

WP1: *Phytophthora*
distribution, diversity and
management in UK nursery
systems



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- UK Horticultural industry success (£120 million)
- But vulnerability to pathogen spread remains (Jung *et al.*, 2015 review)
- Project supports industry to limit disease risk

Objectives

- WP1 objective I – Using metabarcoding to analyse community structure in nurseries and associated ecosystems
 - Providing a detailed insight into *Phytophthora* problems to improve disease management and advise ‘best practice’
- WP1 objective II – *Phytophthora* community modelling
 - Seeking explanations for variation in *Phytophthora* community richness among nurseries – trade, management and ecology

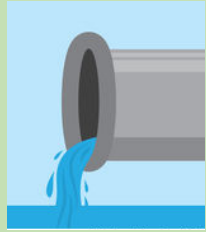
Methods

- Questionnaire – simple (6 questions) to collect basic data on nursery practices
- Sampling nurseries
 - Broad-scale – sampling alongside statutory plant health testing
 - Fine-scale – testing by project staff for more detailed breakdown of problems and solutions
- *Phytophthora* detection and metabarcoding
- Computational biology to process large sequence datasets
- Interpretation and provision of feedback to owners
- Use of data for Community modelling

Field



Roots



Capture spores on filter



Lab



Amplify DNA of pest/pathogen



Sequence DNA barcode



CCACACTGAGCTAAGGCCTTTAA
CCACACAGAGGTAAGGCCATTAA



Computer



Bioinformatics to identify species in sample

Results to inspectors & project team

Does *Phytophthora* metabarcoding work?

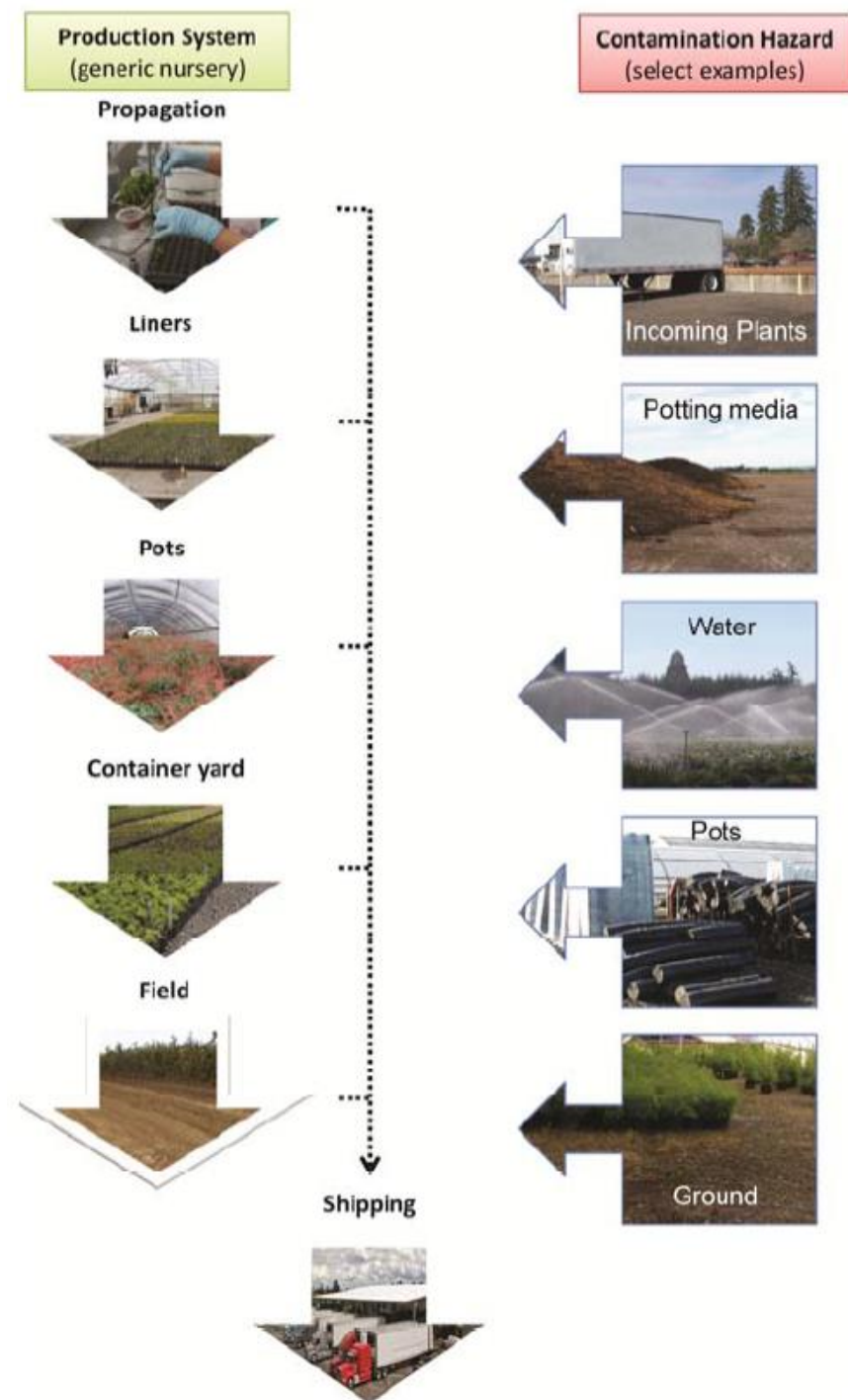
- Primers highly specific to *Phytophthora* and downy mildews
 - Scibetta et al 2012
- Several publications now indicating success sampling
 - Nurseries (roots and soil) (Prigigallo et al., 2016)
 - Soil and water (Catala et al., 2015)
 - Forests (Catala et al., 2016; Vannini et al., 2013)
- Scottish Government funded study in four Scottish streams detected 45 ‘species’ of *Phytophthora*; Many new to science and some unreported in UK to date
- Provides broad ‘baseline’ to help define what is ‘natural’

