Forest Research

Identifying *Phytophthora* risk in plant nurseries - an international collaboration between science and industry that is helping to shape best practice

Partner countries;

UK, **USA**, **Greece**, **Italy**, **France**, **Ireland**, Austria, Russia, NZ

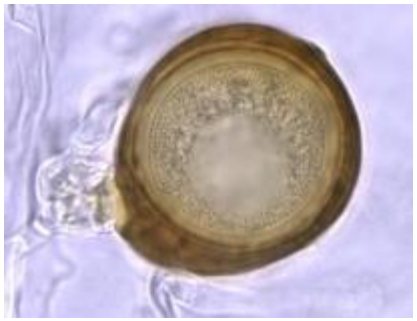
The James Hutton Institute

Euphresco

CEH Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

A few *Phytophthora* facts

- *Phytophthora* means 'plant destroyer'
- Oomycetes – 'water molds' spread via free swimming zoospores
- Borne in water and soil – can persist in soil
- Mainly infect plants through roots but some species also infect stems and foliage
- > 180 species described worldwide on broad range of hosts
- Emergent invasive *Phytophthora* species causing severe damage to landscapes globally
- Strong evidence for spread in trade



Objectives

- Six partner countries surveyed 2 plant nurseries twice (2020-2022)
- Identify *Phytophthora* pathogens using a standardised set of proven baiting and metabarcoding methods
- Identify and survey key industry stakeholders in each country to understand perspectives on biosecurity
- Develop and share best practice guidance across partner countries

Nursery sampling

Water samples filtered on site and collected for baiting from:

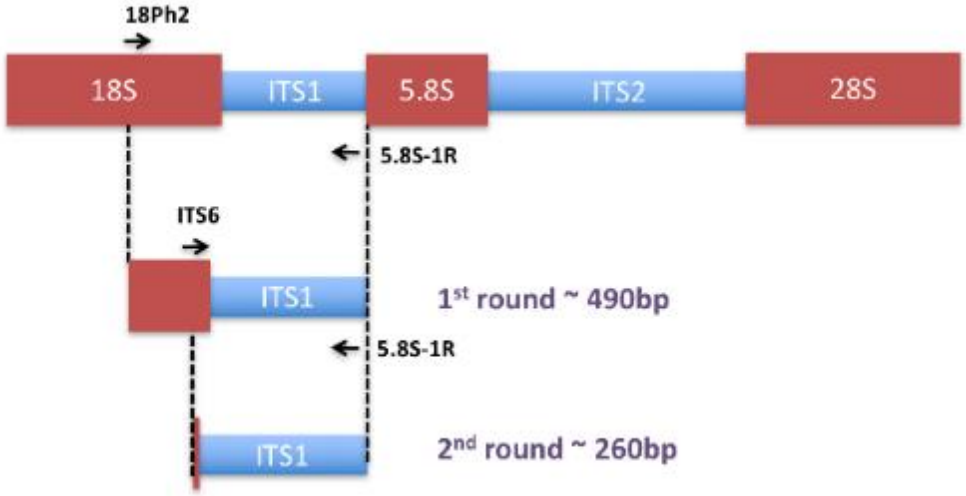
- Irrigation sources
- Drainage ditches, ponds and puddles
- Batches of plants watered and water flow-through sampled

Root sampling

- Roots sampled from batches of plants
- Also sampled roots from dumped plants

Metabarcoding testing outline

- **Sample Prep**
  - Water filter in buffer
  - Roots freeze-dried
- **DNA Extraction**
  - Buffer – kit
  - Roots & soil bead beating and kit
- **PCR** (Kappa polymerase)
  - Round 1 18PH2 & 5.8S1R
  - Round 2 ITS6 & 5.8S1R
- **Various steps to prepare Illumina plate**
- **Sequenced on an Illumina MiSeq**

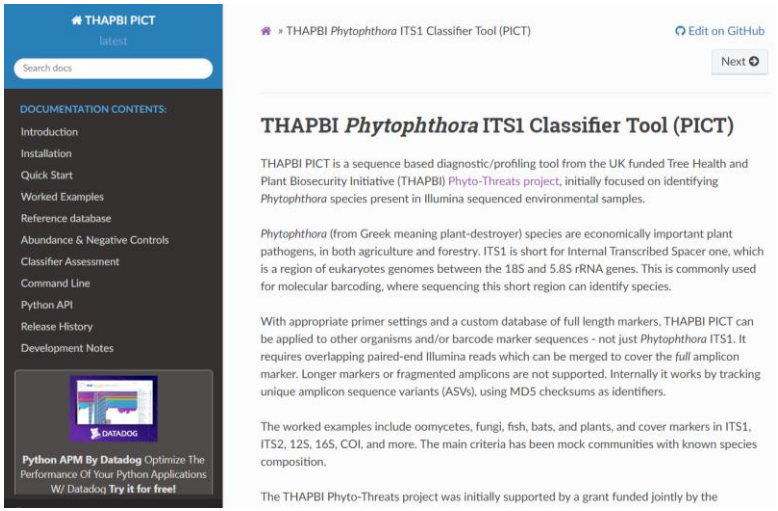


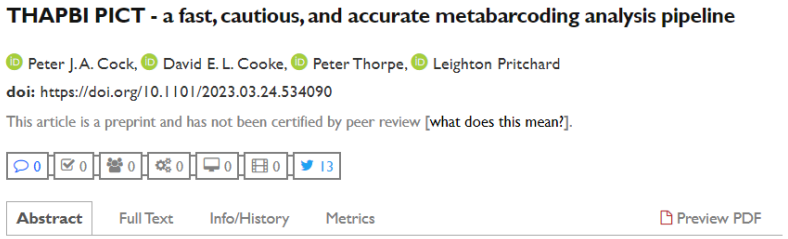
A molecular method to assess *Phytophthora* diversity in environmental samples

Silvia Scibetta [a,b], Leonardo Schena [a], Antonio Chimento [b,c], Santa O. Cacciola [b], David E.L. Cooke [d,e]

