Latency, seed testing, Pathogenesis and the management of soft rot and blackleg of potato

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Agenda

This is a grass root level discussion targeted to deliver a take home message on essential concepts and practices for success in seed testing and thereby management of blackleg and soft rot.

- Latent (Piilevä) meaning and importance
- Length of latency
- Transition from latent to active infection, what are the main factor (s) governing it.
- Understanding the disease process (pathogenesis)
- Seed testing pays off
- Sampling and seed testing- two sides of the same coin
- Predictiveness of the test method
- Seed testing is collaborative work (the players' role)
- The peril (danger, risk) of too much tolerance
- Conclusions

Latent (piilevä)- meaning

A latent infection is an infection that is
- hidden
- inactive
- dormant

As opposed to active infections, where the bacterium is actively replicating and potentially causing symptoms, latent infections are essentially inactive.

Asymptomatic (symptomless) infection capable of showing symptoms under some circumstances or if activated.

Word attached to the name has significance

Pillevä Tyvimätä vs Blackleg

- The naming in Finnish carries an emphasis on ”Latent”

Out of Sight out of mind?

Tyvimätä on iso ongelma
Siementuotannossa koska
Se piilee siemenperunan pinnalla
Paljain SILMIN näkymättomänä

Kotipuutarahurin Perunaopas

Main source of infection

Dickeya and *Pectobacterium* latent in the seed tubers

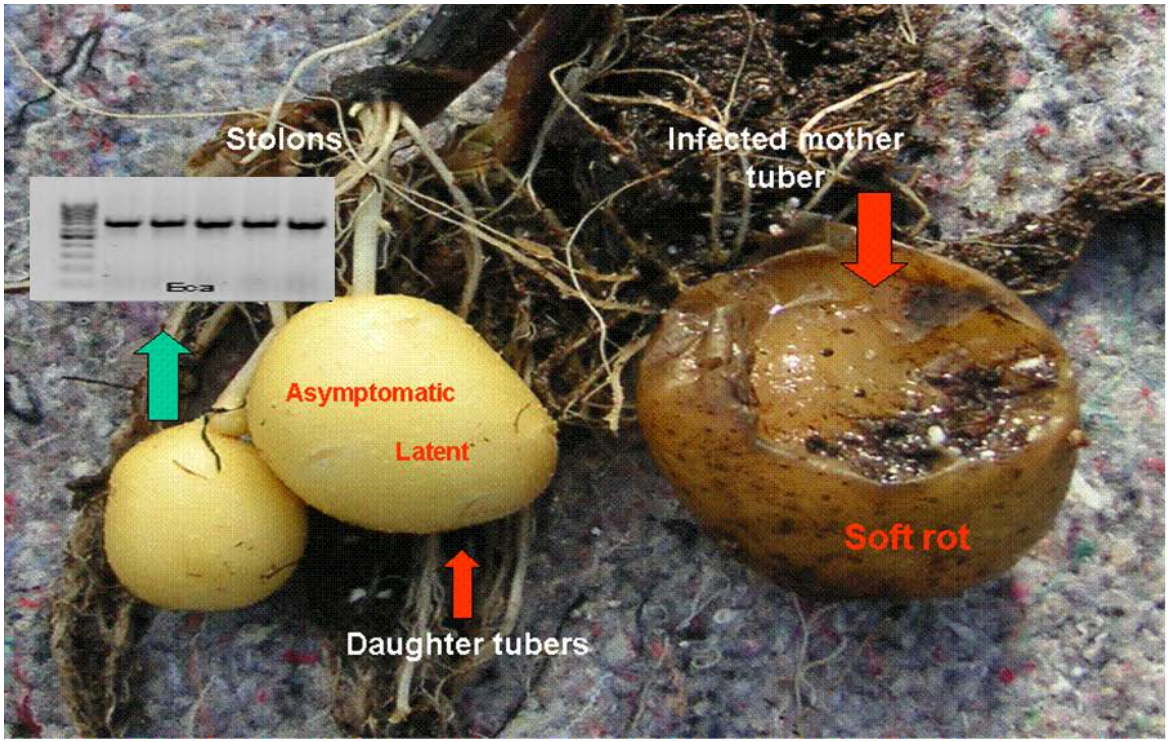
Transition from **latent to active infection** in *Dickeya* and *Pectobacterium*- Pathogensis (disease process and progress in the plant)

The most important factor for symptom expression in potatoes infected with *Pectobacterium* and *Dickeya* species is the titer (number)of the bacterial population present in contaminated seed tubers at the time of planting.

