Controlling Soft Rot Bacteria through Epidemiology and Resistance Screening

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Objectives


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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?

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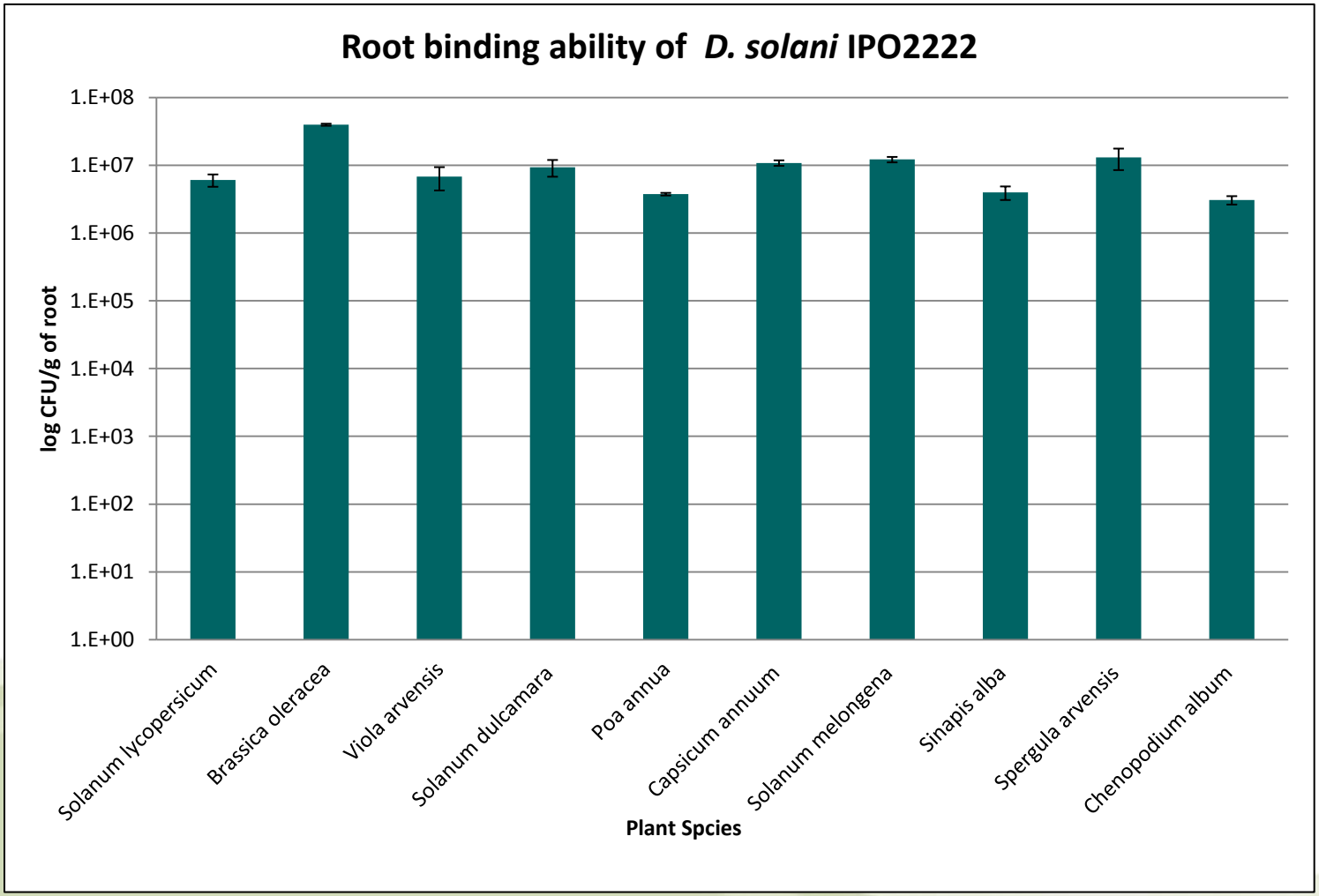
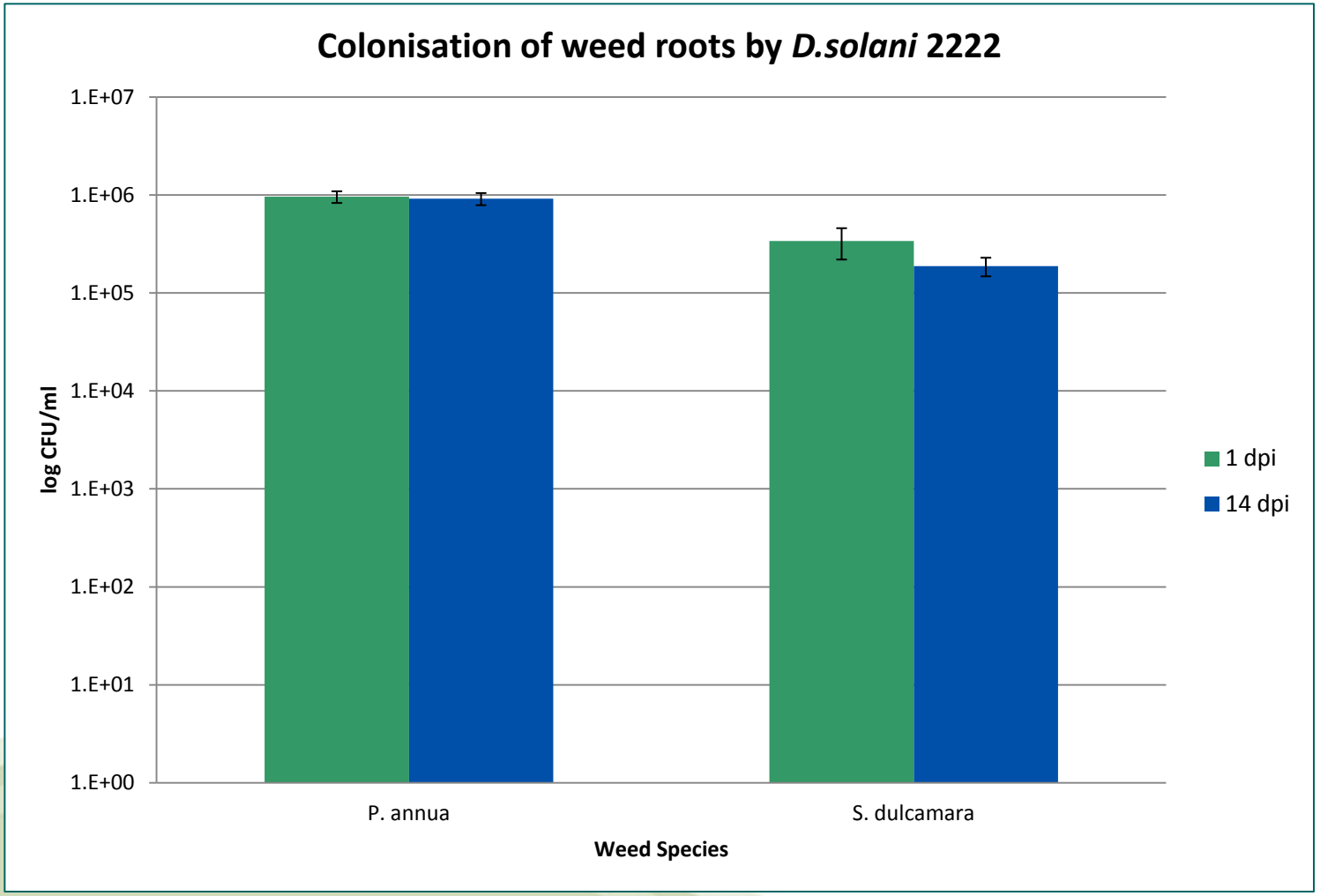