Illumina data analysis pipeline

- *Phytophthora* ITS1 classifier developed at Hutton, Dundee
- Development focused on minimising error
- Quality control and cleaning
- Use of synthetic DNA sequence controls
- Each unique sequence aligned to highly stringent reference database





Water baiting

- Water samples tested for *Phytophthora* using rhododendron and other bait leaves (and pears)
- Lesions plated onto selective media
- *Phytophthora*-like colonies subcultured onto V8 agar
- Colony PCR and sequencing using ITS4 and ITS6 primers
- Sequences identified to species based on 99% or more ID to verifiable Genbank sequence

Two UK nurseries

UK nurseries 1 and 2

- Producers of hardy nursery stock
- Annual turnovers £1.5m and £2.8m
- Mix of home propagated and imported (NL, France, Germany, Italy, Portugal, NZ, Ethiopia, Kenya)
- Mostly landscaping/amenity (80%)
- Each nursery sampled twice by project team

Nursery 1:

- 26 known *Phytophthora* species across two sampling visits
- ***Phytophthora* species of particular concern**:
  - *P. ramorum* (*Rhododendron*)
  - *P. austrocedri* (*Cupressus x leylandii*)
  - *P. uniformis* (roots of *Prunus* and *Tsuga* in plant dump)
  - *Phytophthora macrochlamydospora; quininea* (*Pinus mugo*)

Results UK

- **Hosts of particular concern at Nursery 1**:
  - Rhododendron
  - *Lavendula*
  - *Cupressus x leylandii*
- **Water source of concern**; stream draining into open reservoir
  - 11 *Phytophthora* species in irrigation water
- **Puddle water** also contained *Phytophthora*
- **Plant dumps** a risk factor too

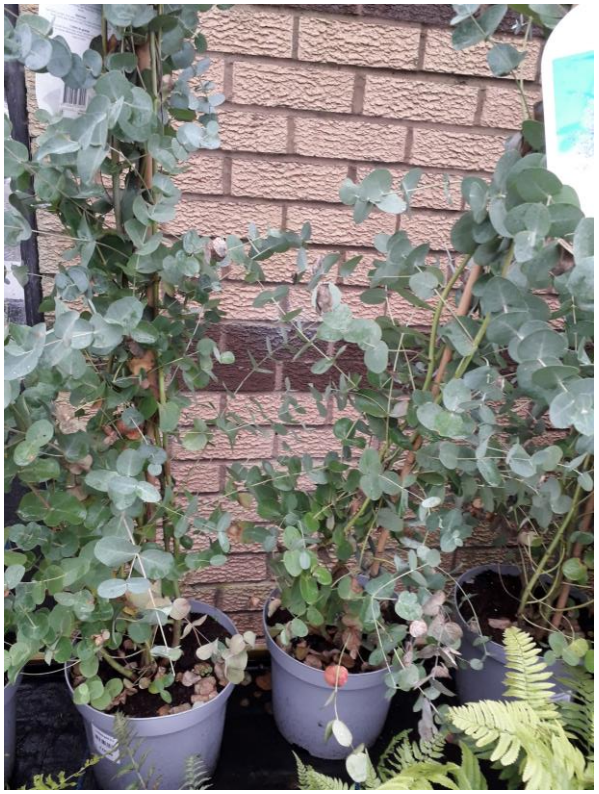
Nursery 2:

- 30 known *Phytophthora* species across two sampling visits
- ***Phytophthora* species of particular concern**:
  - *P. uniformis* (*Lavendula*, *Physocarpus opulifolius*)
  - *P. pachypleura* (*Physocarpus opulifolius*)
  - *P. occultans* (*Buxus*)

Hosts of particular concern at nursery 2:

- *Physocarpus opulifolius*
- *Thuja*
- *Eucalyptus*

- Imported stock had *Phytophthora*
- Puddle water contained DNA of 6 *Phytophthora* species
- Mains water clean
- **Evidence for *Phytophthora* contamination in propagation facilities** (particularly *P. cryptogea/P. pseudocryptogea*)



Results US

- Two large ornamental plant nurseries sampled in California
- 20 *Phytophthora* species detected at Nursery 1
- *7 Phytophthora* species detected in Nursery 2

J. Del Castillo, UC Davis

Risky hosts:

- *6 Phytophthora* pathogens found on *Lycianthes rantonnetii (*blue potato bush)
- *Cistus purpureus* (rock rose) harbored the pathogenic *P. niederhauserii*, and *P. tropicalis*

J. Del Castillo, UC Davis

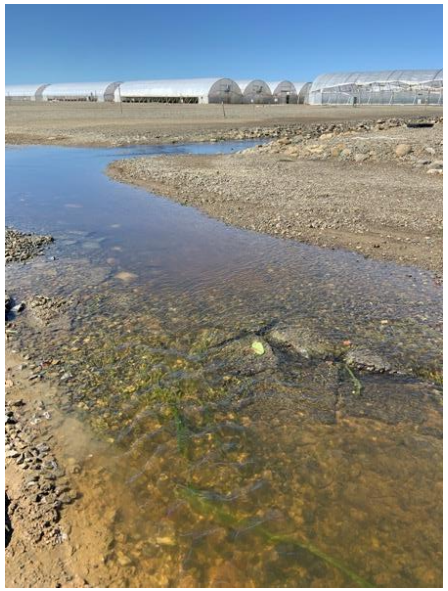
Results US

Water risk:

- Nursery 1 uses recycled irrigation water which is not treated prior to reuse
- Recycled water is the main source of *Phytophthora* contamination of nursery stock
- Run-off water contained *Phytophthora*