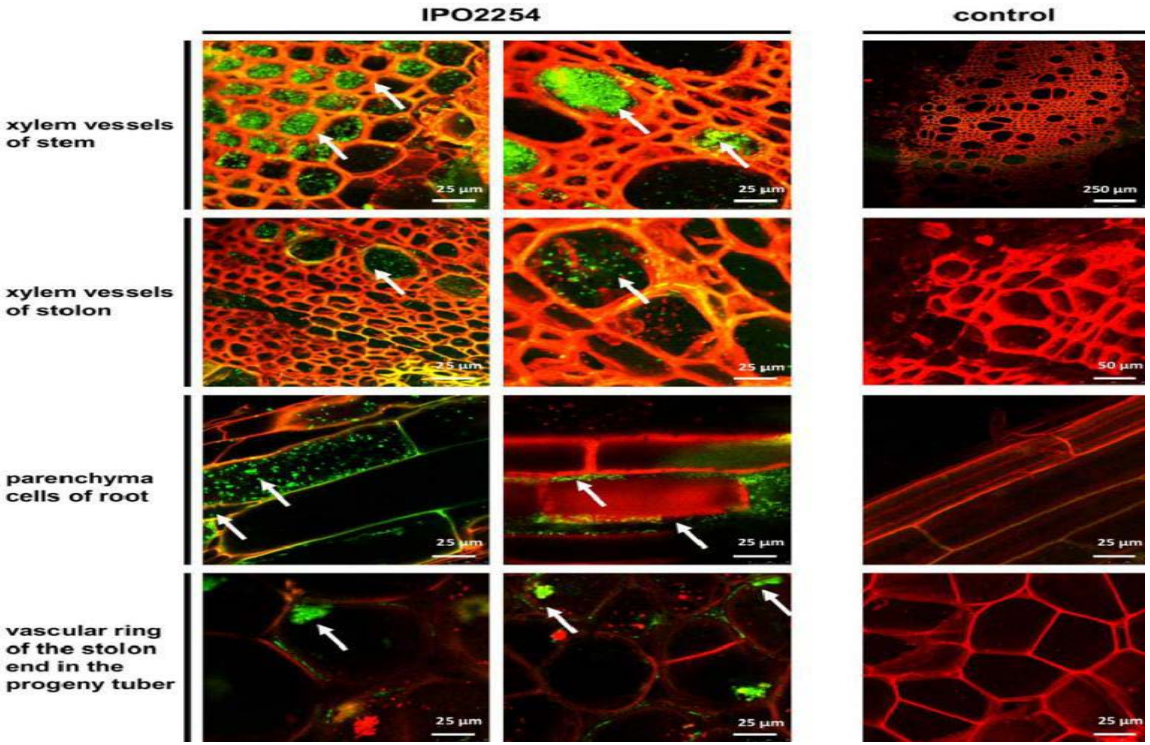
The bacteria multiply until a critical cell density between 10^6 and 10^8 cfu ml−1 is reached; the rotting process is initiated in the seed tubers, which subsequently moves up into the stem through the vascular system. Here the bacteria remain quiescent until environmental conditions are favourable, after which they become active and cause disease expression in the above-ground plant parts.

