Controlling Soft Rot Bacteria through Epidemiology and Resistance Screening

*Sonia Humphris*, *Emma Campbell*, *Leighton Pritchard*, *Kath Wright*, *Sophie Mantelin*, *John Elphinstone*, *Gerry Saddler* and *Ian Toth*

1The James Hutton Institute, Invergowrie, Dundee, DD2 5DA
2The Food and Environment Research Agency, York, YO41 1LZ
3SASA, Roddinglaw Road, Edinburgh, EH12 9FJ
Objectives

1. Gain a greater understanding of the epidemiology of *D. solani* in relation to host range, its ability to survive and spread in the environment after introduction of infected crops.

2. Identify susceptible and resistant pre-breeding material in the potato collections held at James Hutton Institute to produce genetic crosses. Screen these crosses for co-segregation of resistance to *Pba* and *D. solani.*
Role of alternative hosts in the establishment and spread of *D. solani* in the environment?
**D. Solani Colonisation of Roots**

**Colonisation of weed roots by D. solani 2222**

![Bar chart showing colonisation of weed roots by D. solani 2222](chart.png)

- **P. annua**
  - 1 dpi: log CFU/ml
  - 14 dpi: log CFU/ml

- **S. dulcamara**
  - 1 dpi: log CFU/ml
  - 14 dpi: log CFU/ml

The chart indicates the colonisation level of two weed species, **P. annua** and **S. dulcamara**, by *D. solani* at 1 and 14 days post-inoculation (dpi).
Survival of *Dickeya solani*

- Four weeds species chosen:
  - Annual Nettle; Field Pansy; Shepherd’s Purse and OSR

- Five replicate pots of compost set up with the following:
  1. 4 week old seedlings from 4 weed species inoculated with *D. solani*
  2. Soil inoculated with *D. solani*, no weeds
  3. Un-inoculated control soil containing 4 week old seedlings

- Pathogen free tuber planted into all pots 3 weeks after inoculation

- Both roots and stems were tested for the presence of *D. solani*
Survival of *D. solani* on Weed Roots

**D. solani Weed Root Colonisation**

![Bar chart showing survival of *D. solani* on various weed roots and soil over different time periods.](chart_image)

- **Plant Species**: Shepherd's Purse, Annual Nettle, Field Pansy, OSR, Soil
- **Time Periods**: Day 1, 1 month, 2 month, 3 month
- **CFU/ml**: Log scale

**Legend**:
- Day 1
- 1 month
- 2 month
- 3 month
Survival of *D. solani* in Weed Stems

*D. solani* Weed Stem Colonisation

- **Shepherd’s Purse**
- **Annual Nettle**
- **Field Pansy**
- **OSR**

Log CFU/ml

- 1 month
- 2 month
- 3 month
Spread of *D. solani* to Potato Plants/Tubers

- Samples were taken from:
  - Potato stems
  - Potato roots
  - Progeny tubers
  - Mother tubers

- *D. solani* was not detected on the potato plants or tubers
Spread of *D. solani* from weeds to potato plants/tubers under waterlogging conditions

- Three weeds species chosen:
  
  Annual Nettle; Field Pansy and Shepherd’s Purse

- The individual weed species planted into smaller 25cm pots.

- After 3 weeks, a pathogen free tuber was planted into the centre of all pots.

- Pots were submerged in boxes filled with water for 1 week and then removed for 1 week.
Spread of *D. solani* From Weeds to Potato Plants/Tubers

*D. solani* spread from inoculated weeds to potato plants in water-logged soil

![Graph showing the spread of *D. solani* from inoculated weeds to potato plants in water-logged soil.

- **Shepherd's Purse**: Log CFU/ml of potato stems, potato roots, daughter tubers, and mother tubers.
- **Annual Nettle**: Similar data as Shepherd's Purse.
- **Field Pansy**: Similar data as Shepherd's Purse.