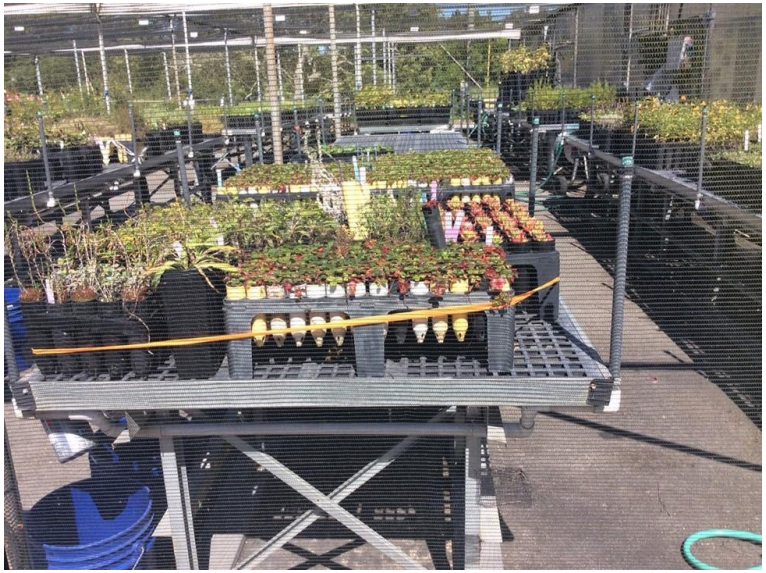
Key management advice:

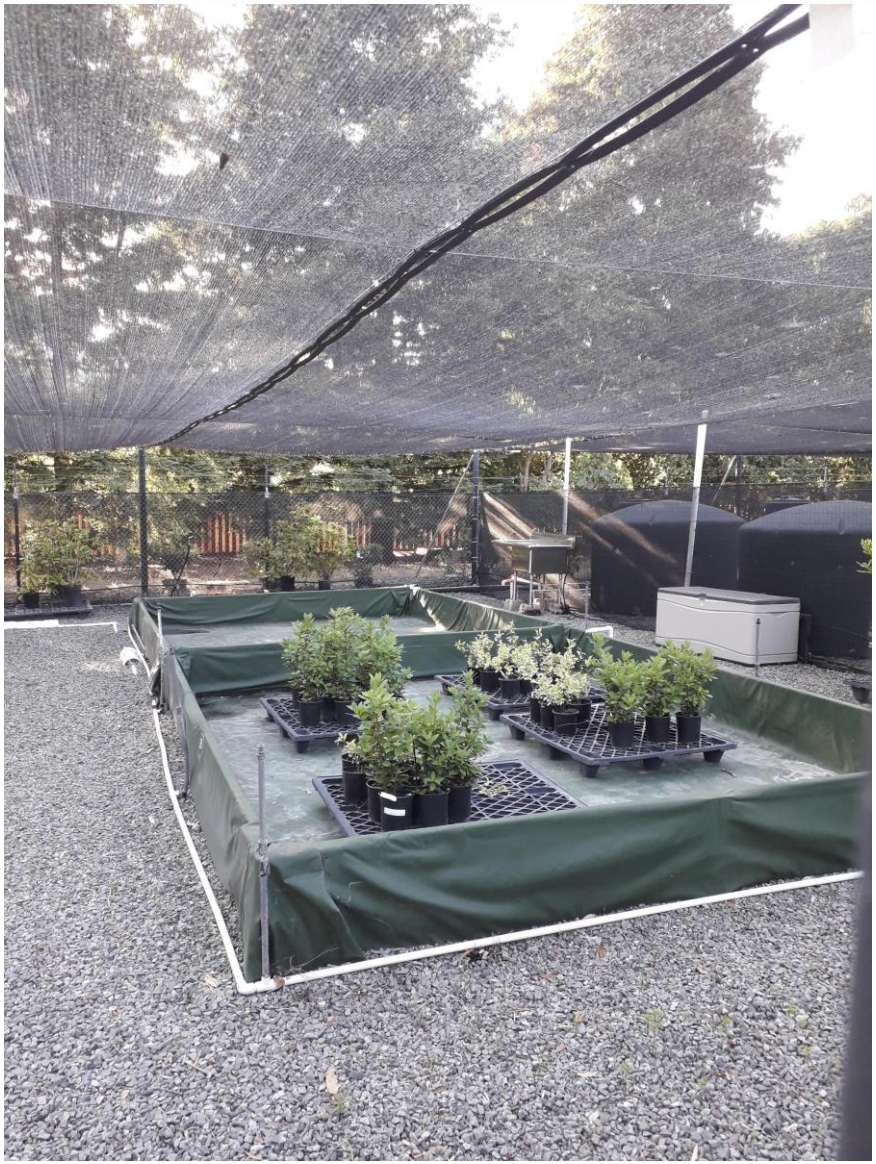
- Treat water prior to use – variety of options discussed with manager



J. Del Castillo, UC Davis

Native plant nursery - California





Results Italy

Nursery 1:

- Small experimental nursery
- Produces stock for field trials
- 99% stock propagated on site

Water risk:

- 11 *Phytophthora* species detected, mainly in water samples
- 9 species baited into culture from reservoir which stores mains and borehole water

D. Migliorini, CNR

Results Italy

Nursery 2

- Large ornamental nursery
- 95% stock bought in, 30% imported from NL, Belgium, France
- 21 *Phytophthora* species detected across range of water and root samples

High risk hosts:

- *Choiysa, Viburnum* and *Ligustrum* harbour high diversity of pathogens

D. Migliorini, CNR

Results Greece

- Sampled two ornamental plant nurseries
- Trade within EU and third countries including Kenya, China, Australia, Costa Rica, USA
- 8 and 16 known *Phytophthora* species detected at Nurseries 1 and 2, respectively
- Findings included some rare, warm temperature pathogens

D. Kiziz, BPI

Results Greece

Water risk:

- *Phytophthora* found in irrigation and run-off water

High risk hosts:

- *Myrtus, Lavendula* and *Azalea* hosted high *Phytophthora* diversity

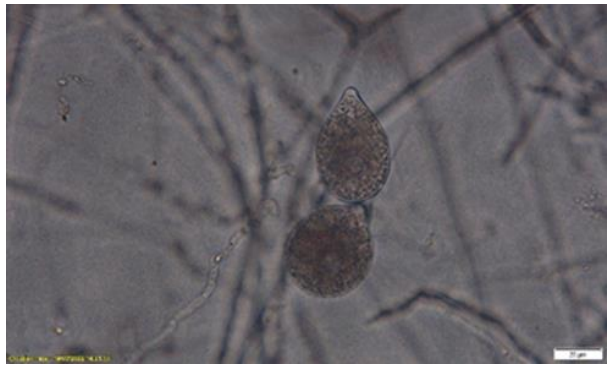
Management change:

