Understanding the ecology and epidemiology of *Pythium violae* causing cavity spot in carrot

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Overview

- The problem…
- Background: UK cavity spot
- PhD aims
  - *Pythium* identification
  - *P. violae* capture and detection
  - Artificial inoculation
  - *P. violae* dynamics
- Future work

The problem

- Identified by UK carrot growers as their biggest disease problem
- 2012 home production marketed at £126.4 million (DEFRA, 2014)
- Cavity spot disease causes losses estimated at £3-£5 million per annum (Martin, 2013)

The problem:

What causes cavity spot?

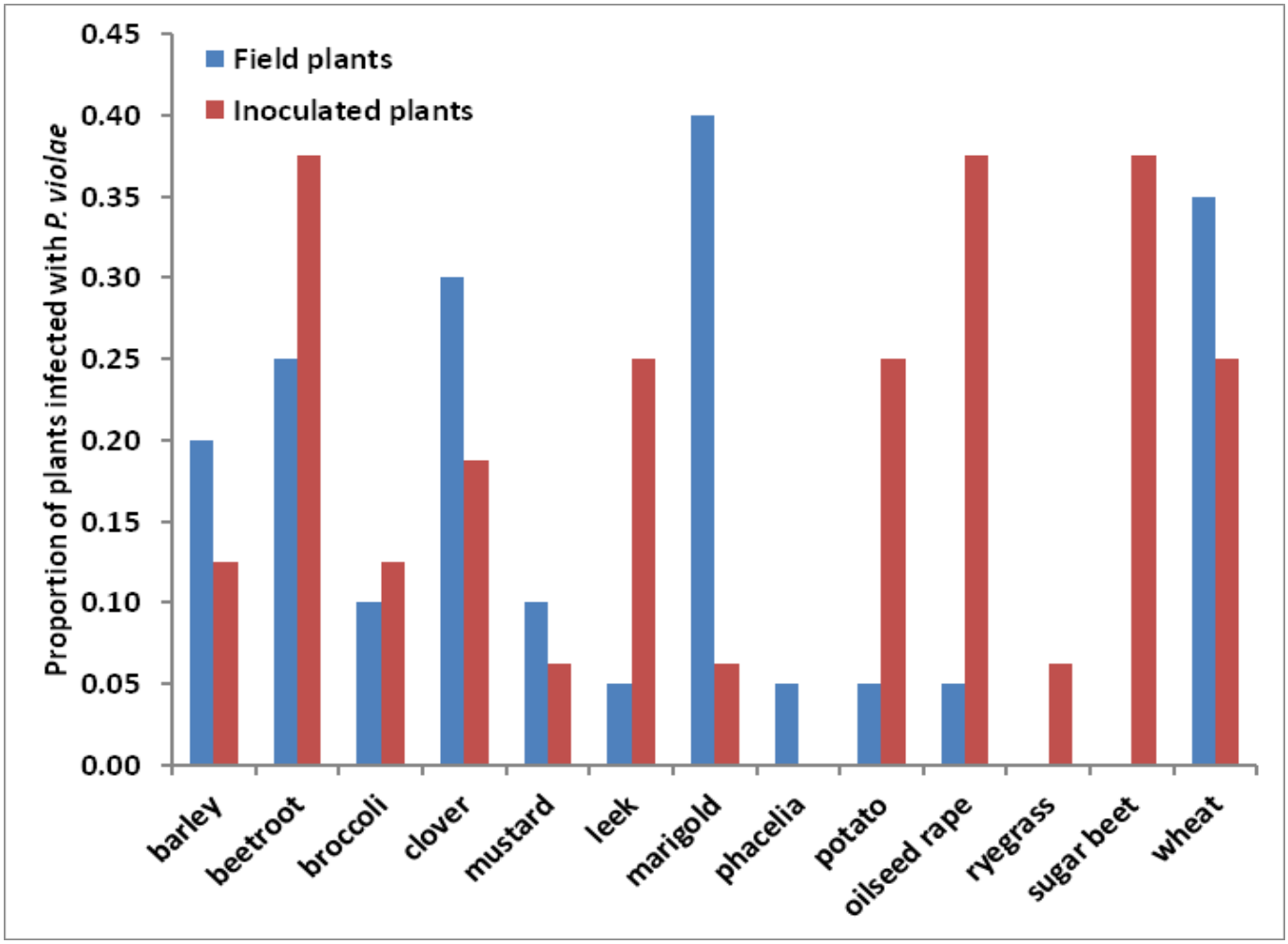
- Primarily the oomycete ***P. violae*** (in the UK)
- Can colonise many plant species, but does not cause disease in the majority

- Control relies on the fungicide metalaxyl, but levels of control are diminishing with growers reporting a less than 50% success rate.

