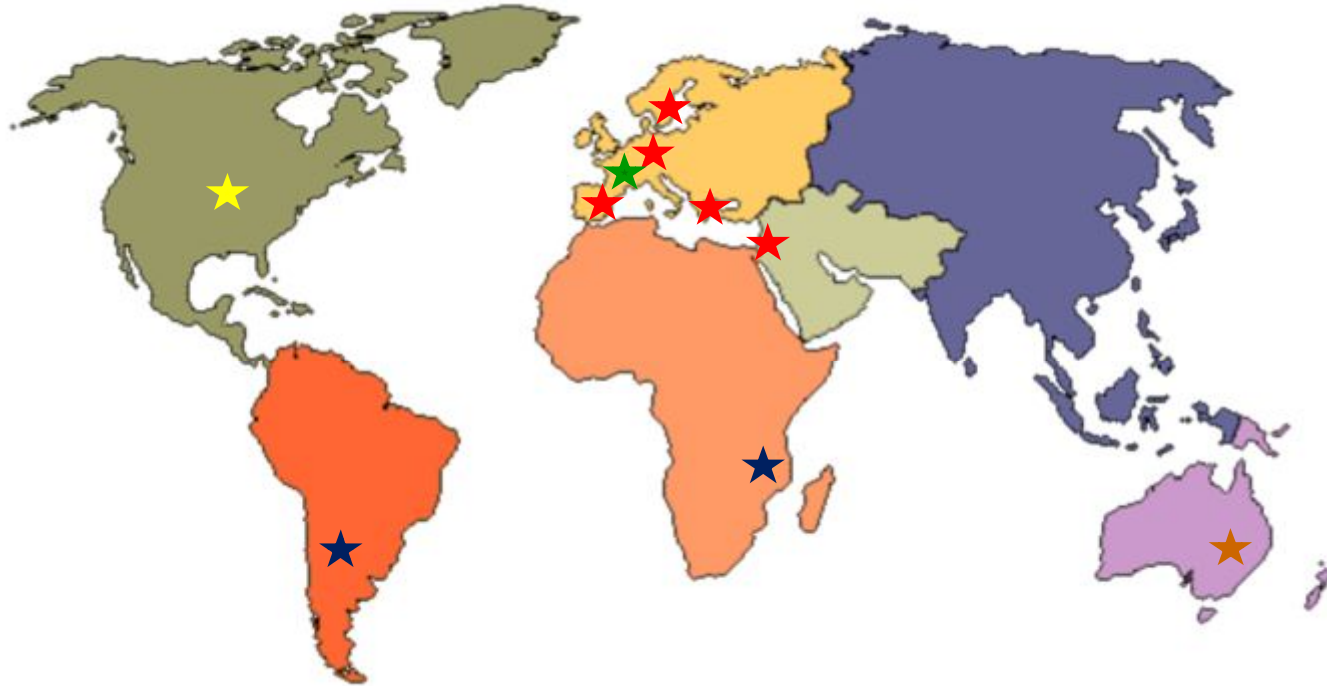
Dickeya & Pectobacterium (2005)



Nieuwe *Dickeya* variant in aardappel (2009)



De wereld van *Dickeya* in aardappel



Noord-Amerika

D. chrysanthemi ★

Zuid-America

D. dadantii ★

Afrika

D. dadantii ★

Australië

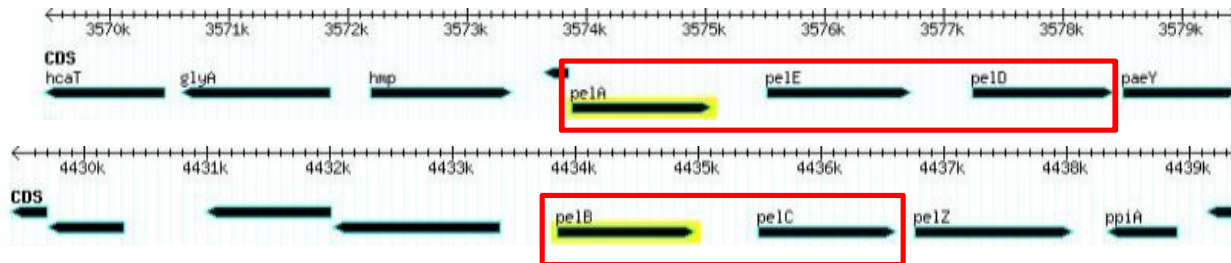
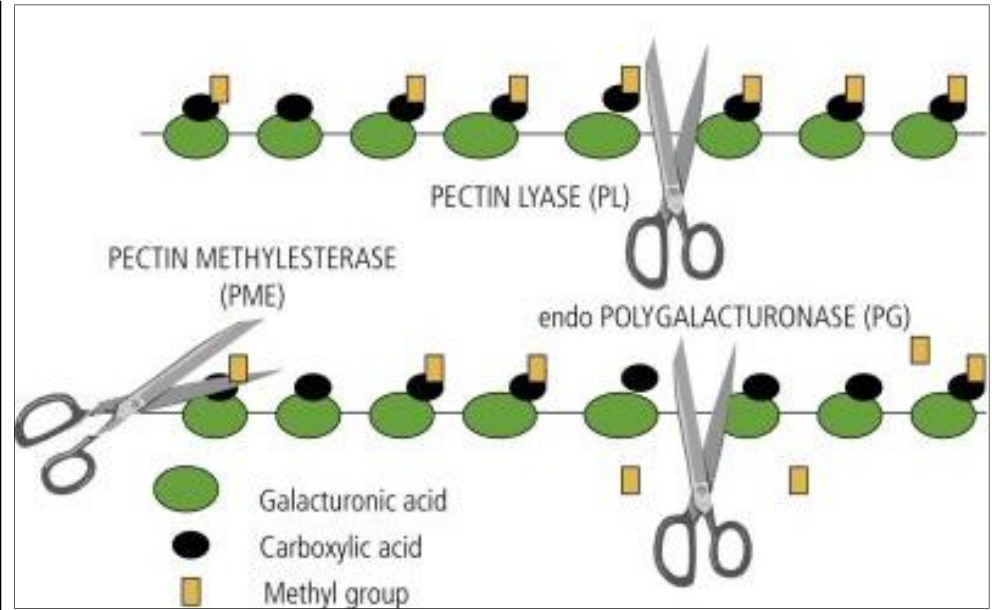
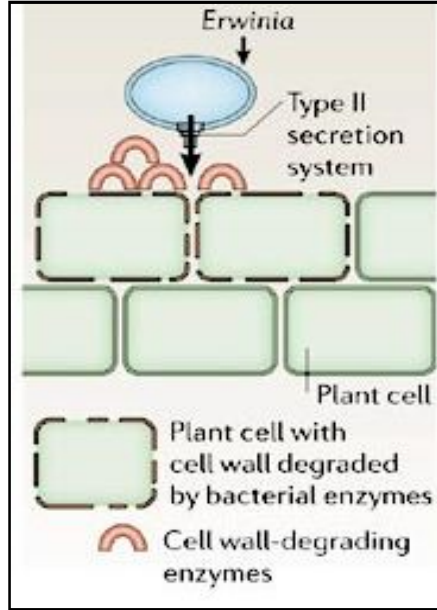
D. zeae ★

Europa, Israël

D. dianthicola ★ *D. solani* ★

De belangrijkste eigenschappen van *Dickeya*

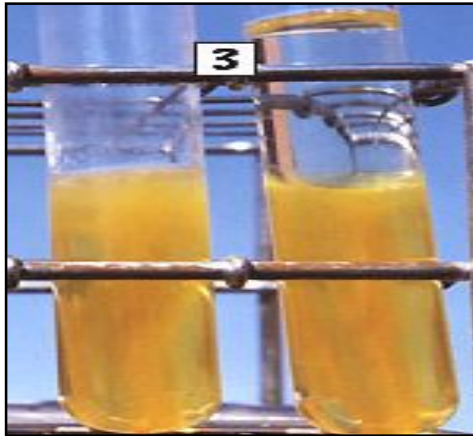
Pectinolytisch



5 pectinelyasen (A – E): pH4 – pH10

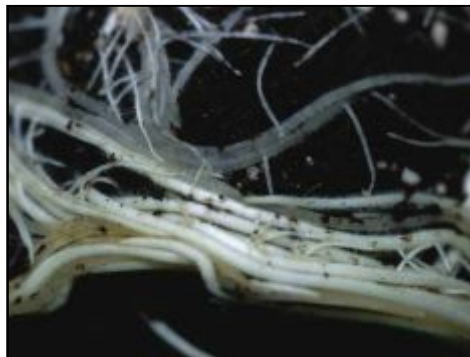
Pectobacterium: ook pectinolytisch maar andere pectinelyasen

De belangrijkste eigenschappen van *Dickeya*



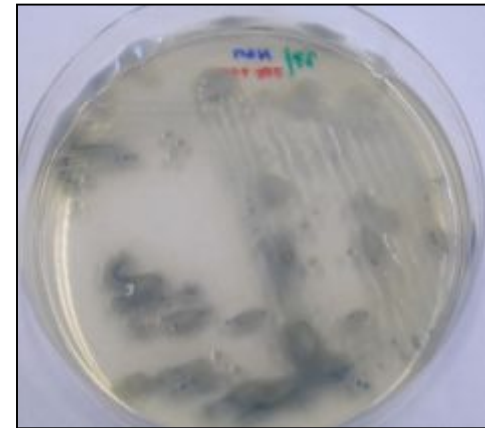
facultatief
anaeroob

Pectobacterium ook



peritriche
flagellen

Pectobacterium ook



produceert
indigoidine

Pectobacterium niet

Dickeya in aardappel: verwelking



Dickeya in aardappel: zwartbeen



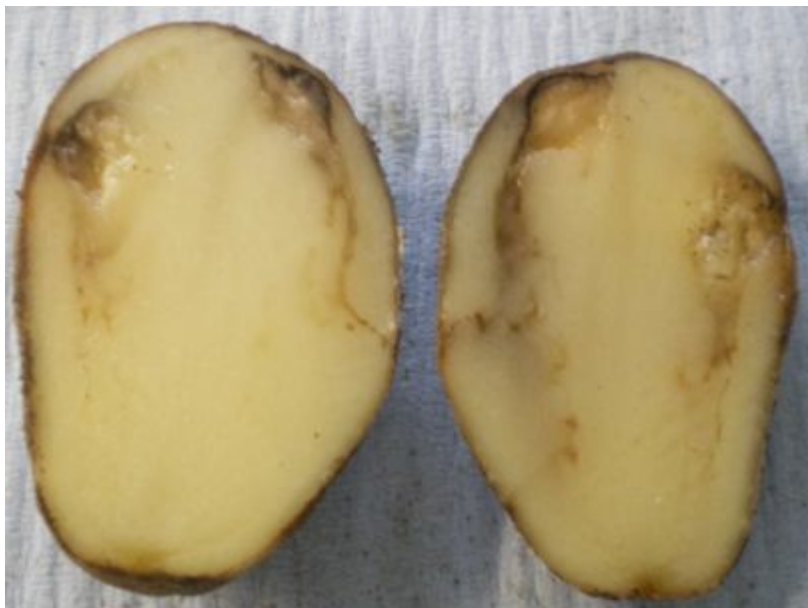
Dickeya in aardappel: maceratie



Pectobacterium in aardappel: *P. atrosepticum*



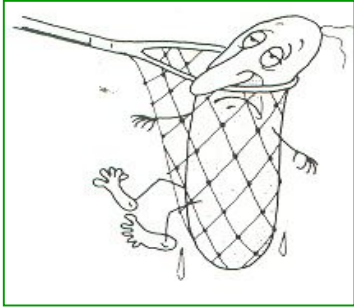
Pectobacterium in aardappel: *P. carotovorum* Pcc type 1