- One nursery implemented a copper-based and desalination treatment prior to irrigation
- Water tested after treatment was <u>negative</u> for *Phytophthora*

D. Kiziz, BPI

Combined analyses

- Analyses by Louise Barwell, CEH
- Data from 1011 samples from 13 nurseries in 6 countries (UK, US, IT, GR, FR, IE)
  - 647 root samples
  - 364 water samples
- 65 unique *Phytophthora* species identified
- 86 samples contained unknown/unidentified *Phytophthora* species



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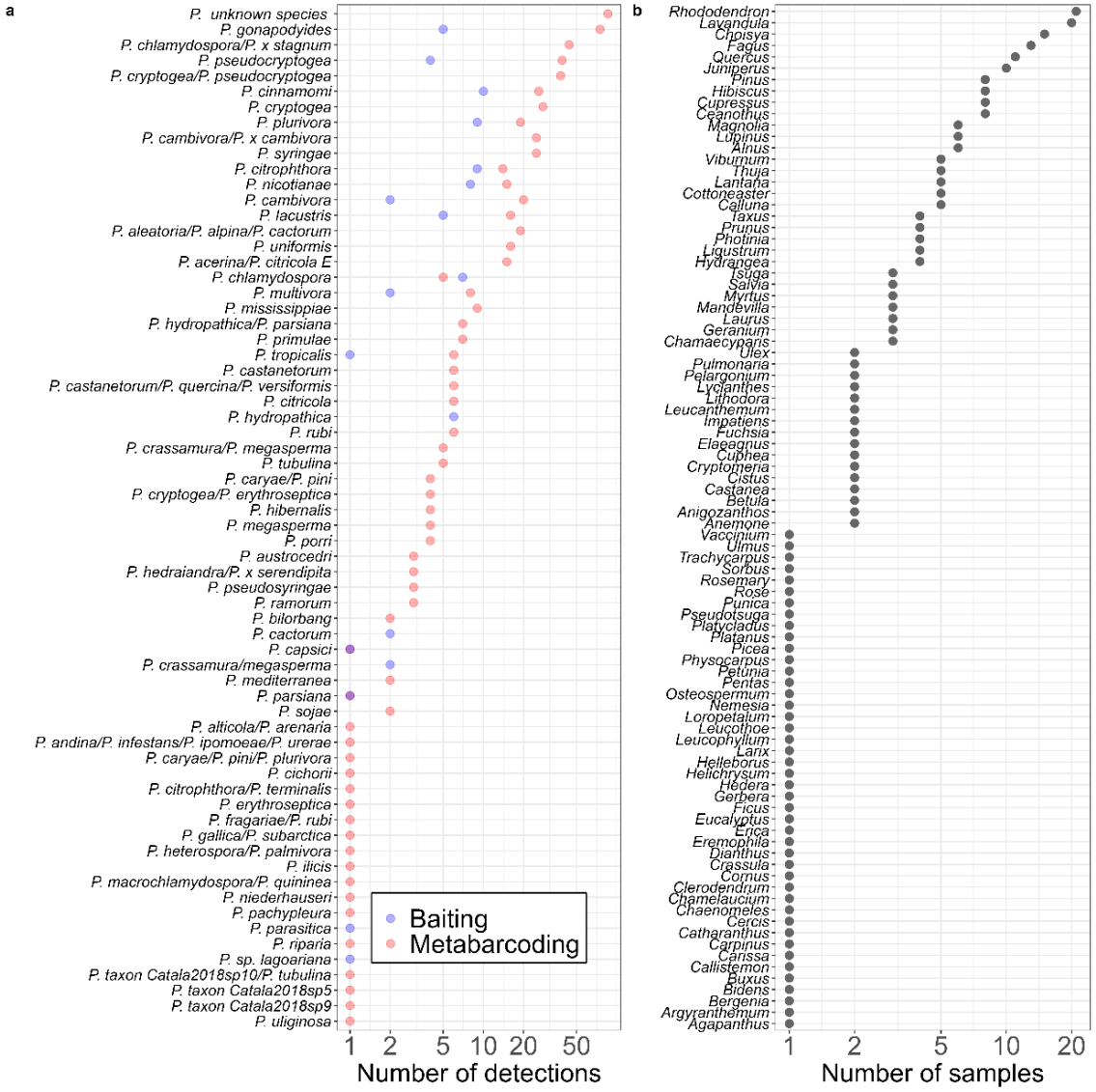
Overview of species/hosts



Modelling framework

Questions tested by analyses:

- Effect of selected nursery management practice on *Phytophthora* positive samples
- Whether *Phytophthora* communities differ among water and plant samples
- Effect of host genus and latitude

Nursery attribute	Potential relationship with phytosanitary risk	variable type (units)
Size	Larger sites may incur greater costs in implementing best practice	numeric (Hectares)
Latitude	Regional differences in attitudes to plant health relating to, for example, different agencies responsible for plant health inspections and advice, or proximity to recent high impact outbreaks. *Phytophthora* species differ in their thermal tolerances and some warm-adapted species may be absent or rarer at higher latitudes.	numeric (decimal latitude)
Turnover	Rapid turnover of stock and larger businesses potentially with greater financial resources for phytosanitary investment	numeric (£)
Spend on buying in plants	Bringing infected material into the nursery. May also reflect greater financial resources for phytosanitary investment, as above	numeric (£)
Irrigation sources	Water taken from open or closed sources. Open sources include ponds, river, streams, springs, canals and reservoirs. Closed sources include mains and borehole. If closed sources were stored in an open tank these were classed as open sources.	binary (0, 1)
Water treatment	Water is untreated before use. Treatment includes sodium hypochlorite, chlorine dioxide, sand filters and screen filters	binary (0, 1)
Growing medium	Own mix used rather than commercial compost. The latter requires a high temperature phase to reduce risk of contamination.	binary (0, 1)
Reuse pots	Re-use of pots (with or without disinfection in between).	binary (0, 1)
Raised benches	No raised bench facilities to keep containers off the ground and away from standing water and soil contact	binary (0, 1)
Disinfection mats/stations	No facilities for the disinfection of plants and containers	binary (0, 1)
Import whole plants	Whole live plants are imported from outside of the UK	binary (0, 1)
Quarantine holding area	If live plants are imported, are there quarantine areas for holding away from main stock?	binary (0, 1)
Plant disposal	Plants and organic material dumped on site	binary (0, 1)