D. Solani Colonisation of Roots



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

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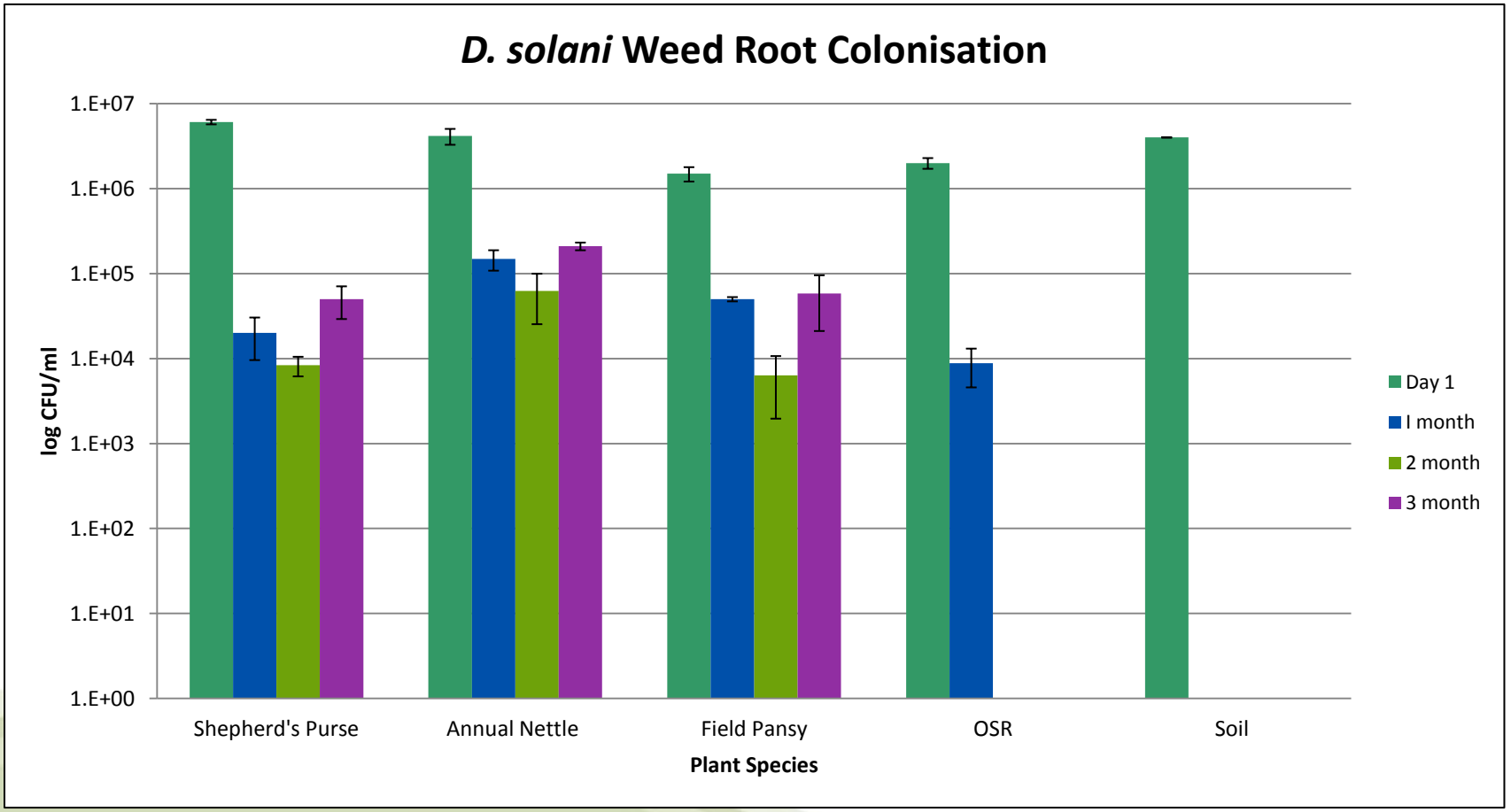
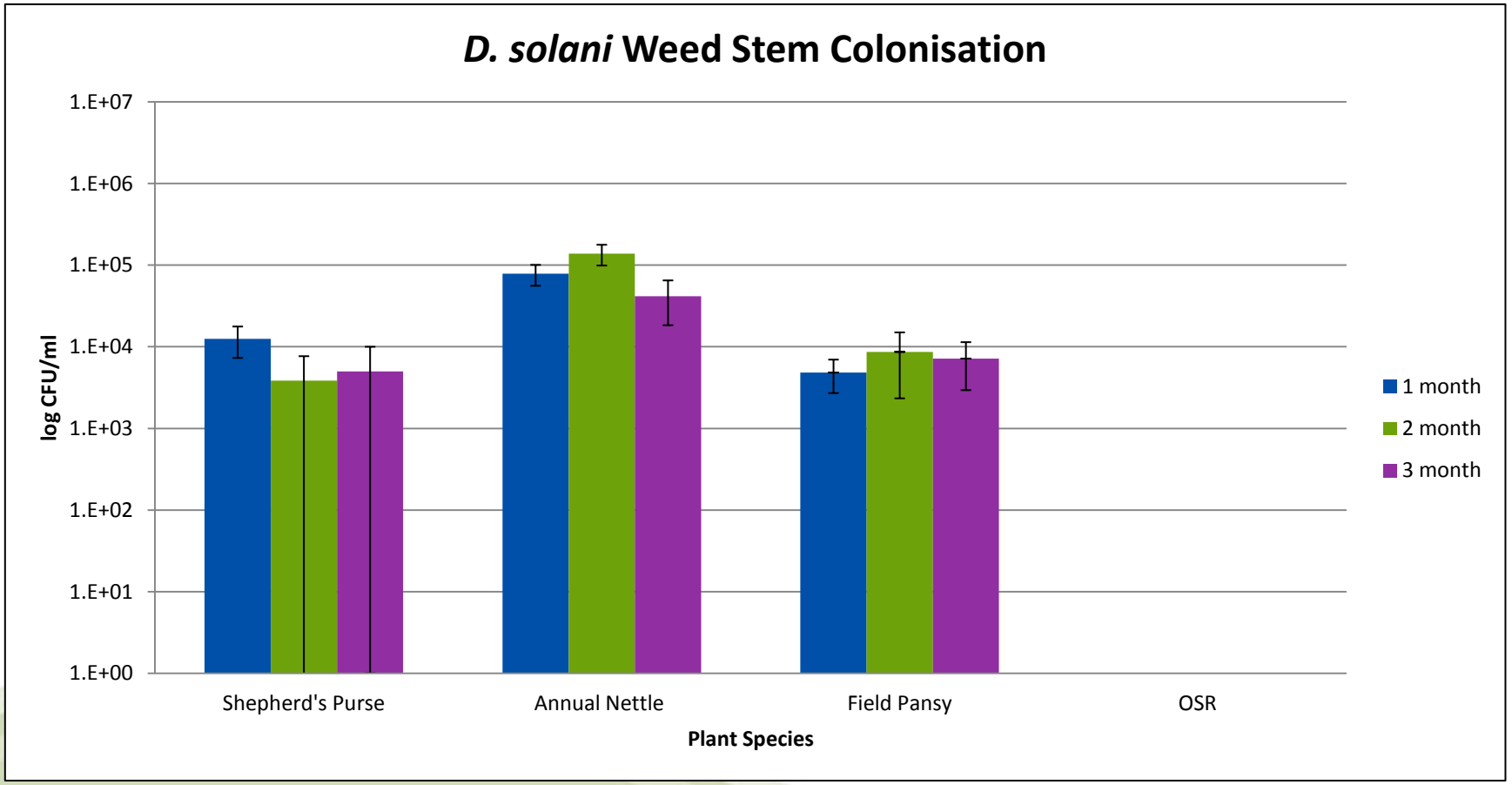