The graph indicates higher log CFU/ml values for potato roots compared to potato stems, daughter tubers, and mother tubers in all three weed species tested. The error bars represent the variability in the data.
Confocal Microscopy

- Nettle and Meadow Grass seedlings grown in sterile conditions for 4-5 weeks.
- Fluorescent dyes were added to the buffer 1 day before infection to stain the plant cells.
- The staining solution was removed and the plants infected with *D. solani*+GFP in fresh buffer.
Images of Nettle Roots
Images of Meadow Grass Roots
Nettle Colonisation

- Are higher levels of *D. solani* responsible for colonisation in weed roots?
  - Set up experiment with nettle using 3 different concentrations ($10^7$, $10^5$, $10^3$) of *D. solani*. 
Colonisation using lower inoculum levels

![Graph showing bacterial concentration and nettle colonisation at 1 dpi.](image)
Colonisation of Nettle Root and Stem – 28dpi

10^7 CFU/ml
Summary – Objective 1

- *D. solani* can survive on the roots of actively growing weeds for at least three months and appears to be systemically colonising Nettle and Field Pansy.

- Although weed species became colonised, spread to potato plants or daughter tubers in the soil was only detected under heavily waterlogged conditions using a high inoculum of bacteria.

- Lower concentrations of *D. solani* (10^5, 10^3 CFU ml⁻¹) did not result in detectable colonisation of the roots or stems of nettle.

- Confocal microscopy of nettle and meadow grass roots showed *D. solani* within epidermal and cortex cells.
Objective 2

1. Gain a greater understanding of the epidemiology of *D. solani* in relation to host range, its ability to survive and spread in the environment after introduction of infected crops.

2. Identify susceptible and resistant pre-breeding material in the potato collections held at JHI to produce genetic crosses. Screen these crosses for co-segregation of resistance to *Pba* and *D. solani*.
Resistance Screening

Resistance of Phureja Tubers to Pba 1039

Weight of Rot (g)

Tuber rot mean weight

Population ‘A’
Resistance Segregation - \textit{Pba} 1039?

Resistance of population ‘A’ clones to \textit{Pba} 1039

14 dpi

Average Lesion Length (mm)
New Genetic Crosses

- Four new populations have been produced using clones that are resistant and susceptible to both *D. solani* and *Pba*.
- These populations will be screened for co-segregation of resistance to *D. solani* and *Pba*.
Development of a Seedling Test

One-month-old soil grown potato plants (cv Estima) were inoculated using five different methods:

1. Dipped in *Dickeya* solution.
2. Dipped in *Dickeya* solution and plant placed under vacuum.
3. Two leaves damaged and plant dipped in *Dickeya* solution.
4. Two leaves damaged, dipped in *Dickeya* solution and plant placed under vacuum.
5. Blunt syringe infiltration of one leaf.

Plants were incubated in a growth room at 21°C.
Plants remained asymptomatic even after 2 weeks. Same result if vacuum applied after dipping.
Leaves developed maceration symptoms one day post inoculation. The lesions co-localized with the damage.

After 7 days the tissues were necrotic but the symptoms did not spread further. Same result if vacuum applied after dipping.
Seedling Test – Blunt syringe infiltration

After 1 day there was complete maceration of the leaf tissue at the infiltration spot which then expanded to the entire leaf.

After 7 days the leaf fell off but the plant looked completely healthy.
Found segregating resistance in 07.H.128 population after stem inoculation with *Pba* 1039.

Have identified another four clones for genetic crosses, two of which are resistant to both *Pba* and *D. solani* and 2 which are susceptible.

Have seen promising results using a new seedling test with blunt syringe infiltration of leaves.
Future Work

- Establish if *D. solani* can move systemically from the roots to the stems of weeds.
- Test the stems of the remaining 07.H.128 clones with *Pba* 1039.
- Test the tubers of 07.H.128 population with *Pba* for co-segregation of resistance.
- Repeat seedling tests using blunt syringe infiltration to test different cultivars of potato and concentrations of bacteria.
Acknowledgments

Ian Toth
Kath Wright
Emma Campbell
Leighton Pritchard
Sophie Mantelin

Gerry Saddler
Greig Cahill
Rachel Kelly

John Elphinstone
Neil Parkinson