Simultaneously, bacteria can move downwards in the vascular bundles, infecting progeny tubers. Depending on the bacterial concentration and rate of multiplication, symptoms will be expressed immediately or during the next growing season. Therefore, the most important means of dissemination of inoculum is movement of latently-infected seed tubers
Pérombelon and Kelman 1987, Laurila et al. 2008, Czajkowski et al. 2009

The Disease progress



THE POWER OF MOLECULAR DETECTION (DIAGNOSTIC PCR): Latent bacteria in healthy looking tubers revealed by the powerful PCR technology. "Out of sight may not out mind" (Degefu, Luke Oulu)



Czajkowski *et al*. 2010- Phytopathology (2010), 100: 1128-1137

Figure 6. Colonization of xylem vessels of potato stems, stolons, parenchyma cells of roots and stolon end containing xylem elements of progeny tubers by GFP-tagged *Dickeya* sp. IPO2254 at 30 days post stem inoculation analyzed with confocal laser scanning microscopy. Plant parts were embedded in PT agar for 24 h prior to analyses. Plant cells were counter-stained with the redfluorescentdye propidium iodide. Bacteria were found inside xylem vessels in stems and stolons, inside and between parenchyma cells in roots and inside xylem cells of the vascular ring of the stolon end in progeny tubers (white arrows). Bacteria in green

Czajkowski et al., 2010
Phytopathology 100: 1128-1137

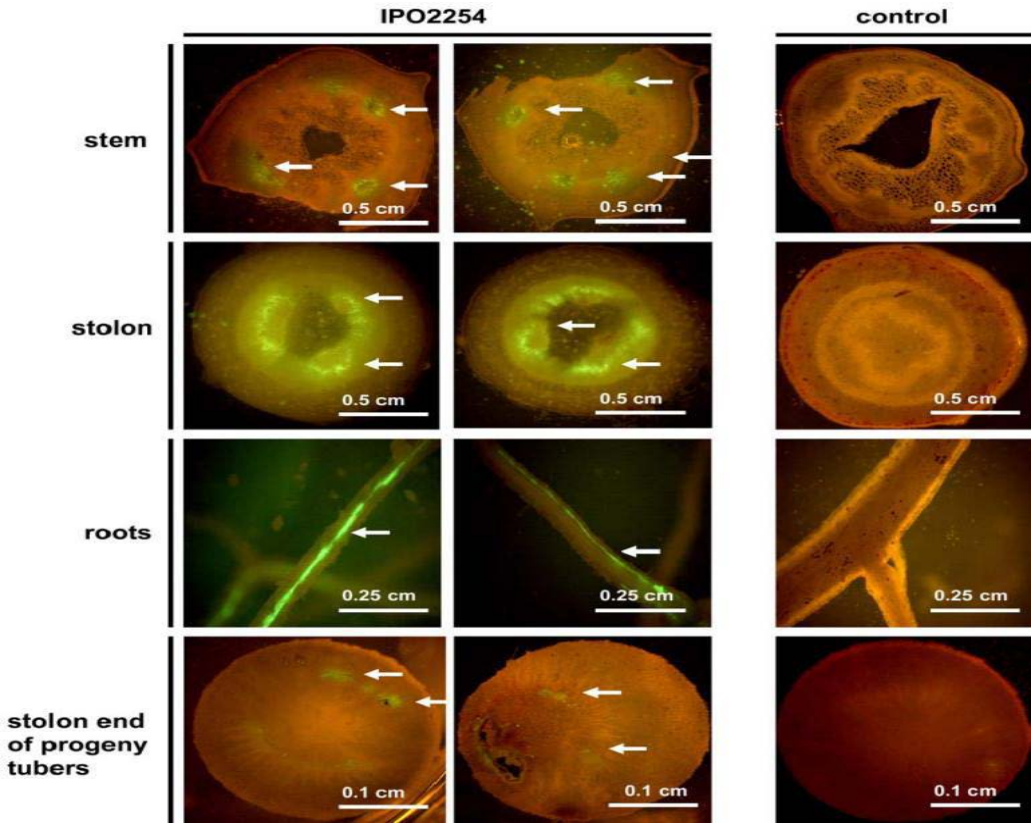


Fig. Colonization of potato stems, stolons, roots and progeny tubers with GFP-tagged Dickeya sp. IPO 2254 at 30 days post stem inoculation analyzed with epifluorescence stereo microscopy. Plant parts, embedded in PT agar for 24 h at 28 C were screened for the presence of a GFP signal. TheGFP signal was found in vascular tissue of stems, stolons and stolon end of progeny tubers, and in pith tissue of roots (white arrows). Control – water inoculated plants. Bacteria in green color