Survival of *D. solani* in Weed Stems



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

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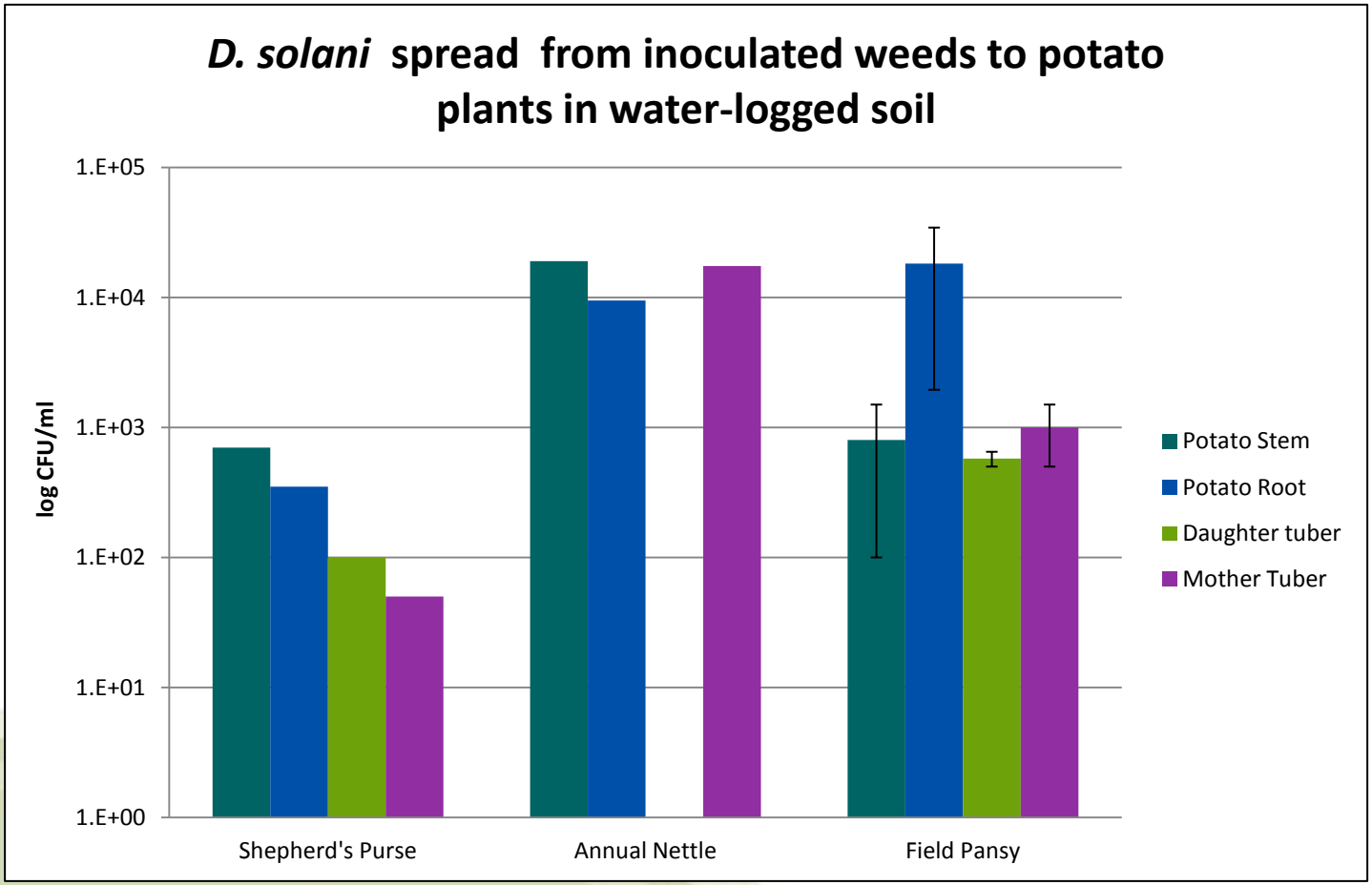

Confocal Microscopy

- 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

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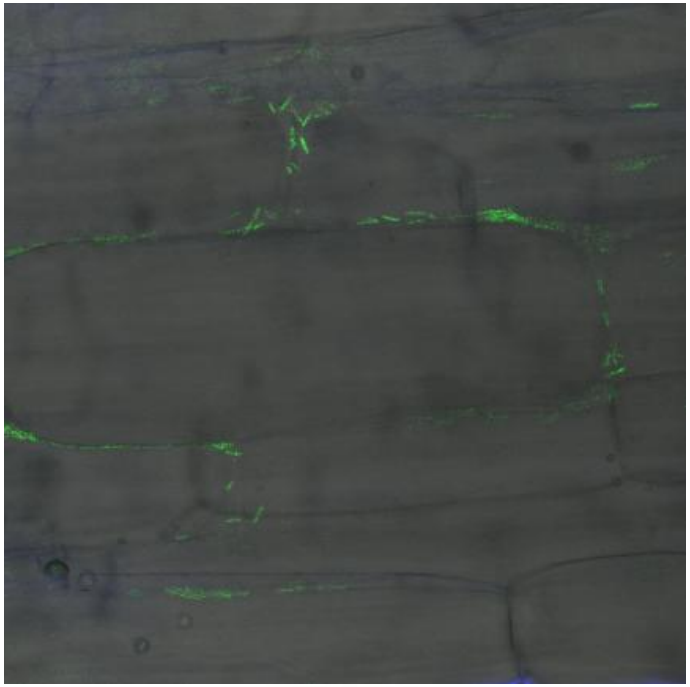