Host range of *P. violae*

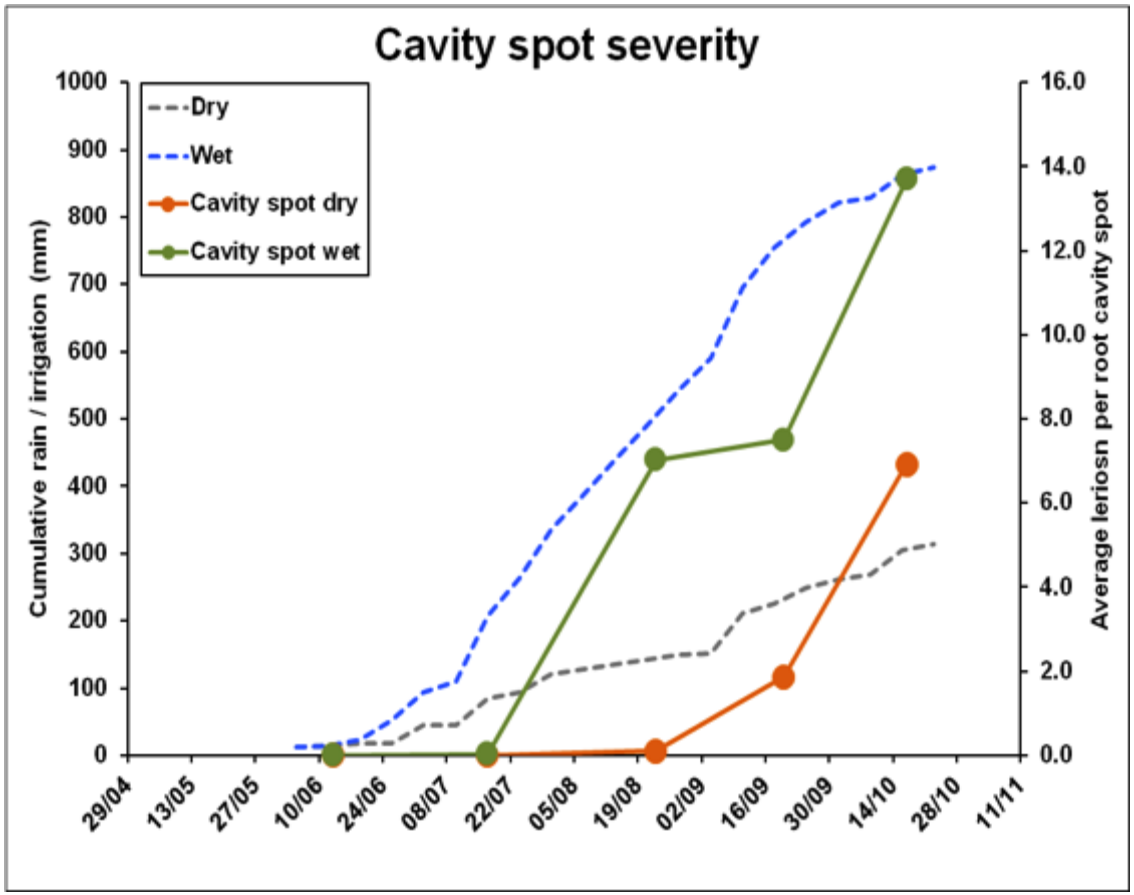
- **Anne Kretzschmar PhD thesis 2009**

- Other crop plants can sustain *P. violae*



Effect of soil moisture

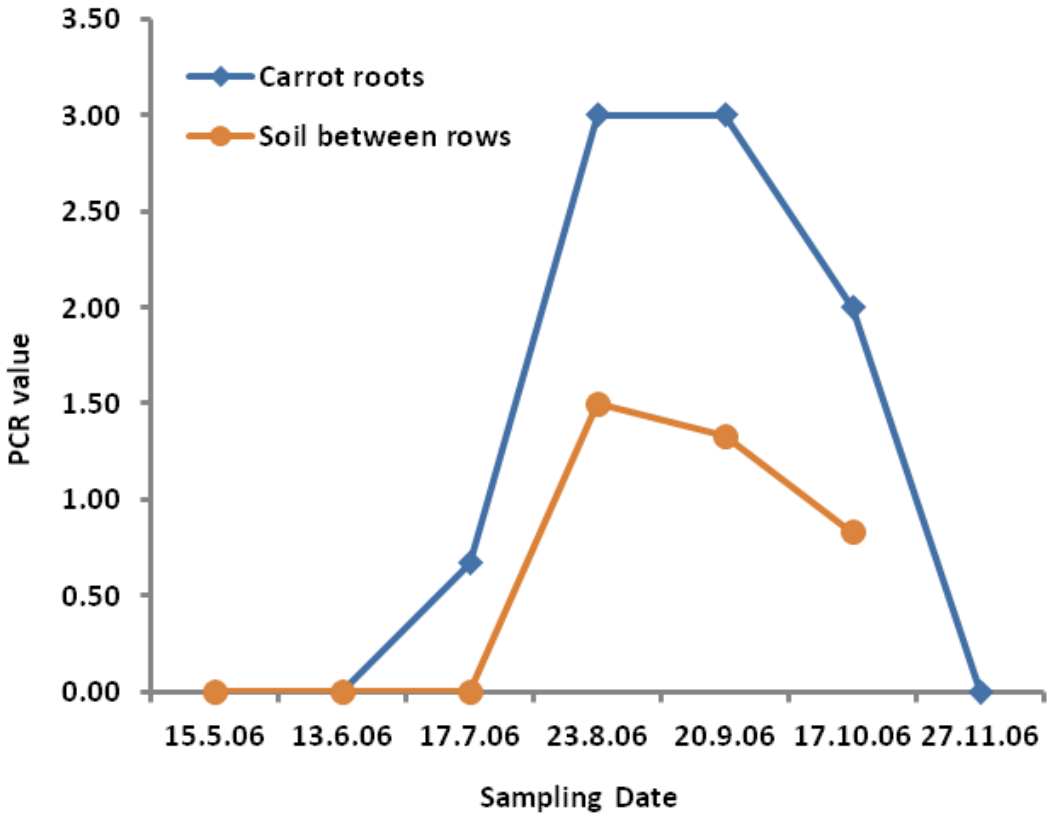
- Waterlogged / poorly drained soils usually increase levels of cavity spot
- Correlation of total water input and disease levels in some years (Grower funded project, AHDB)
- 30-45 mm / week of trickle irrigation increased cavity spot (Grower funded project, AHDB)



Current problems:

Lack of research tools

- DNA based PCR detection needs refining
  - would enable dynamics of pathogen to be better assessed
- No artificial inoculation system
  - major barrier to assessing new treatments and effects of environment