Pectobacterium in aardappel: *P. carotovorum* Pcc type 2



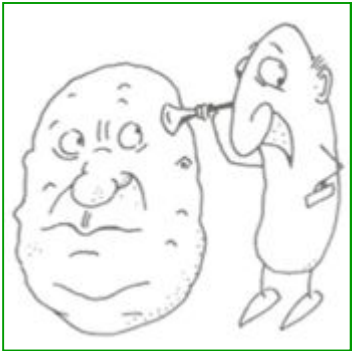
Dickeya @ ILVO projecten (1)



- 2008 - lopend



- Survey in pootgoed (diagnose, detectie)
- Epidemiologie
- Symptoomexpressie in relatie tot het levels of contamination in the seed lot
- Symptoomexpressie in aardappelrassen
- Beheersing: verhoogde weerstand



Dickeya @ ILVO projecten (2)



- 2012 – 2013



federale overheidsdienst

VOLKSGEZONDHEID, VEILIGHEID VAN DE VOEDSELKETEN EN LEEFMILIEU

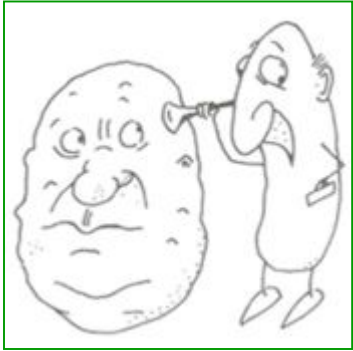
- Moleculaire identificatie & detectie
- Barcodes
- Virulentie
- Taxonomie & evolutionaire



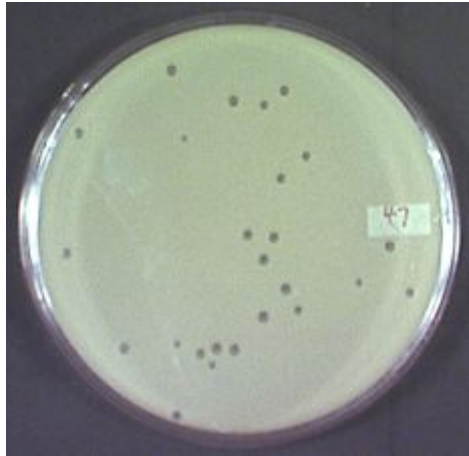
Dickeya @ ILVO projecten (3)



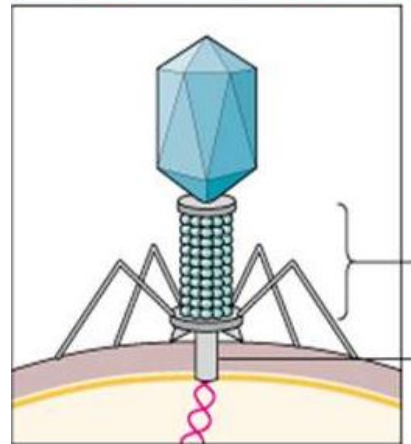
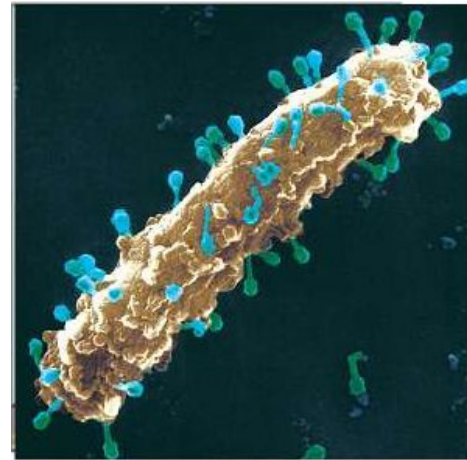
Dickeya @ ILVO projecten (4)

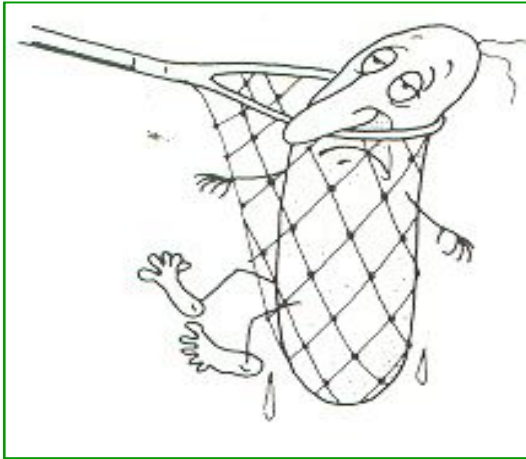


- *Dickeya* fagen



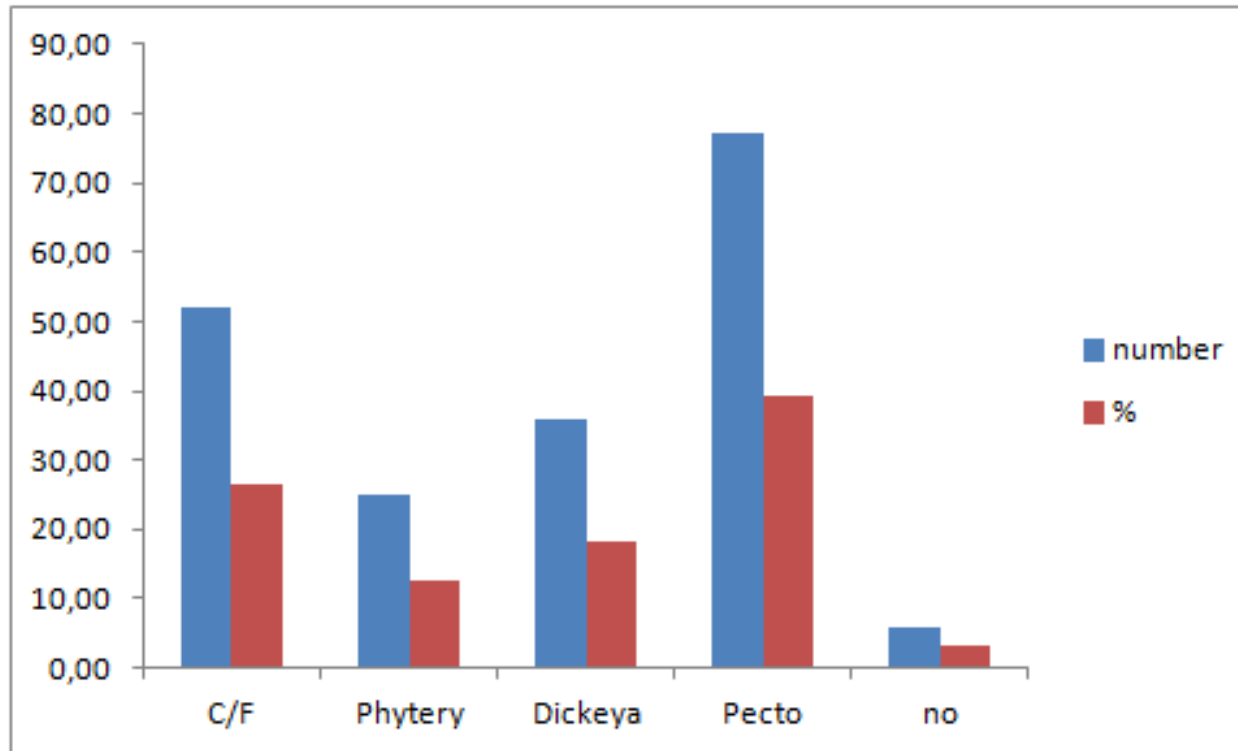
KATHOLIEKE UNIVERSITEIT
LEUVEN





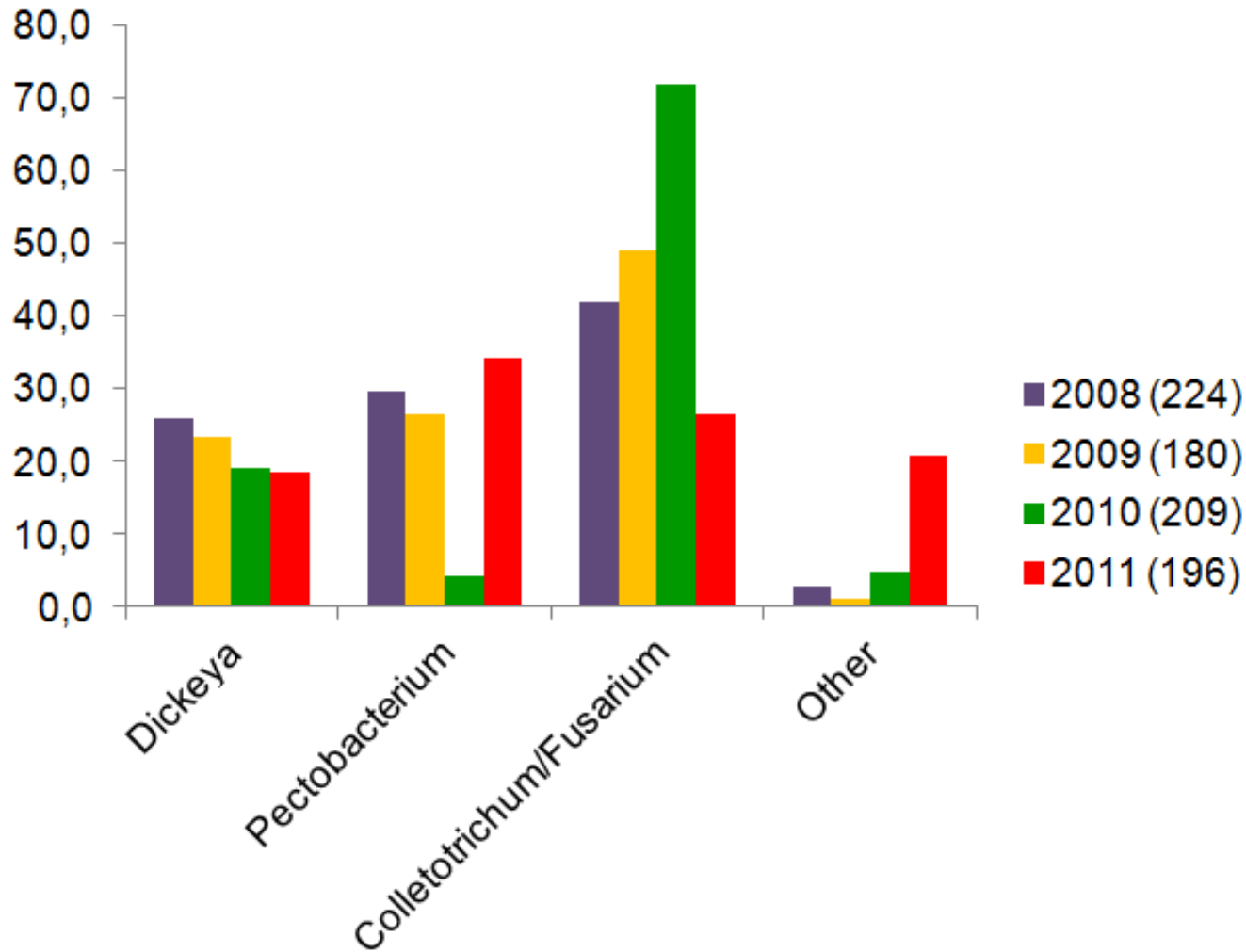
- Survey in pootgoed

Belangrijkste ziekten van pootgoed in Flanders' fields (196 analyses in 2011)