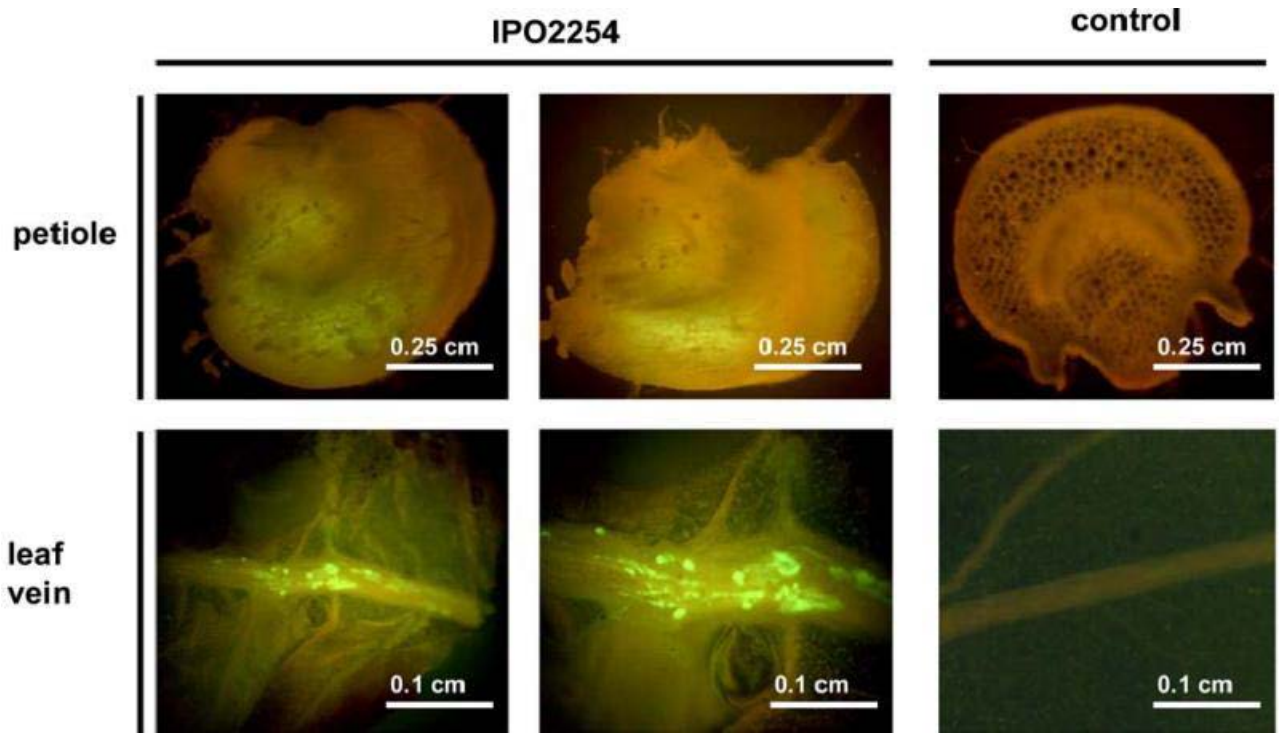
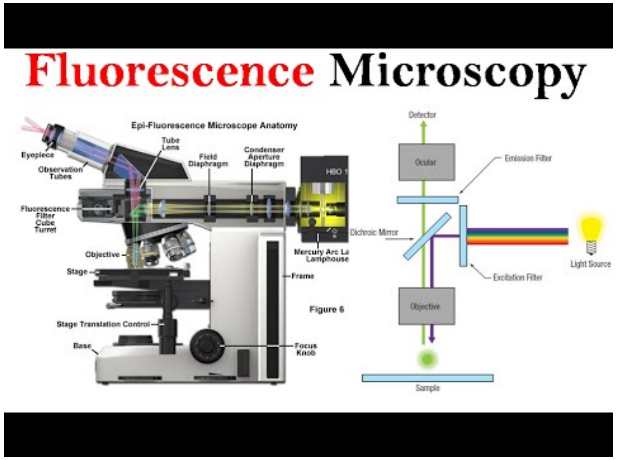