Sampling - theory

- Chance of interception depends on rate of epidemic growth, sample size and sampling frequency
- “Discovery prevalence” – (Parnell *et al.*, 2015)
- More samples taken at high frequency increases probability of early detection
- Validation important – what are the rates of false positives and false negatives?
- Current methods – tending to test for single quarantine species in specific samples

Sampling – practical issues

- What host plants ?
- Which plant parts?
- Water flowing through pots?
- How many samples per batch?
- Symptomatic or asymptomatic plants?
- Critical control points and contamination hazards (Parke et al 2012)
- Water supply – source and run-off
- Balance between time available and need for detail
- Draft protocols to be circulated for comment and nursery visit arranged



Variation between nurseries

- Parke *et al.*, 2014 found differences in *Phytophthora* diversity between nurseries in Oregon
- Prigigallo *et al.*, 2016 Metabarcoding from roots and soil in Italian nurseries

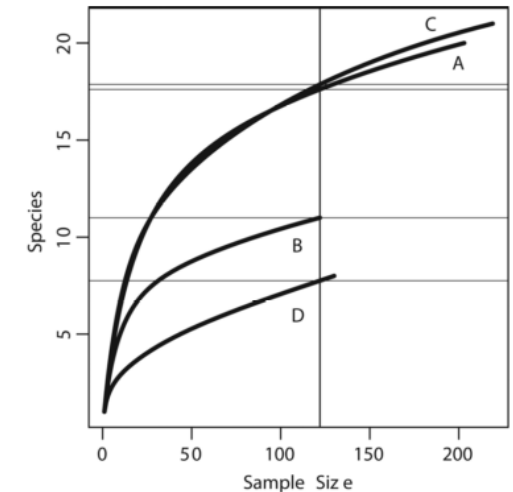
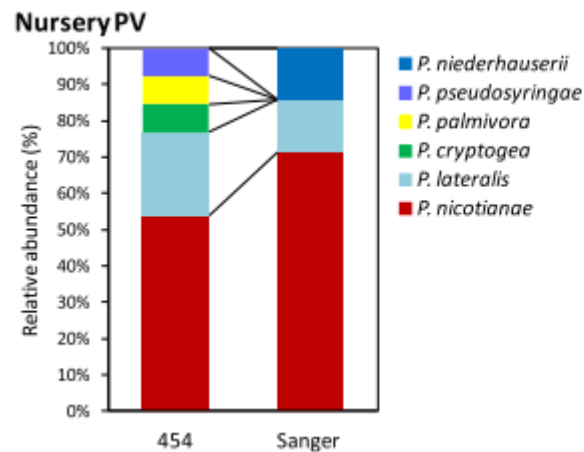
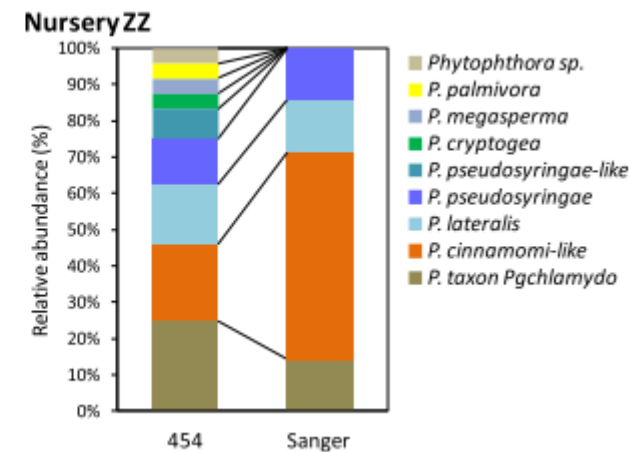


Fig. 1. Rarefaction curves for sampling *Phytophthora* isolates in nurseries A, B, C, and D.

- Related to hosts & management (e.g. water source)



Cyclamen, Tagetes, Petunia



Cercis, Punica, Arbutus, Rosa, Grevillea, Bougainvillea

Work programme

- **Nursery survey** – questionnaires and leaflets passed out
- **Broad scale sampling** as part of statutory testing by PMU and APHA
 - Approx 200 samples from 50 nurseries/garden centres England & Wales and 25 in Scotland to be sampled twice
- **Fine-scale sampling** of 10 ‘partner nurseries’
 - Critical control points sampled over three years, feedback provided and the effect of mitigations examined
- OPAL project – co-operation with David Slawson and staff associated with this project – **community sampling and engagement** in particular areas of recent planting/regeneration