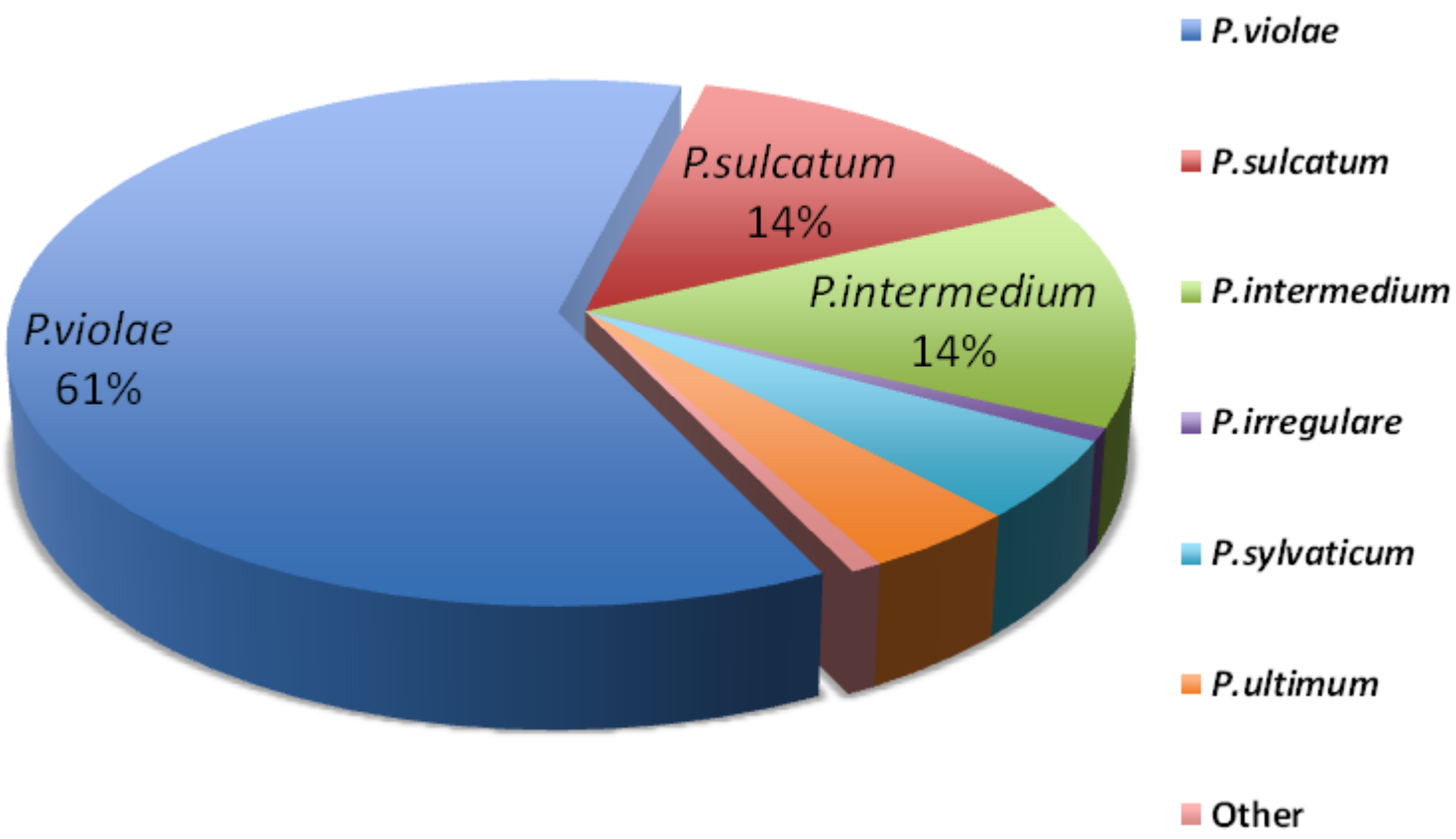


PhD Aims

- **Develop effective tools for *P. violae* research:**
  - Collect and **characterise multiple isolates** of *Pythium* from carrot
  - Develop an **improved sampling and/or DNA extraction** method from soil
  - Develop a more **robust and accurate PCR test** for *P. violae*
  - Develop a **reproducible *P. violae* inoculation system** for carrot
- **Investigate year-round dynamics and ecology of *P. violae***
  - Assess the **dynamics of *P. violae*** on carrot crop throughout the year
  - Explore the **community of microbes** associated with cavity spot in lesions and/or soil