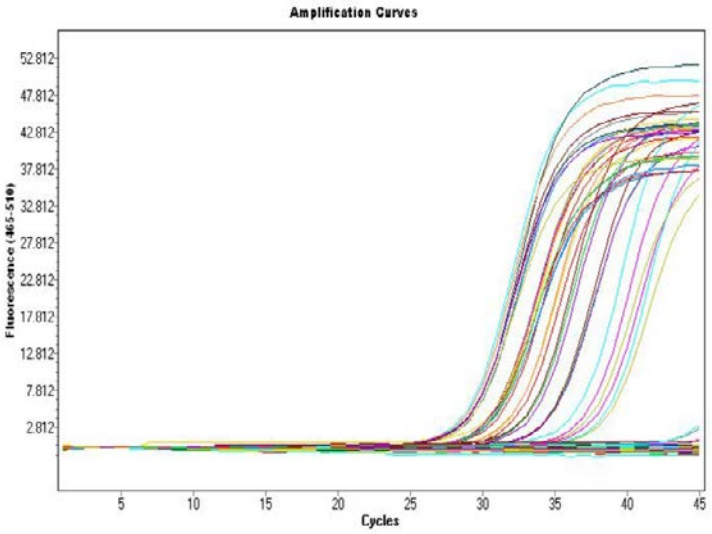
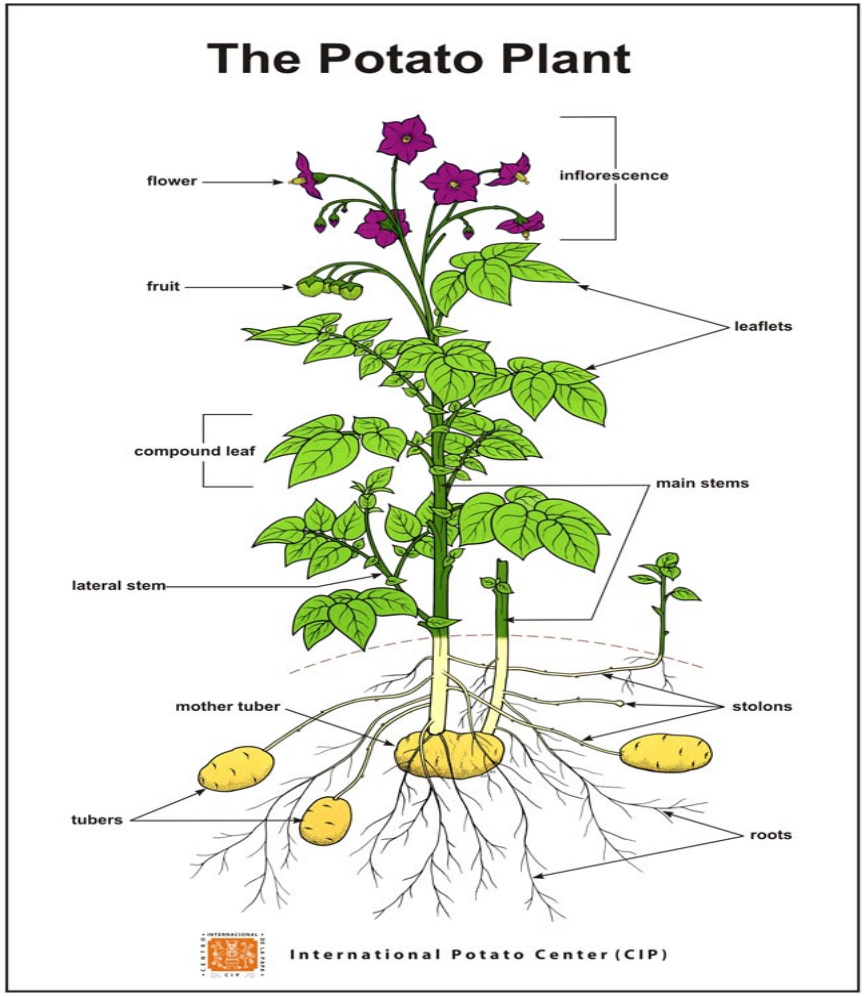