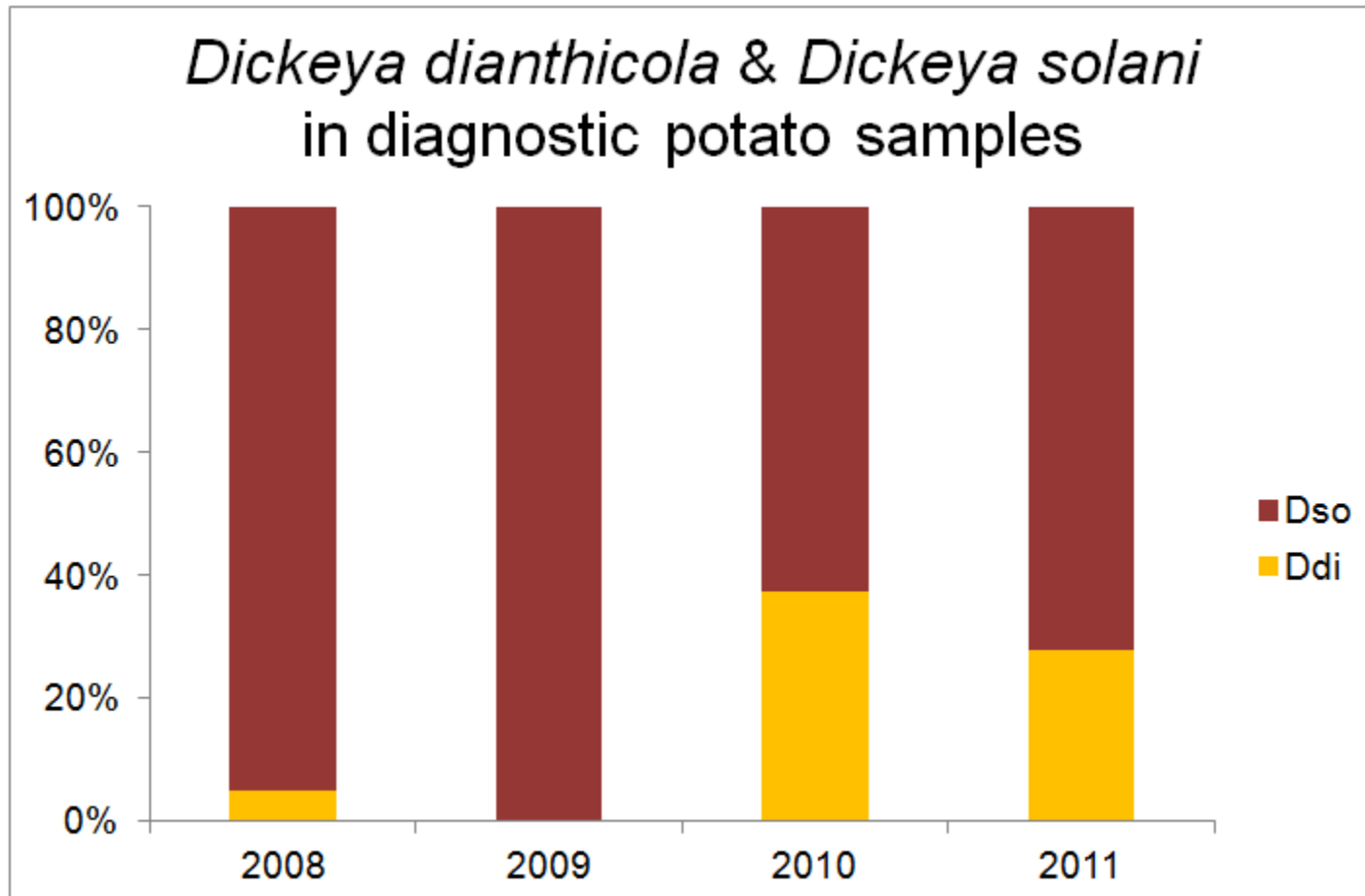


C/F	52	26,5
Phytery	25	17,9
Ddi	10	5,1
Dso	26	13,3
Pecto	77	34,2
geen	6	3,1

Belangrijkste ziekten van pootgoed in Flanders' fields (2008-2011)



Belangrijkste ziekten van pootgoed in Flanders' fields (2008-2011)





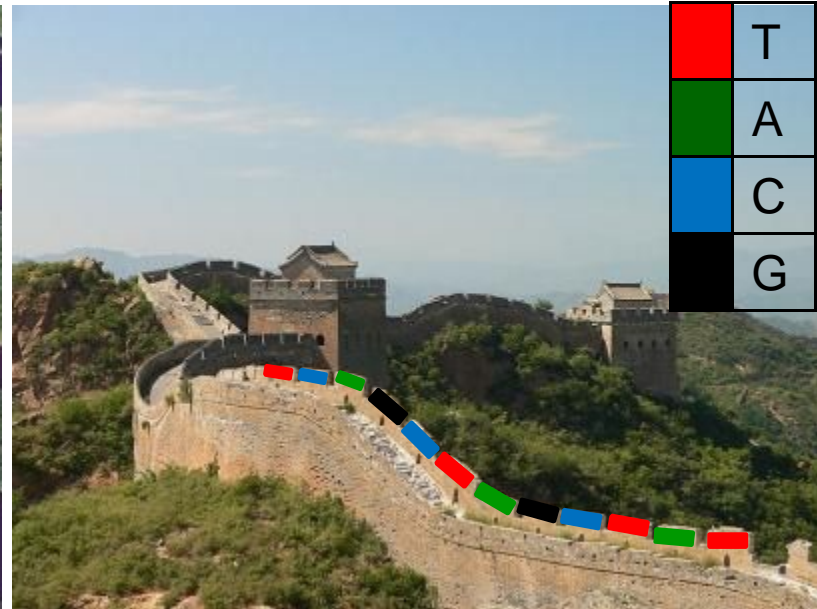
- Identificatie & opsporing

Identificatie van *Dickeya* (1)

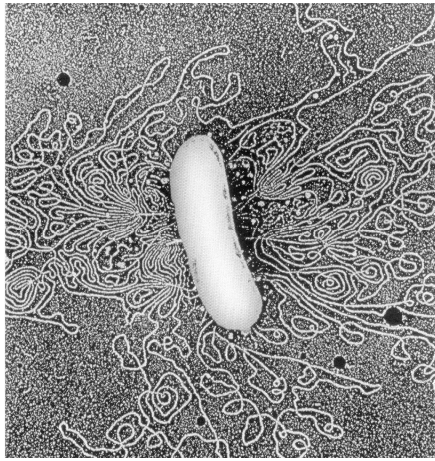
- DNA test
- Alle *Dickeya*: pel ADE



- Onderscheid tussen de *Dickeya* varianten?



Identificatie van *Dickeya* (2)

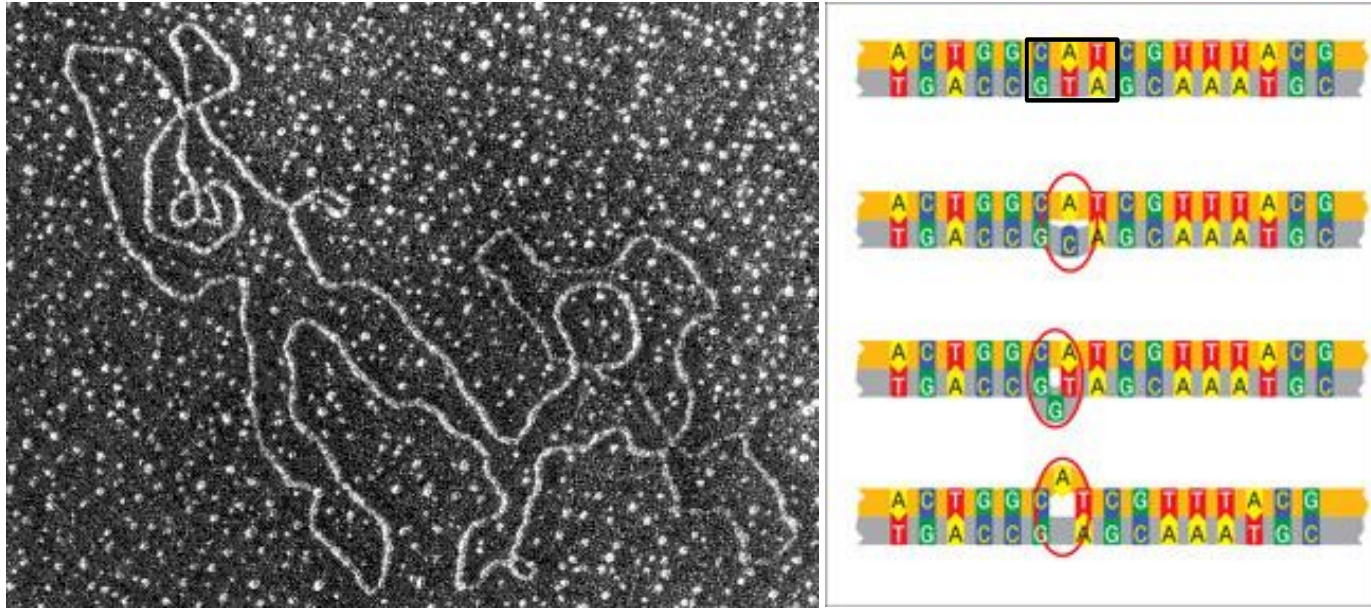


Dickeya DNA



Opeenvolging van bouwstenen = **barcode**
Verschillen in *Dickeya* barcodes \Rightarrow identificatie

Identificatie van *Dickeya* (3)



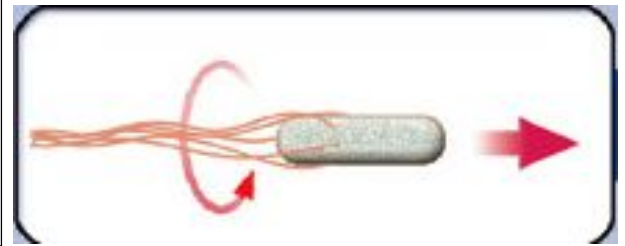
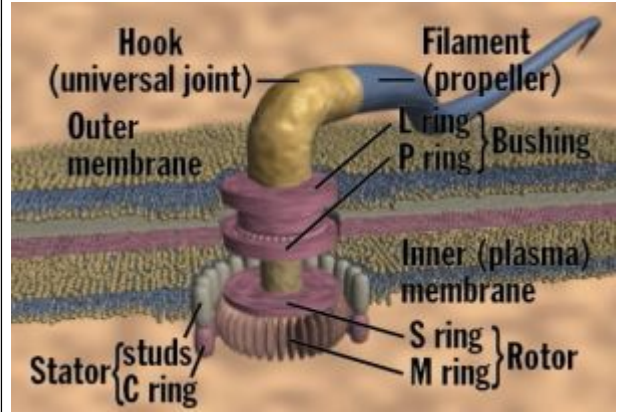
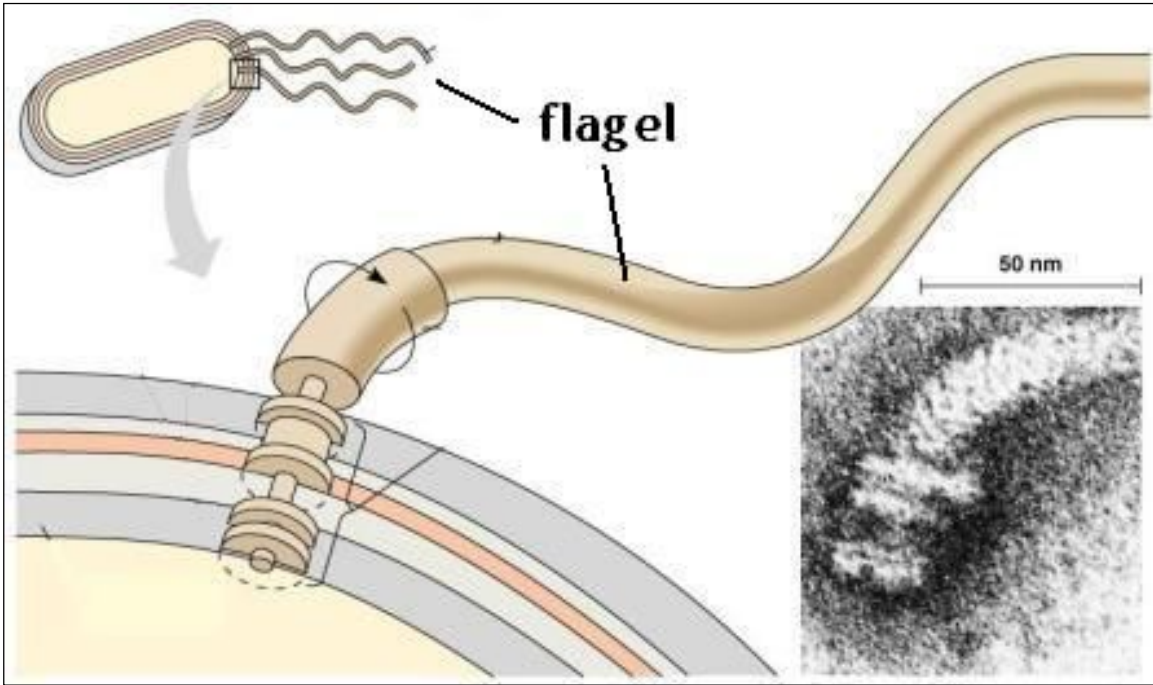
DNA van *Dickeya* = ~4.500.000 bouwstenen

Stukje DNA gebruiken dat in elke *Dickeya* variant aanwezig is maar toch voldoende verschillend is om de varianten te onderscheiden.