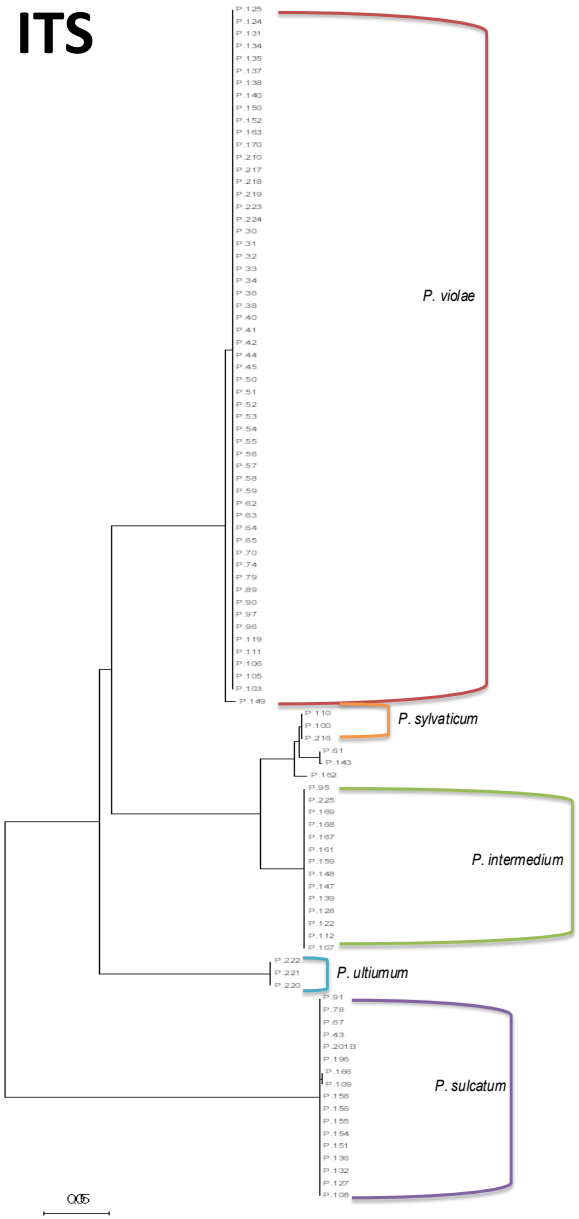
Pythium isolate collection

Pythium isolate identification

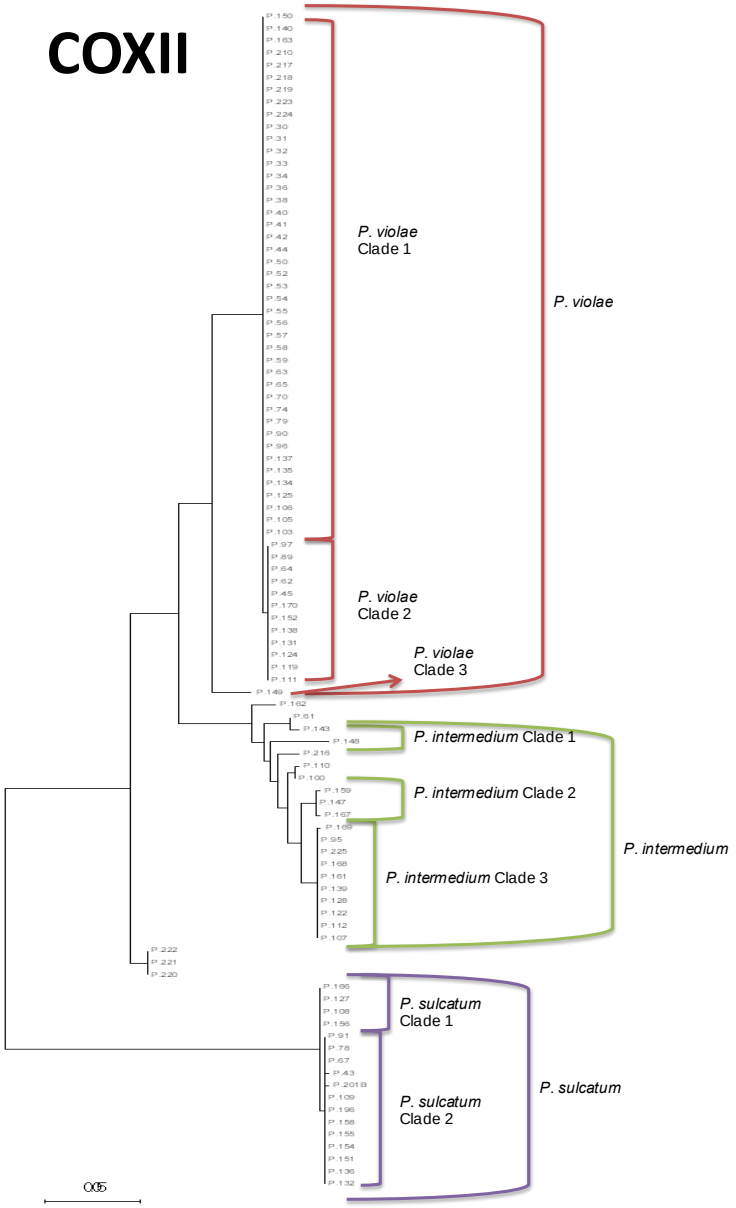


Characterisation: Phylogenetic analysis

ITS



COXII



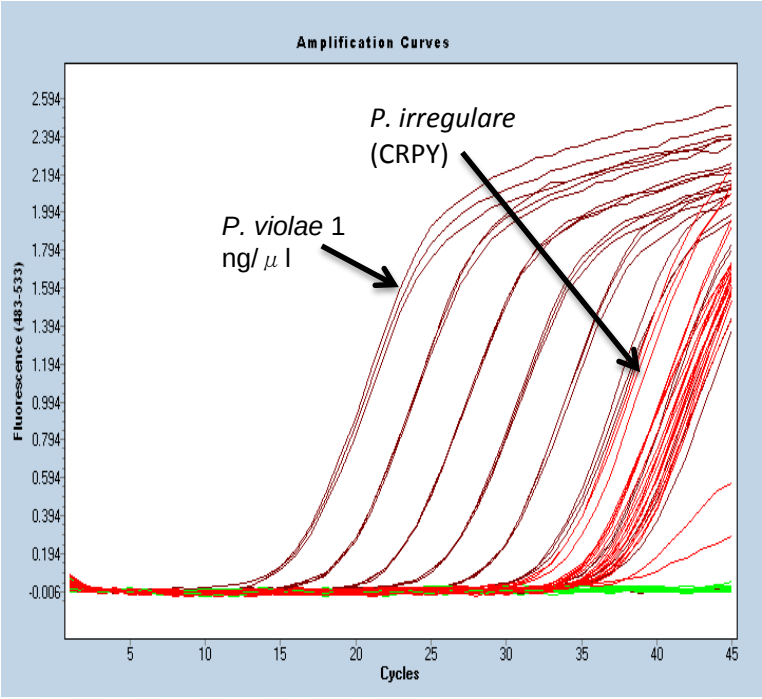
Improvement of *P. violae* capture

- Previous methods to detect *P. violae* DNA from soil use 0.25g soil
- Due to patchy nature of *P. violae*, it may be missed during sampling
- A method of capturing oospores from soil based on flotation of spores in sucrose solution has been developed

P. violae detection and quantification

Sul: *P.sulcatum*, Ult: *P.ultimum*, Lut: *P.lutarium*. Irr: *P.irregulare*, Syl: *P.sylvaticum*, Int: *P.intermedium*, Viol1/2: *P.violae*