Effect of management practice

Preliminary results:

Nurseries with;

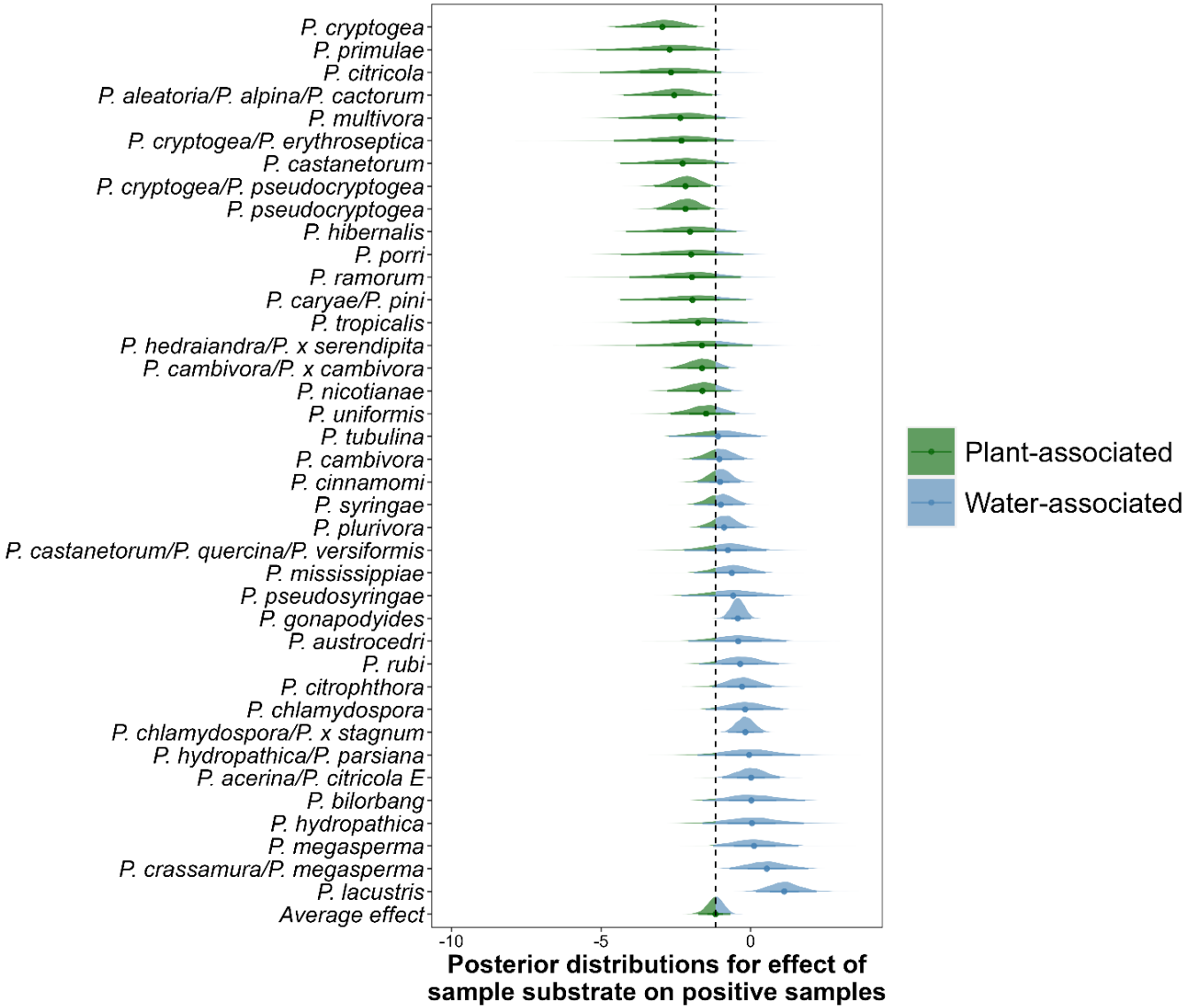
- Diverse plant stock
- >50% imported plants

had more;