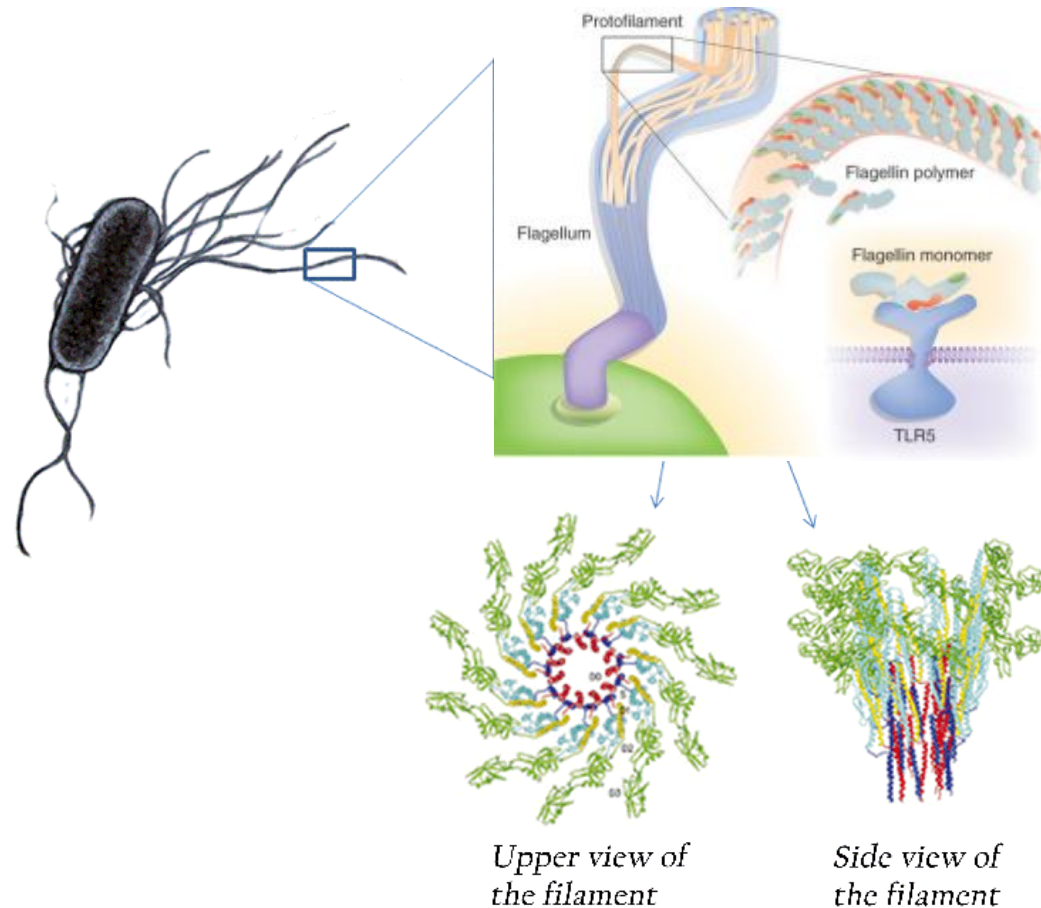
⇒ flagelline gen

gen ⇒ eiwit

Identificatie van *Dickeya* varianten met het flagelline gen

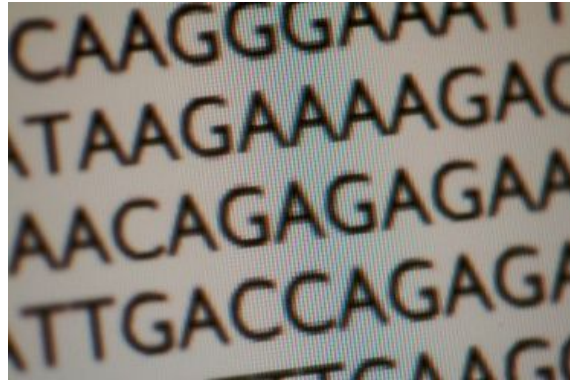


Identificatie van *Dickeya* varianten met het flagelline gen

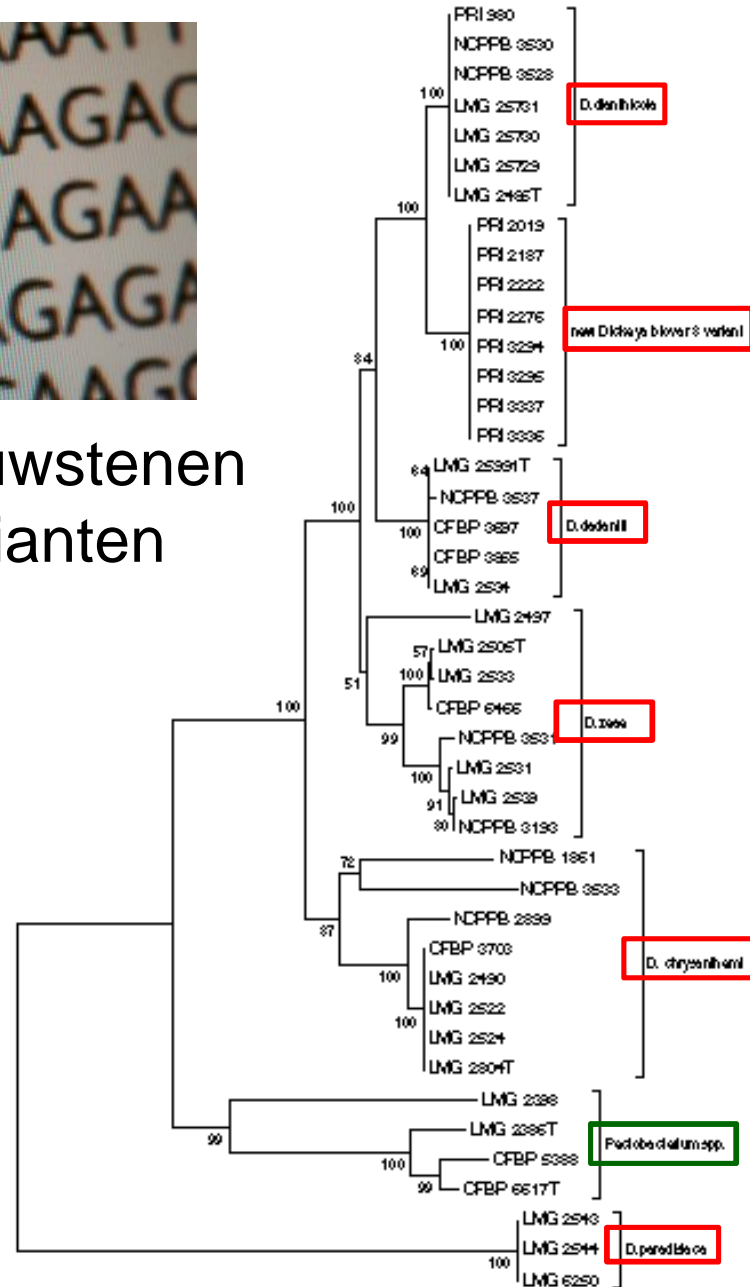


Flagelline = polymeer met vaste ruggengraat en variabele componenten aan de buitenoppervlakte
Flagelline gen = *fliC*

fliC verwantschap & identificate van *Dickeya*

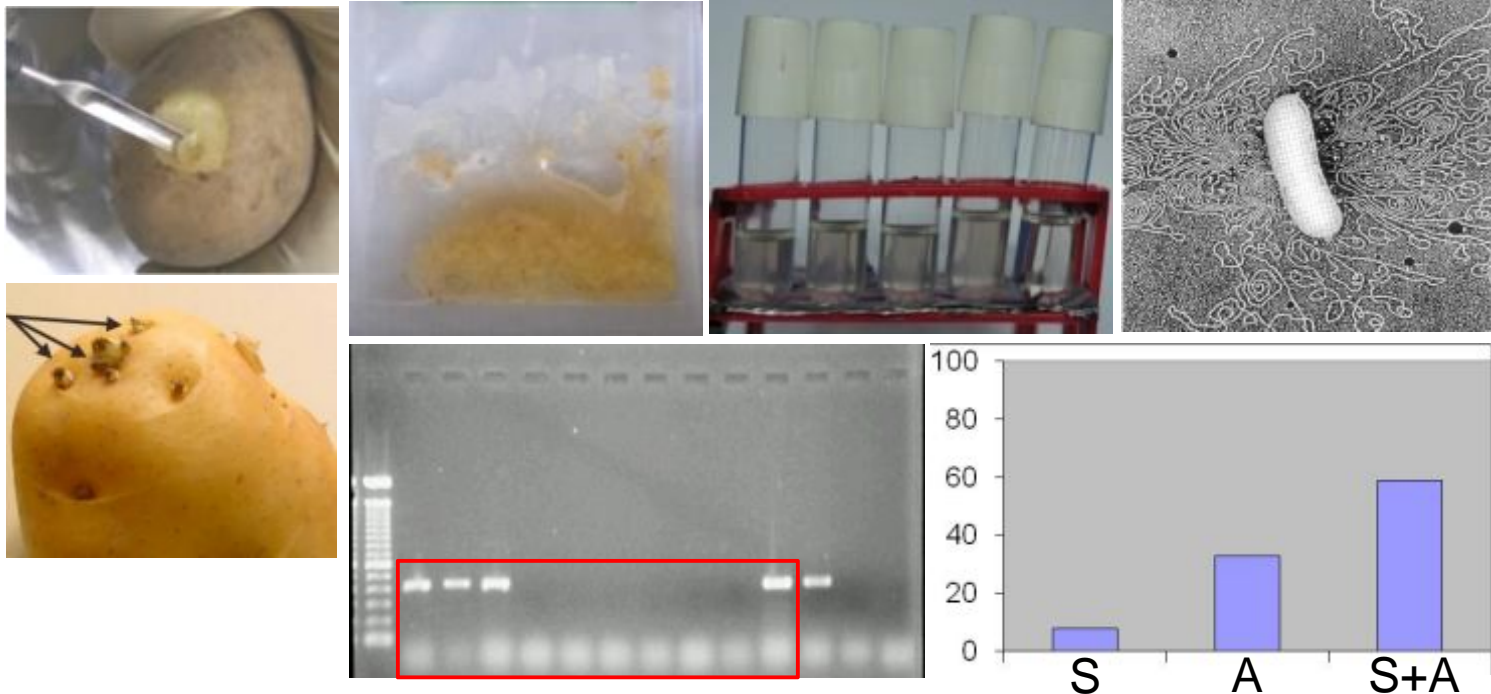


Consensus fragment = 621 bouwstenen
40 stammen van 6 *Dickeya* varianten
4 *Pectobacterium* varianten