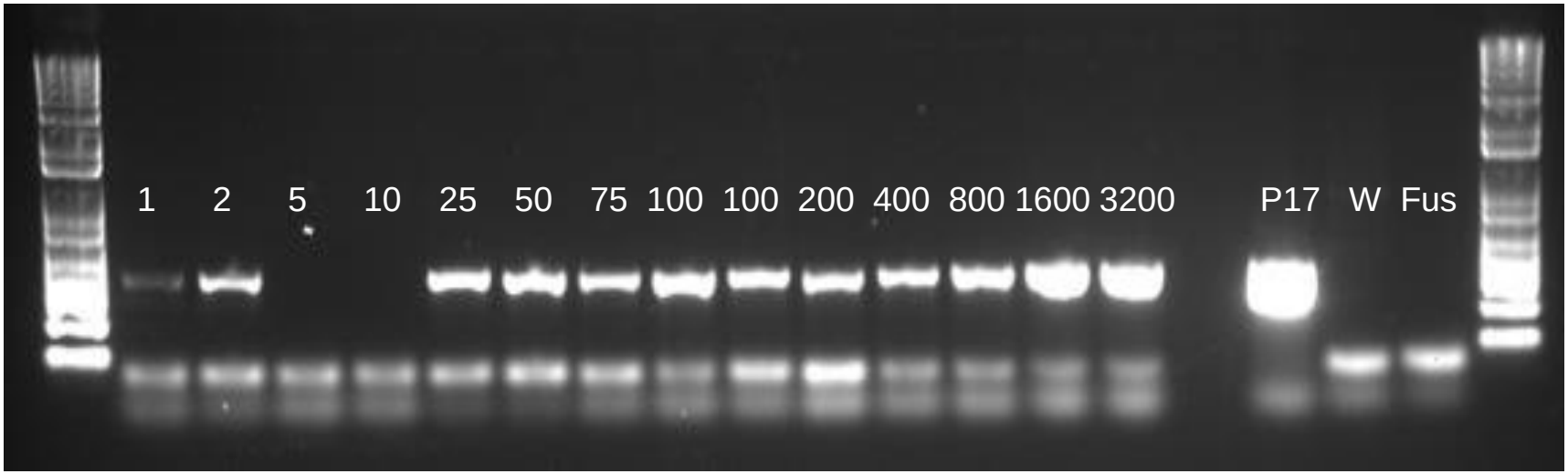
- Published primers were found to cross-react with other *Pythium*'s
- Development of new primers reduced amplification of other *Pythium* species



- The primers were successful with qPCR
- This allows the numbers of spores in soil samples to be correlated with the quantity of DNA

P. violae quantification

- Initial sensitivity of PCR detection revealed reliable detection of less than 25 oospores in 10 grams