Figure 3. Colonization of potato leaves by GFP-tagged *Dickeya* sp. IPO 2254 at 30 days post leaf inoculation analyzed with epifluorescence stereo microscopy. Plant parts were sterilized and embedded in PT agar for 24 h. Control: water inoculated plants. GFP signal was found inside main veins and inside petioles. Bacteria in green (Czajkowski, *et al*., (2010), 100: 1128-1137)

What happens inside the potato as a result of invasion by the bacteria



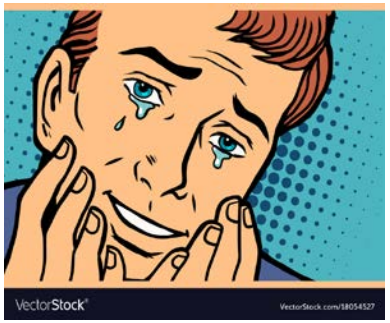




PCR Reveals bacteria latent in the tuber

What we observe in worst case scenario when all conditions for disease development are met

- Inoculum (number, virulence, etc.)
- Temperature and moisture & other environmental factors
- Susceptible potato or potato with compromised resistance



Blackleg and soft rot outbreak proportion – Symptoms and damage

Field Outbreak: Rapidly spreading

Multiplication of *Dickeya* and *Pectobacterium depends*

- Nutrient availability
- Water
- Host resistance

From Latency to disease development

The main environmental factor that drives the shift from latency to disease development is the presence of free water on tubers, which trigers a cascade of events leading to the onset of rotting.

Water film → Anaerobiosis → Swelling of cortical cells → Breaching of the phelloderm layer in lenticels which tends to open.

Pérombelon 2002. Plant Pathology
51: 1-12

Essentials of of seed testing & Predictiveness

REPRESENTATIVE SAMPLE

- ✓ Predictive (**Ennakoiva**) test result
- ✓Lab test correlate to field infection
  - ➢ Lab positive vs Field infection (positive)
    - ➢ Lab negative vs no field infection (negative)
      - ➢ Lab negative vs Field infection (positive)

Seed testing- a collaborative work

Coordinated and close cooperation among players along the seed potato value chain.

- Seed producers
- Seed distributing companies
- Seed testing laboratories

Diagnostics is Dynamic and evolving

Disease Diagnostics: dynamic and evolving.
”We need better methods for diagnosis: none of the methods given are to be considered as ’standardised’ to think of them in such a way would put an end to efforts of improvement. They are useful only until better procedures can be developed” ((Riker & Riker, 1936)

Introduction to research in plant diseases- a guide to the principles and practices for studying various plant disease problems)

A valid saying after more than 80 years

LUKE Oulu consistently working on standardizing and improving the diagnostic methods