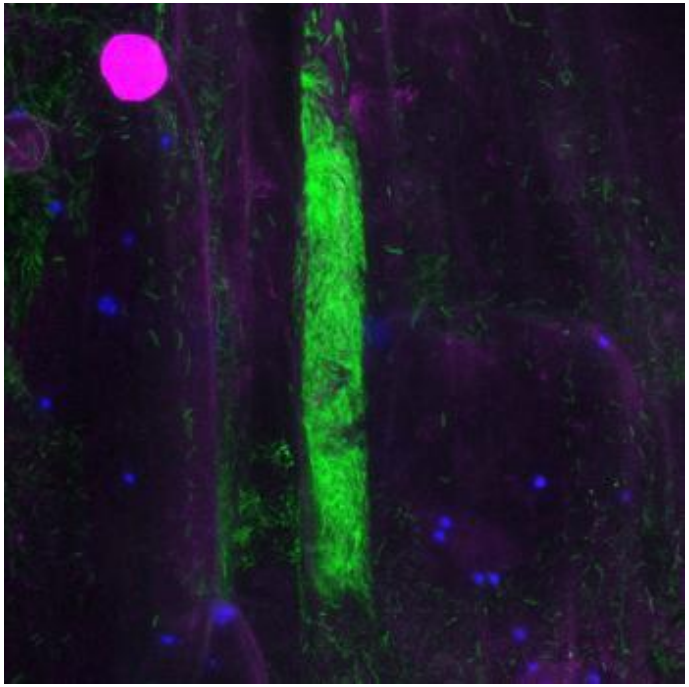

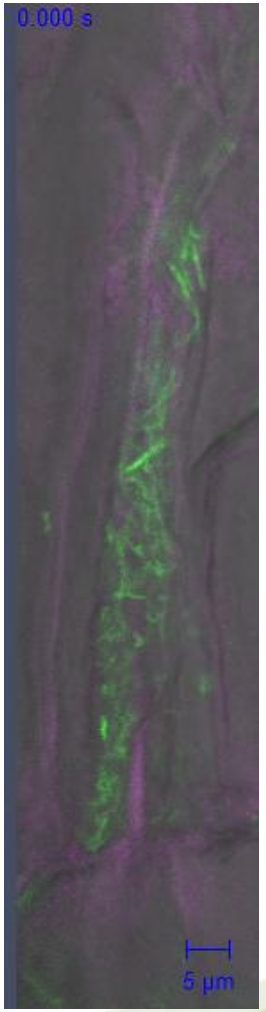

Images of Meadow Grass Roots

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