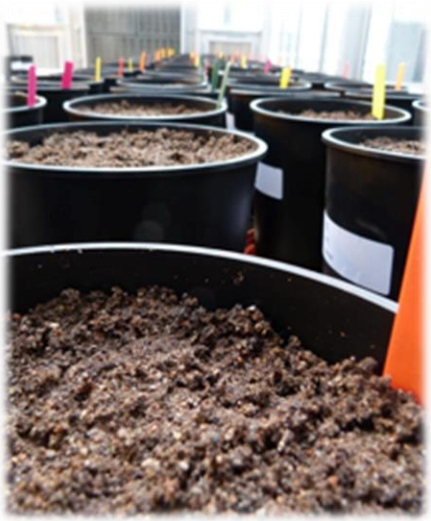


Artificial inoculation: Early infection

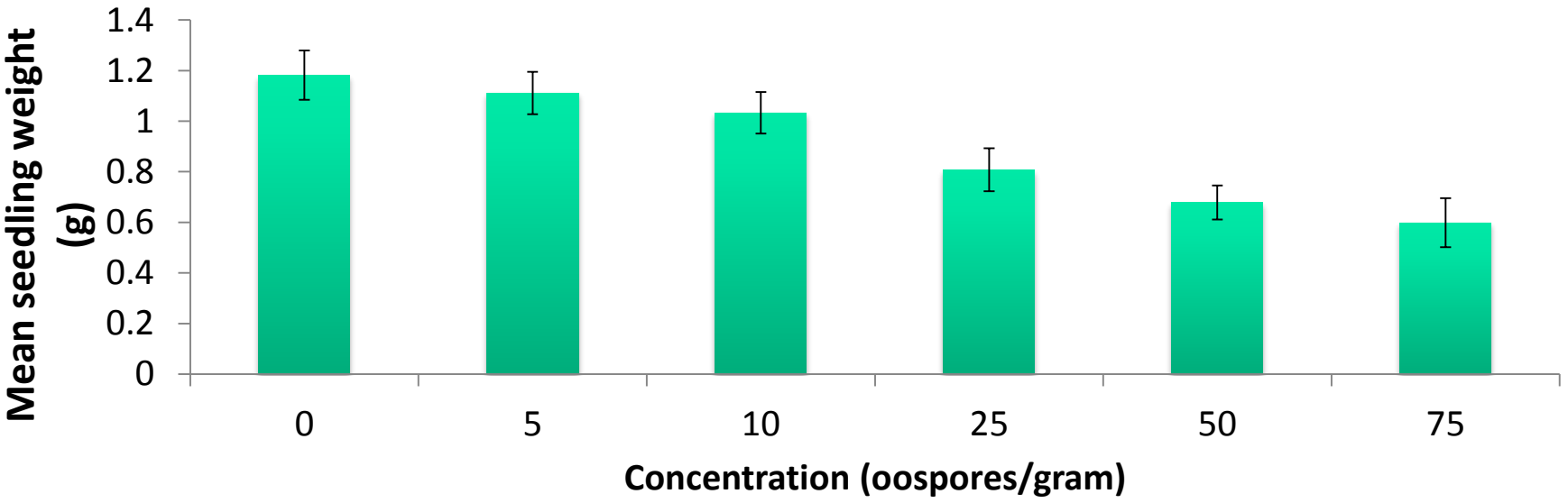
- A sand-based *P. violae* inoculum has been developed to try to produce dose-dependent damping off of carrot seedlings

Artificial Inoculation: Pot experiments

- A range of inoculum concentrations were mixed with soil and sand into large pots
- 10 carrots sown, thinned to 5 seedlings after six weeks
- Carrots grown in glasshouse controlled conditions to maturity for 22 weeks

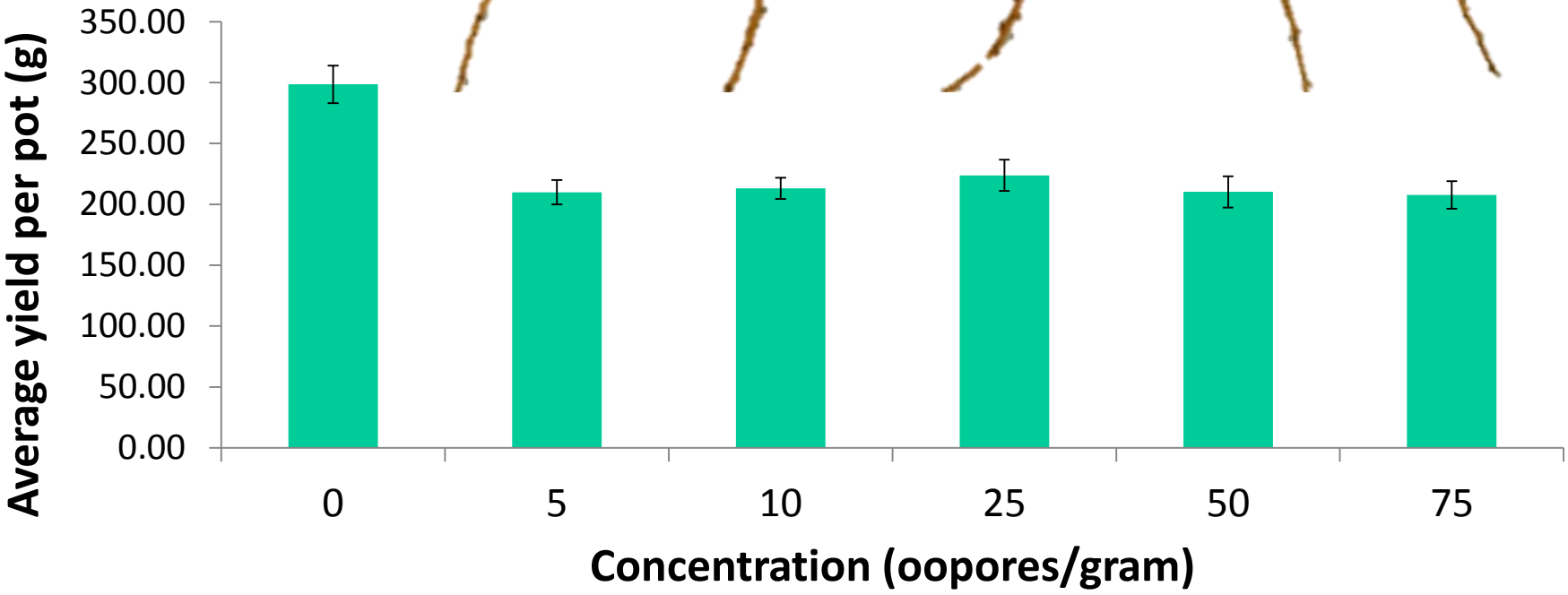


Artificial Inoculation: Pot expts. Top growth



Artificial inoculation: Pot expts. Root growth

- All inoculated treatments resulted in a failure of carrot roots to properly form compared to the inoculated control.