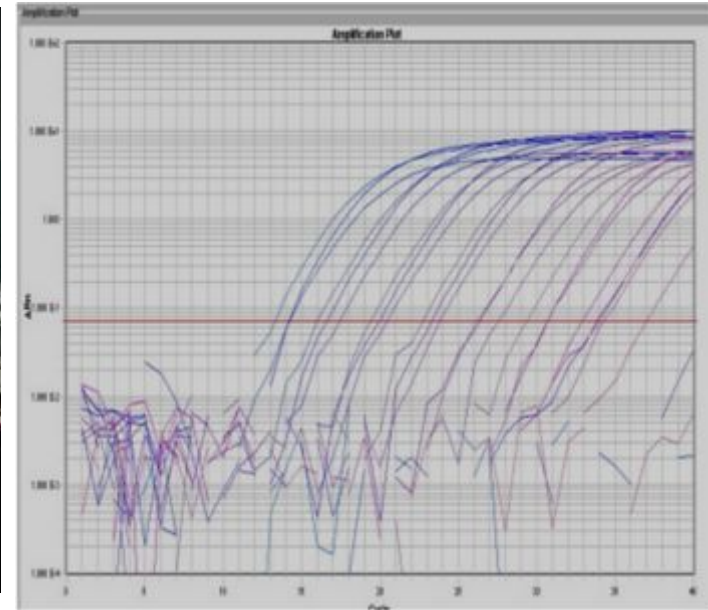
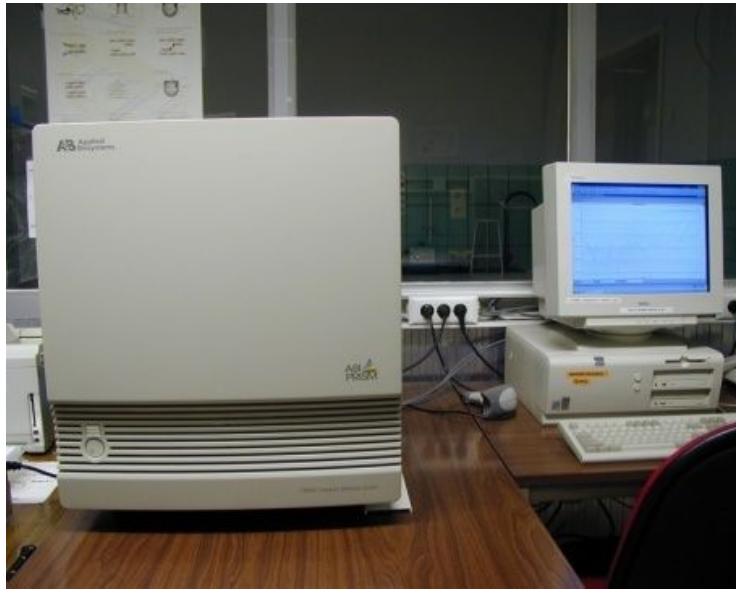


Opsporing van *Dickeya* & *Pectobacterium* in pootgoed

- 1 monster = 100/200 knollen
- 10 subsamples of 10/20 knollen
- navelpitje (en apikaal pitje)
- 72 uur anaërobe aanrijking in pectinemedium
- *Dickeya* = PCR van het *pe/ADE* gencluster
- (*Pectobacterium* = PCR van het *pe/Y* gen)



TaqMan real-time PCR voor *Dickeya solani*



TaqMan real-time PCR voor *Dickeya dianthicola*

Ministerial Decree
No. (1703) for year 2011

Import Phytosanitary Requirements and Specifications for the
Importation of Seed Potatoes for Growing Year (2012)

(B) Diseases

1- Imported potato seeds shall be originated in places of production free from the following diseases and its pathogens; these places should be established according to the relevant international standards of phytosanitary measures:

- *Synchytrium endobioticum*
- *Globodera* spp.
- *Trichodorus* spp. and *Paratrichodorus* spp.
- Potato Yellow Dwarf Virus
and Phytoplasma
- *Clavibacter michiganensis* sub. sp.
sepidonicus
- *Ralstonia solanacearum*
- *Dickeya solani*

d- *Pectobacterium carotovorum* :

It is not allowed to enter infected tubers with infection percentage exceeding 0.5%.

e- *Pectobacterium atrosepticum* :

It is not allowed to enter infected tubers with infection rate exceeding 1%.

f- *Phytophthora erythroseptica*:

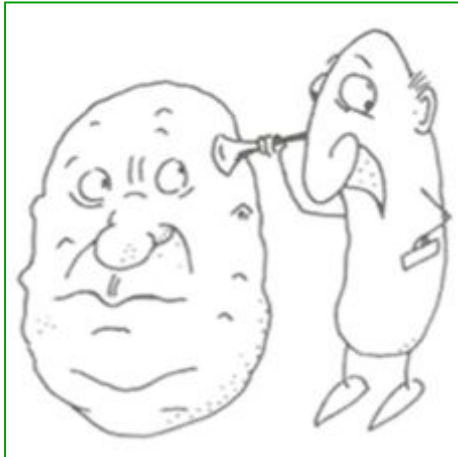
It is not allowed to enter infected tubers with infection rate exceeding 0.1%.

Opsporing van *Dickeya* & *Pectobacterium* in pootgoed



100 monsters	navelpitjes	kapjes
Pca (15)	5	10
Pcc (25)	8	17
D (60)	27	37

Bacterieziek in vermeerdering	2010	2011
Labtest positief	70%	52%
Labtest negatief	94%	97%

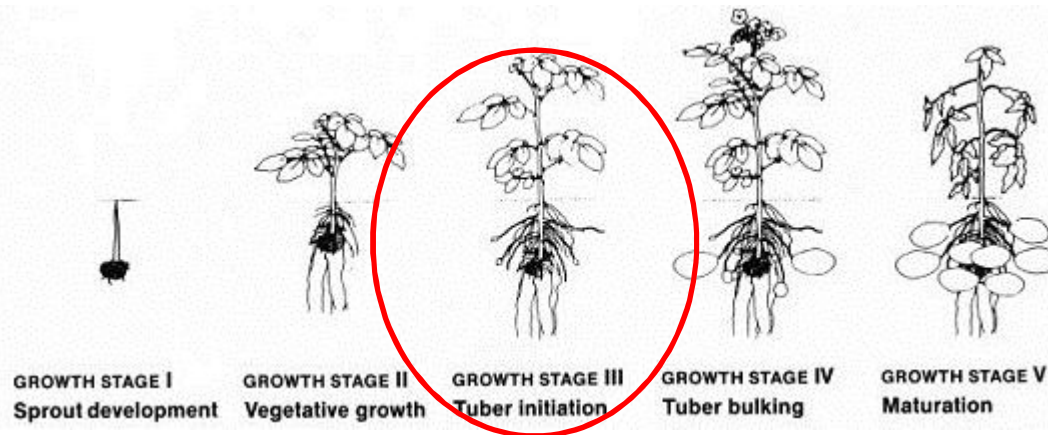


- Virulentie

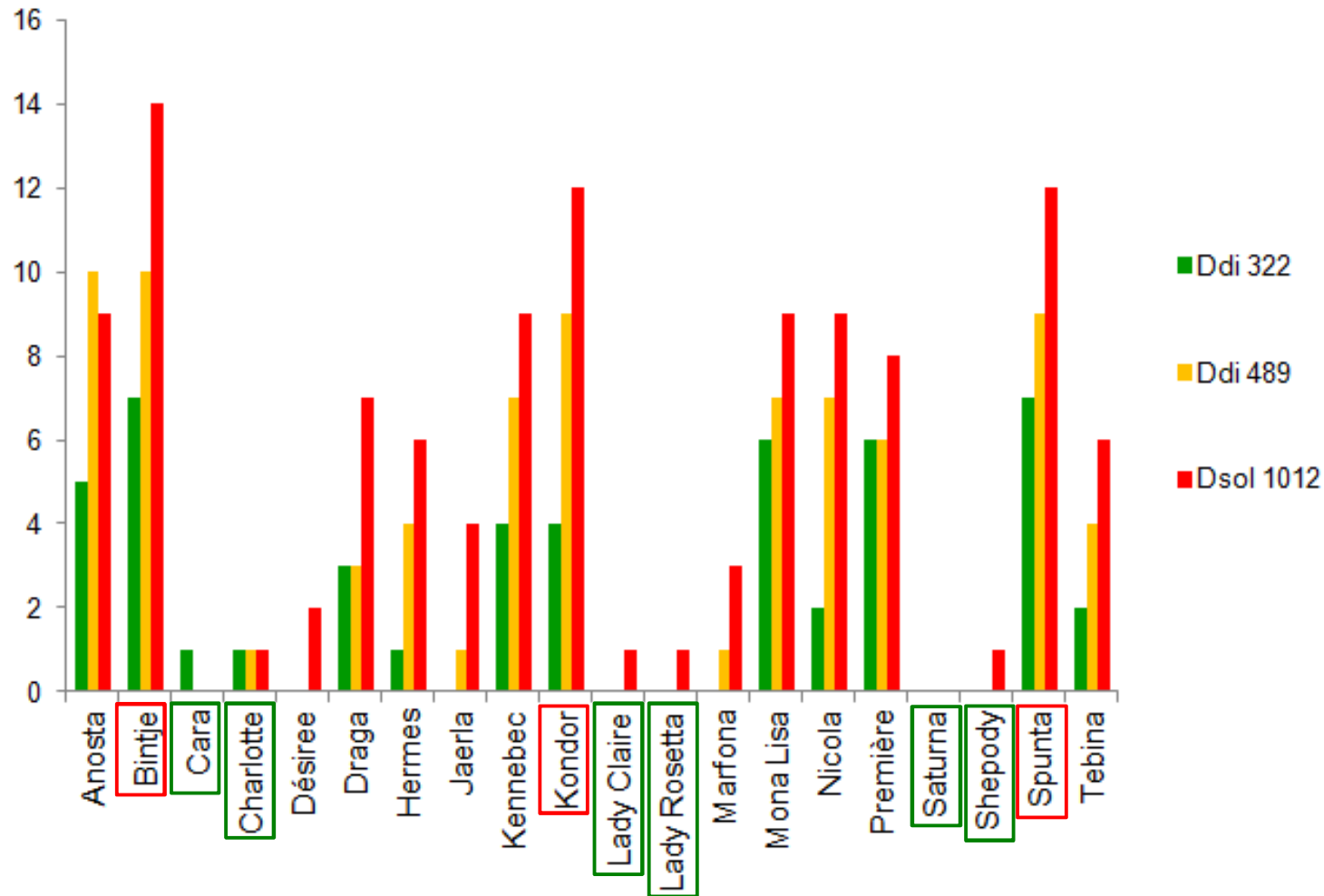
Dickeya virulentie

- Aardappel
- Andere cultuurplanten

Aardappel



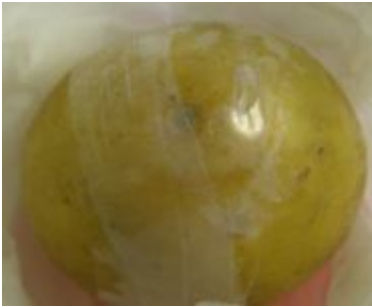
Dickeya virulentie in aardappelrassen (2)