Advances Required

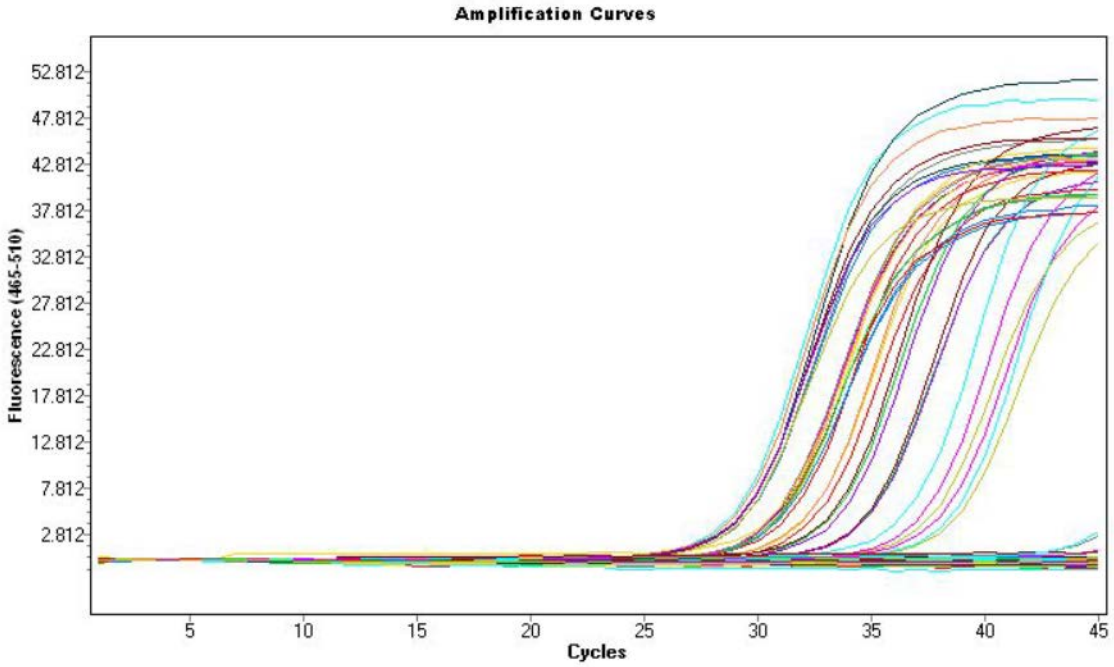
Technical and Economic Demands

- Sensitivity and specificity
- Discrimination between Viable & Dead pathogen
- Multiplexing (as many pathogens/reaction)
- Short diagnosis time (speed) and cost effectiveness
- High throughput
- Quantification
- Robustness
- Validation and standadization

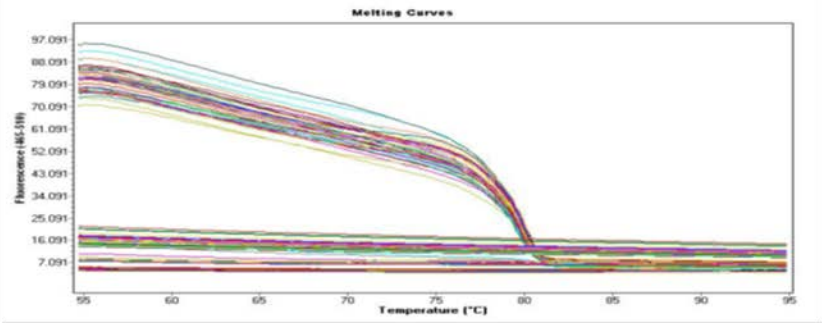
Moving from End Point to Real Time PCR at LUKE OULU Molecular Biology Laboratory

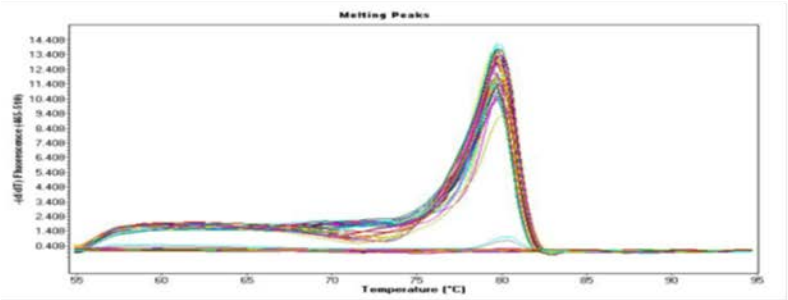
- Reduced work flow
- Cost effective
- Specific
- Sensitive

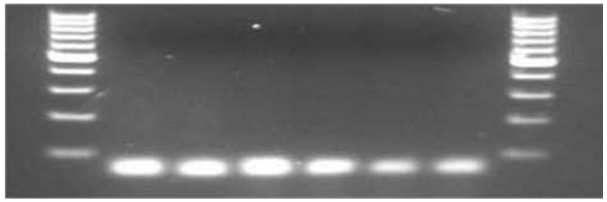
Detection in Real Time: Quatification, speed and specificity



Melting curve analysis- Testing specifity







Conclusions

- *Dickeya* and *Pectobacterium* cause soft rot and blackleg on potatoes
- Latent infection is widespread in tubers and stems
- Bacterial multiplication is initiated and disease develops when host resistance is impaired
- Succeess in seed testing and predictabilty of results depends on process coordination and networking along the value chain
- Seed testing pays off
- There is risk in too much tolerance.

Thank you for your attention