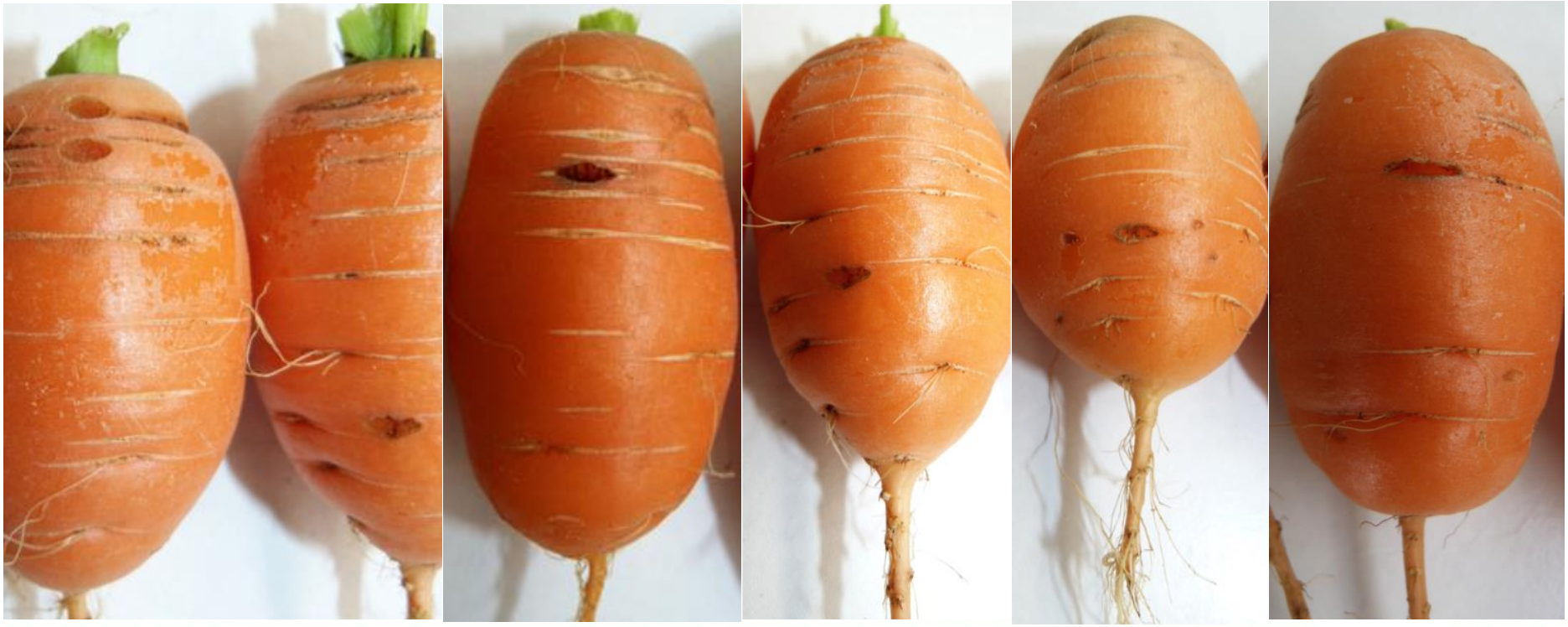
Artificial inoculation: Pot expts. Root colonisation

- Tap roots of inoculated carrots were darker than those of uninoculated control with collapsed lateral roots
- *P. violae* was isolated from the tap roots

Inoculated (top) and uninoculated control (bottom) roots

Artificial inoculation: Pot expts. Cavity spot

- Despite root stunting, cavity spot symptoms were observed on inoculated roots
- The surprising symptoms may be due to inoculation dose and/or time. Further trials are in progress to address this.



Monitoring a commercial carrot field

- Growers site in Yorkshire where significant cavity spot developed
- Samples taken for a 12 month period
- This temporal progression will be used to study *Pythium* development with the molecular techniques being developed

Early August

Late September

Summary and future work:

- Characterisation: over 120 isolates have been characterised; the major species causing cavity spot is *Pythium violae.* Isolates have been genetically characterised using housekeeping gene sequences.
- Method development: an 'oospore capture' and specific qPCR has been developed for *P .violae* to allow for more confident capture, detection and quantification from soil.
- Artificial inoculation: cavity spot has been induced in an artificial inoculation system producing some promising results, however this needs further development.
- Dynamics: samples collected from monitoring will be used with qPCR to quantify *P. violae* in field samples. Further analysis using 'whole amplicon' sequencing will be carried out to examine the whole soil community.

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