Aantal door *Dickeya* aangetaste knollen

Dickeya virulentie in aardappelrassen (3)

- 36 aardappelrassen
- 3 methoden:

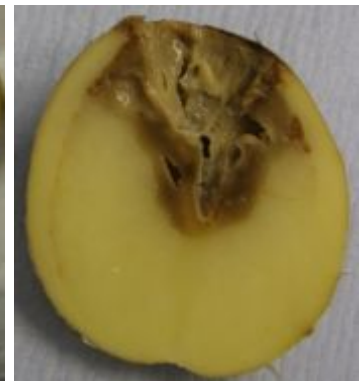
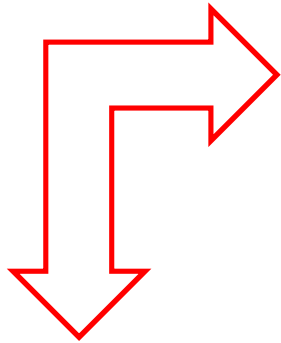


0,1 ml

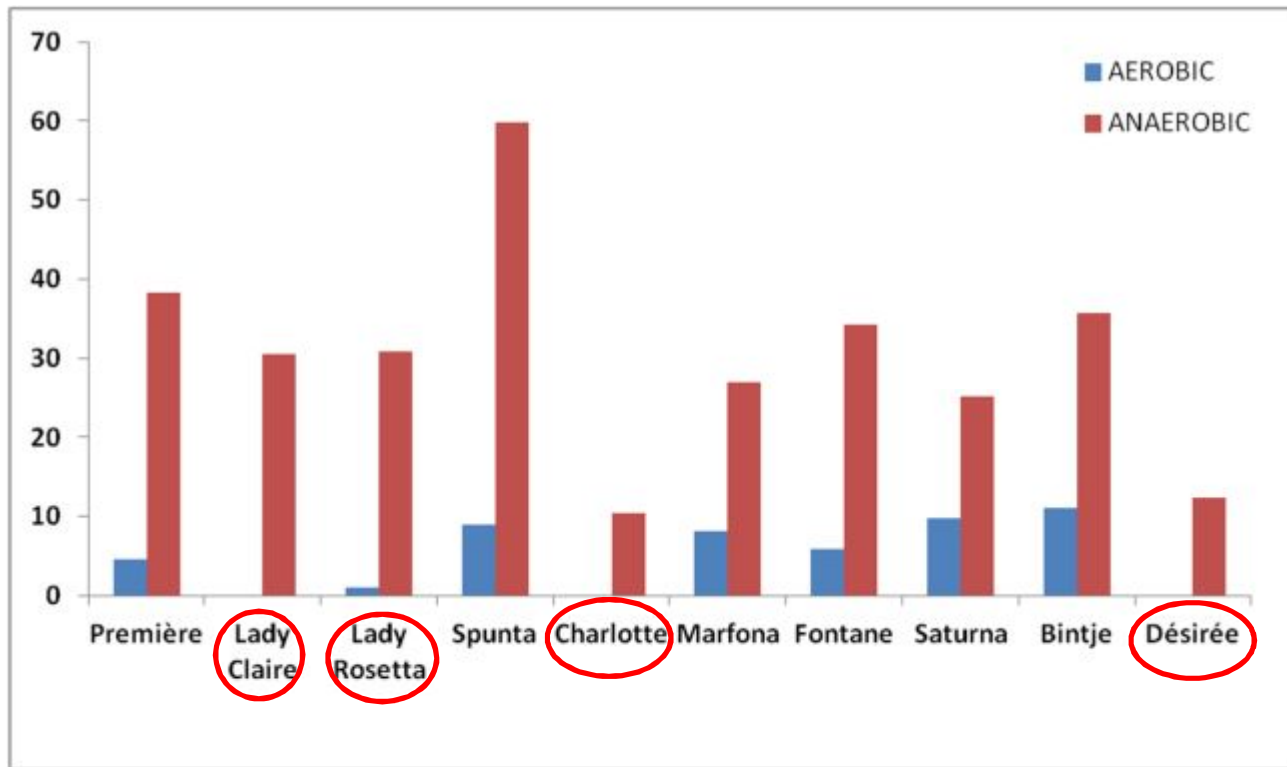


Dickeya virulentie in aardappelrassen (4)

- 2 celconcentrations: ~ 1 miljoen & ~ 50.000 cellen per knol
- 3 dagen aërobe en anaërobe incubatie bij 28°C (20°C)

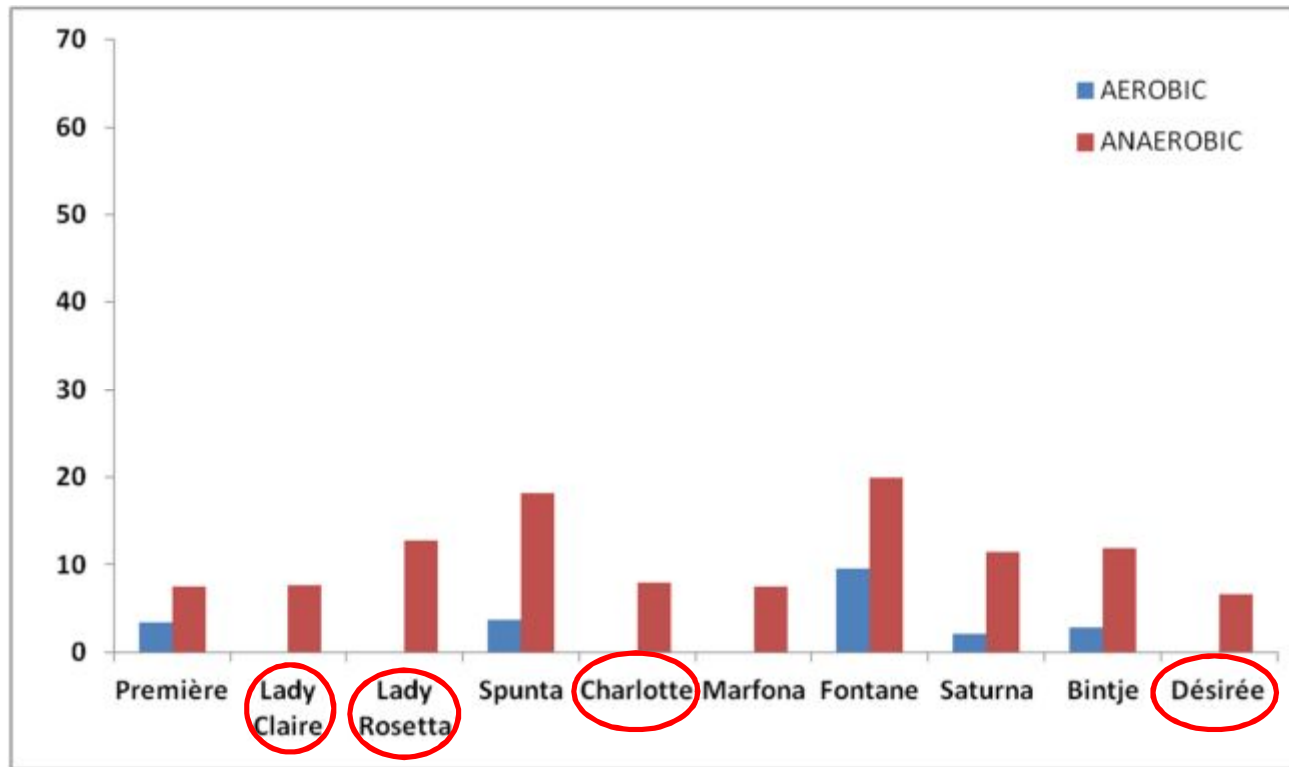


Dickeya virulentie in aardappelrassen (5)



D. solani GBBC 1012, % gemacereerd knolweefsel

Dickeya virulentie in aardappelrassen (6)



D. dianthicola GBBC 489, % gemacereerd knolweefsel

Dickeya virulentie in aardappelrassen (7)

Samenvatting	aëroob, 28°C	anaëroob, 28°C
Ddi 489	2,99	11,39
Dso 1012	4,66	28,10

% gemacereerd knolweefsel na 3 dagen



- *Dickeya* in waterlopen

Dickeya in waterlopen (1)



Dickeya zea: VK, Finland, Spanje
Dickeya solani: Schotland, Wales
nieuwe, onbenoemde *Dickeya*



Dickeya in waterlopen (2)

Dickeya infecteert langs de wortels van de aardappelplant.

Beregening is een risico voor insleep.

Maar hoeveel *Dickeya* cellen per liter?

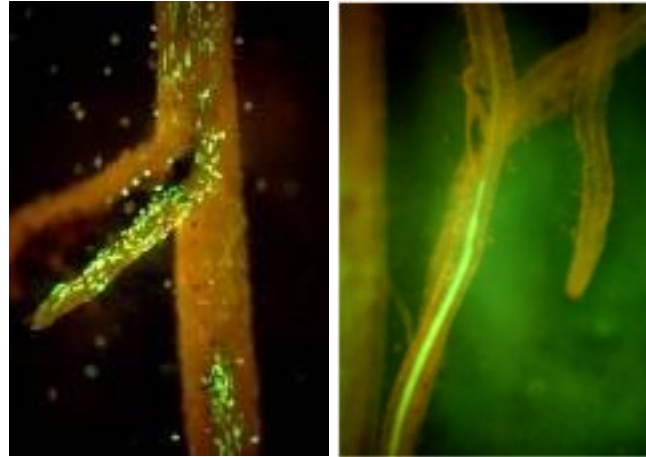
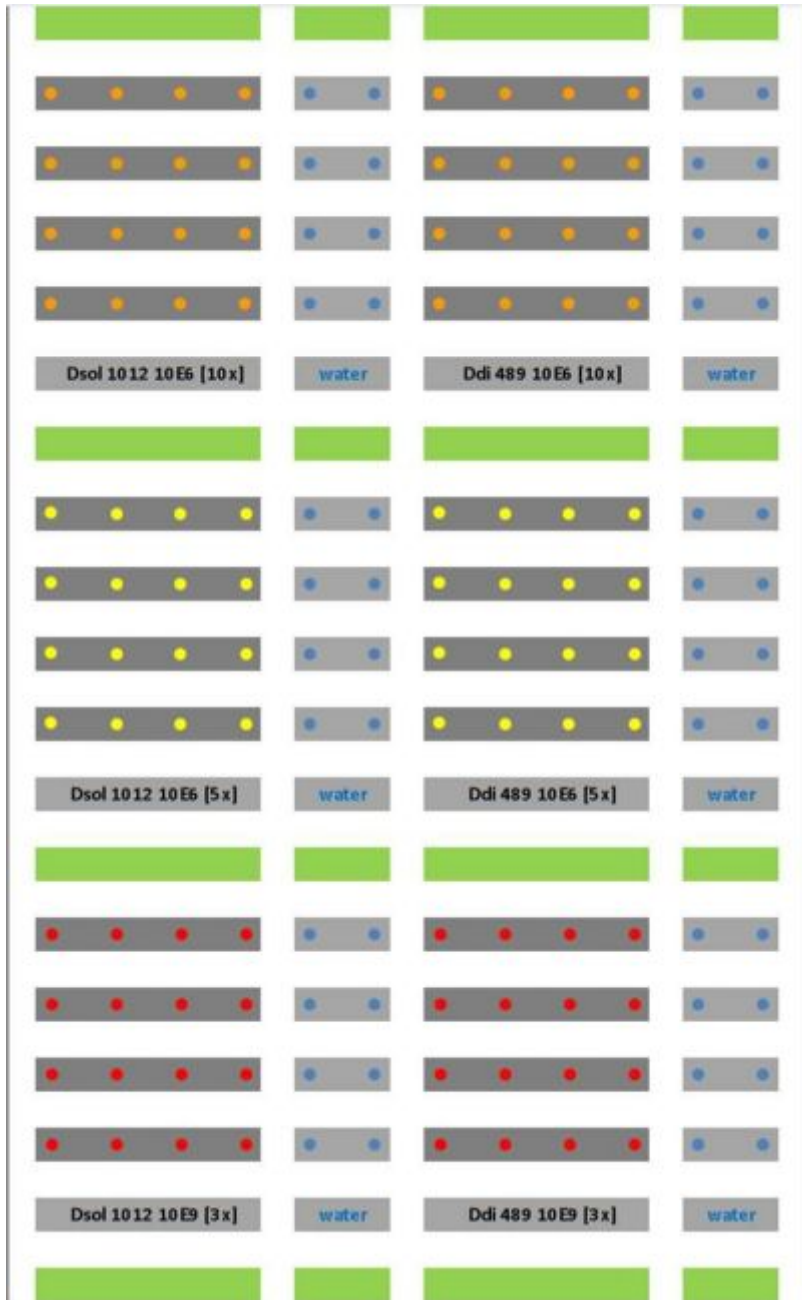
Moeilijk exact te bepalen want voor de analyse wordt *Dickeya* eerst aangerijkt.