- Positive samples
- Diverse *Phytophthora* communities

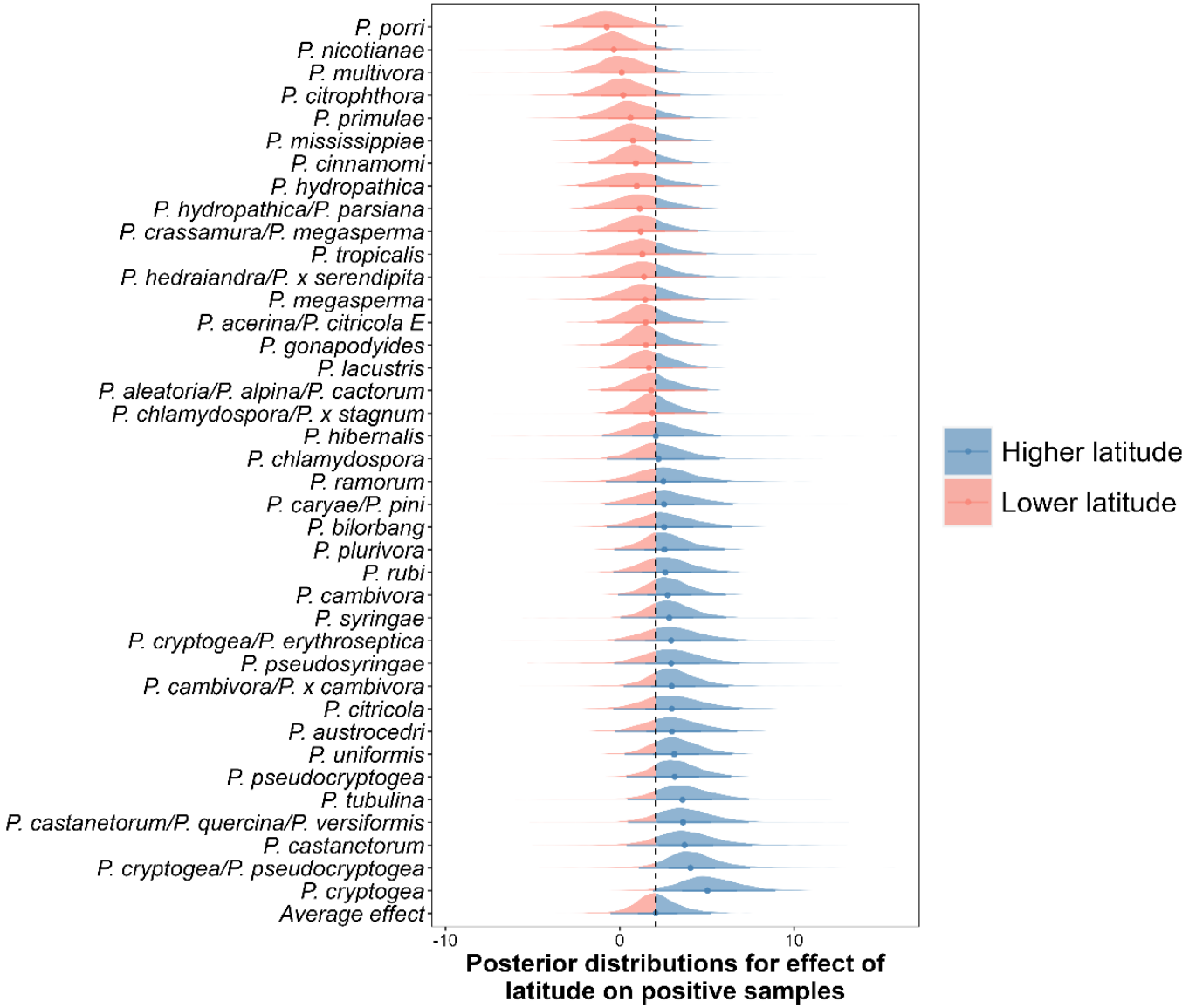
D. Kiziz, BPI

Species sensitivity to sample substrate



Prevalent host/*Phytophthora* associations

Species sensitivity to latitude



Summary of overall results

- Very high diversity of *Phytophthora* spp. across countries
- Baiting and metabarcoding data reveal *Phytophthora* in irrigation water
- Some species able to thrive in both roots and water
- Reliance on importation from other countries a risk factor
- High diversity of stock a risk factor

Management recommendations

Reports with advice to nursery managers;

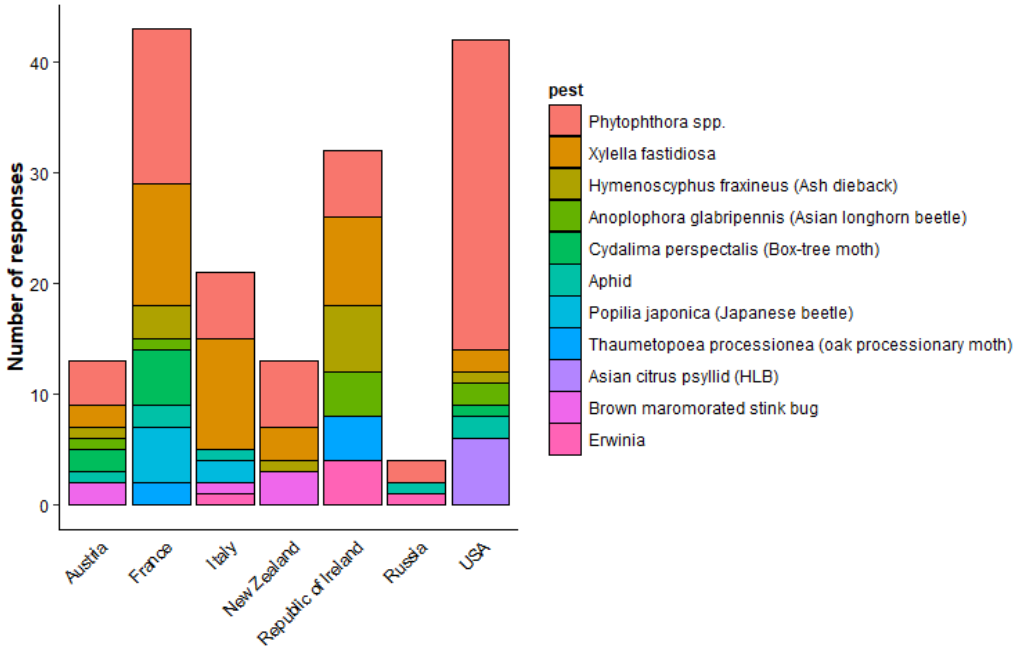
- Use of open water supplies is high risk, especially if recycled – treatment needed
- Sanitise surfaces between batches
- Disinfect all propagation tools regularly
- Raise more plants off ground
- Control irrigation/improve drainage
- Reconsider trading in high risk hosts
- Inspect and quarantine imports away from other stock
- Identified need for staff training
- Advice needed on safe waste disposal options

Stakeholder survey

- 94 responses from plant trade stakeholders
- 115 different pests/pathogens named as being of most concern

Message for communicating best practice:

- Emphasise benefits of best practice for tackling *many* pests and pathogens

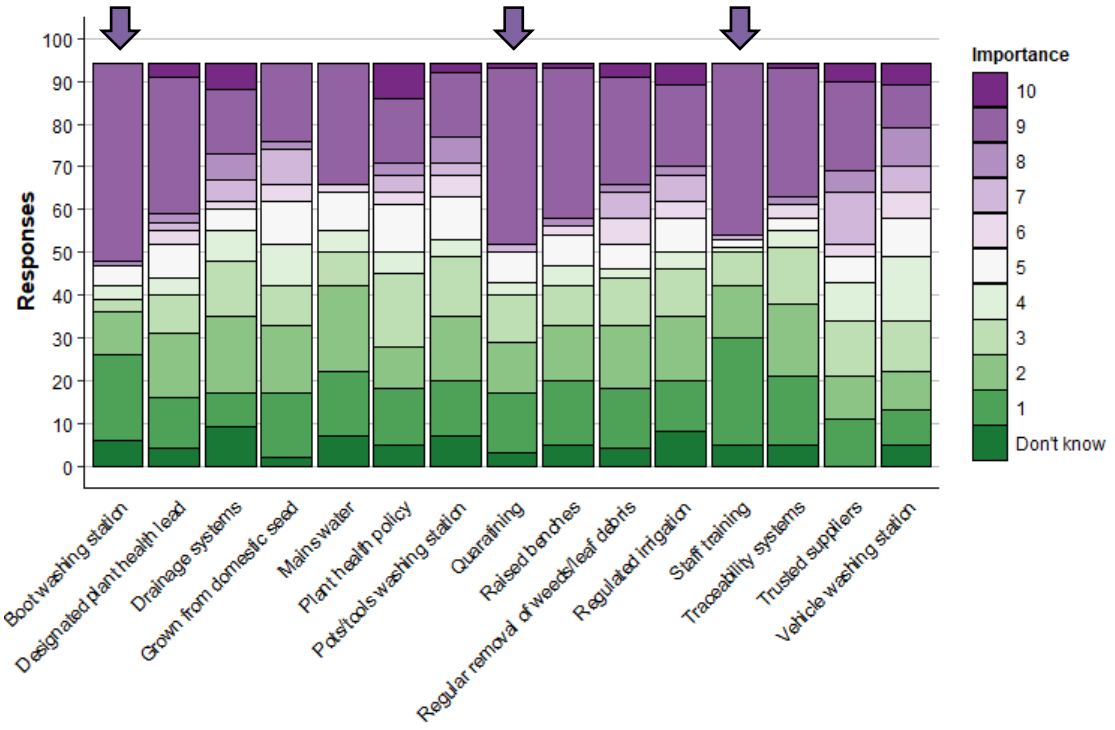


Perceptions on usefulness of biosecurity actions

- Boot washing station, quarantining plants and staff training seen as most important
- However, few respondents thought any of these actions would work!

Message for communicating best practice:

- Linking actions and impacts – showing that taking action does work!



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Reducing *Phytophthora* risk in nurseries – key considerations

Globally, environments are threatened by increasing numbers of invasive plant pathogens which can be spread in infested planting material. Invasive *Phytophthora* species in particular are responsible for many plant disease epidemics around the world. Their life cycles allow them to thrive in nursery environments, in plants, water and soil; and spread to wider landscapes.

A recent Euphresco project involving nine countries found that *Phytophthora* pathogens were widespread in many of the plant nurseries surveyed in participating countries. The risk from *Phytophthora* and other pests and pathogens can be reduced by ensuring good biosecurity practices are applied throughout plant supply chains.

Symptoms of *Phytophthora ramorum* on Rhododendron.

This guidance aims to help those growing or handling live plants to follow good biosecurity practices and reduce their *Phytophthora* risks from trade pathways. By considering these points, plant health will be protected in your nursery and in the landscapes your plants are placed into.

Key plant biosecurity considerations

Plant origin

Any movement of plant material through trade carries the risk of introducing *Phytophthora* into new areas. Reliance on imported stock increases *Phytophthora* prevalence and poses additional risks of introducing new *Phytophthora* species to a country or region.

Plant hosts

Phytophthora can infect any live plant material, including woody plants, and persist in growing media/soil. Assess the biosecurity procedures of all your suppliers and check that all stock arriving on site is 100% healthy.

Be aware of symptoms

All symptoms, including foliage discoloration or desiccation (wilting), however minor, should be investigated by a diagnostic lab and any affected plants removed from growing areas. See below for recommendations on plant disposal.

Phytophthora can be present in the absence of visible symptoms. Following the recommendations in this document will reduce this risk.

Assess your site(s) and know your suppliers

- Water source: Water is a carrier of many pathogens. Mains or underground water supplies tend to be low risk. Sourcing water from open reservoirs, ponds, rainfall butts or extracting from rivers, or using recirculated water can carry a higher risk, unless the water is treated using a method proven to kill damaging microorganisms.

Open irrigation reservoirs are often contaminated with *Phytophthora*.

- Drainage: Puddles and excess run-off can harbour and spread waterborne pathogens. Containerised plants should be grown on a clean, free-draining surface, raised above the ground. Persistent puddles in nurseries and plant retail areas – especially on roadways – are high-risk for pathogen movement.
- Potting mix: Potting mix should be stored in a covered area to prevent contamination by air-borne pathogens.
- Quarantine areas: 'Quarantine areas' should be used to ensure that imported stock is well separated from other plants. These areas should be monitored over several months for symptoms of *Phytophthora* on foliage such as black-brown leaf or stem lesions, bronzing and wilting.
- Surroundings: Shelterbelts and landscape trees/shrubs growing in and around the nursery premises should be monitored for disease symptoms.
- General nursery hygiene: The nursery site should be free of weeds, spilt soil/potting mix and piles of soiled pots. There should be facilities for disinfestation of tools, pots and boots.
- Plant disposal: Dumping unhealthy or unwanted plants close to the nursery premises carries a high risk of spreading *Phytophthora*. Plants should be disposed of through a contained composting system and isolated from stock or natural ecosystems. Reuse of growing media is high risk and should be avoided.

Burning of infected stock.

- Plant health knowledge: Every nursery should have a biosecurity management plan and a staff member trained in plant health and symptom awareness. All staff and visitors should be informed of site biosecurity procedures.