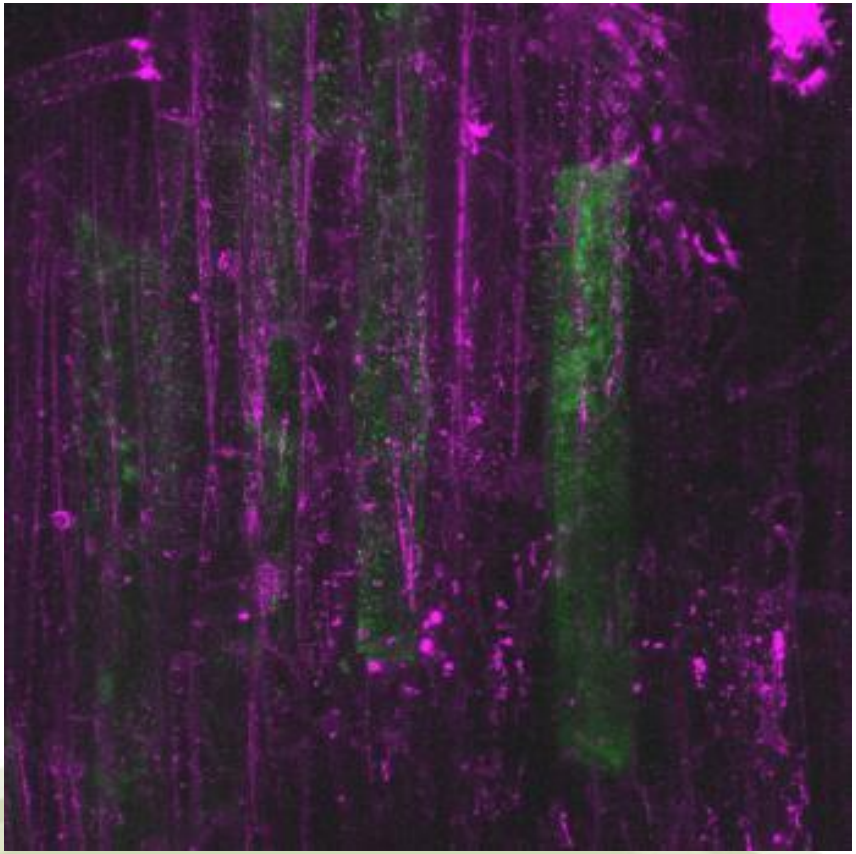
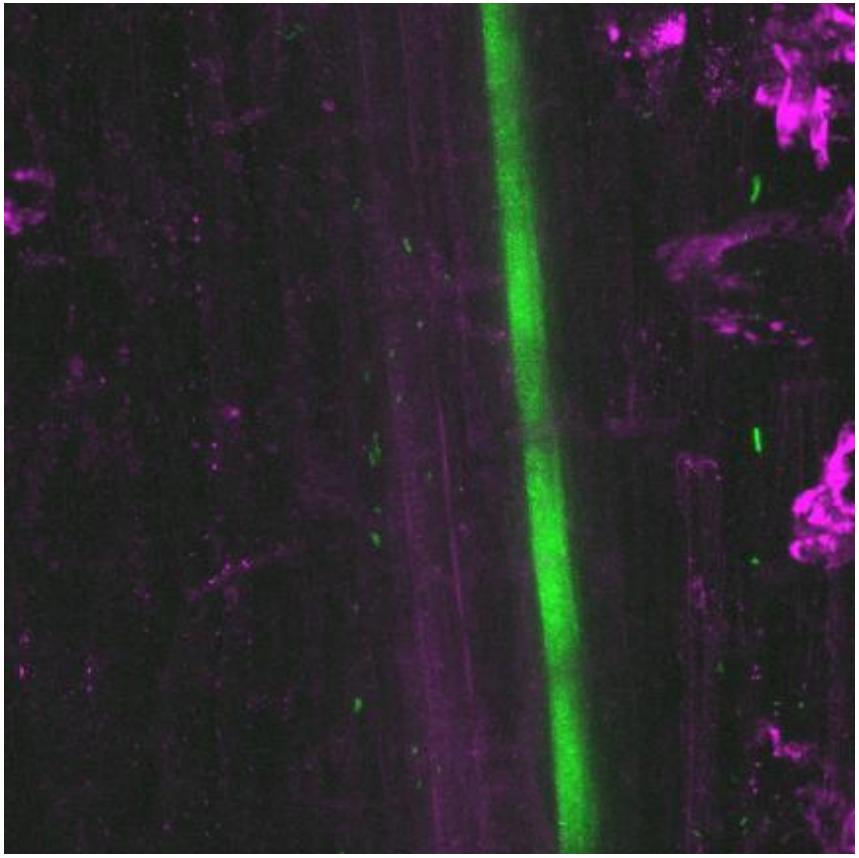
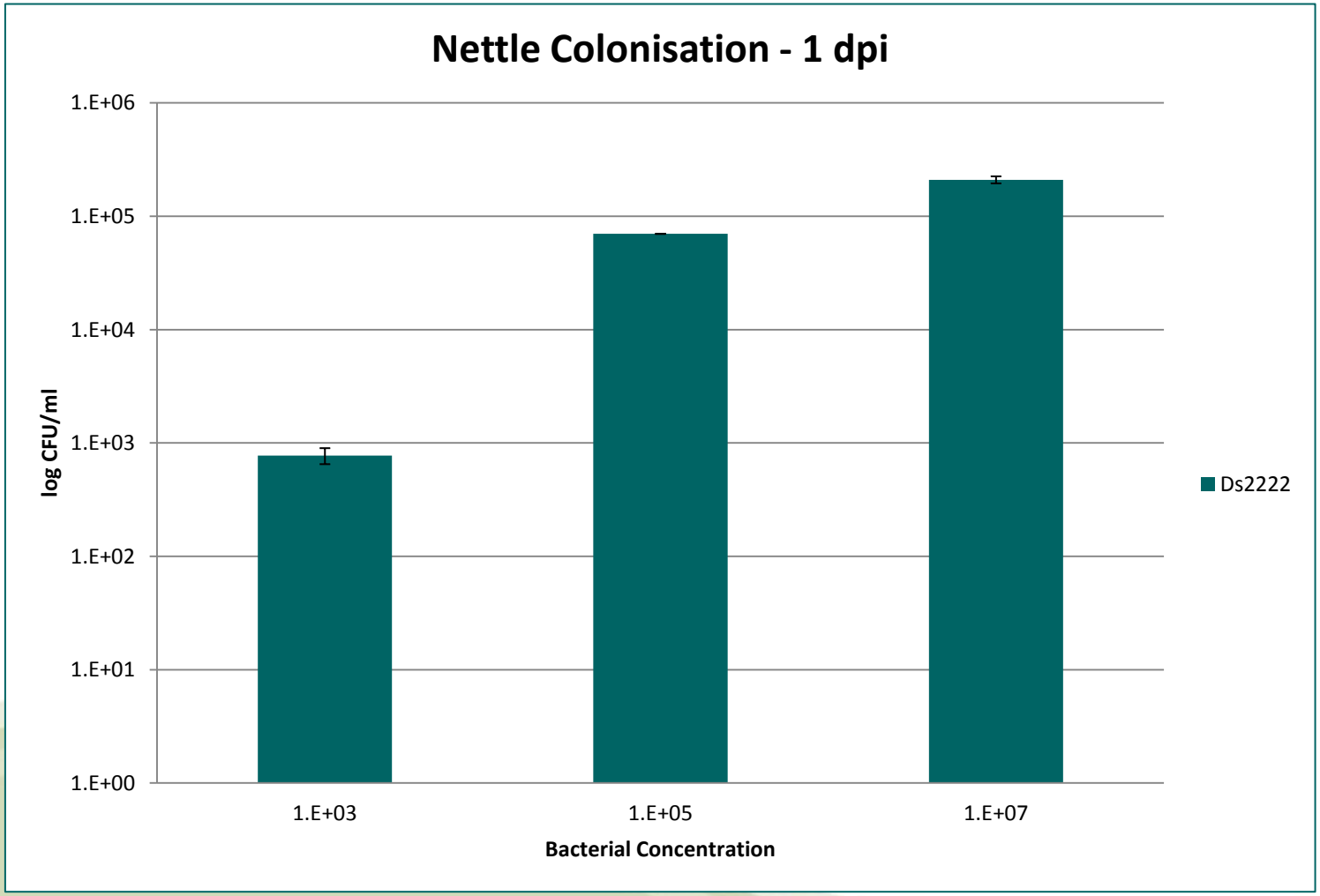