Als *Dickeya* werd uitgekweekt: 10000 – 100000 cellen/liter

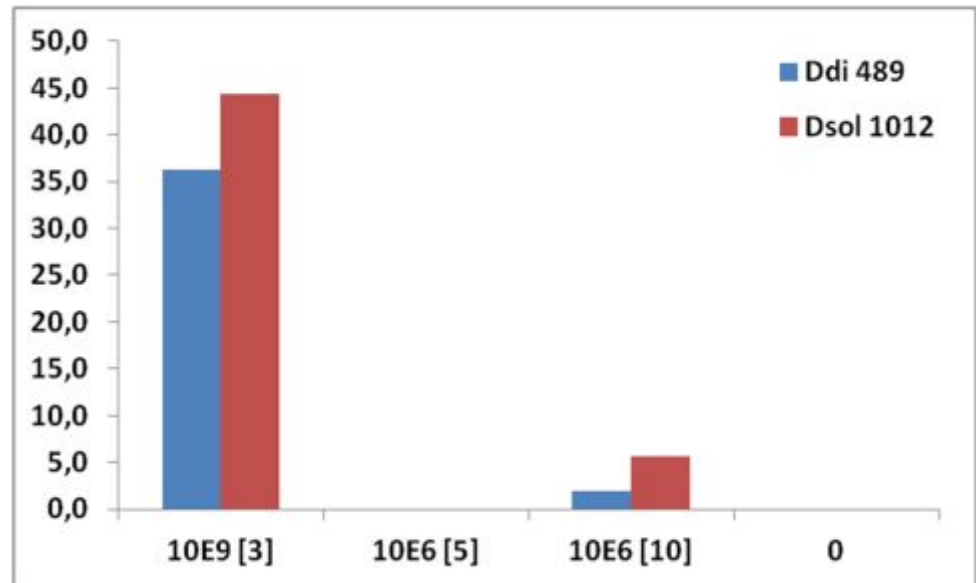
Risico?

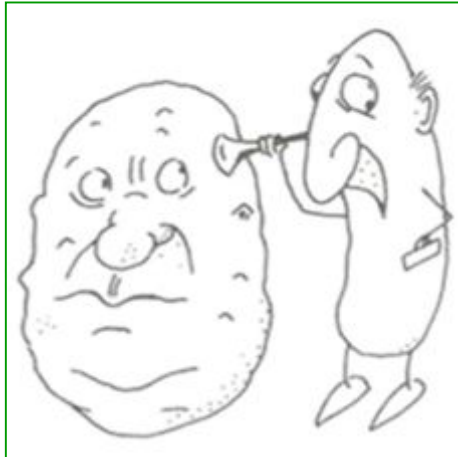


Dickeya in waterlopen (3)



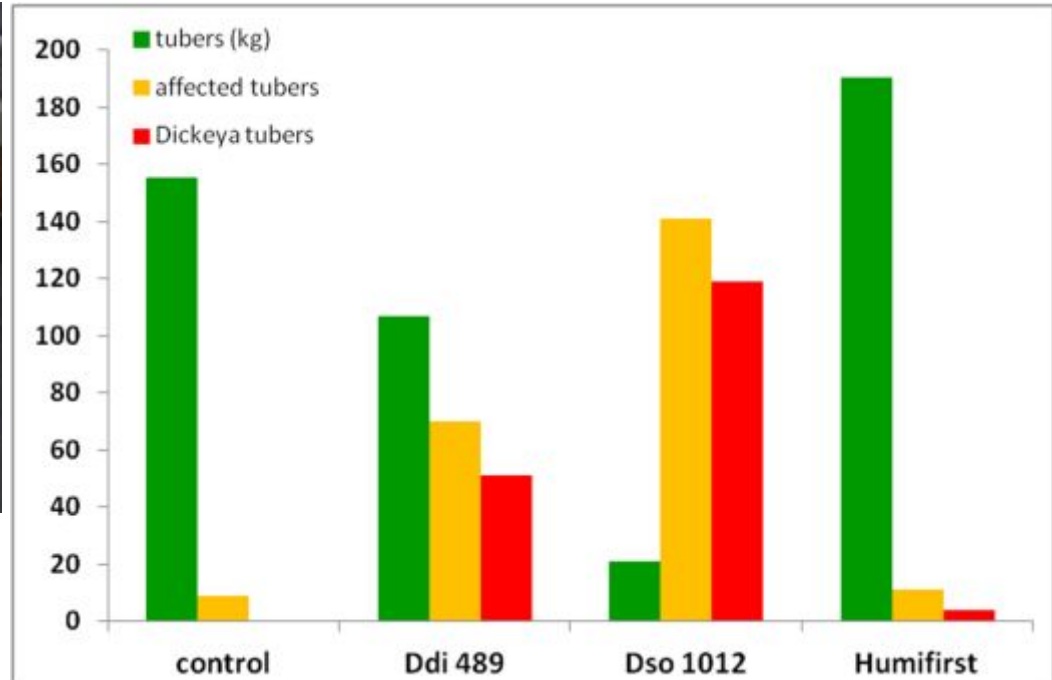
10 mm = 10 miljoen *Dickeya* per m²

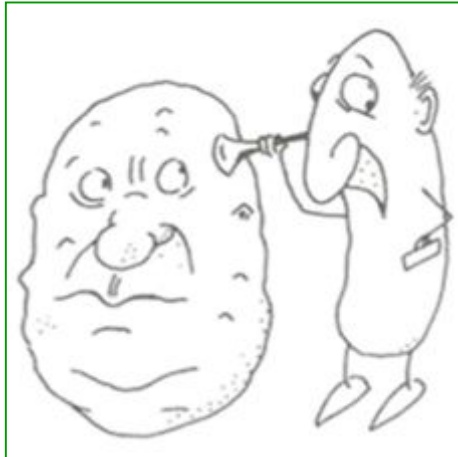




- Contactbesmetting

Dickeya: contact contamination





• Akkerkwesties

Akkerkwesties: *Dickeya* aan wortels van onkruiden



VK, Finland, Israël

Akkerkwesties: *Dickeya* na maïs, tarwe of wortelen



12%



1,4%



3,9%

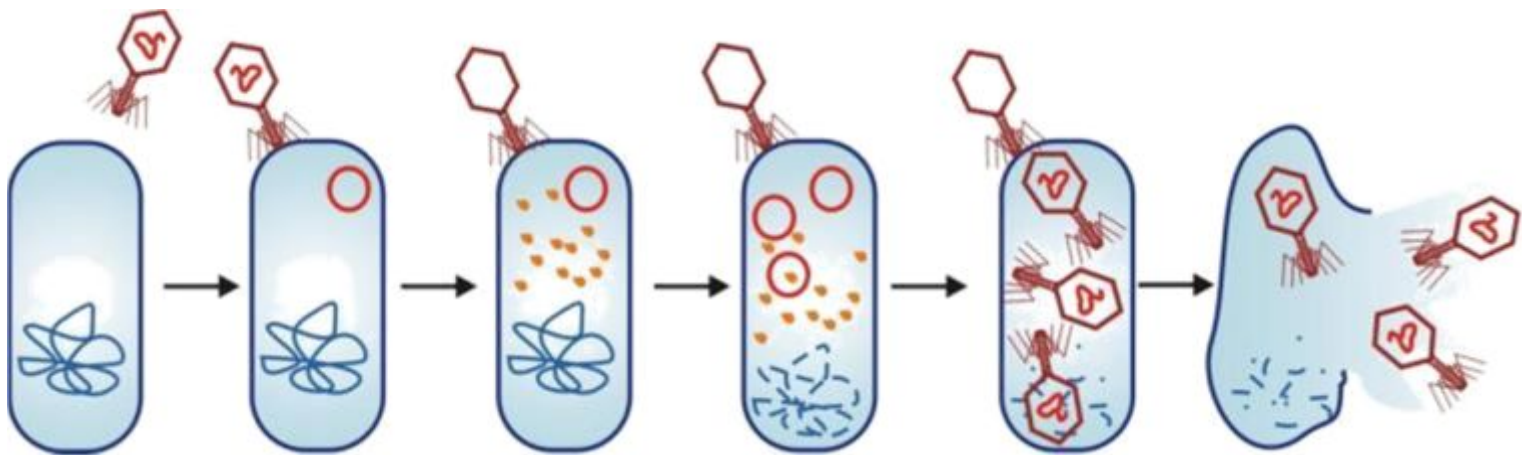
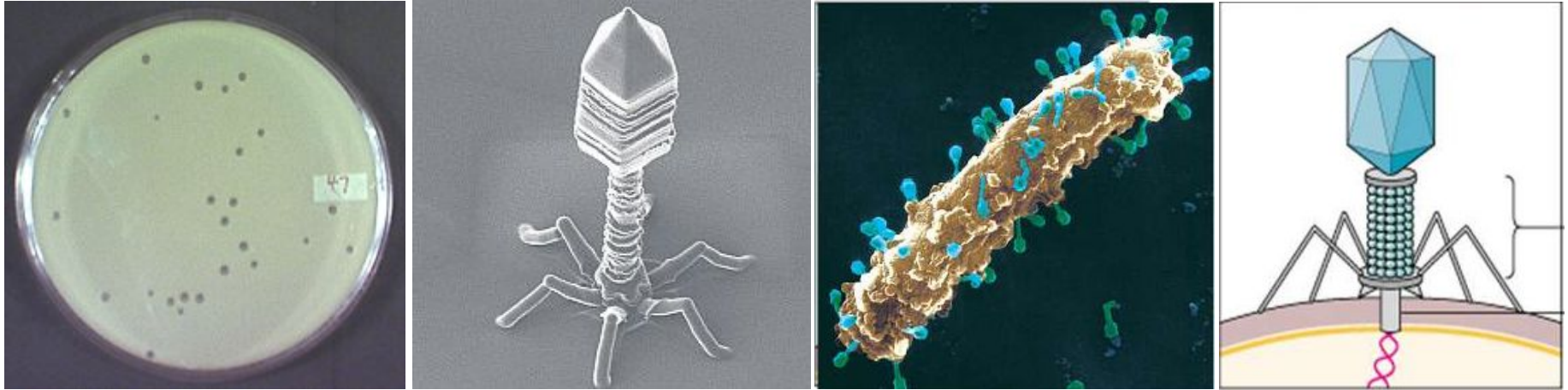
Frankrijk, Zwitserland