More information

Further details of our work on biosecurity is available at:
Insert link to each science team website
To discuss any aspect of insert institution name work on biosecurity threats, contact:
Insert main contact email address

Euphresco

The James Hutton Institute

www.forestresearch.gov.uk

Guidance on symptoms and high risk hosts

Forest Research

Symptoms of Ill Health in Plants: An Introductory Guide for the Horticultural Sector

Invasive plant pests and pathogens are having increasing impacts on UK landscapes, often due to being spread by infested planting material via trade pathways. These risks can be mitigated by ensuring that only healthy stock is grown and planted.

This guidance aims to help the professional operators responsible for growing or handling live plants to identify and act upon symptoms of ill health. A key purpose is to avert potential new pest and pathogen outbreaks that can damage horticultural businesses, trade and wider landscapes.

Symptom awareness

Know what a healthy plant should look like

Know what the habit and healthy colour of each plant species that is grown and handled should look like, so that malformed or 'off-colour' plants can be identified and assessed. It is also essential to know the main pests and pathogens affecting the plant species grown or handled, as well as the typical symptoms to look out for.

Build your plant health knowledge

Every nursery or horticultural site should have at least one staff member trained in plant health and symptom awareness.

Monitor stock regularly

A plant health 'crop walk' should be carried out across the entire nursery or horticultural site on a regular basis. Action should be taken to confirm the presence of suspected pests and pathogens and deal with symptomatic stock. This action includes the isolation of symptomatic plants away from remaining stock and seeking an accurate diagnosis of the cause from a diagnostics lab (see below). If the pest or pathogen is suspected to be notifiable then immediate contact should be made with the relevant plant health authority (see below).

Diagnose the cause

Organisations such as Forest Research (in case of tree nurseries) and the Royal Horticultural Society offer diagnostic services to growers. APHA and SASA are the competent plant health authorities and will investigate concerns around statutory pests and pathogens. A correct diagnosis is crucial to effective control.

Forest Research

Understanding high risk hosts for *Phytophthora*

Invasive *Phytophthora* spp. are responsible for many plant disease epidemics around the world. Their lifecycles allow them to thrive in nursery environments and spread to wider landscapes via infected propagation material.

A recent scientific study investigating *Phytophthora* diversity in plant nurseries identified certain plant species that are of higher risk in terms of harbouring *Phytophthora*.

This guidance aims to help those growing or handling live plants to manage their *Phytophthora* risk by understanding which hosts pose the greatest threat. Bear in mind however that *Phytophthora* can infect any live plant material, including woody plants, and persist in growing media/soil. To avoid spreading these plant pathogens, assess the biosecurity procedures of all your suppliers and check that all stock arriving on site is pest and pathogen free.

High risk hosts for *Phytophthora*

Hosts at higher risk of carrying quarantine regulated or newly invasive species of *Phytophthora*

- *Rhododendron* and *Viburnum* are the two most common hosts for *P. ramorum*
- *Chamaecyparis, Cupressus x leylandii* and *Juniperus* are hosts for *P. austrocedri* and *P. lateralis*

Symptoms of *P. ramorum* on *Rhododendron*.

Cupressus x leylandii infected with *P. austrocedri*.

- *Buxus* is frequently infected with *P. occultans*

Buxus with symptoms associated with *P. occultans*.

Plant Health Centre
Scotland's Centre of Expertise

Scottish Government
Riaghaltas na h-Alba
gov.scot

Biosecurity best practice for safe disposal of plant waste and spent growing media

Research has shown that plant waste and spent growing media can harbour pests and pathogens, including quarantine-regulated species such as *Phytophthora ramorum*. These organisms have the potential to spread from untreated waste piles to cultivated plants and the wider environment. This guidance aims to help the horticultural sector understand and mitigate these risks by providing some practical advice on waste management.

*Waste and environmental legislation is a devolved issue so please note that if you are using this guidance outside Scotland, the relevant national licencing authorities will need to be contacted.**

Minimise waste and risk of infected waste material by growing clean plants

The best way to limit waste volumes and to minimise the risk of pests and pathogens proliferating in waste is to ensure that plant stock is sourced and grown to high standards of biosecurity and is monitored frequently to ensure that plants remain visibly healthy/ symptom-free. Various types of growing media ingredients and mulches (e.g. pot tops) can also harbour and be sources of pests/pathogens.

not therefore be used as a component of growing media used to grow plants which are then sold off the nursery. In any case, it is very difficult to manufacture growing media of sufficient quality when using home-produced compost as one of the main constituents.

- As an additive to soils in ornamental and stock beds to add fertility and improve soil structure
- A surface mulch in ornamental and stock beds

Understand your obligations

Follow plant health regulations, e.g. the notification scheme for importing some high-risk plant species, which, along with Plant Passports and Phytosanitary Certificates, aim to protect against the introduction and spread of pests. More details are available on the Plant Health Portal.

Plant Healthy Certification Scheme

One way of having confidence in the health of the plants you grow and handle is by becoming certified. The voluntary Plant Healthy Certification Scheme, now being rolled out in the UK, is based on the Plant Health Management Standard.

A focus of the Standard is identifying key notifiable pests and their life-cycles that present a threat to a business or organisation. The requirements present a pest management framework to minimise pest risk throughout a site and the associated operations.

Help safeguard our cultivated and native flora by promoting biosecurity across the supply chain by becoming Plant Healthy certified and by sourcing from horticultural businesses who are scheme members. More details are available at: planthealthy.org.uk

More information

Further details of our work on biosecurity are at:
https://www.planthealthcentre.scot/

*National licencing authorities:
Environment Agency
Natural Resources Wales
Northern Ireland Environment Agency

Royal Botanic Garden Edinburgh | Earthcare TECHNICAL | Forest Research | Plant Healthy

Training in plant health for horticultural sector

- Two training days planned for autumn 2023
- Ideas/suggestions/comments?

UK participants and partners

Forest Research

- Sarah Green, Debbie Frederickson-Matika, Mariella Marzano, Chris Pollard, Mike Dunn

The James Hutton Institute

- David Cooke, Eva Randall, Beatrix Keillor, Peter Cock

CEH Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

- Lousie Barwell, Beth Purse