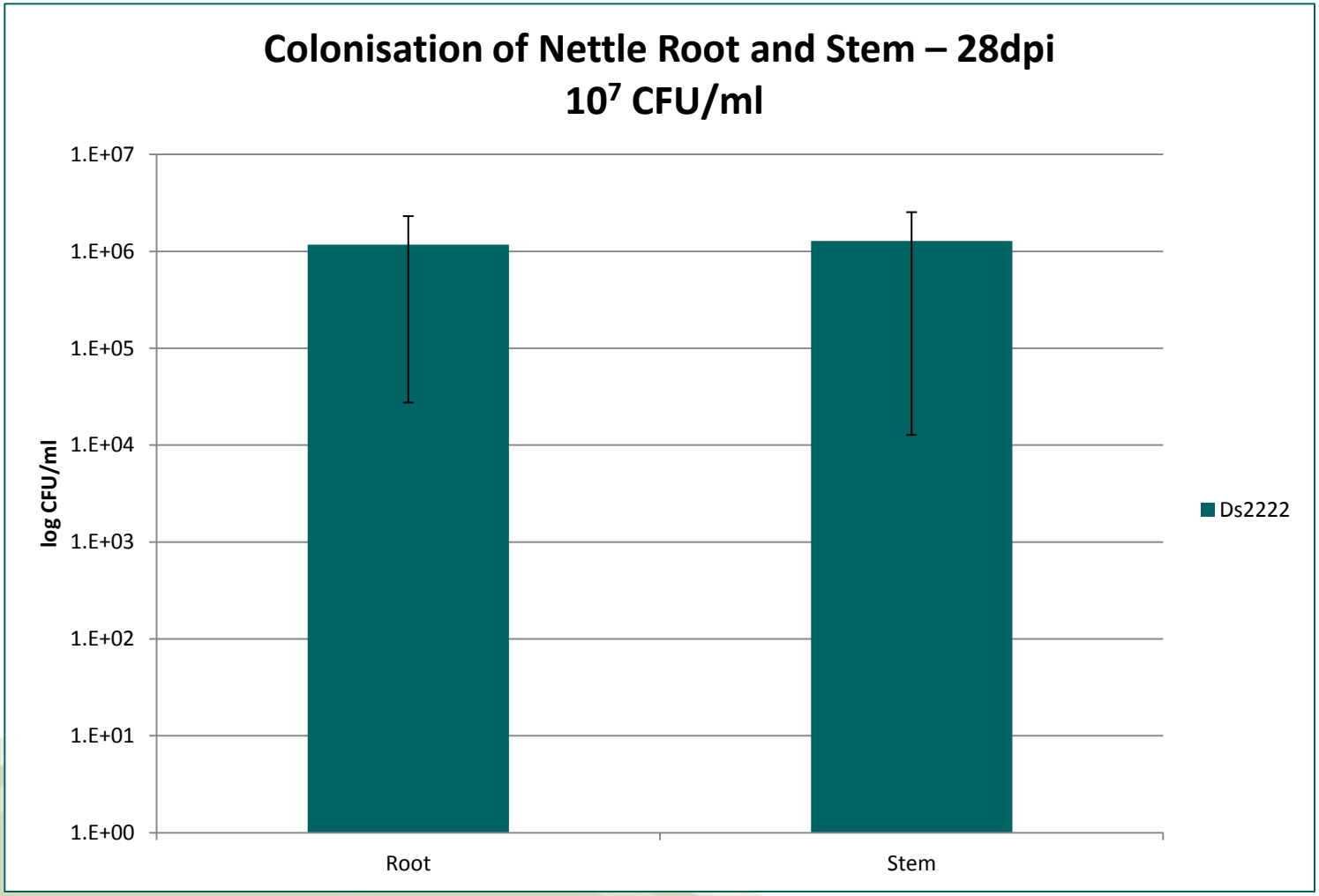
Nettle Colonisation

- 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



Colonisation using lower inoculum levels



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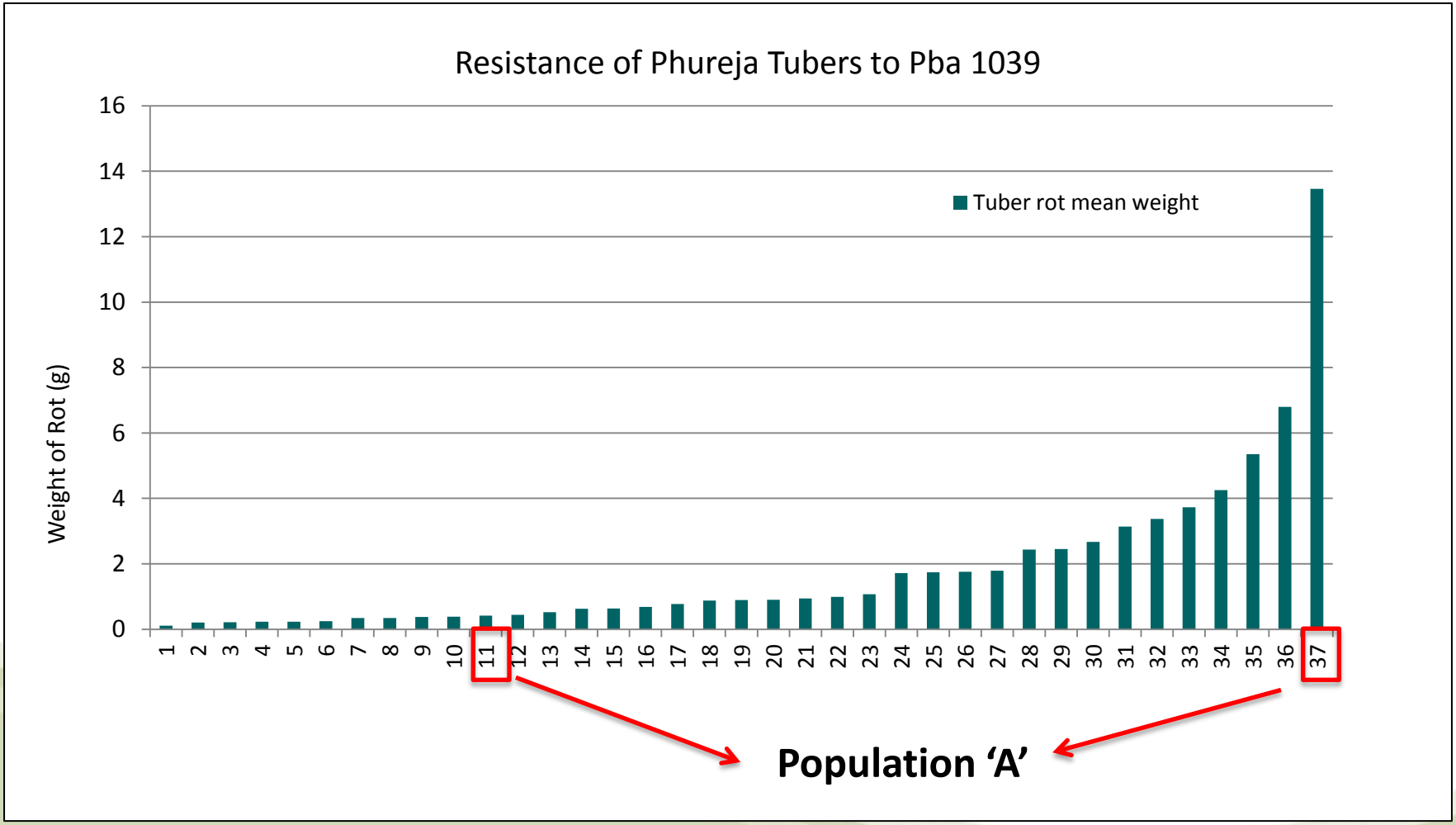
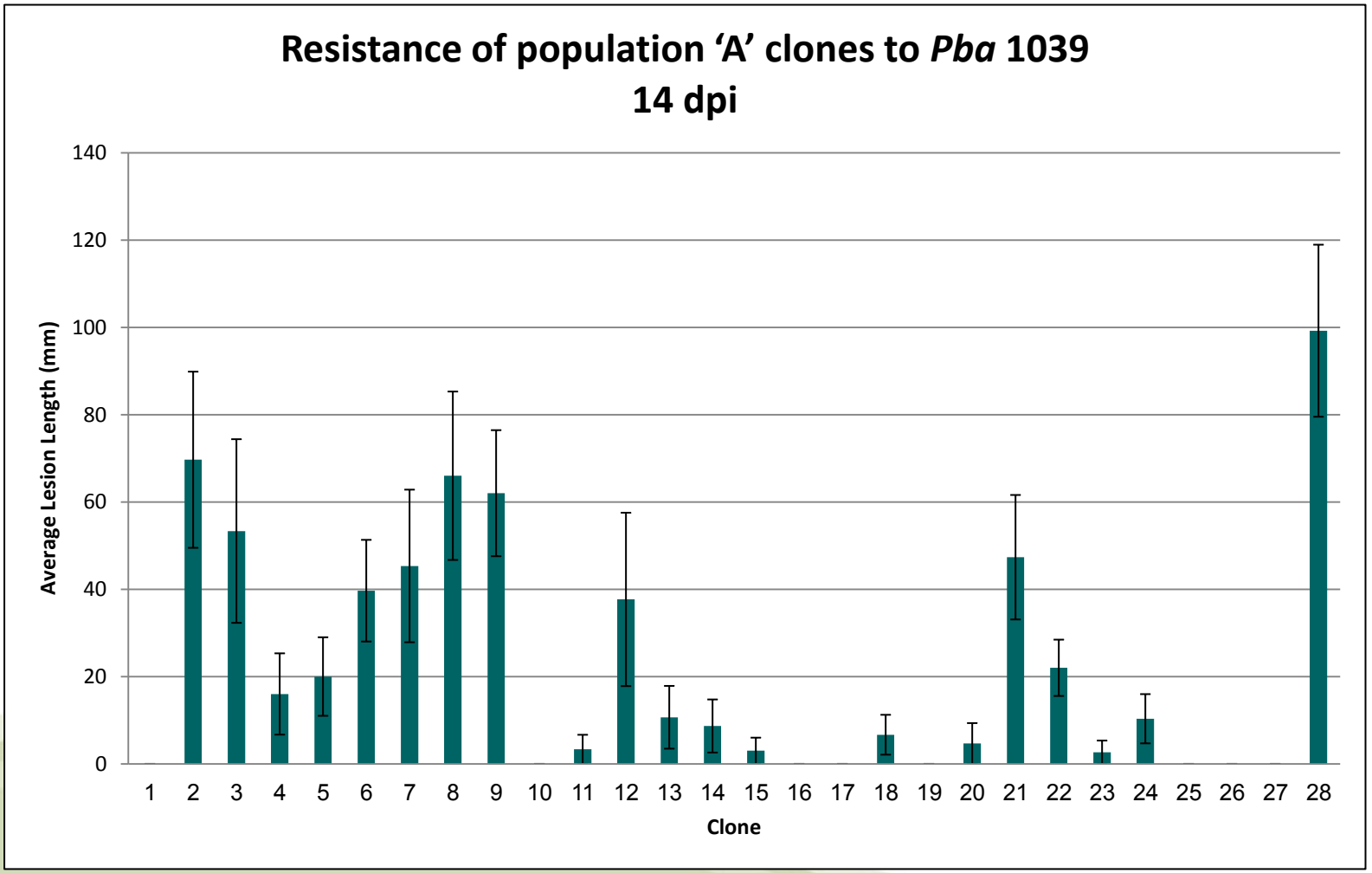
1. Gain a greater understanding of the epidemiology of *D. solani* in relation to host range, its ability to survive and spread in the environment after introduction of infected crops

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 Segregation - *Pba* 1039?



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.

Seedling Test - Dipping

Plants remained asymptomatic even after 2 weeks. Same result if vacuum applied after dipping.

Seedling Test – Damage and Dip

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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

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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.

Summary – Objective 2

- 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

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