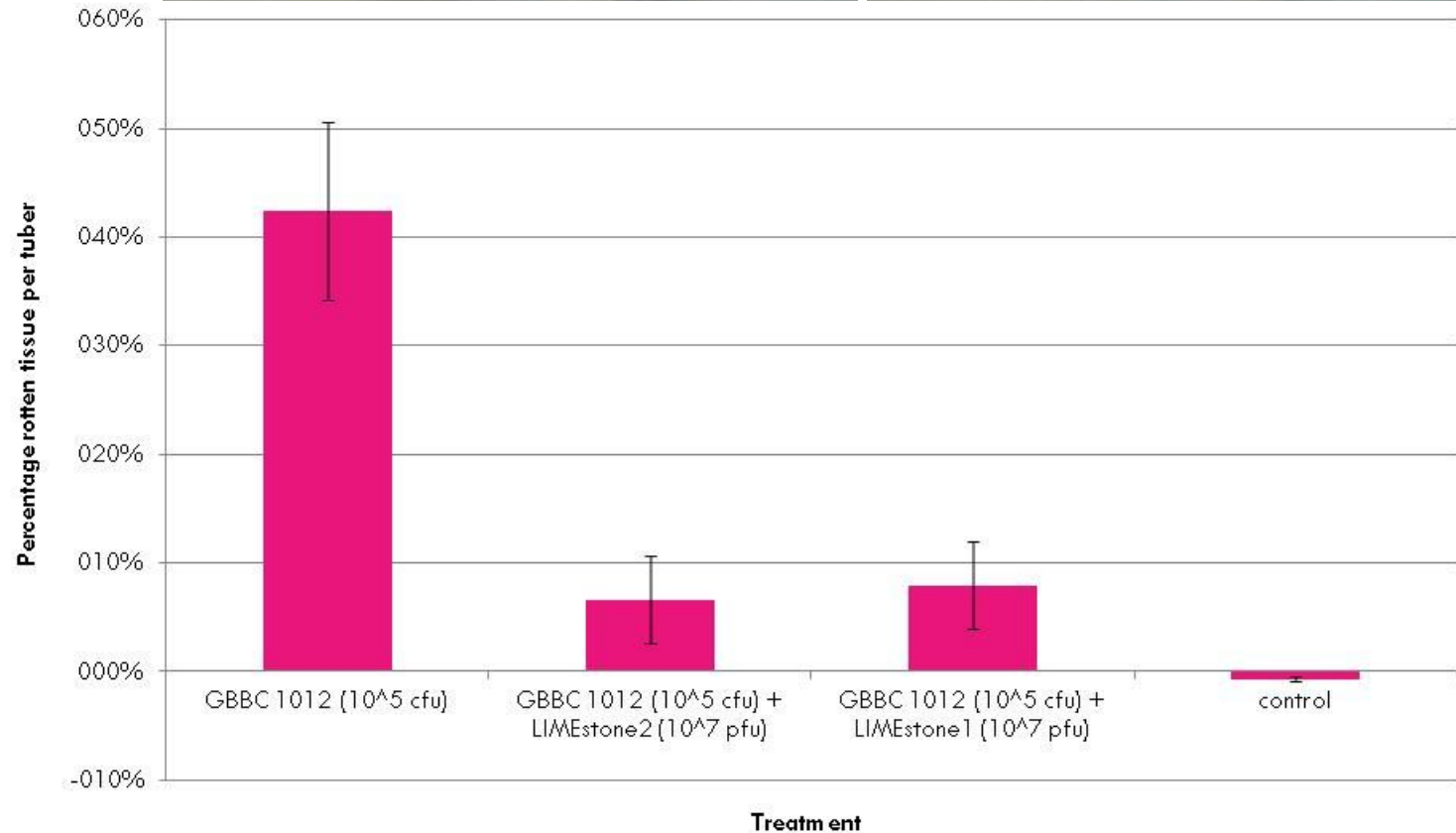
- Bio control

Biocontrole van *Dickeya*: bacteriofagen (1)

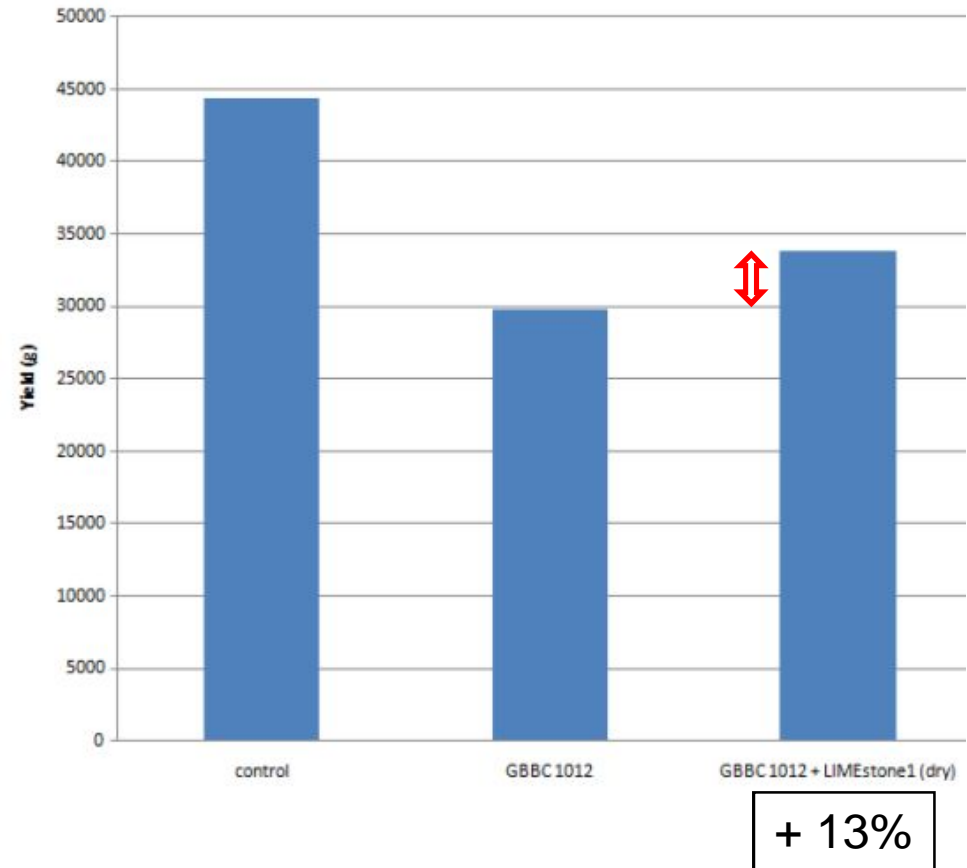
Bacteriofagen = virussen van bacteriën



Biocontrole van *Dickeya*: bacteriofagen (2)

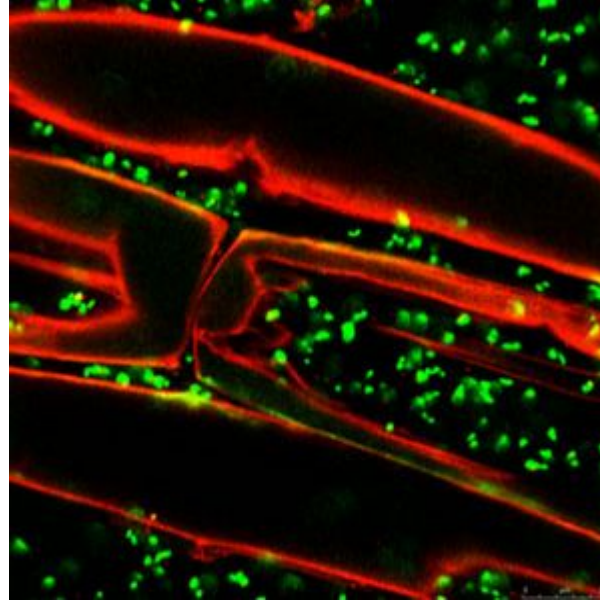
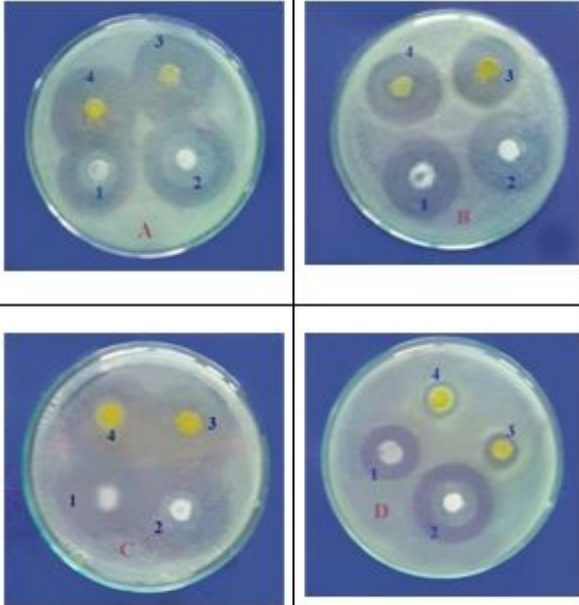


Biocontrole van *Dickeya*: bacteriofagen (2)

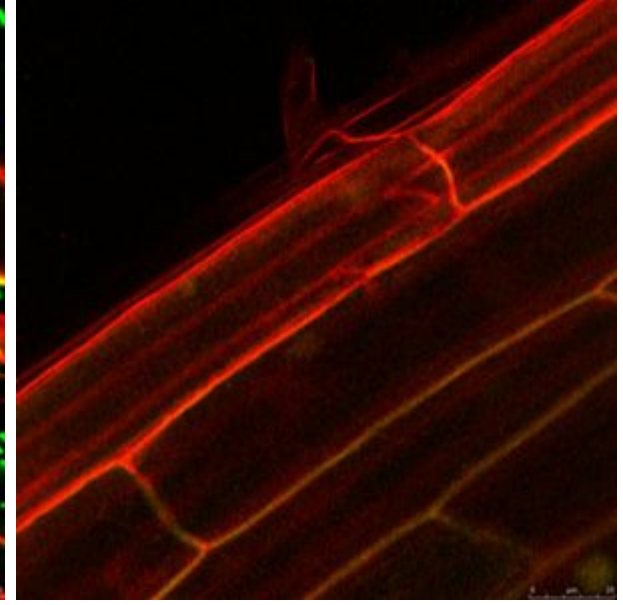


Biocontrole van *Dickeya*: antagonist bacterie

Serratia plymuthica



55%



0%

PRI, NL

Gezond pootgoed @ ILVO 2011: samenvatting

- Survey in pootgoed
 - ~52% bacterieziek in survey
 - Meer *Pectobacterium* dan *Dickeya*
 - Colletotrichum*: ~ 25%
- knolbehandeling
- Identificatie en opsporing
 - DNA test voor *Dickeya* varianten
 - Real-time opsporing van *D. solani* (*D. dianthicola*)
 - Partijtoets keuringsreglement
 - 100 knollen
 - navelpitjes met schil
 - enkel *Dickeya*
 - 'nagenoeg vrij'

Gezond pootgoed @ ILVO 2011: samenvatting

- Rasgevoeligheid

D. solani > *D. dianthicola*

anaëroob > aëroob

+ = chipsrassen, Désiree, Charlotte

- = Spunta, Bintje, Fontane

- *Dickeya* in waterlopen

meestal andere *Dickeya* dan in aardappel (uit kasteelt?)

risico van insleep door beregening is gering

- Contactbesmettingen

30% - 85% reductie

D. solani > *D. dianthicola*

Gezond pootgoed @ ILVO 2011: samenvatting

- Akkerkwesaties

D. solani hecht zich aan wortels van akkeronkruiden
akkerkers, ganzevoet, knolcyperus, kleine brandnetel

voorvrucht: maïs < wortelen < tarwe

- *Dickeya* biocontrol
faagtherapie
antagonist bacterie

- Project 4/2012 – 3/2013
survey & opsporing in pootgoed
rasverschillen

Einde